SECONDARY METABOLISM OF LICHENS AS THE CYTOTOXIC AGENT TO KILL THE CANCER CELLS

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Abstract
Cancer is competing with cardiovascular diseases to rank as the number one cause of fatality worldwide. The distinctive nature of cancer cells is to grow and multiply regardless of the inhibitory signals to avoid apoptosis. Lichens are organisms formed from a combination of fungus and algae or cyanobacteria, and these components are in a mutual relationship. Lichens produce primary and secondary metabolites and hormones due to environmental changes or infection. These metabolites have an essential role in protecting the plants from exogenous invaders. For this reason, these creatures can survive drastic environmental changes and severe infections. Over the years, many researchers have tested the secondary metabolites in vivo and in vitro and found promising antitumor, antifungal, antiviral, antibacterial, and antipyretic properties. These beneficial activities were enticing to many pharmaceutical companies. These secondary metabolites can be classified according to their biochemical structure, biosynthetic pathway, and maturation. In this particular study, we are focusing on the structure of Lichen secondary metabolites, biosynthesis, and anticancer activity. Secondary metabolites can inhibit carcinogenesis by targeting multiple stages of growth and angiogenesis signaling mechanisms and induction of apoptosis and autophagy in tumor cells. The programmed cell death is induced by activating mTOR and JNK signaling pathways and promotes the expression of p53, p38, Bax, and cleaved PARP genes. At the same time, autophagic tumor cell death is instigated by stimulation of the LC3II protein. On the other hand, secondary metabolites activate cell cycle arrest by hindering cyclin-dependent kinases 2, 4, cyclin D1, cyclin B1, and Cdc25C gene expression. Lichen increases the production of reactive oxygen species, which causes suppression of tumor cells by negatively regulating the biosynthesis of ATP and nucleotides. Secondary metabolites provoke the immune response against cancer cells. Clinically, despite the evolution of cancer medicines, a wide range of complications are documented.
Keywords: Lichens, Secondary metabolites, anti-cancer therapy, in vivo, invitro

INTRODUCTION

Cancer is a fatal disease around the world. In 2020, 19.3 million new cases were reported, and approximately 10 million deaths were announced. Elevating cancer is the first or second most common cause of death in more than half of the world's nations by the age of 70 (Ferlay *et al*., **2019**). Despite continued progress in cancer therapy, cancer is still one of the most health problems in the world. Because of the side cytotoxic effect of synthetic compounds of drugs, researchers recently increased their attention to using plants in pharmacological research and medicine development as an alternative therapy for disease (Zeeshan *et al*.,2022). The plant secondary metabolisms are a reservoir of active compounds that can be used in many scientific areas in pharmacy, medicine, and biology. The natural compounds present in plants contribute actively to the improvement of the treatment of cancer in experiments in vitro and in vivo studies using animal models (Zhang *et al*.,1999). Plant-derived products are used as potential cancer therapies alone due to their potent anticancer activities and less likely to cause side effects (Sevastre *et al*., 2022; Padmapriya, 2017), or combined with chemotherapy or radiotherapy to increase the therapeutic efficacy or reduce side effects for conversational anticancer drugs. (Kapinova *et al*.2017). There are a lot of secondary metabolism compounds figured out in plants each year. They are used as therapy for many diseases, opening the field to many researchers to use them as anticancer (Ceramella *et al*.,2019). Nowadays, more than half of the anticancer drugs are derivatives of plant metabolites. Plants derived produce secondary metabolites that can target multiple signaling mechanisms of cancer cells. Secondary metabolites have revealed promising antitumor effects in vitro and in vivo studies (Bray *et al*.,2018).

Lichens, symbiotic creatures compositing of mycobionts, which are fungal partners and photosynthetic that can be either green algae or cyanobacteria, produce a wide range of secondary metabolites, including xanthones, dibenzofurans, depsides depsidones, dibenzofurans, and anthraquinones (Solárová *et al*.2020). These compounds represent a class of more than 10,000 symbiotic organisms traditionally used since the ancient era in diverse chemical and biological functions such as antioxidant, antifungal, antiviral, antibacterial, antifungal, and anticancer. (Baĉkorová *et al*.2011; Bhatti *et al*., 2022). The

therapeutic applications of lichen extracts in traditional medicine are commonly presented in treating digestive system, respiratory, skin disorders, wounds, and gynecological and obstetric issues (Crawford,2019). In Japan, compounds extracted of lichen are widely utility in industry of cosmetics, medicine and food (Kumar *et al*., 2020). The several types of crude secondary metabolite compounds isolated from liches demonstrated high cytotoxic activity in induction apoptosis against different human cancer cell lines such as breast (MCF-7), lung (H1975), and colon (HCT-116) (Bhat *et al*.,2022). Also, the mycotoxin, a photoprotective molecule in lichens, is used as an anticancer agent for treating melanomas (Roullier *et al*.,2011). The inhibitory effects of the secondary metabolite of lichens were higher in the inhibitory growth of cancer cells compared with normal cells (Nguyen *et al*.2019).

This article aims to provide modern information about the structure, classification, and biosynthesis of lichen secondary metabolites. We discuss the capability to use these secondary compounds as anticancer therapy to prevent cancer development. Also, it will investigate the study effect of these compounds on various signal pathways involved in cancer or carcinogenesis.

1- Lichens definition- structure -classification

Lichens are stable, consistent, and identifiable mutualistic associations between green algae and cyanobacteria (the photobiont) and fungi (the mycobiont) (Dobson, 2011). During this symbiotic relationship between the two partners, the photobiont partner is affected by the presence of the fungus, as its wall becomes an outlet for the carbohydrates it manufactures during photosynthesis. Hence, these materials infiltrate and are absorbed by the fungus. The fungus provides moisture to the photobiont, which is already present within its tissues, by absorbing water through its hyphae. The fungus also works through its tissues surrounding the photobiont and the pigments it produces as a protective shield for the photobiont from exposure to excess light. So, the fungus created new habitats for the photobiont, which it was unable to inhabit in the free-living state; from these habitats, the bark of trees, rocks, and other known habitats for lichens (Brodo *et al*., 2001). The mycobiont is responsible for adhering the lichen to the stone or surface and adsorbing water and minerals (Goga *et al*., 2018). The fungal part of the lichen mostly belongs to ascomycetes, as the latter represents 98 %, while the remaining small percentage belongs to basidia and imperfect fungi. In general, the fungi forming lichens represent 21% of all fungi, which is the highest percentage of mutualism relationships in fungi (Honegger, 1991). There are approximately 25 genera of green algae, a few golden algae, one genus of brown algae, as well as twelve genera of cyanobacteria that can be the photobiont partners in lichens (Brodo *et al*., 2001). The most common genera of photobiont are *Trentepohlia*, *Trebouxia*, and *Nostoc*: *Trebouxia* and *Trentepohlia* are eukaryotic organisms that are part of the family of green algae, whereas *Nostoc* is a prokaryotic cyanobacterium. The eukaryotic photobionts constitute 90% of the lichens, while the prokaryotic organisms represent 10% (Rankovic, 2015).

Lichens are divided, depending on the form of growth, into three sections: crustose (firmly attached to their substrates), foliose (flat and leaf-like, partially linked to their substrate), and fruticose (hair-like or shrubby, based on their substrates, but it grows far from its surfaces), as well as other particular types, such as gelatinous, in which the photobiont partner is always cyanobacteria. Other secondary forms, such as powdery (leprose) and squamulose, are included under the crustose form (Nash, 2008). Lichens grow and colonize many natural substrates, including all documents of rock (Saxicolous), trees (Corticolous), soils (Terricolous), wood (Lignicolous), and leaves (Follicolous), as well as artificial substrates such as rubber, plastics, glass, concrete, and ceramics (*Shukla et al., 2014*). About 10% of the land on Earth is covered by lichens, which are unique in that they can survive in harsh and extreme environments where other organisms cannot, such as the polar regions (Lee *et al*., 2014), hot dry regions, high humidity (Kranner *et al*., 2008), high altitudes where they are subject to intense UV radiation, as well as on rocks and infertile soil (Nguyen *et al*., 2013), as well as tolerating high salinity and concentrations of air pollutants, conditions of nutrient deficiency or nutritional enrichment (Nash, 2008) because of interactions between symbiotic partners (Backor and Fahselt 2008). However, it is susceptible to any change to its natural environment, making them critical indicators of air pollution, as the abundance and diversity of lichen flora are closely correlated with the environmental conditions (Sommerfeldt and John, 2001). Lichens are of great importance in vital fields, and they are often neglected by many mycologists for several reasons, including their slow growth and the difficulty of cultivating them (Crittenden and Porter, 1991).

2- Secondary metabolism of lichens

Secondary metabolites, organic substances built inside of an organism, are not valuable in metabolic activities but play a vital role in protecting symbiotic association against biotic or abiotic stresses (Legouin *et al*., 2017; Kinghorn,1994; Pagare *et al*., 2015). More than 2,140,000 secondary metabolites have been isolated and identified in plants so far, and they are commonly divided into polyphenols, phytosterols, alkaloids, polyphenols, flavonoids, and terpenoids (Zeeshan et al., 2022). However, secondary metabolites are classified according to McMurry's classification into five primary categories: terpenoids and steroids, alkaloids, enzyme cofactors, fatty acid-derived substances, polyketides, and no ribosomal polypeptides (McMurry,2015). The majority of Secondary metabolites in plants have a crucial role in medicine as an antifungal, antibacterial, antiviral and anticancer, antiangiogenetic agent, anti-inflammatory and antipyretic, and analgesic (Wink *et al*.,2012; Compean and Ynalvez, 2014; Ulus, 2021).

The ability of lichens to withstand harsh conditions directly correlates with the production of many unique and diverse metabolites known as lichen substances resulting from symbiosis between fungi and algae or bacteria (Schweiger *et al*., 2022; Ranković,2019). For example, in conditions of nutrient deficiency, lichen growth becomes slow, stimulating them to produce secondary metabolites (Bu'Lock *et al*., 1974). These compounds represent about 20% of the dry weight of the lichen, and most of them are produced by the fungal partner. They are small, complex crystalline compounds that cannot dissolve in water and are isolated using organic solvents. (Zhao *et al*., 2020) There are more than 800-1000 known lichens' secondary compounds, most of them specific to lichens, and a few created via other fungi or developed plants (Bačkorová *et al*. 2012). The

production of compounds in one species' culture differs from those in nature. For example, the fungal partner synthesizes specific compounds under certain conditions away from algae or bacteria, which vary from compounds built in symbiosis (Yoshimura *et al*., 1994; Hager *et al*., 2008; Packiam and Perumal 2022). Lichens secondary metabolites may play essential roles in several bioactivities, such as protection against animals, pathogens, or competing organisms, protection against physical factors such as high exposure to UV rays, etc. The complex process of producing secondary metabolites in lichens is impacted in diverse ways by the environment, including light, UV exposure, elevation, temperature swings, and seasonality (Rankovic, 2015). Aliphatic, cycloaliphatic, aromatic, and terpenic compounds are among the secondary metabolites present in lichens. These substances have biological and pharmacological effects that are noteworthy, such as those that are anti-inflammatory, antiviral, antibacterial, antipyretic, and anticancer. In addition, their importance in the field of industry, cosmetics, and biotechnologies (Cardile *et al*., 2017; Elkhateeb and Daba, 2019). Although several derivatives of secondary metabolites are isolated and used for antitumor activity, none of these compounds have surpassed the activities presented by Usnic and Polyporic acids (Kerboua *et al.*,2022). The experiments have found that Protolichesterinic acid and usnic acid revealed a potent cytotoxic effect and significantly decreased cell survival in the three cell lines: colon, cervical, and breast cancer (Brisdelli *et al*.,2013). Protolichesterinic acid, Lobaric acid, Atranorin, Usnic acid, and Salazinic acid isolated from various kinds of lichen have exhibited significant antibacterial toward Gram-positive bacteria and good activity against fungi. (Poulsen-Silva *et al*.,2023). Other lichen-derived substances, including bianthrones, hypericin, and anthraquinones derivatives, show inhibitory effects on viral enzymes, including the integrase of HIV-1 and HSV-1. (Culberson and Culberson 2001), and is sometimes related to the external appearance and geography of individuals at the level of species and genus (Zhou *et al*. 2006). Furthermore, These secondary metabolism substances of lichens used in traditional medicine for several centuries by Indians, Native Americans, Chinese, Haitians, Chinese, Brazil and Europeans showed great pharmacological potential to treat a variety of illnesses, including eczema, arthritis, kidney diseases, respiratory diseases, pulmonary diseases, pharyngitis, rabies, infection, constipation worm, alopecia, leprosy and infestation (Romagni and Dayan, 2002; Elkhateeb and Daba, 2019). In addition, they use essential resources in the food industry and cosmetics (Elkhateeb *et al.*,2022).

3- Biosynthetic pathway of secondary metabolites in Lichens

Lichens produce over 1,000 bioactive compounds (Shrestha & Clair, 2013; Goga *et al*.,2018). These compounds varied in chemical structure and have very low molecular weight (Muggia *et al*., 2009). Most of them are created in mycobionts of lichens as tiny crystals deposited in either the external surface of the hyphae within the cortex or medullary shell (Türk *et al*., 2003; Goga *et al*.,2018). The photobiont has a site of photosynthesis and could induce the synthesis of the mycobiont's bioactive compounds by supplying carbon dioxide, which is essential for the synthesis of secondary metabolites (Johansson *et al*.,2011; Yoshimura *et al*., 1994). Fungi and algae are associated with biochemical processes and metabolic mechanisms to produce a range of metabolites in lichen. The two major groups of Lichen metabolites are primary and secondary metabolisms. Primary compounds are the essential elements in metabolisms of the lichens which comprise proteins, polysaccharides, amino acids, sugar and vitamins, (Goga *et al*., 2020; **Dar *et al*.,2022**). Several pathways are involved in the synthesis of lichens' secondary metabolites including the Polymalonate, shikimic acid, and Mevalonic acid pathway. These pathways are found in all living organisms as an essential regulator of secondary metabolism production.

Biosynthesis of dibenzofurans (usnic acid), depsidones (salazinic acid), depsides (barbatic acid, atranorin), depsons (picrolichenic acid), lactones (protolichesteric acid, nephrosteric acid), anthraquinones (parietin), chromones, and xanthones produce via acetyl-malonate pathway (Ibrahim *et al*.,2018). The majority of dapsones, depsides, depsidones, dibenzofurans, and usnic acids compounds are created by fusing two or sometimes three orcinol and β-orcinol phenolic rings joined via ester, ether or carbon-carbon bonds. Other cyclic substances of acetyl-polymalonate origin including, anthraquinones xanthones, and chromones may be produced by the internal cyclization of a single-folded polyketide chain (Nash, 2008). On the other hand, steroids, Carotenoids, and terpenes are lichens' secondary metabolites synthesized by the mevalonate mechanism. It found to be effective in the regulation of cell division and development (Goga *et al*.,2020). Pulvinic and Terphenylquinoes derivates, which are produced via combining two molecules of phenylpyruvate units, are the source of the remaining secondary metabolites found in lichens that originate from the Shikimic acid mechanism. (Shukla *et al*.2010). These substances protect the inner layer of the algae from harmful influences of UV radiation and then emit it as heat energy or fluorescence (Chrapusta *et al*., 2017; Nguyen *et al*.,2013). Fungal partners create these substances only when they coexist with algae (lichen symbiosis). These compounds primarily result from the active mutualistic interaction of lichens (Weber and Büdel,2011).

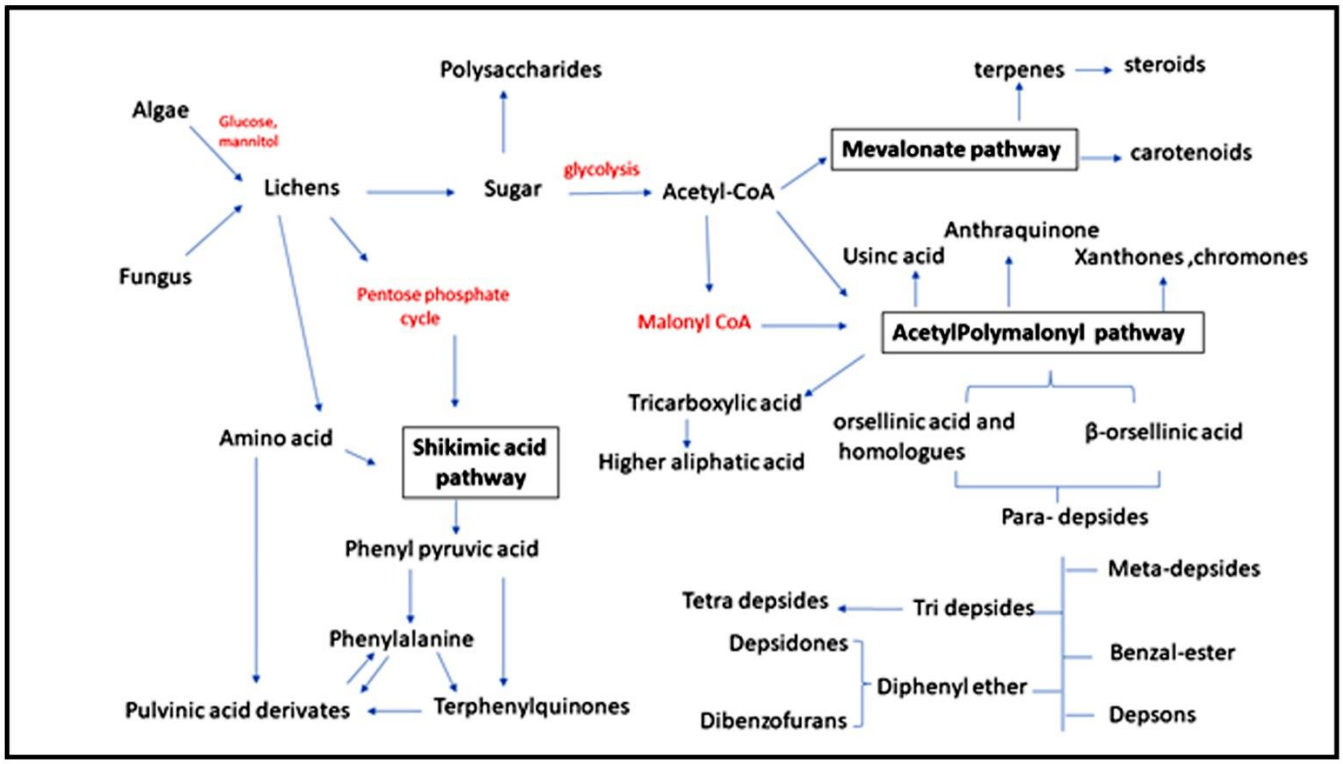

Figure 1: derived from Nash, 2008 demonstrate the secondary metabolites present in lichens derived mainly from three pathways, Acetylpolymalonyl pathway, Mevalonate pathway, and shikimic acid pathway.

4- The mechanism of biological activities of lichens' secondary metabolism as anti-cancer

Secondary metabolites are one of the main sources of medicines derived from plants. Based on experiments in vivo and in vitro, studies showed that lichens have a repository of anticancer compounds (Dar ***et al.*,2022**). These secondary metabolism compounds include Usinic acid, anthraquinones, xanthones, dibenzofurans, depsides, and depsidones. The considerable chemical diversity of the second compound of lichens provided sources distinct used as anticancer drugs in vitro and in vivo studies. These compounds are highly capable of protecting against carcinogenesis by modulating several mechanisms' pathways that lead to the growth and development of cancer. (Solárová *et al*., 2020; Zambare & Christopher,2012).

The inhibitory effect of lichens was noticed in various cancer cell lines compared to intact cells. The lichens demonstrate the cytotoxic activity in control cancer cell growth represented by inhibition proliferation, migration, invasion, tumor-friendly inflammation and angiogenesis of cancer cells and activation of cell cycle arrest, ant-tumor immune activity, or metabolism of energy (Nguyen *et al*.,2019; Thakur *et al*., 2023). First, lichens' antiproliferative activity against human cancer cells is the induction of cell death through processes including apoptosis and autophagy (Yurdacan *et al*., 2019; Dar ***et al.*,2022**). The ability of the secondary metabolism of lichens to promote apoptosis is observed through activating the mTOR-induced JNK signaling pathway (Chen *et al*., 2014). The antiproliferative effect by lichens might also be mediated by an increase in the expression of p53, p38, Bax, and cleaved PARP (Hong *et al*., 2018; Dinçsoy and Duman., 2017). Furthermore, promoting apoptosis in lichens is associated with inhibiting other mechanisms, such as ERK1/2 and AKT, that lead to apoptosis (Sigurbjörnsdóttir *et al*., 2014). Metabolic secondary of lichens induced Autophagic death cells in cancer cells through increased LC3II protein responsible for a compound of autophagosome formation (Yurdacan *et al*., 2019). Lichens affect the cell cycle arrest via a variety of processes linked to cyclin-dependent kinases (CDK4, CDK6) or cyclin D1 (Singh *et al*,2013), reduction in expression of cyclin B1, Cdk-2, and Cdc25C as well as a little reduce in the level of cyclin A1, Cdk-1 (Ghate *et al*.,2013). In contrast, lichen products combined with CQ treatment could inhibit autophagy-induced apoptosis. (Kumari *et al*.,2023). Autophagy inhibition significantly increases ROS generation and dysregulation of the redox machinery in cancer cells (Aggarwal *et al*. 2019). Some secondary metabolism compounds of lichens could induce reactive oxygen Species (ROS). Massive accumulation ROS performs a pivotal role as an antioncogene in suppressing cancer cell growth by inhibiting the biosynthesis of ATP and Nucleotide, which leads to cell cycle arrest and inhibits cancer cell growth. ROS also causes cancer cell death by controlling apoptotic pathways. (Huang *et al*., 2021; Bačkorová *et al*., 2012). As well as Lichens and their secondary metabolites have been investigated as anti-migrative and anti-invasive agents. The effect of lichens on inhibiting cell motility and migration of cancer cells is associated with decreasing the GTP-Cdc42, RhoA, Rac1, and Cdc42 involved in the mesenchymal migration mode. The secondary compound also showed similar inhibitory activity against the invasion of cancer cells. These compounds decreased the expression of KITENIN-mediated in invasion cancer cells and targeted β-catenin or its downstream effectors that consequently led to target genes cell migration (MMP7) (Zhou *et al*.,2017; Paluszczak *et al*., 2018). Also, the extraction lichens treatment reduced cancer cell motility. It inhibited the

invasion of cancer cells by significantly suppressing the expression of epithelial-mesenchymal transition (EMT) markers and inhibiting phosphor-Akt (Nguyen *et al*.,2014). The effectiveness of lichen's secondary metabolism on the angiogenesis of cancer cells has been estimated to be a potent anti-angiogenesis agent against cancer cells. Endothelial tube formation was disrupted when treated with a secondary metabolism compound (Koparal *et al*., 2010). Or inhibition of vascular endothelial growth factor receptor2 (VEGFR-2_mediated in regulating the activation of Endothelial Cells (Dinçsoy and Duman, 2017). Recent findings demonstrated that lichens' anticancer properties are also linked to controlling inflammatory reactions by IL-1, IL-6, TNF-, and TGF. Some secondary compounds were also isolated from the lichens plant and found to have anti-angiogenic activities; these compounds could inhibit endothelial tube formation of breast cancer cells in vitro (Varol *et al*., 2018). The effects of secondary metabolism isolated from lichens were identified as cancer Immunotherapy agents. The extraction of lichens led to the development of strategies to activate T-cells by inhibiting multi-immune checkpoint ligands, including PD-L1, ICOSL, and GITRL, in cancer cells (Varlı *et al*., 2022). Interestingly other inflammatory molecules supported by the secondary metabolism of lichens, the content of immune cells such as IL-1β, IL-6, IL-8, MDA, TNF-α, HYP, and TGF-β1 in lung cancer cells residue when treated with the secondary metabolism of lichens could improve SOD, GSH and IL-10 level (Su- *et al*., 2017).

5- The clinical studies of the secondary metabolism of lichens in vitro and in vivo

Usnic acid (UA), also known as lichenol, is one of the main secondary metabolism compounds synthesized in lichens, figured out for the first time in 1884. It is one of a dibenzofuran derivative that has a yellow color pigment (Ingólfsdóttr, 2002). It is widely distributed in a number of species of lichens including *Alectoria, Cladonia, Usnea, Lecanora, Ramalina, and Evernia*. According to the studies, UA possesses a wide range of biological and physiological properties, including antitumor, anti-oxidative, antimicrobial, anti-inflammatory, and anti-cancer (Geng *et al*., 2018: Bray *et al*., 2018). Recent researchers have found that Usnic acid was able to influence different stages of cancer (anti-proliferative, anti-angiogenesis, and inhibition of metastasis via different signaling pathways in various types of cancer (Geng *et al*., 2018). The proliferation of several types of cancer cell lines is inhibited by Using acid through promoting the expression of the genes responsible for apoptosis Bax (proapoptotic protein), p53 (tumor suppressor gene), and caspase 3(tumor suppressor protein) (Zuo *et al*.,2014). The research examined the molecular pathways linked to the antiproliferative properties of usnic acid against two varieties of human gastric cancer cell lines, gastric adenocarcinoma AGS and gastric carcinoma SNU-1.

Usinc acid inhibited growth and induced apoptosis by an increase in the level of Bax: Bcl-2 expression and cleaved-PARP. Also, Usnic acid caused DNA damage response through increasing expression of reactive oxygen species (ROS)mediated in upregulation of p53, Chk-2, and γH2AX that led to apoptosis induction in gastric cancer cell lines. while the study observed that Usinc acid does not affect normal cells (Kunal *et al*.,2020). Another study observed that Usnic acid (UA)has antiproliferation in gastric cancer cells via activating autophagic cell death. Usinc acid could increase the activation of autophagy-associated proteins (LC3-II) while p62 level was decreased in BGC823 and SGC7901 cell lines. (Geng *et al*., 2018). Additionally, data suggested that low molecular of antiproliferation (Unsic acid) triggered autophagy activation in breast cell lines while was quite safe with non-tumorigenic MFC-10 mammary breast cell line. Unsic acid induced autophagy through inhibiting of PI3K/AKT/mTOR pathway and activating c-Jun N-terminal kinases (JNK). The molecular mechanism in suppression of rapamycin (mTOR) summarized through binding Unisc acid at pose of mTOR pocket and target deep hydrophobic pocket at the core of the kinase cleft (Ebrahim *et al*., 2017).

Unsic acid has a good growth condition activity against the migration of cancer cells; inhibitory activity was elucidated through regulating many singling pathways to inhibit the metastasis of cancer cells. In lung cancer cells, Usnic acid showed a high inhibitory effect against the motility of cancer cells by level involved in the mesenchymal mode of migration in the A549 cell line; also Unsic acid could reduce adhesion and cell movement through decreasing activity of RhoA in A549 cells. Inhibition of migration of colorectal cancer cells is also associated with treating cells with Usnic acid. The results showed that UA inhibits SCF-dependent activation of C-KIT mediated in the migration of C-KIT positive cells in colorectal cancer cells (Wu *et al*., 2018). Usnic acid also suppresses the migration of colorectal cancer cells through upregulating ATMs to induce the DNA damage singling mechanism in RKO colorectal cancer cells (Wu *et al*., 2021). Usnic acid is defined as anti-angiogenesis therapy against cancer cells. The effective strategy for Unisc acid in the inhibition of angiogenesis is represented in blocking vascular endothelial growth factor receptor 2(VEGFR2) mediated ERK1/2 and AKT signaling pathway of breast cancer cells (Song *et al*.,2012). The antiangiogenic activity of Usnic acid on the growth of HepG2 hepatocarcinoma cells NS2oy neuroblastoma cells, and HUVEC endothelial cells was investigated by suppressing tube formation vessel network. It showed a potent anti-angiogenesis potential (Koparal*,*2015).

Similarly, the natural compound Depsides or Barbatic acid (BA) isolated from Usnea longissimi was reported. It demonstrated a strong cytotoxic effect against lung cancer cell lines (A549), cervical cell line (HeLa), and prostate cancer cell line (DU-145). BA cause death of cell through induction of apoptosis and cell cycle arrest in concentrations (1.0 and 2.0mM) with 70.9% and 74.4% of cell accumulation in the G0/G1 phase. Expression of Poly ADP-ribose polymerase (PARP) cleavage and caspase-3 activity responsible for induction of apoptosis increased as well as CDK4 and Cyclin D1 protein levels accumulated in cell cycle after treatment the cancer cell with Barbatic acid (Reddy *et al*., 2019). A recent report of depsides found that two molecules of depsides isolated from *P. millegrana aurulenta* have high toxicity against diverse kinds of cancer cell lines including A549 lung cancer cells, the HepG2 liver cancer, and

HL-60 leukemia cell lines, while the mono-depsides showed low cytotoxic activity toward cancer cells (Nugraha *et al*. 2020). Truong *et al*.2014 identified and isolated three types of new depside from *Usnea aciculifera*, including depside aciculiferin

A, barbatinic acid, and diffractaic acid. These three types of depsides were investigated for their cytotoxic activity against NCI-H460 (human lung cancer), HeLa (human epithelial carcinoma), and MCF-7 (human breast cancer) cell lines and demonstrated that diffractaic acid was a potent, cytotoxic solid activity more than depside aciculiferin A, barbatinic acid in promoting apoptosis with unknown mechanism in inhibition of cancer cells. Barbatic acid derived from Bryoria Capillaris was studied as antiangiogenetic activity on human umbilical vein endothelial cells (HUVEC), human breast ductal carcinoma cell line (T-47D) Adenocarcinoma cancer cell line (HCC1428). The result has also found that barbaric caid completely suppressed the tube formation and blocked the migration of cancer cell lines with unclear mechanisms (Varol *et al*., 2018).

The depsidones include (salazinic acid and hypostitics acid) an isolated *psudoparmiedia sphaerospora,* and *P. sphaerospora,* respectively. Both types of depsidones exhibited potent antiproliferative effects toward a wide variety of cancer cell lines, including the Murin melanoma cell line (B16-F10), renal cancer cell (786-0), and chronic myelogenous leukemia cell line (K562), Prostate cancer line (PC-03), hepatocellular carcinoma cell line (HepG2), breast cancer cell line (MCF7). The finding confirmed that the hypostatic could induce apoptosis in a percentage of 72% through increasing expression of caspases -3 mediated apoptosis in cancer cells. In contrast, salazinic acids were more active in inhibiting cancer cells and promoting apoptosis compared with hypostitics acid, which reached a percentage of inhibition of 88% of tumor volume in both in vivo and in vitro studies (Alexandrino *et al*.,2019).

The results observed pro-apoptotic effects of lichen secondary metabolites after the handle with depsidones (salazinic acid, physodic acid) and depsides (Atranorin, lecanoric acid, and squamatic), and a poly-carboxylic fatty acid (caperatic acid). These compounds could regulate cancer-related signaling pathways and showed anticancer properties toward glioblastoma cell lines (T98G, U-138, A-172). The results demonstrated that (squamatic acid, salazinic acid, and lecanoric acid) had the highest cytotoxicity in the induction of apoptosis cells after 84 from treatment compared with other compounds. Whereas number of cells in the G0/G1 phases of the cell cycle is somewhat increased. However, cell numbers in the S phase are lowered when atranorin and salazinic acid are added to cancer cells. These secondary metabolites, combined with TMZ-induced inhibition of the Wnt/β-catenin pathway, mediated the regulation of various biological processes, including the proliferation, differentiation, and migration in glioblastoma. In addition, the data found that physodic and squamatic acids were able to induce oxidative stress in the T98G cell line. At the same time, other secondary metabolites did not show any effect as an antioxidant (Majchrzak-Celińska *et al*.,2022). The Effect of lichens-derived salazinic acids and physodic acid against colorectal colon cancer cells was evaluated by studying secondary metabolites of lichens. As a result, an increase in the activity of transcription factor Nrf2 that vital play in protecting cells from DNA damage was observed after cancer cells were treated with salazinic acids and physodic acid. Also, both secondary metabolites compounds demonstrated decreased expression of STAT3 and NF-κB signaling pathways promoting cancer growth in both colon cancer cell lines HCT116 and DLD-1 cells. The reduction of expression of STAT3 largely contributed to the downregulation of the anti-apoptotic protein Bcl-xl (Papierska *et al*.,2021).

Physciosponin is one of the secondary metabolites of lichens derived from isolated Pseudocyphellaria granulata. Physciosponin has been demonstrated as an anti-tumor for colorectal cancer cells, including DLD1, Caco2, and HT29, HCT116, and inhibits the proliferation and migration of breast cancer cells by decreasing Bcl-2 proteins and activity caspase pathway. As well as Physciosponin could downregulate B-Calin, C-Myc, HIF-1a, and NFkB, which are found in the genus Pseudocyphellaria (Yang *et al*., 2015).In a similar study, Yang and Colleagues 2019 proposed that Physciosponin has a novel anticancer in the suppression of migration and induces differentiation in human colon cancer stem cell line(CSC221), colorectal cancer cell lines Caco2, DLD, one, and HT29 by inhibiting transcriptional activity of the glioma-associated oncogene homolog zinc finger protein (Gli) Gli1/2 and transcription factor Engrailed 1(ΔEN1) through Sonic hedgehog(SHH) and Notch singling pathway involved stemness potential of Colon cancer. Conversely, physciosponin-isolated Psedocyphelloria coriacea significantly suppressed cell migration and invasion of lung cancer cell lines A549, H1650, and H1975 through the novel pathway. This mechanism is represented in decreasing KITENIN involved in metastasis-enhancing. Also, Physciosponin downregulated Cdc42 and Rac1 protein levels that play a role in the actin cytoskeleton, motility, and adhesion between cells in epithelial cells. Moreover, the study found that Physciosponin upregulated KAL1-mediated AP-1, which is responsible for the suppression activity of metastasis in lung cancer cell lines (Yang *et al*.,2019). In vivo, study physciosponin could inhibit growth tumor growth in a skin xenograft mouse model (İsa Tas *et al*., 2019)

Ramalin is a secondary metabolite of a tratctic lichen species. Recent studies have shown the use of Ramalin as a potential source for cancer treatments. RM downregulated PDGF-induced proliferation of hepatic stellate cells (HSC). RM decreased college accumulation and upregulated erythroid type-1 related factor 2 Nrf2 mediated antioxidant response protein HO-1 and NADP (Kim *et al*., 2018). Ramalin derived from lichen *Ramalina terebrata* was also evaluated as anticancer therapy for colorectal cancer. Ramalin has an essential function in the activation of apoptosis and cell cycle arrest in the Gap 2/mitosis (G2/M) phase through upregulating many genes such as p53, p21, and cyclin-dependent kinase inhibitor1A(CDKN1A) and downregulating cyclin kinase 1 (CDK1) and Cyclin B1(CCNB1). Furthermore, Ramalin restrained colorectal cancer metastasis by suppressing the invasion and migration of cancer cell lines HCT116 in vitro using the Boyden chamber system (Suh *et al*. 2017). Additionally, Ramalin inhibited proliferation and induced cell death program in both breast cancer cell lines (MCF-7, MDA-MB-231) via an increasing expression of proapoptotic proteins Bax and decreasing terms of antiapoptotic proteins Bcl-2; this led to the release of cytochrome c and induce apoptosis through activating the mitochondrial apoptotic pathway. In addition, Ramalin activated caspase-8 and caspase-9 in both types of cells, while caspase-3 only is activated by Ramalin in the MDA-MB-231 cell line. Furthermore, the cells treated with Ramalin upregulated the levels of LC3-II and p62 involved in the induction of autophagy.

Another class of depsides is Protolichesterinic PA (lactones), extracted from lichen. PA isolated by Zopf from different types of *Cetraria* and *Parmelia.* It has attracted significant attention from researchers because of its unique chemical and biological characteristics and potential uses in various fields of scientific research and the drug industry (Murta *et al*.,1993). It was also recorded as an anticancer drug against breast cancer cells. The results showed that Protolichesterinic acid increased the expression of FAN and HER2 by downregulating the ERK1/2 and AKT signaling mechanism, while the PA did influence breast cancer cell line T47D (Bessadottir *et al*., 2014). Protolichesterinic acid also suppressed the growth of the cervical cancer cell line (Hela) and caused induction apoptosis with PA's ability to increase the expression of Caspase 3,8,9. In addition, PA combined with doxorubicin acid caused the accumulated cleaved form of proapoptotic protein (Bid) and increased expression of Bim protein involved in cell death and survival essential to normal tissue homeostasis. In contrast, Protolichesterinic did not exert any cytotoxic activity in neural cell line SH-SYSH and leukemia cell line K562 cells (Brisdelli *et al*., 2016). Johannsson and colleagues studied the inhibitory effectiveness of Protolichesterinic acid against two cell lines: the breast cancer cell line(T-47D) and the pancreatic cancer cell line (AsPC-1). The data has shown increased levels of glutathione in treated cells by PA. Protolichesterinic acid regulated the transcription factor Nfr2, which rescued the cellular resistance of oxidants (Jóhannsson *et al*., 2022).

Epanorin (EP), one of the shikimic acid-derived metabolites, is extracted from *Acarospora lichens*. It represents one Pulvinic acid derivative for lichens (Hauck *et al*.,2010). The Cytotoxic effects of Epanorin against cancer cells were examined via Flow cytometry to evaluate cell cycle progression and TUNEL assay for detecting DNA fragment MCF-7 in breast cancer cells. The study found that EP can inhibit cell viability and induction of cell cycle arrest. EP was more cytotoxicity in the regular cell line HEK-293 and human fibroblasts. The study observed that EP might be a new anticancer drug (Palacios-Moreno *et al*., 2019).

Vulpinic acid (VA) is another Pulvinic acid derivative derived from the shikimic acid pathway for lichens exerted anticancer drug in inhibiting cell growth and induction apoptosis of human breast cancer cell line (MCF-7). The molecular mechanism for the effect of Vulpinic acid on cancer cells represents increasing gene expression of the p53 gene in breast cancer compared to the non-cancerous breast epithelium cell line (MCF-12A) (Kılıç *et al*., 2018 a). Another study found that the therapeutic effect of VA induced apoptosis and inhibited metastasis of prostate cancer cell line (PC-3) cells by increasing cell cycle arrest G0/G1, nuclear blebbing, and activation caspase (Cansaran-Duman *et al*., 2021).

Table (1). Activity of Secondary metabolites of lichen on variety signaling pathways on different types of human cancer cells

Biochemistry categories	Secondary metabolite type	Activity	Site of tumor	Molecules involved in tumor
Dibenzofurans derivative	Usnic acid	anti-proliferative anti-autophagy anti-migration	Colon cancer	p53, Bax, γH2AX Caspase3 ↑ Cleaved-PARP LC3-II, p62 Chk-2, ATMs ↑ ↑ Bcl-2, ↓
		Anti -autophagy	Breast cancer	mTOR, JNK ↑
		Anti-angiogenesis	Liver cancer	VEGF ↓
Depsides	Barbatic acid (BA)	Anti-proliferative	Lung cancer cervical cancer prostate cancer	(PARP) cleavage, caspase-3, ↑ CDK4 and Cyclin D1 ↑
		Anti-angiogenic Anti-migration	breast cancer	Unknown mechanism
depsidones	salazinic acid hypostitics acid	anti-proliferative	Skin cancer kidney cancer leukaemia cancer Prostate cancer liver cancer Breast cancer	Caspase 3 ↑
Depsidones	physodic acid salazinic acid	Anti proliferation, anti-migration anti-angiogenesis Differentiation	Glioblastoma	G0/G1 ↑ Wnt/β-catenin pathway ↓
Depsides	Atranorin, lecanoric acid, and squamatic			
Depsidones	salazinic acids and physodic	Antiproliferation	Colon cancer	Nrf2 ↑ NF-κB ,STAT3,Bcl-xl ↓
Physciosponin		Antiproliferation Antimigration Differentiation	Colon cancer	Bcl-2 ,B-Calin, c-Myc, HIF-1α, ↓ NFkB, ΔEN1 ,Gil1/2 ↓
depsidones	Ramalian	Antiproliferation	Liver cancer	Nrf2 ↑

		Anti-proliferation Anti-migration	Colon cancer	(G2/M) Bax,p53,p21 ,CDKN1A, CDK1, CCNB1 Bcl2
		Anti-proliferation	Breast cancer	Caspases 3,8,9 Bax,Lc3II,P62 ↑ BCL2 ↓
		Anti-migration	Lung cancer	KITENIN, Cdc42, Rac1 ↓ AP-1 ↑
Pulvinic acid derivatives	Epanorin	Anti proliferation	Breast cancer	arrest in G0/G1 ,Nfr2 ↑
depsides	protolichesterinic acid	Anti- proliferation	Brest cancer	FAN , HER2 ERK1/2 , AKT ↑
		Anti- proliferation	cervical cancer	Bax ,Bim, Caspase 3,8,9 ↑
Pulvinic acid derivatives	Vulpinic acid	Anti-proliferation	breast cancer	P53, TrxR1 ↑
		Anti-angiogenesis	Neuroblastoma, liver cancer	VEFG ↓
		Anti-angiogenesis	Liver, colon ,cervix cancer	Bax-p53 bcl2 ↓
Triterpenes	Retigeric acid	Anti- proliferation	Prostate cancer	Bax/Bcl2, p21, caspase -3,DR5 ↑ cleavage PARP ↑, NFkB ↓

Similarly, Kalin and others observed that Vulpinic acid promoted cell cycles arrest and development of breast cancer cells (MCF-7) and (MDA-MB-453) in a dose-dependent manner by downregulating the activity of TrxR1 as well as Vulpinic acid could suppress the cell motility in both cell line (Kalin *et al*.,2022). Furthermore, Vulpinic acid exerted a cytotoxic effect against other cancer cell lines such as cervix carcinoma cell line (Hep2), colon cancer cell line (CaCo2), hepatocarcinoma cell line (HepG2), and Rhabdomyosarcoma (RD) and promoted to resistance of oxidants factor. The data showed that Vulpinic acid induced apoptosis via increased proapoptotic protein (Bax) expression and decreased expression of antiapoptotic proteins (Bcl2). In addition, Vulpinic acid increased the expression of the p53 gene mediated in programmed cell death. In contrast, VP showed no cytotoxic activity on normal cells (L929) (Kilic *et al*.,2018 b). Furthermore, the role of Vulpinic acid as antiangiogenics activity was investigated on the neuroblastoma cell line (NS20Y), hepatocarcinoma cell line (HepG2), and endothelial cells line (HUVEC) in the endothelial tube formation assay. Vulpinic acid is a potent antiangiogenic agent in suppressing endothelial tube formation while less cytotoxic in standard cell lines (HUVEC) with a mechanism not fully understood (Kopara,2015).

A recent study found that Retigeric acid (RAB) is the natural pentacyclic triterpene acid of lichens isolated from *Lobaria kurokawae* and belongs to teraponids derived from the Mevalonic pathway. It has anti-tumor activity on two types of prostate cancer cell lines, PC3 and DU145. Retigeric acid strongly suppressed cell proliferation and triggered cell death of cancer through inhibiting transcription factor NFkB modulated in regulation proteins Survivin, Cyclin D1, Bcl-2, and Bcl-XL. On the other hand, the study observed that IkB6 and nuclear translocation of p65 lead to activation of cell death of cancer in vivo and in vitro (Liu *et al*., 2018). Other studies indicated that Retigeric acid -B(RA-B) induced cell cycle arrest by upregulating of p21 and enhanced apoptosis of PC-3 cells through increasing ration Bax/Bcl2 proteins and activation of caspase -3 and cleavage PARP (Liu *et al*.,2010). Also, Retigeric acid combined with Cisplatin promoted apoptosis of PC3 cells by suppressing DNA repair and activating proapoptotic protein death receptor 5(DR5) induced apoptosis (Liu *et al*.,2018).

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