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Lichens: A promising source of anti-cancerous activity and their molecular mechanisms

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ABSTRACT

The potential of lichens for medicinal use is to have a variety of bioactive anticancer compounds. Several factors drive the issue of lichens as a usual substance of carcinoma chemicals for lichens. In a variety of climates which could be represented by extreme climatic variables, lichens can endure heat intensities, Ultraviolet rays, limited nutrient uptake, humidity, etc. Their results are obtained is due to the possible defence system they have towards severe change, simultaneously generating bioactive substances with distinctive attributes through different biosynthesis pathways. Synthesized lichen compounds may exhibit a range of bioactive constituents in response to their cytotoxic effects, including antioxidant, antibiotic, antimutagenic, antimicrobial, antibacterial, anti-inflammatory, antipyretic, inhibitory proteins, as well as endogenous plant growth impact. In addition, lichen derivatives have a wide range of anti-cancer effects against many forms of cancer cell types, like usnic acid, gyrophoric acid, and lechanoric acid. Furthermore, when functioning together through human cancer cells, lichens compounds can cause synergistic cytotoxic results. Anti-cancer stimulation of molecular lichen derivatives through the process of cytotoxicity by cellular processes and initiation of cell death and by modulation in immune activity, angiogenesis, or metabolism of energy. This review mainly focuses on the potential role of lichens' active constituents as beneficial moieties to exhibit anticancer activities with their molecular mechanism.

1. Introduction

Cancer remains one of the world's greatest chronic illnesses, despite major developments in anti-cancer care (Ferlay et al., 2019; Patafio et al., 2016). Naturally occurring compounds are an abundant source of bioactive metabolites that could be used in several applications in the fields of biology, pharmaceuticals, and medicine, including oncology. Carcinogenic is associated with proliferation and apoptosis inequalities, inadequate epigenetic and transcription factor activity, and inhibition of over-accumulation of free radical's protective factors, as well as increased angiogenesis, resulting in increased invasiveness and production of metastases. Many of these carcinogenic pathways are quite well established in specific molecular mechanisms for bioactive compounds of phytochemicals (Solár et al., 2011; Uramova et al., 2018). Lichens are mutualistic organisms that generate multiple substances that are bioactive. The substantial chemical range of lichen bioactive compounds makes them a good sustainable resource of therapeutic assays that could be used in drug therapy (Stanojković, 2019). Aliphatic, cycloaliphatic, aromatic, and terpenoid substances are secondary metabolites found in lichens, showing important biological and pharmacological effects, such as anti-inflammatory, antiviral, antibacterial, analgesic, antipyretic, anti-proliferative, and cytotoxic effects (Cardile et al., 2017). Cancer is a condition triggered through irregular growth accompanied by the absence of immune suppression, activation of angiogenesis, and deregulation of cellular energy metabolism by cell proliferation of malignant behaviour (Hanahan and Weinberg, 2011). By removing cancerous cells, cancer is characterized through one or more treatments, including chemotherapy, radiation treatment, surgery, as well as targeted therapy. Among others, chemotherapy utilizes chemical compounds that cause cytotoxic effects on neoplastic cells. As a consequence, although these anticancer compounds may also have a non-specific impact on rapidly growing cells in the body, many undesirable adverse effects, especially on haemostasis, the immune response as well as the intestinal system, are typically caused by their usage. To address these issues, targeted clinical strategies have been developed. These are intended to obtain regulated cell signalling mechanisms within cancer cells or accompanying stromal cells. By targeting various carcinoma signal transduction pathways in each

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type of carcinoma or extensive therapy, targeted therapy may potentially work on cancer to less severe symptoms on certain cells. For the treatment of cancer, small molecules and antibodies are widely used, as well as a significant reservoir of these biomolecules has been identified as lichen products (Kim et al., 2015).

2. Anticancer efficacy of the extract of lichens or their isolated compounds

Currently, several scientists have researched the anticancer capacity of lichen extracts in conjunction with isolated lichen metabolites. Through inducing apoptosis at lethal concentrations, the acetone extract of *Flavocetraria cucullata* or even UA has induced cytotoxic effects in cancerous cells. Sublethal concentrations of this extract and UA stimulated tumour growth as well as motility of cancer cells, inhibited epithelial-mesenchymal transformation (EMT), and prevented Akt phosphorylation. Notably, the anticancer activity of the extract was more active than UA (Nguyen et al., 2014). Another research, via tetrazolium analysis, investigated the anti-cancer effects of *Toninia candida* and *Usnea barbata* acetone extract, as well as their key norstictic acid (NA) as well as UA derivatives for FemX and LS174 cells. The results of the study showed the cytotoxic activity of NA, UA, and lichen extracts across both cancer cells. Notably, UA had the greatest anticancer activity, although both NA and UA mediated cell death in FemX and LS174 cells (Ranković et al., 2012). In contrast, anticancer and antioxidant availability of supercritical CO2 extract of *Usnea barbata* opposed towards compounds determined by standard methods (Soxhlet extracts and macerate) has been assessed in mouse skin cancer (B16), rat glioma (C6), and HaCaT cells. On both B16 and C6 cells, SCE induced the maximum cytotoxicity. UA content and production of reactive oxygen species have been well associated with the cytotoxicity of lichen material. The cytotoxic effects of extracts were determined for apoptotic cell death and autophagy pathways by monitoring cellular for cell cycle stages and generation of acidic cytosolic vesicles. As a result, in B16 and C6 cells, both SCE and UA mediated apoptotic cell death and autophagy. Consequently, only the very low toxicity of all the extracts assessed was exhibited toward normal HaCaT cells (Zugic et al., 2016). Furthermore, acetone extracts from lichens such as *Evernia prunastri* and *Pseudoevernia furfuraceae* have been shown to have anticancer effects using the MTT test in FemX and LS174 cells and certain main biological activities, mainly PA. The strongest cytotoxic effect was induced by physodic acid against both cell lines. In addition, a decrease in FemX and LS174 cells in the S and G2/M phase was also found by researchers. On the other side, following treatment with the extracts and compounds mentioned above, there was a rise in the cell sub-G1 population (Kosanić et al., 2013). Similarly, PA has shown the most important cytotoxicity on MCF-7 cells, led through T47D and MDA-MB231 cells (isolated from *Hypogymnia physodes*). The efficacy of MCF-10A cells has not been changed, even at a PA concentration greater than 100 μM (Studzińska-Sroka et al., 2016). The ethanol extract of *Usnea strigosa* and its derivative NA have exhibited cytotoxic effects in MDA-MB-231, MDA-MB-468, MCF-7, T-47D, BT-474, and SK-BR-3 cells. NA exhibited antiproliferative effects in all six mammary cell lines, with the greatest activation seen in MDA-MB-231 and MDA-MB-468 cells. Norstictic acid prevented the proliferation of MDA-MB-468 cells as well as the intrusion of MDA-MB-231 cells through the extracellular matrix. No major cytotoxic activities have been observed on non-tumorigenic human MCF-10A cells, on the other hand. This acid has also inhibited C-Met, STAT3, paxillin/Rac-1and FAK phosphorylation in MDA-MB-231 cells (Ebrahim et al., 2016). Moreover, the acetone extract of *Melanelia subaurifera* and *Melanelia fuliginosa* and their metabolites of LeA and 2′-O-methyl anziaic acid (2′-O-MA) were inhibited by HeLa, A549, and LS174 cells. As a result, LeA and 2′-O-MA derivatives had lower cytotoxic activity compared with both extracts, although both extracts and derivatives have not shown any anticancer impact on normal MRC5 cells (Ristić et al., 2016). In the ether extract of *Cladonia salzmannii*, anticancer effects were observed on human acute promyelocytic leukaemia (HL-60), human laryngeal carcinoma (HEP-2), human lung mucoepidermoid carcinoma (NCI-H292), and murine macrophage (RAW-264.7) cells. In this respect, acetone extract indicated cytotoxic activity on MCF-7 and NCI-H292 cells, while distilled BA induced cytotoxicity on HEP-2, MCF-7, and RAW-264.7 cells (Gonçalves et al., 2018). Barbatic acid, a secondary metabolite of Cladia aggregate, was treated for HEp-2, human squamous cell lung cancer (NCI-H292), and human nasopharyngeal squamous cell carcinoma (KB). Lichen extract and the isolated compound have shown cytotoxic effects on all cancer cell lines tested, though HE-p2 cells are the most susceptible (Barroso Martins et al., 2016). *Everniastrum vexans* acetone extract prevented the proliferation (10 μg/mL) of A549 cells. At concentrations greater than 5 μg/mL, Atranorin, known as an active bioactive compound of the extract, exhibited a cytotoxic activity on A549 cells. Atranorin prevented the activity of TOP FLASH mediated by β-catenin, decreased nuclear imports of β-catenin as well as inhibited the expression of c-jun/AP-1 target genes. In addition, ATR decreased KITENIN's kinase activity or raised KAI1 mRNA activity. The amounts of GTP-Cdc42, GTP-RhoA and STAT factors have been reduced (Zhou et al., 2017). Another anticancer effect of lichens extracts and compounds is shown in Table 1.

2.1. Molecular mechanism of anti-cancerous activity

Lichens bioactive compounds, assert significant capability in the protection toward carcinogenesis (Zambare and Christopher, 2012), that is owing to their cytotoxic, pro-apoptotic, antioxidant, anti-migrative, anti-invasive, antiproliferative, and overall anti-tumorigenic capacities (Fig. 1), (Zambare and Christopher, 2012; Nguyen et al., 2013; Ristić et al., 2016). The enhanced free radical scavenging ability of phenolic compounds is due to the antioxidant activity of most lichens (Nguyen et al., 2013;). Lichens resist mutagenesis and/or carcinogenesis by the avoidance of the oxidation of cellular macromolecules (Studzińska-Sroka et al., 2016). Correspondingly, the protective effects of lichens or their metabolites against oxidative harm can be determined by monitoring oxidative stress indicators along with superoxide dismutase (SOD) or malondialdehyde (MDA) (Paluszczak et al., 2018). The cytotoxic effect of lichens has been documented in various cancer cell lines, so found that the anticancer potential of lichens in carcinoma cells is greater than in non-cancer cells (Nguyen et al., 2013; Zambare and Christopher, 2012). First of all, the strong cytotoxic ability of lichens for carcinoma cells is regulated by pathways such as cell death, necrosis, or autophagy, along with step G2/M, S, or G0/G1 cell cycle arrest (Yurdacan et al., 2019a). Lichens often act as promoters of the cell cycle by various methods, like cyclin-dependent kinase-associated (CDK4, CDK6) or cyclin D11-associated kinases (CDK4, CDK6) or cyclin D11-associated lichens (Singh et al. 2013). In access to regulated apoptosis, lichens act as modulators of cell death in various cancerous cells (Nguyen et al. 2013; Paluszczak et al., 2018) by modulating the gene expression of apoptosis-related substances such as caspases, p53, p38, or Bcl-2 family anti-/pro-apoptotic proteins (Dincsoy et al., 2017). Stimulation of lichen cell death can also be associated with increased cleaved PARP, an antioxidant defence enzyme that restores damaged DNA and regulates chromatin function (Hong et al., 2018), with inhibition of rapamycin mammalian target (mTOR) or c-Jun N-terminal kinase (JNK) signalling activation (Yurdacan et al., 2019b).

By controlling other signalling pathways, like ERK1/2 and AKT (Bessadóttir et al., 2014) or the replication protein marker Ki-67 (El-Garawani et al., 2019), the anti-proliferative influence of lichens could be mediated. Consequently, the anticancer ability of lichens may also be preserved by modulating cancer-invasive mechanisms like c-Met that acts as a mesenchymal-epithelial transition variable that controls the signalling cascades of PI3K/Akt/mTOR, Paxillin/Rac-

Table 1
Anticancerous activities of Lichens species.

Lichens Species	Cell Line	Mechanisms	References
Flavocetraria cucullata	HT29, AGS, A549, CWR22Rv-1	Through using acetone extract, increased selective cytotoxicity and anticancer activity and decreased tumorigenesis and motility or EMT and Akt phosphorylation.	Nguyen et al., 2014
Hypogymnia physodes	MCF-7, T47D, MDAMB-231, MCF-10A	By using acetone extract, increased cytotoxicity of MCF-7 (IC50 72.4 μg/mL), T47D (IC50 75.4 IC50 μg/mL), MDA-MB-231 (IC50 93.9 μg/mL)	Studzińska-Sroka et al., 2016
Pseudoevernia furfuraceae	FemX, LS174	By carrying out acetone extract, raised cytotoxicity LS 174 and FemX and declined FemX and LS174: S and G2/M arrest PA	Kosanić et al., 2013
Extracts of endolichenic fungus EL002332	AGS, TMK-1, CT26	Cytotoxicity (on AGS and CT26) EL002332 + docetaxel: synergistic effects (on AGS and TMK-1)	Y. Yang et al., 2018
Cetraria islandica	FemX, LS174	Through using methanol extract, raised cytotoxicity: FemX (IC50 22.68 μg/mL); LS174 (IC50 33.74 μg/mL)	Grujičić et al., 2014
Xanthoria parietina	MCF-7, MDA- MB-231	By using acetone extract, reduced proliferation or cell cycle, and increased apoptosis	Basile et al., 2015
Xanthoparmelia chlorochroa and ***Tuckermannopsis ciliaris***	Human Burkitt's lymphoma (Raji)	Induced apoptosis or cell arrest and increased p53 expression	Shrestha et al., 2015
Parmotrema gardneri, Pannaria* sp., *and Canoparmelia aptata	AGS, A549, MDCK	By using acetone extract, *P. gardneri:* enhanced cytotoxicity of AGS (IC50 39.1 μg/mL), A549 (IC50 20.24 μg/mL), MDCK (IC50 66.35 μg/mL); *Canoparmelia aptata*: AGS (IC50 167.9 μg/mL), A549 (IC50 200 μg/mL)	De Jesus et al., 2016
Pleurosticta acetabulum **(cytochalasin E)**	HT-29	By using acetone extract, raised cytotoxicity (IC50 after 48 h, 6 μg/mL) and declined proliferation, and induced apoptosis	Delebassée et al., 2017
Cladonia furcata **and** ***Cladonia foliacea***	HeLa, Human lung carcinoma A549, Human colon carcinoma LS174	Through using acetone extract, increased cytotoxicity of A549 (IC50 13.58 μg/mL), LS174 (IC50 28.98 μg/mL) [112] Human lung carcinoma A549 *C. furcata*: enhanced cytotoxicity of HeLa (IC50 11.69 μg/mL)	Kosanić et al., 2018
Usnea intermedia	A549, H1299 MCF7, MDA-MB231	By using methanol extract, decreased proliferation of H1299 (IC50 10.2 μg/mL) and MDA-MB-231 (IC50 3.0 μg/mL) induced apoptosis (phosphatidylserine translocation, raised caspase 3/7 activity, loss of mitochondrial membrane potential, formation of pyknotic nuclei)	Ozturk et al., 2019
Parmelia arseneana	FemX, LS174, A549, K562	Through using acetone extract, increased cytotoxicity (IC50 11.61–47.06 μg/mL)	Kosanic et al., 2014
Cladonia pocillumon	MCF-7	By using acetone extract, stimulated apoptosis (concentration-dependent)	Ersoz et al., 2017
Umbilicaria esculenta	A875, A375, HUVEC	Increased cytotoxicity of A875 and A375 and Annexin-V positive and TUNEL positive A875 and stimulated apoptosis of A875 (ROS generation followed by enhanced caspase-3 and −9)	Sun et al., 2018
Physcia millegrana, Parmelia dilatata and Parmeila aurulenta	HepG2, A549 HL-60	lowest toxicity of the depsides towards human A549 lung cancer cells. Importantly, the di-depsides showed greatest toxicity, indicating that these structures are biologically more active than the mono-depsides against the HepG2 liver cancer, A549 lung cancer and HL-60 leukaemia cell lines.	Nugraha et al., 2020
Dirinaria aegialita and Parmotrema praesorediosum	MCF-7 and MDA MB-231	*D. aegialita* showed the higher inhibitory activity against MCF-7 and MDA MB-231 breast cancer cell line as compared to *P. praesorediosum*.	Pradhan et al., 2022
Pseudevernia furfuracea	(PA-1), (MCF-7)	the capacity to act as a potential EGFR inhibitor *via* occupying the ATP binding pocket of EGFR and making favourable electrostatic interactions and van der Waals interaction with its key residues.	Kalra et al. 2022
Pseudevernia furfuracea, Lobaria pulmonaria, Cetraria islandica, Evernia prunastri, Stereocaulon tomentosum, Xanthoria elegans and Umbilicaria hirsuta	cell lines in 2D (monolayer) and 3D (spheroid) models	the most cytotoxic were physodic acid (2D model) and gyrophoric acid (3D model). Moreover, the 3D model compared to the 2D model showed increased cytotoxicity	Kello et al., 2023

1, and STATs (Ebrahim et al., 2016). Despite the control of the STAT3 function, lichens target β-catenin and its downstream effectors (Zhou et al., 2017; Yang et al. 2018), resulting in the regulation of Wnt/β-catenin target genes, especially cell cycle-regulating genes (c-myc, cyclin D1) and cell migration-related genes (MMP7), cell death (BIRC5) or other regulatory agents such as Axin2 (Zhou et al., 2017; Paluszczak et al., 2018).

In addition, modulation of c-Jun and c-fos, members of the AP-1 family of moderators of essential gene expression, and reduction of KITENIN-regulated AP-1 activity are also correlated with lichen cytotoxic pathways (Zhou et al., 2017). The anti-invasive, as well as anti-migration potential of lichens, is still related to the regulation of different transcription factors, such as the Ras superfamily's small GTPase (RhoA, Rac1, Cdc42, and KITENIN) components, which play an important role in tumour advancement and proliferation. The targets of lichens in this regard may also be metastasis-related factors such as CAPN1, CDC42, CFL1, IGF1, or WASF1 and epithelial-mesenchymal markers (Twist, Snail, and Snug). On the other hand, lichen cancer's angiogenesis-inhibitory function is correlated with endothelial tube formation stimulation (Varol, 2018) or vascular endothelial growth factor receptor (VEGFR)−2-mediated signalling Akt as well as an extracellular signal-regulated kinase (ERK) (Dincsoy et al., 2017).

2.2. Anticancer efficacy of lichens in an animal model

The anticancer effects of containing natural components or extracts obtained from lichens are endorsed by numerous research that has used animal xenograft/allograft models. Some research has

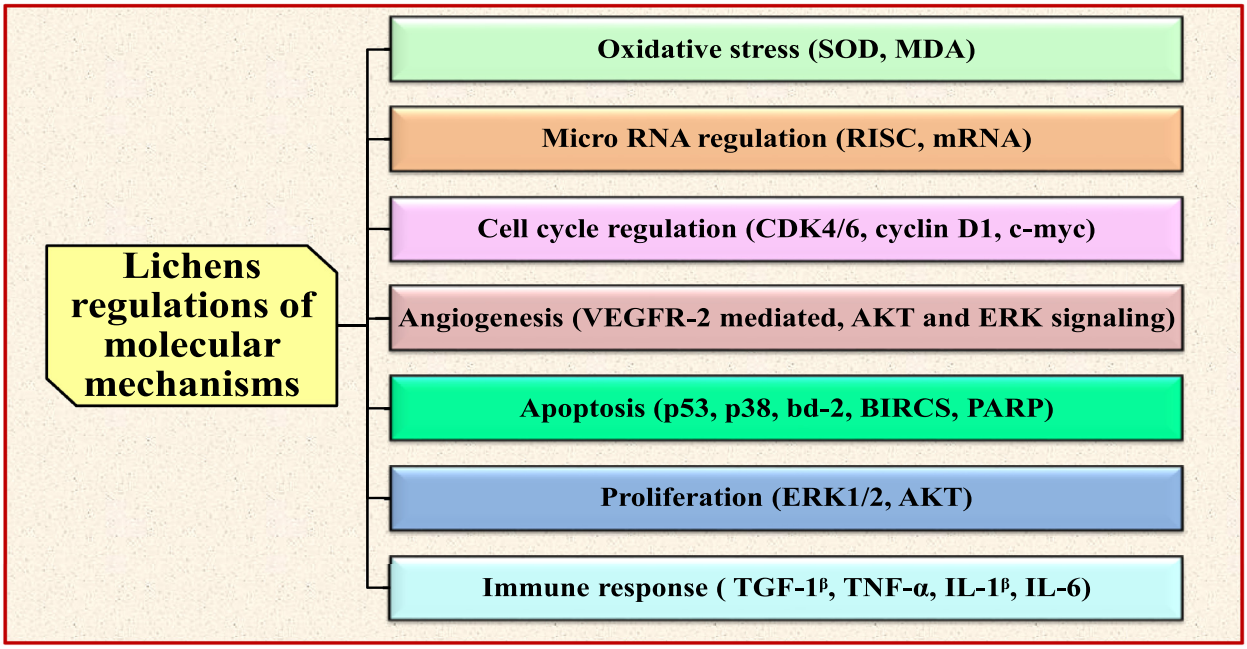

Fig. 1. The role of lichens in the regulation of cancer-associated molecular mechanisms.

investigated the anticancer ability of UA in vivo. Usnic acid prevented angiogenesis in both the chick embryo chorioallantoic membrane and the VEGF-induced mouse corneal angiogenesis method. In addition, in C57BL/6 female nude mice, UA decreased the production of infected human Bcap-37 BC cells as well as prevented cell growth of tumour tissue. The prevention of cell proliferation was investigated by anti-CD31 and showed a reduction in the optical transmission concentration of tumour blood vessels in the treatment community of the UA (Solárová et al., 2020). The in vivo experiments was followed via an in vitro simultaneous evaluation in which UA reduced HUVEC cell growth, differentiation as well as canal production, disrupted ERK1/2 and AKT regulated VEGFR2 signalling as well as mediated cell death by decreasing Bcl-x1 or integrin concentrations or by increasing caspase 3 activity and PARP cleavage activity (Song et al., 2012). In addition, by generating ROS and JNK stimulation relevant to the mitochondrial/caspase mechanism induction of apoptosis of cancerous cells, UA prevented tumour growth of human MCF-7 breast cancer-bearing mice dose-dependently. UA was indeed well controlled and had no adverse effect on animals (Zuo et al., 2015). This metabolite also decreased the sensitivity of bleomycin therapy in Kunming mice to immunized mouse H22 hepatocellular carcinoma cells and the synthesis of these substances was more successful compared to single bleomycin cells for H22 cancer. Nevertheless, the mixture of UA and bleomycin halted tumour cells at the G0/G1 stage and induced cell death through caspase-3 and −8 activation. In addition, UA affected the amount of MDA, hydroxyproline, TNF-alpha, IL-1β, IL-6, and TGF-β1 and its mixture with bleomycin raise the amount of SOD in H22-bearing mouse lung tissues, likely via inhibition of p-Smad2/3 and Smad7 protein up-regulation (Su et al., 2017). In addition, the UA benzylidene analogue exhibited in vivo therapeutic potential on two models of MDA-MB-231 and MCF-7 xenograft mouse mammary cancer. Autophagy and modulation of the mTOR signalling cascade were mediated by both UA and its benzylidene analogue, followed by a substantial decline in the amount of mTOR downstream effectors p-S6K and p-4E-BP1 in the treatment categories of both designs (Ebrahim et al., 2017). Without any animal weight loss, UA decreased the volume and weight of tumours in the mouse model of human intestinal carcinoma BGC823. Additionally, when evaluating the Bax/Bcl2 ratio, UA demonstrated greater pro-apoptotic activity relative to 5-FU. Although 5-FU had the same effect on the volume and weight of the tumour, major animal weight loss was associated with it (Geng et al., 2018). The therapeutic efficacy of *Flavocetraria cucullata* acetone extract, UA, and LiA on human A549 lung cancer cells was evaluated in another study by Nguyen et al. (2014) using the xenograft Balb/c mouse model. In F, the maximum number of survivors free from tumours was reported. In comparison to the DMSO (zero out of eight) group, the UA (four out of eight) group, or the LiA (two out of eight) group, the pretreated cucullata (six out of eight tumour-free mice) group (Nguyen et al., 2014). Interestingly, in the CT26-Fluc syngeneic mouse tumour model, PU showed better tumour, liver, and plasma bioavailability as compared to UA. Notably, ethyl acetate extract from *Usnea longissima* prevented esophagogastric adenocarcinoma caused by oral administration of N-methyl-N-nitro-N-nitrosoguanidin in males *Albino Wistar* rats. At 50 and 100 mg/kg quantities, the extract of *U. longissima* demonstrated a conspicuous capacity for cancer as well as the effectiveness of cancer tissue in animals without adverse effects. While Mammadov et al. (2019) were tested at high doses (500, 1000, and 2000 mg/kg), this extract had no lethal impact. In the mouse 4T1 allograft model of breast cancer in BALB/c mice, the study showed substantial anti-cancer effectiveness of ATR.

2.3. Lichens as a source of cytotoxic chemotherapeutic

Since these preliminary studies, several other lichen elements are being examined for their cytotoxicity on various cancer cells (Shrestha and St. Clair, 2013). Usnic acid, cristazarin, protolichesteric acid, polyporic acid, depsidone, and licheninine are case studies of findings including aqueous extract and processed active substances on various cancer cells, including skin cancer, blood cancer, breast cancer, prostate cancer, and stomach cancer (Zambare and Christopher, 2012). The main methods of preventing cell growth by regulating the cell cycle and of increasing apoptosis by apoptosis induction or necrosis have been developed for this anticancer therapeutic potential. The processes of cytotoxicity of the lichen metabolite vary, as expected, between cancer cells. In a study of transcriptional regulation and apoptosis, Singh et al. (2013) have previously noted that usnic acid prevents the activity of lung cancer cells (A549) by stopping the cellular

proliferation at the G0/G1 stage by altering the levels of cyclin D1 protein expression, cyclin-dependent kinases (CDKs) or cyclin-dependent kinase inhibitor (CDKI) and inducing mitochondrial membrane cell death. Einarsdottir et al. (2010) also suggested that both (+) and (-) usnic acid prevented the passage into the S phase of the cell cycle and led to a decrease in the cell size of the breast cancer cells of T47D and the pancreatic cancer cell line of Capan-2; in addition, no cell death was recorded in these cells and necrosis was only seen in Capan-2 cells. Lichen acids such as usnic acid, atranorin, and gyrophoric acid have been found to contribute to cell cycle accumulation in the S process in nine distinct human cancer cell lines (Backorova et al., 2011). During cell apoptosis, the death receptor-mediated (extrinsic) mechanism as well as mitochondria-mediated (intrinsic) mechanism activates 202 H. Kim et al. (2011) cleaved the caspase pathway and activated caspase with several cysteine residues like poly (ADP-ribose) polymerase (PARP) or Lamins. Lin et al. (2003) suggested that lichenin *Cladonia furcata* polysaccharide-2 (CFP-2) induced up-regulation of Fas and FasL transcription as a death receptor-induced cell proliferation in the cell lines of HL-60 promyelocytic leukaemia. CFP-2 also diminished telomerase activity in these cells, showing its possible therapeutic benefits for cancer. Furthermore, Russo et al. (2012) stated that the expression of TNF-related apoptosis-inducing ligand (TRAIL) in LNCaP prostate cancer cells was significantly enhanced by vicanicin and protolichesterinic acid. In this study, the potential of activity of Hsp70 in the initiation of intrinsic pathway-mediated cell death by vicanicin and protolichesterinic acid was also increased, because Hsp70 can induce main effectors of mitochondrial apoptotic mechanism (Rerole et al., 2011). As an endogenous cell death mechanism, Backorova et al. (2012) stated that usnic acid and atranorin mediated huge loss of mitochondrial membrane potential in an ovarian cancer cell line (A2780) and colon cancer cell line, causing apoptotic cell death.

2.4. Active constituents of lichens in cancer research

In traditional medicine, lichen constituents and related products have been used for several decades to treat many health problems; modern vaccination being used in vitro cancerous cell types has demonstrated their ability as therapeutic drugs (Table 2). Although the mechanisms of cellular activity of lichen bioactive compounds on existing and cancerous cells are still not fully understood, results show that certain bioactive substances have cytotoxicity on certain cancerous cells, especially carcinomas of the mammary, lung as well as colon, as demonstrated by their ability to reduce cell proliferation in a dosage way (Koparal et al., 2010; Bačkorová et al., 2011; Nguyen et al., 2014; Yang et al., 2015). Data from other findings have shown that cell proliferation, regulation of angiogenic as well as control of many signalling pathways, cellular cycles as well as regeneration are influenced by bioactive compounds (Bessadóttir et al., 2014; Zakharenko et al., 2016; Zhang et al., 2016; Zhou et al., 2017; Yang et al., 2018c). A range of molecular and biochemical approaches have been used to test the possible mechanisms of activity of lichens constituents in cancerous cells. In context, the cytotoxic effect of lichen-derived substances is determined either by trypan-blue exclusion assay or the MTT assay, which measures cellular biological pathways. To assess the molecular pathways of lichen compounds in regulating cell death, cell proliferation kinetics, as well as transcriptional regulation, multiple endpoints such as p53, cyclin A and D1, caspases, BAX, BCL-2, and others were evaluated (Azmir et al., 2013; Ghate et al., 2013; Aravind et al., 2014; Gonçalves et al., 2018; Triggiani et al., 2009). It should be noted that the heterogeneity of the observed responses to specific secondary compounds inside or between the different carcinoma cells could be attributable to the physical and chemical properties of the specific substances as well as the biochemical variations of the molecules (Tas et al., 2017). It has been shown that lichens constituents exhibit different degrees of cancerous cell tolerance. Tumour growth, separation, cell apoptotic nuclear proliferation, cell cycle transformation, and specific signal transduction pathways have assessed the mechanisms of lichens compound's activity on cancerous cells. It addresses evidence supporting the therapeutic agent's activities and potential mechanisms through which lichens chemicals operate on various cancer cell lines.

2.5. Breast cancer

Numerous mammary cell types have been tested to assess the effectiveness of lichens against breast carcinoma cells. The key concern in this research was the progression of cancerous cells, the mechanisms of activity of constituents of lichens and molecular pathways, particularly cell cycle inhibition, programmed cell death and autophagy induction, angiogenesis control, and the regulation of many signalling pathways. For instance, the acetone extract and bioactive compound parietin of lichen *Xanthoria parietina* prevented angiogenesis and cell proliferation in the MDA-MB231 cancer cell. The gene-regulating cell cycle expression regulation for protein components like p16, p27, cyclin D1, and cyclin A was followed by both mechanisms (Basile et al., 2015). There was over-expression of fatty acid synthase (FASN) and human epidermal growth factor receptor 2 (HER2) in the presence of protolichesterinic acid (PA), a lichen-extracted compound that stimulated programmed cell death and also modulation of the ERK1/2 and AKT mechanisms in SK-BR-3 cell However, no alterations in T-47D cells were claimeder, because PA seems to not affect ERK and AKT signal transduction (Bessadóttir et al., 2015). FASN is an important biomarker responsible for catalysing long-chain fatty acids in mammalian cells (Long et al., 2014), which regulates the expression of HER2, a tyrosine kinase transmembrane receptor. In the case of breast cancer, both HER2 and FASN seem to be over. Any target drug which prevents the function of FASN could also be efficient for HER2 inhibition (Cheng et al., 2014). Exposure to usnic acid and rapamycin of various breast cancer cell lines caused marked angiogenesis, proliferation, and invasive inhibition (Ebrahim et al., 2017). Usnic acid has been reported to cause DNA damage and induce p53 tumour suppressor action on MCF-7 cells (Mayer et al., 2005; Brisdelli et al., 2013). Furthermore, the use of usnic acid, as well as the chemotherapy drug bleomycin, has improved caspase-3 and 8 toxicity and function, enhancing programmed cell death and p53/p21 pathways. Programmed cell death and regulation of cell multiplication in MCF-7 breast cancer cells were induced by *Hypogymnia physodes* lichens compounds (Ari et al., 2014); however, no morphological or microtubular variations were seen in MCF-7 cells via usnic acid therapy. This finding completely contradicts other studies in which substantial morphological and microtubular changes in MCF7 cells containing usnic acid have been documented, indicating that the effects on mammary carcinoma cells may be due to the active quantity of usnic acid in such analyses (O'Neill et al., 2010).

2.6. Colon cancer

There were different IC50 values for lichen extracts and lichens constituents tested on various colon cancer cells, indicating the effect on the physical and chemical characteristics of lichen substances and their amounts on targeted cancer cells. Methyl-β-orcinolcarboxylate (1.5 μg/ml) as well as usnic acid (2.3 μg/ml) along with acetone extract from *Endocarpon pusillum* (1.84 μg/ml) was the lowest IC50 values, although it is difficult to compare directly because different colon cancer cell lines were included. In the different phases of the cell cycle, both cell types displayed controlled levels of apoptosis and seizure. Diagnosis of lobastin and lobaric acid with HCT116 led to changes in cellular proliferation from polygonal to circular, which may be due to apoptotic cell death along with separation as well as breakage of cells. In addition, PARP cleavages, as well as a decrease in Bcl-2 gene regulation, have also been reported (Hong et al., 2018).

Table 2
Anticancer potential of isolated lichen constituents.

Lichen acid	Cell line	Mechanisms	References
Usnic acid	HepG2, SNU-449	Induction apoptosis and autophagy, G0/G1, G2/M arrest	Yurdacan et al., 2019a
	BGC823, SGC7901	Decreased proliferation and G0/G1 and G2/M arrest of BGC823 (IC50 236.55 μM) and SGC7901 (IC50618.82 μM) induction of apoptosis, increased autophagy, Increased Bax/Bcl-2 ratio, caspase-3, and PARP.	Geng et al., 2018
	A549, H460, H1650 and H1975	motility of A549, decreased invasion of H1650 and H1975 (decreased β-catenin-mediated TOPFLASH and KITENIN-mediated AP-1 activity) lowest expression of CD44, c-myc and Cyclin D1 in all cell lines and GTP-Rac1 and RhoA	Y. Yang et al., 2016
Synthetic derivatives of Usnic acid	MCF-7, PC-3, HeLa	Derivatives 2a, 2b: decreased proliferation of PC-3, MCF-7 (IC50 value 3 μM), HeLa (IC50 1 μM), G0/G1 arres, induction apoptosis of MCF-7Activation of cytoplasmic vacuolisation.	Pyrczak-Felczykowska et al., 2019
PU	HCT116, DLD1, SW480, HT29, SW620, Caco2, COLO320, CT26	Enhance the cytotoxicity (lower IC50 than UA, except of SW480 and CT26 cells), minimize the invasion of Caco2 and HCT116, Caco2 motility (CAPN1, CDC42, CFL1, IGF1, WASF1, WASL)	Yang et al., 2018d
Usnic acid and Atranorin	HTB-140, DU-145, PC-3	Decreased proliferation, migration, actin organization	Galanty et al., 2017
Atranorin and Gyrophoric acid	A375	PA (concentration, 6.25–50 μM): decreased A375, enhance apoptosis, ATR and GA (high concentrations): increased A375	Cardile et al., 2017
Barbatic acid	HeLa, A549, MCF-7, DU-145, HEK293	HeLa (IC50 3.2 μg/mL), A549 (IC50 1.8, 3.2 μg/mL), MCF-7 (IC50 3.2 μg/mL), DU-145 (IC50 9.0 μg/mL) BA (concentration, 1 μM): G0/G1 arrest, increased apoptosis,caspase-3 activity, PARP cleavage, annexin V staining and chromatin condensation (A549)	Reddy et al., 2019
Physodic acid	TRAIL-resistant LNCaP cells in combination with TRAIL	It was observed that physodic acid showed a dose–response relationship in the range of 12.5–50 μM concentrations in LNCaP and DU-145 cells, activating an apoptotic process. In addition, physodic acid sensitizes LNCaP cells to TRAIL-induced apoptosis.	Cardile et al. 2022
Evernic acid	MCF-7 and MDA-MB-453	The cytotoxicity results indicated that evernic acid suppressed the proliferation of MCF-7 and MDA-MB-453 cells in a dose-dependent manner and the IC_{50} = 33.79 and 121.40 μg/mL, Migration assay revealed the notable antimigratory ability of evernic acid against both cell types. The expression of apoptotic markers Bcl2 associated X, apoptosis regulator, Bcl2 apoptosis regulator, and tumour protein p53 Evernic acid showed its anticancer effect via inhibiting TrxR1 enzyme activity rather than mRNA and protein expression levels in both cell lines.	Kalin et al., 2023

Kosanić et al. (2014) reported the action of antiproliferation and reduction of the G2/M cell line also as two profound effects of atranorin or fumarprotocetraric acid on the LS174 cancer cell using propidium iodine cytofluorometric assay. In addition, atranorin was more efficient in accumulating cells in the sub-G1 step than fumarprotocetraric acid. The acetone extract of *Lethariella zahlbruckneri* induced programmed cell death in HT-29 cancerous cells or triggered caspase-8 and caspase-9 activity in the Western blot analysis (Ren et al., 2009), showing upregulation in Bax and a decrease in Bcl-2 proteins. A study on two certain lines of colon cancer cells (HCT116 and DLD-1) also reported modulation of dose and time dependence signalling involving different lichen substances. In these, lecanoric acid exhibited a marginal impact on Axin2 induction in HCT116 cells compared with physodic acid. Caperatic acid also decreased the expression of Axin2 in both cell lines (Paluszczak et al., 2018). Ramalin, another secondary lichen biomarker, showed G2/M cell cycle progression as a sign of HCT116 apoptotic cell death. In addition, there has been a rise in the synthesis of the TP53 protein and a decline in CDK1 and CCNB1 (Suh et al., 2017). A further successful anti-colorectal agent, tumidulin, showed decreased spheroid emergence in CSC221, DLD, and HT29 mRNA transcription and cancer assays, such as aldehyde dehydrogenase1, CD133, CD44, and Lgr5. Besides, tumidulin inhibited the gene expression operation of oncogene-linked glioma (Gli1 and Gli2) (Yang et al., 2018a).

2.7. Skin cancer

Melanoma cancer cells UACC-62, FemX, and HTB-140 (Hs 294T) were all used to study the anti-cancer efficacy of lichens compounds. The bioactive compounds demonstrated bioactivity through apoptosis and DNA damage against skin cancerous cells. For example, melanoma A375 cancer cell therapy with physodic acid at amounts of 6.25–50 μM consisted in inhibition of cell proliferation and cell death. Physodic acid has been suggested to induce underexpression of the 70 kDa protein Hsp70 (Cardile et al., 2017), a heat shock protein located in tumour cells including such malignant melanoma cells (Farkas et al., 2003). In another study, cell proliferation, as well as actin cytoskeleton in melanoma cells HTB-140, was reduced significantly by both atranorin and usnic acid (Galanty et al., 2017).

Usnic acid and SFE extract from *Usnea barbata* lichen influenced cell death as well as proteolysis in cancer cells of the B16 cell line, resulting in morphological characteristics in the cytosolic acid vesicles which promote vesicular movement throughout the cells as well as endocytosis processes (Zugic et al., 2016). Two lichen derivatives, sphaerophorin, and pannarin have been reported to induce cell death in cancer cell M14 or DNA damage through substantial caspase-3 activity and inhibition of free radicals (Russo et al., 2008). Cytotoxicity was documented by the diagnosis of usnic, gyrophoric, and diffracteic acid in human keratinocyte cells (Kumar et al., 1999). Usnic acid was also particularly toxic to malignant mesothelioma of MM98, A431 human epidermoid carcinoma cells, as well as HaCaTT keratinocyte cells (Burlando et al., 2009).

2.8. Lung cancer

Lines A549, NCI-H460, and NCI-H2923T3 of human non-small cell lung cancer cells were used to analyse the antitumor activity of different constituents of lichens. Therefore, physciosporin has been shown to have a major effect against lung cancer cell invasion and metastasis by minimizing the protein and mRNA concentrations of N-cadherin while increasing the KAI1 gene suppressor tumour expression. In lung cancer cells A549, H1650, and H1975 (Yang et al.,

2015), the activities of gene expression control component 42 (Cdc42) and Rac1 were also prevented by physciosporin. Usnic acid concentration-dependent diagnosis of non-small lung cancer cells has been used to relieve the β-catenin protein and oncogene metastasis-enhancing action of KITENIN-mediated AP-1 involved in cancer control (Yang et al., 2016b). Usnic acid also decreased the mRNA levels of CD44, Cyclin D1, and c-myc in those cells. Low doses of usnic acid (6.25 and 12.5ug/mL) in another study induced an excitatory mitochondrial effect through raising the number of reactive oxygen species (ROS) that lead to decline apoptosis (Koparal et al., 2010). Similarly, the problem of atranorin-containing A549 cells decreased the action of both β-catenin and AP-1 mediated KITENIN and the Rho family of GTPase proteins, which are signal transducers as well as transcription stimulators (STAT) (Zhou et al., 2017). XD8, a chemical-produced variant of the natural sources xanthone XD8 lichens (Liu et al., 2016), induced a dosage inhibitory effect at the G0/G1 cell cycle level in the lung cancer cell line A549 and cell aggregation. Antineoplastic action with non - toxicity towards healthy cells has been observed within ductal lung cancer cells diagnosed by lichen *Cladonia* aggregate extracts and barbatic acid. (Martins et al., 2016).

2.9. Liver cancer

Lichen bioactive constituents and lichen isolated compounds have shown important anticancer effects on HEPG2, C3A, WRL68, and human liver cell lines. An approach was used to explain the cytotoxic activity of *Umbilicaria tornata* and its two derivatives, UTP-1 and UTP-2, on HEPG2. Both demonstrated antiproliferative activity on HEPG2 cells and significantly suppressed cell proliferation (Shang et al., 2018).

3. Conclusions

Lichens are a rich source of bioactive components that, in cancer diseases, have huge potential for their therapeutic efficacy. The bioactive components of lichens, including gyrophoric acid, usnic acid, and lecanoic acid, have a wide variety of anticancer effects on different forms of carcinoma cells. Modern biomedical analytical methods that facilitate the isolation and characterization of lichen derivatives and thereby explain the rather important physiological cellular effects of lichen derivatives establish a suitable environment for advancing studies to determine future medical testing and the use of lichen-derived anticancer drugs in medical practice. Researchers have identified the significant anti-cancer effectiveness of isolated lichen metabolites, lichen extracts, or isolated compounds through association with lichen crude extract in carcinoma cells and animal cancer models. Despite the above extreme view of the research as a cytotoxic compound of lichen extracts or isolated lichen molecules, no specific scientific study has been conducted to provide an overview of the therapeutic potential of lichen species to date in humans. Therefore, more studies are still aimed at evaluating the potential therapeutic application and describing its beneficial effects on cancer patients or at-risk individuals. However, in terms of the therapeutic utility of lichens' bioactive compounds in cancer disease, there are methodological drawbacks that must be explored with scientific oncologists/researchers. The data available to date indicates such a need to pursue the quest for new, more accurate, and more effective active lichen compounds; boost technological development on the production of quality and hence more enhancing the capacity for their high yield cultivation, isolation, or separation; as well as improve scientific insight into the underlying factors accounting for stimulating them on various cancer cells.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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