CHAPTER 2-2

MEDICAL USES: BIOLOGICALLY ACTIVE SUBSTANCES

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Figure 1. Bryophytes and other herbs on sale in a Yunnan, China, market. ***Rhodobryum giganteum*** (upper bag) and ***Leucobryum*** (lower bag), both called Hui Xin Cao. Photo by courtesy of Eric Harris.

Antibiotics and Other Biologically Active Substances

Bryophyte species actually produce broad-range antibiotics (Asakawa 2007a, b, 2008; Asakawa *et al*. 2013). Their usage in surgical dressings, diapers, and other human medicinal applications is well known. And their use has not been confined to Asia (Frahm 2004), but is known in Brazil (Pinheiro da Silva *et al*. 1989), England (Wren 1956), North America (Pejin *et al*. 2011a, b), and Germany (Frahm 2004), as well as in China (Ding 1982; Wu 1982) and India (Watts 1891). Frahm (2007) has reviewed the literature on bryophytes and their antibiotic activity.

Bryophytes discourage the feeding by a variety of organisms, as discussed in the chapters on terrestrial insects, arthropods, and other interaction chapters. Frahm and Kirchhoff (2002) showed that extracts of the epiphytic

moss ***Neckera crispa*** (Figure 2) and leafy liverwort ***Porella obtusata*** (Figure 3) both discouraged feeding by the Portuguese slug ***Arion lusitanicus*** (Figure 4).

Figure 2. ***Neckera crispa***, a species that discourages the Portuguese slug from eating it. Photo by Jan-Peter Frahm, with permission.

Figure 3. ***Porella obtusata***, a species that discourages the Portuguese slug from eating it. Photo by Michael Lüth, with permission.

Figure 4. ***Arion lusitanicus***, a slug that avoids eating extracts of the moss ***Neckera crispa*** and leafy liverwort ***Porella obtusata***. Photo by Ondřej Zicha, through Creative Commons.

One indication of the presence of unique and potentially important pharmaceutically and anti-feedant chemicals in bryophytes is the presence of unique odors. This is especially true for liverworts, with more than several hundred new compounds identified among them (Asakawa 2012). In addition, more than 40 new carbon skeletal acetogenins, phenolic compounds, and terpenoids were identified. Testing of these compounds has led to the commercial development of a natural pesticide (Frahm 2004).

Leptolejeunea (Figure 5) and ***Moerckia*** (Figure 6) are distinctly aromatic (Schuster 1966), ***Lophozia bicrenata*** (Figure 7) has a pleasant odor, species of ***Solenostoma*** (Figure 8) smell like carrots, ***Geocalyx graveolens*** (Figure 9) has a turpentine-like odor, and ***Conocephalum conicum*** (Figure 10) smells like mushrooms. The tropical ***Plagiochila rutilans*** (Figure 11) smells like peppermint, caused by several menthane monoterpenoids (Heinrichs *et al*. 2001).

Figure 5. ***Leptolejeunea elliptica***, a species with a distinct odor. Photo by Yan Jia-dang, through Creative Commons.

Figure 6. ***Moerckia flotoviana*** female, a species with a distinct odor. Photo by Michael Lüth, with permission.

Figure 7. The leafy liverwort ***Lophozia bicrenata***. Photo by Michael Lüth.

Figure 8. ***Solenostoma hyalina***, a species that smells like carrots. Photo by Janice Glime.

Figure 9. The leafy liverwort ***Geocalyx graveolens***. Underleaf is indicated by the red star. Photo by Michael Lüth.

Figure 10. ***Conocephalum conicum***, a species that smells like mushrooms. Photo by Hermann Schachner, through Creative Commons.

But can you imagine using mosses to lower your cholesterol? Yes, mosses contain polyunsaturated fatty acids that are already known to have important potentials in human medicine, such as preventing atherosclerosis and cardiovascular disease, reducing collagen-induced thrombocyte aggregation, and lowering triacylglycerols and cholesterol in plasma (Radwan 1991).

Figure 11. ***Plagiochila*** sp. ***Plagiochila rutilans*** smells like peppermint. Photo by Lin Kyan, with permission.

It appears that these unique odors result from a combination of many compounds, including monoterpene hydrocarbons such as α-pinene, ß-pinene, camphene, sabinene, myrcene, alpha-terpinene, limonene, fatty acids, and methyl esters of low molecular weight (Hayashi *et al.* 1977). For example, ***Isotachis japonica*** (Figure 12) has at least three aromatic esters: benzyl benzoate, benzyl cinnamate, and B-phenylethyl cinnamate (Matsuo *et al.* 1971).

Figure 12. ***Isotachis*** sp. ***Isotachis japonica*** has at least three aromatic esters. Photo by George Shepherd, through Creative Commons.

But progress in purifying and identifying bryophyte biochemical components and demonstrating their antibiotic effects has been slow. As early as 1952, Madsen and Pates found inhibition of microorganisms in products of bryophytes, including ***Sphagnum portoricense*** (Figure 13), ***S. strictum*** (Figure 14), ***Conocephalum conicum*** (Figure 10), and ***Dumortiera hirsuta*** (Figure 15) (see also Sabovljević *et al*. 2011; Chandra *et al*. 2017). Pavletic and Stilinovic (1963) found that ***Dicranum scoparium*** (Figure 16) strongly inhibited all bacteria tested but Gram-negative *Escherichia coli* (Figure 17). McCleary and Walkington (1966) considered that non-ionized organic acids and polyphenolic compounds might contribute to the antibiotic properties of bryophytes and found eighteen mosses that strongly inhibited one or both of Gram-positive and Gram-negative bacteria, the most active being ***Atrichum*** (Figure

20), ***Dicranum*** (Figure 16), ***Mnium*** (Figure 18), ***Polytrichum*** (Figure 19), and ***Sphagnum***. Reminiscent of ***Dicranum scoparium*** (Figure 16), ***Atrichum undulatum*** (Figure 20) was effective on everything tested except *Enterobacter aerogenes* (drug resistant and infectious to people with weak immune systems; Figure 21) and *E. coli*.

Figure 13. ***Sphagnum portoricense***, a species that inhibits microorganisms. Photo by Blanka Shaw, with permission.

Figure 14. ***Sphagnum strictum***, a species that inhibits microorganisms. Photo by Jan-Peter Frahm, with permission.

Figure 15. ***Dumortiera hirsuta,*** a species that inhibits microorganisms. Photo by Michael Lüth, with permission.

Figure 16. ***Dicranum scoparium***, a species that inhibited all bacteria tested but Gram-negative *Escherichia coli*. Photo by Janice Glime.

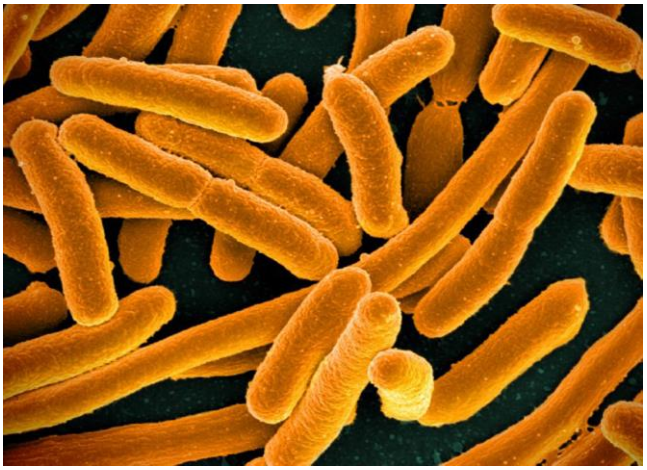

Figure 17. *Escherichia coli*, a species that is inhibited by acetone-soluble extracts of several thallose liverwort species. Photo by NIAID, through Creative Commons.

Figure 18. ***Mnium spinulosum***. Members of the genus ***Mnium*** are among the most active against one or both of Gram-positive and Gram-negative bacteria. Photo by Michael Lüth, with permission.

Figure 19. ***Polytrichum commune***. Members of the genus ***Polytrichum*** are among the most active against one or both of Gram-positive and Gram-negative bacteria. Photo by Michael Lüth, with permission.

Figure 20. ***Atrichum undulatum*** is a moss that is very effective against a wide range of bacteria. Photo by Michael Lüth.

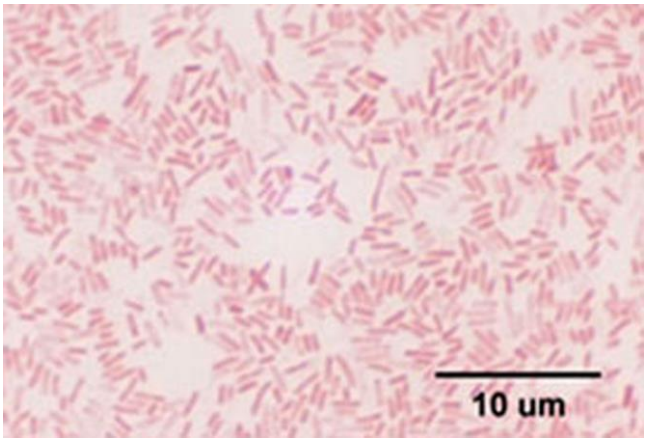

Figure 21. *Enterobacter aerogenes*, a bacterium that seems to be resistant to extracts of 18 moss species that negatively affect other bacteria. Photo by Alexa Rakusin Muna, through Creative Commons.

Gupta and Singh (1971) found high occurrence of antibacterial activity in extracts of ***Barbula*** species (Figure 22), reaching as high as 36.2%, whereas it was only half that in ***Timmiella*** species (Figure 23) (18.8%). In 1982, Asakawa *et al.* (1982) isolated three prenyl bibenzyls from ***Radula*** spp. (Figure 24) and demonstrated that these bibenzyls could inhibit growth of *Staphylococcus aureus* (Figure 25) at concentrations of 20.3 µg ml^{-1}. Out of more than 80 species tested, Ichikawa (1982) and coworkers (1983) found antimicrobial activity in nearly all. Acyclic acetylenic fatty acid and cyclophentenonyl fatty acid extracts from the mosses completely inhibited the growth of the rice blast fungus *Magnaporthe grisea* (Figure 26). Belcik and Wiegner (1980) reported antimicrobial activity in extracts of the liverworts ***Pallavicinia*** (Figure 27) and ***Reboulia*** (Figure 28), and Isoe (1983) reported it from ***Porella*** (Figure 29).

Figure 22. ***Barbula convoluta***, member of a genus with high antibacterial activity. Photo by Michael Lüth, with permission.

Figure 23. ***Timmiella*** sp., a genus with high antibacterial activity. Photo by Ken-ichi Ueda through Creative Commons.

Figure 24. ***Radula complanata***, a species with bibenzyls that could inhibit growth of *Staphylococcus aureus*. Photo by Michael Lüth, with permission.

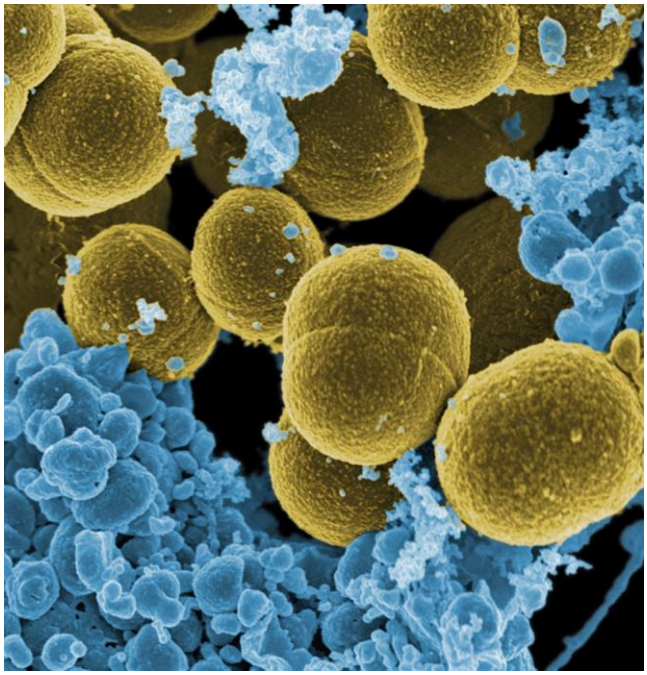

Figure 25. *Staphylococcus aureus*, a bacterium that is not inhibited by the thallose liverwort ***Riccia fluitans***. Photo by NIAID, through Creative Commons.

Figure 26. *Magnaporthe grisea*, a plant pest that is inhibited by several liverworts. Photo by IRRI Photos, through Creative Commons.

Figure 27. ***Pallavicinia lyellii***, member of a genus with reported antimicrobial activity. Photo by Jan-Peter Frahm, with permission.

Figure 28. ***Reboulia hemisphaerica***, member of a genus with reported antimicrobial activity. Photo by Malcolm Storey <www.discoverlife.org>, with online permission.

Figure 29. ***Porella platyphylla***, member of a genus with reported antimicrobial activity. Photo by Tim Waters through Creative Commons.

Another three species of mosses [***Anomodon rostratus*** (Figure 30), ***Plagiomnium cuspidatum*** (Figure 31), ***Orthotrichum rupestre*** (Figure 32)] produce substances that inhibit bacteria and fungi, but these inhibitors seem to be unstable products that vary considerably among species and possibly also among seasons (McCleary *et al*. 1960). Indeed, it would appear that some of these antibiotic compounds are the very ones that bryophytes produce in response to stress. However useful they may be, it seems that these discoveries have not yet found their way into medical practice.

Figure 30. ***Anomodon rostratus***, a species that inhibits bacteria and fungi. Photo by Dale A. Zimmerman Herbarium, Western New Mexico University, with permission.

Figure 31. ***Plagiomnium cuspidatum***, a species that inhibits bacteria and fungi. Photo by Štěpán Koval, with permission.

Figure 32. ***Orthotrichum rupestre***, a species that inhibits bacteria and fungi. Photo by Michael Lüth, with permission.

Scientists have found innumerable kinds of biological activity in compounds from bryophytes. Even in a single species, one might find multiple kinds of activity. For example, the liverworts ***Plagiochasma japonica*** and ***Marchantia emarginata*** subsp. ***tosana*** (Figure 33) exhibit antitumor activity, antifungal and antimicrobial activity, inhibition of superoxide release, inhibition of thrombin activity, and muscle relaxation (Lahlou *et al.* 2000). As is often the case with herbal medicine, the effect of the total extract is better than that of the isolated compounds, perhaps due to a synergistic effect (Frahm 2004).

Figure 33. ***Marchantia emarginata*** subsp. ***tosana***, a subspecies with a wide range of medicinal properties. Photo by Taiwan Mosses, through Creative Commons.

On the other hand, some researchers claim that antibiotic properties of some mosses, including ***Sphagnum*** (Figure 13, Figure 14), may actually be the work of associated microorganisms. In some cases, *e.g.* ***Sphagnum***, it may be *Penicillium* sp. (Figure 34) effecting this antibiotic ability (Lewington 1990). Or is it the closely associated **Cyanobacteria**, such as ***Nostoc*** (Figure 35) (Spjut *et al*. 1988; Solheim & Zielke 2002)?

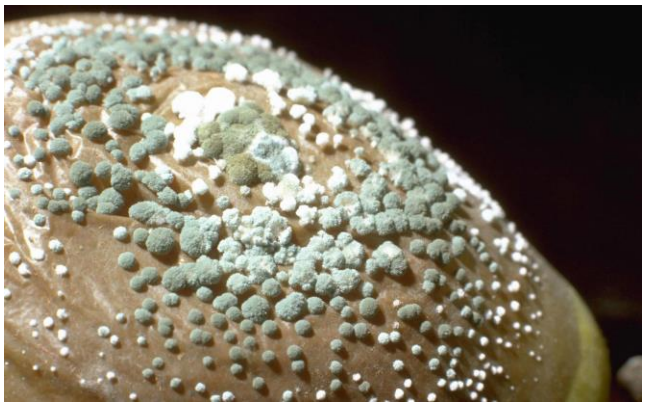

Figure 34. ***Penicillium expansum*** on pear, in a genus that can grow on ***Sphagnum*** and may contribute to its antibiotic effects. Photo by H. J. Larsen, through Creative Commons.

Figure 35. ***Nostoc pruniforme***, in a genus with close associations with ***Sphagnum*** and other bryophytes and could contribute to antibiotic properties. Photo by Lairich Rig, through Creative Commons.

Painter (2003) notes that ***Sphagnum*** (Figure 13, Figure 14) can be 3-4 times as absorbent as cotton equivalents. But its call to fame seems to be its ability to react chemically with all sorts of proteins. ***Sphagnum*** species have the potential to immobilize whole bacterial cells, enzymes, exotoxins, and lysins that are secreted by most of the invasive pathogens. Once these are immobilized, they are inactivated by a **Maillard reaction**.

The **Maillard reaction** makes this story complex. It is known to suppress the virulence gene expression operon in the bacterium *Listeria monocytogenes* (Figure 36) (Sheikh-Zeinoddin *et al*. 2000), so that is a good thing. On the other hand, a variety of foods form potential cancer-causing acrylamides, especially fried foods (Stadler *et al*. 2002). Such acrylamides can be released by thermally treating certain amino acids such as asparagine, especially in combination with reducing sugars through the Maillard

reaction. The early Maillard reaction products are N-glycosides. Painter (1998) found that the Maillard reaction inhibits microbial growth in animal products preserved in bogs by sequestering ammonia, amino acids, and peptides, whereas the polymeric end-products (melanoidins) inhibit their growth by cross-linking the polypeptide chains and sequestering essential multivalent metal cations. In short, the Maillard reaction appears to be an important component of the ***Sphagnum*** (Figure 13, Figure 14) antibiotic activity. Furthermore, its preservative ability correlates with α-keto-carboxylate groups in a glycuronoglycan (**sphagnan**) that comprises ~60% of the holocellulose in the ***Sphagnum*** hyaline cell walls.

Figure 36. *Listeria monocytogenes*, a bacteria species that is inhibited by extracts of the leafy liverwort ***Porella cordaeana***. Photo from CDC, through public domain.

Harris (2009) considered phylogenetic, elevational, and latitudinal relationships of the production of flavonoids in medicinal mosses. He was unable to show any significant correlation between phylogenetic independent contrasts of total phenolic content, number of flavonoids, or percent luteolin derivatives. He furthermore found no correlation with elevation or latitude. He could not rule out the possible correlation with fine-scale ecological features, and he considered flavonoid variation to reflect recent evolution.

Labbé *et al*. (2007) tested the thallose liverwort ***Riccardia polyclada*** for potential pesticidal properties. They identified four compounds that contributed to lethality in the brine shrimp (*Artemia salina*; Figure 37). Two of the compounds had moderate activity as an antifeedant for the African cotton leafworm (*Spodoptera littoralis*; Figure 38). They also inhibited culture growth of the fungal plant pathogen *Cladosporium herbarum* (Figure 39).

Figure 37. ***Artemia salina***, a species that is killed by extracts from the liverwort ***Riccardia polyclada***. Photo by Hans Hillewaert, through Creative Commons.

Figure 38. ***Spodoptera littoralis***, a species that is discouraged from eating the liverwort ***Riccardia polyclada*** by its chemical compounds. Photo from Forestry Images, through Creative Commons.

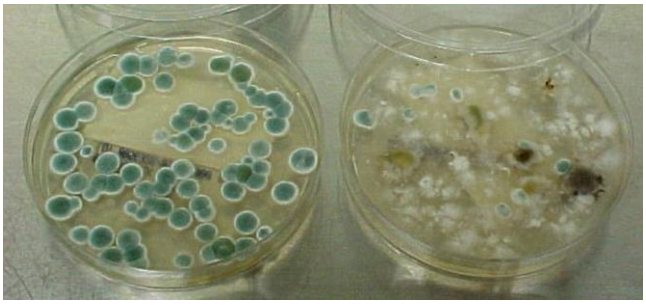

Figure 39. ***Cladosporium*** spp., fungal pathogens that are inhibited by extracts from the liverwort ***Riccardia polyclada***. Photo from Mold Treatment Centers of America, through Creative Commons.

Antimicrobial Activity

Our knowledge of antimicrobial activity of bryophytes is mostly based on ***Sphagnum*** (Figure 13, Figure 14). However other bryophytes are now known to have antibiotic properties. Some of these may just be folklore as they have not been tested experimentally. For example, Cheng *et al*. (2008) reported the folk use of ***Polytrichum*** to treat pneumonia. And we have already seen that ***Polytrichum commune*** (Figure 19) is used in a tea for treating colds (Gulabani 1974; Beike *et al*. 2010).

Nevertheless, antibiotic properties have been demonstrated in the laboratory. Moss activity against Gram-positive and Gram-negative bacteria have been demonstrated (Basile *et al.* 1999; Merkuria *et al.* 2005; Zhu *et al.* 2006). Asakawa (2007a, b) has demonstrated many effects of liverworts.

Kumar *et al*. (2007), in their review of classical ethnobotanical Indian uses, reported the antibacterial value of the mosses ***Anomodon rostratus*** (Figure 30), ***Atrichum angustatum*** (Figure 40), ***A. undulatum*** (Figure 41), and ***Hyophila involuta*** (Figure 42) and the thallose liverworts ***Conocephalum conicum*** (Figure 10) and ***Dumortiera hirsuta*** (Figure 15). These bryophytes reportedly produce antibiotics. The leafy liverwort ***Radula complanata*** (Figure 24) similarly has antimicrobial properties. To these we can add the antiseptic properties of ***Frullania tamarisci*** (Figure 43) (Asakawa 2007b; Chandra *et al*. 2017) and antimicrobial activity of ***Pallavicinia*** sp. (Figure 27) (Azuelo *et al*. 2011; Chandra *et al*. 2017).

Figure 40. ***Atrichum angustatum***, a species with antibacterial properties. Photo by Janice Glime.

Figure 41. ***Atrichum undulatum***, a species with antibacterial properties. Photo by Brian Eversham, with permission.

Figure 42. ***Hyophila involuta***, a species with antibacterial properties. Photo by Michael Lüth, with permission.

Figure 43. ***Frullania tamarisci***, an epiphytic species with antiseptic properties, having both allergenic and medicinal properties. Photo by Michael Lüth, with permission.

Recent tests on the floating thallose liverwort ***Riccia fluitans*** (Figure 44) from Florida indicated no ability to inhibit growth of the tested bacteria [*Pseudomonas aeruginosa* (Figure 45), *Staphylococcus aureus* (Figure 25)] or yeast (*Candida albicans*; Figure 46) (Pates & Madsen 1955). Vashistha *et al*. (2007) determined the antimicrobial activity of three other thallose liverworts. They found that water soluble extracts from ***Conocephalum conicum*** (Figure 10), ***Marchantia polymorpha*** (Figure 47), and ***Plagiochasma appendiculatum*** (Figure 48) had no effect on any of the pathogens tested [Gram-negative bacteria *Escherichia coli* (Figure 17) and *Salmonella typhi* (a variant of *S. enterica*; Figure 49)] and fungi *Aspergillus niger* (Figure 50) and yeast *Candida albicans* (Figure 46). However, acetone-soluble extracts of all three bryophyte species were inhibitory against the pathogens. They were more effective against the growth of *S. typhi* than against *E. coli*. ***Plagiochasma appendiculatum*** had a strong inhibitory effect against *A. niger* and ***Conocephalum conicum*** was strongly inhibitory to *Candida albicans*.

Figure 44. ***Riccia fluitans***, a species that was unable to inhibit the growth of several tested bacteria. Photo by Štěpán Koval, with permission.

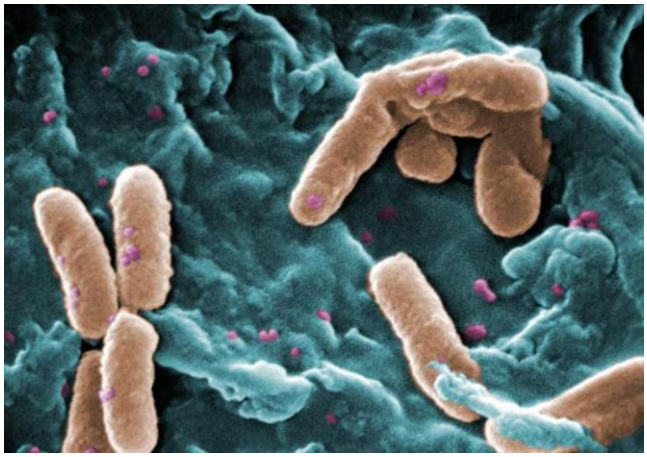

Figure 45. ***Pseudomonas aeruginosa***, a species that is not inhibited by ***Riccia fluitans***. Photo by Janice Haney Carr, through Creative Commons.

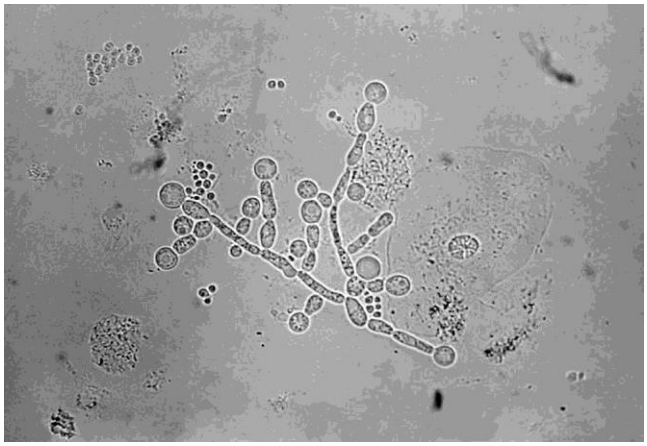

Figure 46. *Candida albicans*, a yeast species that is inhibited by extracts of the liverworts ***Conocephalum conicum***, ***Marchantia polymorpha***, and ***Plagiochasma appendiculatum***, but not ***Riccia fluitans***. Photo from Public Health Image Library, through public domain.

Figure 47. ***Marchantia polymorpha*** subsp. ***ruderalis***. This species had no effect on the fungi or Gram negative bacteria tested by Vashistha *et al*. Photo by Malcolm Storey, through Creative Commons

Figure 48. ***Plagiochasma appendiculatum***, a species that is effective against some pathogens. Photo by Ying Jia-dong, through Creative Commons.

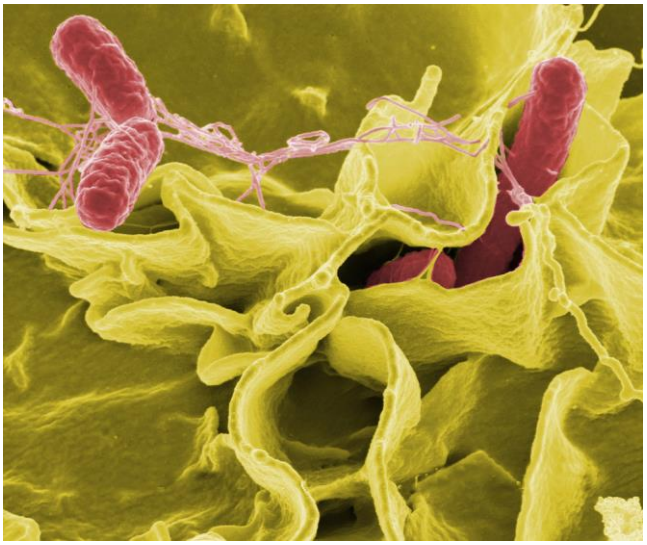

Figure 49. ***Salmonella enterica*** invading human cells. Photo by NIAID, through public domain.

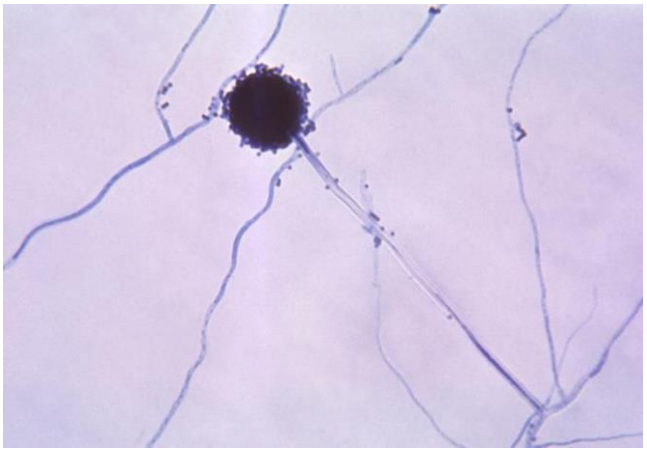

Figure 50. *Aspergillus niger*, a fungal species that is inhibited by extracts of the liverworts ***Conocephalum conicum***, ***Marchantia polymorpha***, and ***Plagiochasma appendiculatum***. Photo from Public Health Image Library, through public domain.

Bukvicki *et al*. (2012), using solid-phase microextraction-gas chromatography mass spectrometry, explored the volatile components of the leafy liverwort ***Porella cordaeana*** (Figure 51). Using methanol, ethanol, and ethyl acetate to extract terpenoids, they were able to identify sesquiterpene hydrocarbons and monoterpene

hydrocarbons. These same hydrocarbons were active against the eleven food microorganisms tested, but at different concentrations among the microorganisms. The fungi among these will be discussed blow. Affected bacterial strains were *Salmonella enteritidis* (food poisoning that causes diarrhea, fever, and abdominal cramps; Figure 52), *Escherichia coli* (commonly found in lower intestine of warm-blooded organisms with some strains causing food poisoning; Figure 17), and *Listeria monocytogenes* (very virulent food pathogen that causes the infection listeriosis; Figure 36). Methanol extracts showed the best activity.

Figure 51. ***Porella cordaeana***, a species that is able to inhibit a variety of yeasts and bacteria. Photo by J. C. Schou, with permission.

Figure 52. *Salmonella enteritidis*, a bacteria species that is inhibited by extracts of ***Porella cordaeana***. Photo through OGL (public domain).

It appears that differences in bryophyte extract activity among various pathogens may be common. Extracts of the liverworts ***Marchantia polymorpha*** (Figure 47), ***Porella platyphylla*** (Figure 29), and the moss ***Dicranum scoparium*** (Figure 16) showed antimicrobial effects on the Gram-positive bacteria *Bacillus subtilis* (Figure 53), *Staphylococcus aureus* (Figure 25), and *Micrococcus luteus* (Figure 54) (Pavletic & Stilinovic 1963; Frahm 2004). These same bryophytes exhibited no activity against Gram-negative *Escherichia coli* (Figure 17).

Figure 53. SEM of ***Bacillus subtilis***, a species that is inhibited by extracts of several bryophyte species. Photo by Davehwng, through Creative Commons.

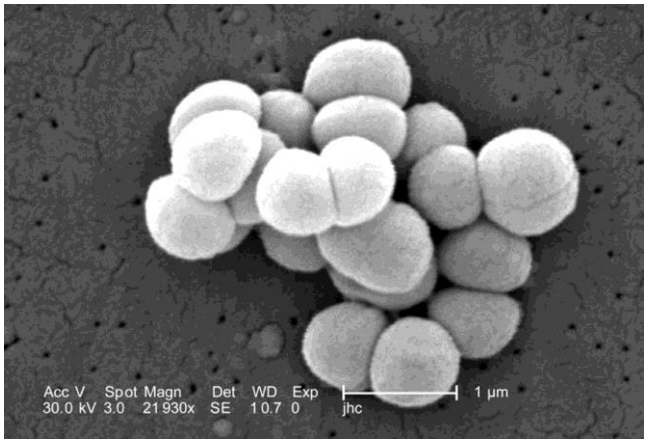

Figure 54. *Micrococcus luteus*, a bacteria species that is inhibited by extracts of several bryophyte species. Photo by Janice Carr, through public domain.

Basile *et al*. (1999) isolated seven pure flavonoids from five species of mosses. Some of these exhibited strong antibacterial effects against the bacteria *Enterobacter cloaceae* (Figure 55), *E. aerogenes* (Figure 56), and *Pseudomonas aeruginosa* (Figure 45). They were mainly active against Gram-negative bacteria that caused severe opportunistic infections and were at the same time resistant to commonly used antibacterial therapy. This means that the bryophyte products could become important tools in treating some bacterial infections.

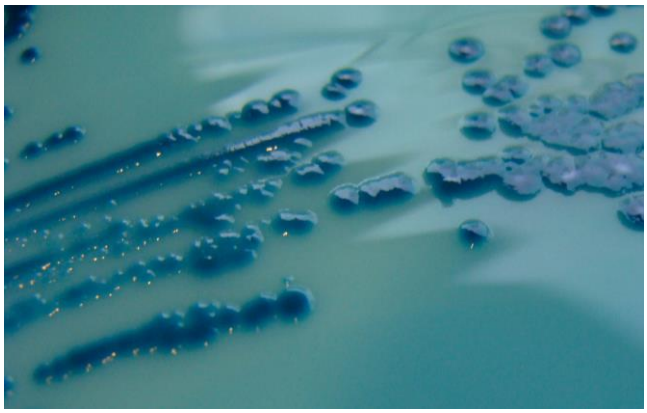

Figure 55. ***Enterobacter cloaceae***, a species that is active against Gram-negative bacteria. Photo by Nathan Reading, through Creative Commons.

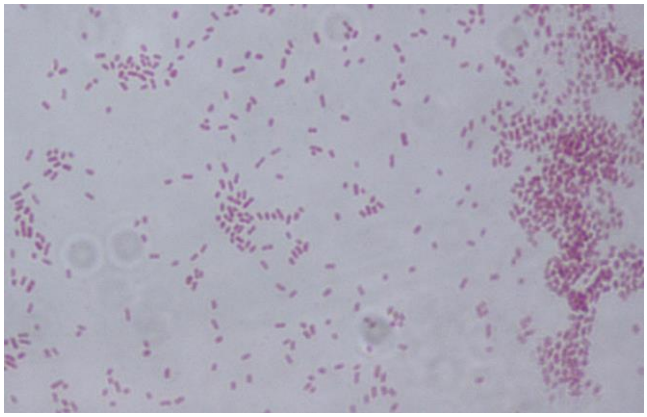

Figure 56. ***Enterobacter aerogenes***, a species that is active against Gram-negative bacteria. Photo by Riraq25, through Creative Commons.

Ariyo *et al*. (2011) compared the efficacy of extracts from the Nigerian thallose liverwort ***Riccia nigerica*** as antimicrobial agents. These extracts were tested against the bacteria *Bacillus subtilis* (Figure 53), *Pseudomonas aeruginosa* (Figure 45), *Shigella dysenteriae* (Figure 57), and *Staphylococcus aureus* (Figure 25) and fungi *Rhizopus* spp. (Figure 58), *Aspergillus flavus* (Figure 59), *A. niger* (Figure 50), *Penicillium* spp. (Figure 34), and and demonstrated strong significant antibacterial and antifungal activity.

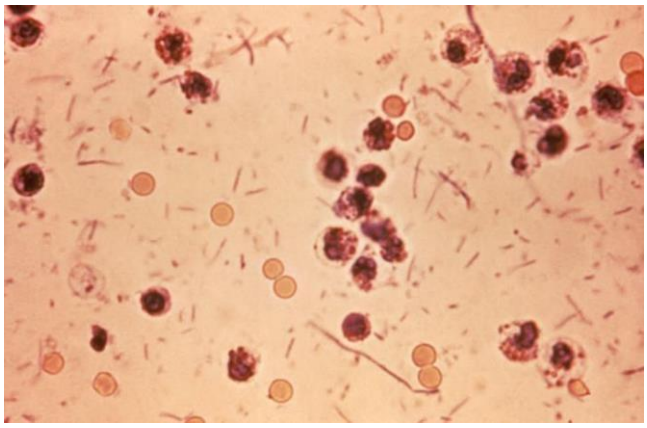

Figure 57. *Shigella dysenteriae*, a bacterial species that is inhibited by extracts of ***Riccia nigerica***. Photo by Public Health Image Library, through public domain.

Figure 58. ***Rhizopus*** on yam, a fungal species that is inhibited by extracts of ***Riccia nigerica***. Photo by Charles Averre, through Creative Commons.

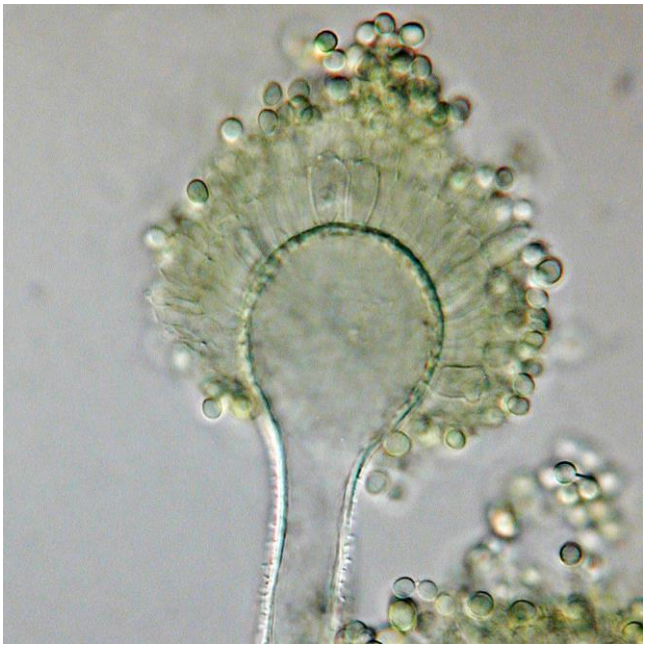

Figure 59. ***Aspergillus flavus***, a fungal species that is inhibited by extracts of ***Riccia nigerica***. Photo by Medmyco, through Creative Commons.

The leafy liverwort ***Ptilidium pulcherrimum*** (Figure 60) exhibits antimocrobial activity against both Gram + bacteria and Gram negative bacteria, but the effect is greater on the Gram positive bacteria (Veljić *et al*. 2010).

Figure 60. ***Ptilidium pulcherrimum***, a species that is effective against both Gram positive and Gram negative bacteria. Photo by Hermann Schachner, through Creative Commons

Antifungal Activity

Although mosses are known to harbor fungi and will quickly become infected if kept moist in a plastic bag, some fungi are inhibited by many species of bryophytes, including many that cause skin infections. Jennings (1926) reported moss immunity to molds as early as 1926, but the possibility of using them as a source of antifungal activity seems to have been largely overlooked. Among these, the cosmopolitan moss ***Hypnum cupressiforme*** (Figure 61) has remarkable antibacterial and antifungal effects. Ven Hoof *et al*. (1981) demonstrated strong antibacterial and antifungal effects by extracts of ***Hypnum cupressiforme***.

Figure 61. ***Hypnum cupressiforme***, a species that is effective against fungi that cause skin infections. Photo by Michael Lüth.

Kumar *et al*. (2007) report antifungal properties for the widespread leafy liverwort ***Porella platyphylla*** (Figure 29). Ando and Matsuo (1984) demonstrated antifungal effects of bryophytes on human pathogenic fungi, but they warned that while the bryophyte extracts have fungicidal and antifeedant effects, they also may cause allergic reactions and dermatitis for some humans.

Bukvicki *et al*. (2012), using solid-phase microextraction-gas chromatography mass spectrometry, explored the volatile components of the related liverwort ***Porella cordaeana*** (Figure 51). Using methanol, ethanol, and ethyl acetate to extract terpenoids, they were able to identify sesquiterpene hydrocarbons and monoterpene hydrocarbons. These same hydrocarbons were active against the eleven food microorganisms tested, but at different concentrations among the microorganisms. These included the yeasts *Saccharomyces cerevisiae* (yeast used in wine making, baking, and brewing, but antibodies against *S. cerevisiae* are found in 60-70% of patients with Crohn's disease; Figure 62), *Zygosaccharomyces bailii* (species causing significant spoilage in the food industry; Figure 63), *Aureobasidium pullulans* (an inhabitant of humidifiers or air conditioners that can lead to hypersensitivity pneumonitis; Figure 64), *Pichia membranifaciens* (species causing grey mold of fruits; Figure 65) (2 strains), *Pichia anomala* (used in winemaking) (2 strains), and *Yarrowia lipolytica* (used in industrial microbiology for production of specialty lipids). Methanol extracts showed the best activity.

Figure 62. *Saccharomyces cerevisiae* SEM, a yeast species that is inhibited by extracts of the leafy liverwort ***Porella cordaeana***. Photo by Mogana Das Murtey and Patchamuthu Ramasamy, through Creative Commons.

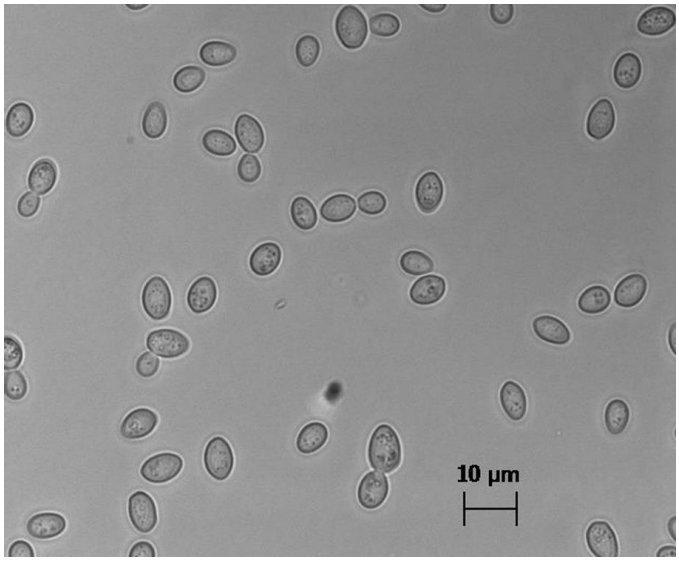

Figure 63. *Zygosaccharomyces bailii*, a yeast species that is inhibited by extracts of ***Porella cordaeana***. Photo by DTDT, through Creative Commons.

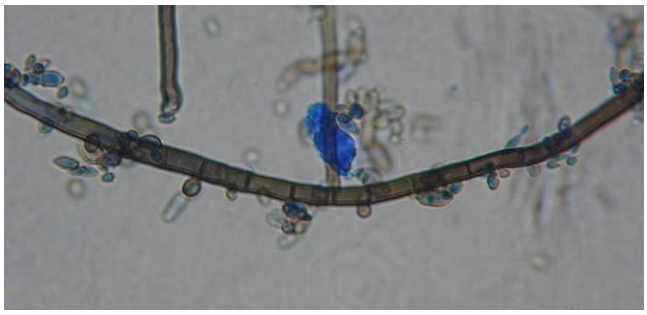

Figure 64. *Aureobasidium pullulans*, a yeast species that is inhibited by extracts of the leafy liverwort ***Porella cordaeana***. Photo by Tom Volk, through Creative Commons.

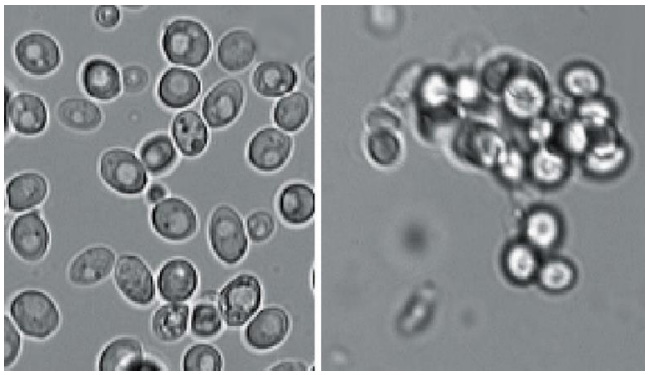

Figure 65. *Pichia membranaefaciens*, a yeast species that is inhibited by extracts of ***Porella cordaeana***. Photo by Luciana Francisco Fleuri & Hélia Harumi Sato, through Creative Commons.

The leafy liverwort ***Ptilidium pulcherrimum*** (Figure 60) is not only effective against bacteria, but also against fungi (Veljić *et al*. 2010). Its best antifungal activity was against *Trichoderma viride* (Figure 66), compared to the activity of the synthetic bifonazol.

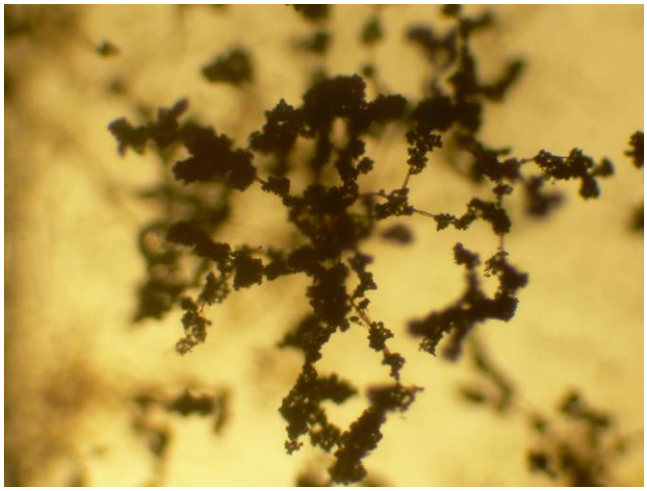

Figure 66. ***Trichoderma viride*** conidiophores, a fungal species that is inhibited by ***Atrichum undulatum***, ***Physcomitrella patens***, and ***Marchantia polymorpha***. Photo by Ninjatacoshell, through Creative Commons.

The absence of fungal diseases in liverworts led Pryce (1972) to suggest that **lunularic acid**, a hormone that affects aging in liverworts but not in mosses, might be responsible for liverwort antifungal activity. Banerjee and Sen (1979; Bannerjee 1974) found that the degree of antibiotic activity in a given species may depend on the age of the gametophyte; Matsuo *et al*. (1982a, 1982b, 1983) supported this conclusion by demonstrating that antifungal activity against *Botrytis cinerea* (Figure 67), *Pythium debaryanum* (Figure 68), and *Rhizoctonia solani* (Figure 69) by the liverwort ***Herbertus aduncus*** (Figure 70) was age-dependent. They subsequently isolated three aging substances from it: (-)-alpha-herbertenol; (-)-Beta-herbertenol, and (-)-alpha-formylherbertenol.

Figure 67. ***Botrytis cinerea*** on grapes. The liverwort ***Herbertus aduncus*** can exercise antifungal activity against this fungus, but activity is age-dependent. Photo by Alexandre Dulaunoy, through Creative Commons.

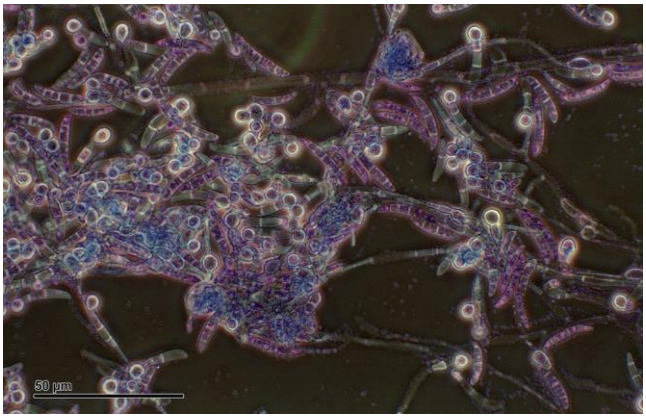

Figure 68. ***Pythium*** sp. The liverwort ***Herbertus aduncus*** can exercise antifungal activity against this fungus, but activity is age-dependent. Photo by Josef Reischig, through Creative Commons.

Figure 69. ***Rhizoctonia solani*** infecting leaves. The liverwort ***Herbertus aduncus*** can exercise antifungal activity against this fungus, but activity is age-dependent. Photo by Howard F. Schwartz, through Creative Commons.

Figure 70. ***Herbertus aduncus***, a leafy liverwort that can exercise antifungal activity against several fungi, but activity is age-dependent. Photo by Martin Hutten, with permission.

Vashistha *et al*. (2007) determined the antimicrobial activity for the thallose liverworts ***Plagiochasma appendiculatum*** (Figure 48), ***Marchantia polymorpha*** (Figure 47), and ***Conocephalum conicum*** (Figure 10). Acetone-soluble extracts of all three bryophyte species

were inhibitory against the fungal pathogens tested. ***Plagiochasma appendiculatum*** had a strong inhibitory effect against *Aspergillus. niger* and ***C. conicum*** was strongly inhibitory to *Candida albicans*. When Niu *et al*. (2006) tested ***Marchantia polymorpha*** (Figure 47) for antifungal activities against the yeast *Candida albicans* (Figure 46), they found **plagiochin** E, 13,13'-O-isoproylidenericcardin D, and neomarchantin A were active against the yeast. The other identified compounds had only weak effects.

Sabovljević *et al*. (2011) used DMSO extracts of both cultured and wild grown mosses ***Atrichum undulatum*** (Figure 20) and ***Physcomitrella patens*** (Figure 71-Figure 72) and thallose liverwort ***Marchantia polymorpha*** ssp. ***ruderalis*** (Figure 47) to test for antifungal activity. Using *Aspergillus versicolor* (Figure 73), *Aspergillus fumigatus* (Figure 74), *Penicillium funiculosum* (see Figure 34), *Penicillium ochrochloron*, and *Trichoderma viride* (Figure 66), these researchers demonstrated antifungal activity by all three bryophytes against all five fungal species. Most of the bryophytes grown in culture had greater antibiotic activity than the wild-grown ones.

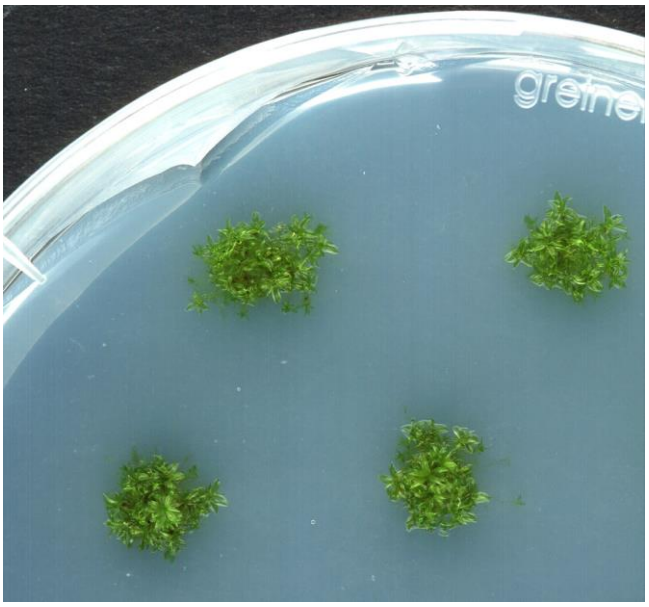

Figure 71. ***Physcomitrella patens*** growing on agar plates. Photo by Sabisteb, through Creative Commons.

Figure 72. ***Physcomitrella patens***, a source of human proteins and blood-clotting factor IX. Photo by Michael Lüth.

Figure 73. ***Aspergillus versicolor***, a fungal species that is inhibited by ***Atrichum undulatum***, ***Physcomitrella patens***, and ***Marchantia polymorpha***. Photo by James Scott, through Creative Commons.

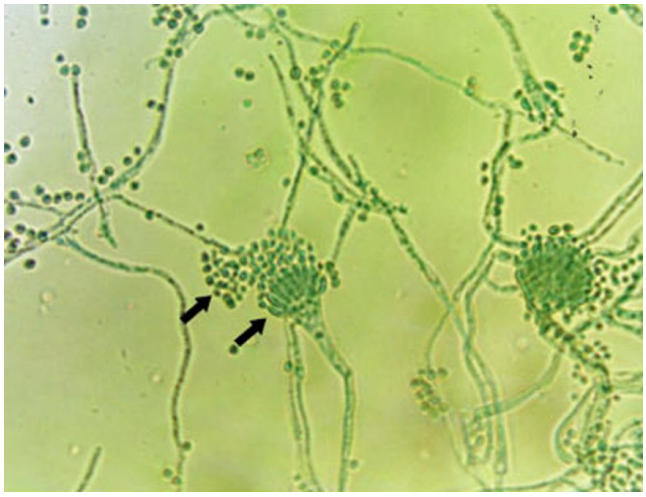

Figure 74. ***Aspergillus fumigatus***, a fungal species that is inhibited by ***Atrichum undulatum***, ***Physcomitrella patens***, and ***Marchantia polymorpha***. Photo through Creative Commons.

Alcoholic extracts of all twenty bryophytes tested at Bonn University had antifungal activity on infected crops (Frahm 2004), as demonstrated in a Petri dish (Figure 75). Frahm reports curing a fungal infection of the skin with a bryophyte extract. The success was reported in a TV magazine and a published book, causing a number of people to use the extract for fungal infections, mostly with favorable results. However, Frahm warns that the biologically active substances are terpenoids, and these may cause allergic effects to some people (Ando & Matsuo 1984). One reputedly can cure athlete's foot by walking through a peat bog, presumably because of these same terpenoids (Frahm 2004).

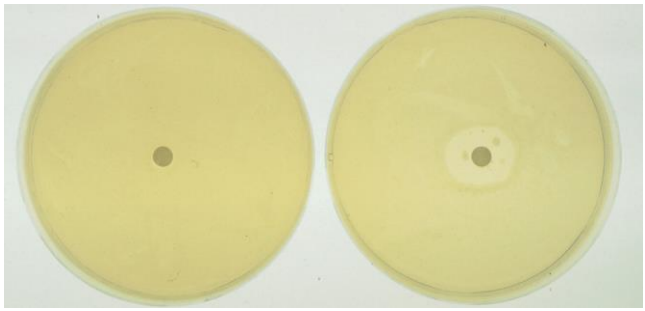

Figure 75. Bryophytes are known to inhibit growth of some kinds of bacteria and fungi. **Left:** Microbes grow uninhibited around a disk with only extraction fluid. **Right:** A zone of inhibition occurs around the disk with bryophyte extract. Photo by Jan-Peter Frahm.

One extract has actually been patented to cure fungal infections of horses (Frahm 2004). An industrious horse owner was inspired by what he read about the Bonn experiments and made a paste of ***Ceratodon purpureus*** (Figure 76) and ***Bryum argenteum*** (Figure 77). The fungus disappeared from the horse in 24 hours! This same extract is also sold as a human foot cream to refresh and fight odor. Unfortunately, the use for curing fungal infections cannot be mentioned in advertising because then it would require the extensive testing necessary to meet medical approval, which might be difficult because it can cause allergies and dermatitis in some people. It also works as an antifeedant against slugs. Unfortunately, to date it must be extracted from field-collected material, creating conservation concerns.

Figure 76. ***Ceratodon purpureus***, a species was used with ***Bryum argenteum*** and killed a fungal infection on a horse. Photo by Janice Glime.

It appears that some bryophytes may contribute to antifungal compounds by hosting a fungus that manufactures both antifungal and antitumor compounds (Guo *et al*. 2008). The leafy liverwort ***Scapania verrucosa*** hosts the fungus ***Chaetomium fusiforme*** (Figure 78). Not only does the latter produce both antifungal and antitumor compounds, but the liverwort itself likewise produces them. However, the fungus compounds provide superior properties and the liverwort might contribute to the medicinal field through this fungal **endophyte** (organisms growing within cells of plant, ranging from symbiotic to parasitic).

Figure 77. ***Bryum argenteum***, a species that was used in a paste with ***Ceratodon purpureus*** and killed a fungal infection on a horse. Photo by Martin Hutten, with permission.

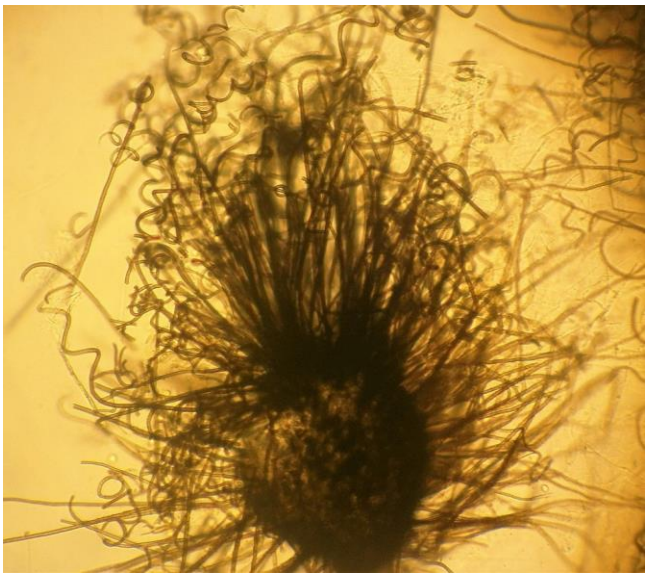

Figure 78. ***Chaetomium globosum***. The fungus ***Chaetomium fusiforme*** occurs on ***Scapania verrucosa*** and produces both antifungal and antitumor compounds. Photo by Ulitca, through Creative Commons.

Antiviral Activity

Even viruses may some day be cured by extracts of mosses, but we cannot simply identify them as "moss" as many of our ecologist friends have been wanton to do in reporting the ground cover. The Maoris of New Zealand have used bryophytes to treat venereal disease by packing wet plants on the infected organs (Frahm 2004). Nevertheless, van Hoof and coworkers (1981) found no effect of 20 species of moss extracts on the herpes virus, but earlier Klöcking *et al.* (1976) found that at least some peat humic acids possess antiviral activity against herpes simplex virus types 1 and 2, interfering primarily with the adsorption of viruses to host cells.

Sphagnum (Figure 13, Figure 14) produces several antivirally active humic acids, and ***Camptothecium*** (Figure 79) extracts can inhibit growth of the poliovirus (Witthauer *et al.* 1976; van Hoof *et al*. 1981). Nevertheless, actual usage of bryophytic extracts has not developed outside of Asia.

Figure 79. ***Camptothecium lutescens***, in a genus that can inhibit growth of the poliovirus. Photo by David T. Holyoak, with permission.

Cancer and Anti-tumor Properties

In the same year as the Madsen and Pates (1952) report of antibiotics in bryophytes, Belkin *et al*. (1952-53) reported anticancer activity against Sarcoma 37 in mice, using extracts of ***Polytrichum juniperinum*** (Figure 80). But application of the antitumor activity fared no better and was apparently not rediscovered in bryophytes until the next century. Finally, Anterola *et al*. (2009) considered the anticancer drug precursors in mosses to be so important that they titled their presentation on them "Turning precursors into gold: Production of anticancer drug precursors in moss."

Figure 80. ***Polytrichum juniperinum*** with antheridial splash cups, a species that produces anticancer compounds. Photo by Paul Slichter, with permission.

Fu *et al*. (2009) attempted to show anti-cancer capabilities of the moss ***Polytrichum commune*** (Figure 19). To this end, they isolated two "unusual" flavones and other compounds from this moss. However, when tested against a small panel of cancer cell lines, they failed to find any activity.

Kumar *et al*. (2007) found that Indians have used ***Polytrichum juniperinum*** (Figure 80) to treat cancer. The anti-tumor use of bryophytes in India included ***Chiloscyphus polyanthos*** (Figure 81), ***Diplophyllum albicans*** (Figure 82), ***D. taxifolium*** (Figure 85), ***Marchantia palmata***, and ***M. polymorpha*** (Figure 47). ***Frullania tamarisci*** (Figure 43) is used as an antileukemic agent. To this list, others added ***Riccardia*** sp. (Figure 84) (Azuelo *et al*. 2011; Alam 2012; Chandra *et al*. 2017) and ***Plagiochila*** sp. (Figure 11) (Asakawa 2007; Alam 2012; Chandra *et al*. 2017).

Figure 81. ***Chiloscyphus polyanthos***, a species that may cause allergic reactions. Photo by Barry Stewart, with permission.

Figure 82. ***Diplophyllum albicans***, used as an anti-tumor treatment in India and as an agent against epidermoid carcinoma. Photo by Michael Lüth, with permission.

Asakawa (1981) has shown that several compounds from leafy liverworts exhibit antileukemic activity. From the thallose species, Marchantin A from ***Marchantia palacea*** (Figure 83), ***M. polymorpha*** (Figure 47), and ***M. emarginata*** subsp. ***tosana*** (Figure 33), riccardin from ***Riccardia multifida*** (Figure 84), and perrottetin E from ***Radula perrottetii*** all show cytotoxicity against the leukemic KB cells (Asakawa *et al*. 1982).

Figure 83. ***Marchantia paleacea***, a thallose liverwort known for its antileukemic activity. Photo by Jan-Peter Frahm, with permission.

Figure 84. ***Riccardia multifida***, a thallose liverwort known for its antileukemic activity. Photo by Michael Lüth, with permission.

In 1976, Adamek reported that peat preparations hold some promise against some types of human cancer. In 1977, Ohta and coworkers (1977) reported that diplophyllin, isolated from the liverworts ***Diplophyllum albicans*** (Figure 82) and ***D. taxifolium*** (Figure 85), shows significant activity (ED50 4-16 μg/ml) against human epidermoid carcinoma (KB cell culture).

Figure 85. ***Diplophyllum taxifolium***, a species that produces diplophyllin, a compound that is active against human epidermoid carcinoma. Photo by Hermann Schachner, through Creative Commons.

Hughes and Anterola (2010) attempted to transplant genes for producing **Taxol** (a potent anticancer agent) into the moss ***Physcomitrella patens*** (Figure 71, Figure 72). They found evidence of small amounts of the anticancer precursors in the moss. If the moss can be taught (genetically) to produce Taxol, it could become a laboratory means to manufacture this important anti-cancer drug without destroying the diminishing number of *Taxus* (Figure 86) shrubs that produce it naturally. Bryophytes are ideal organisms for such gene transplants because of their dominant state with a single set of chromosomes and the relative ease with which genes can be put into them.

Figure 86. ***Taxus baccata***, member of the genus that produces the anticancer agent Taxol. Photo through Creative Commons.

When Asakawa (1981, 1982) entered the arena, he isolated the sesquiterpenoids costunolide and tulipinolide from ***Conocephalum supradecompositum***, ***Frullania monocera***, ***F. tamarisci*** (Figure 43), ***Marchantia polymorpha*** (Figure 47), ***Wiesnerella denudata*** (Figure 87) and ***Porella japonica*** (Figure 88). To this list, Matsuo and coworkers (1980, 1981a, b, c, 1984) added ***Lepidozia vitrea*** (Figure 89) and ***Plagiochila semidecurrens*** (Figure 90). These substances, already known from higher plants, have activity to combat carcinoma of the nasopharynx, at least in cell culture.

Figure 87. ***Wiesnerella denudata***, a species that produces sesquiterpenoids that are likely to have antibiotic properties. Photo by Ying Jia-dong, through Creative Commons.

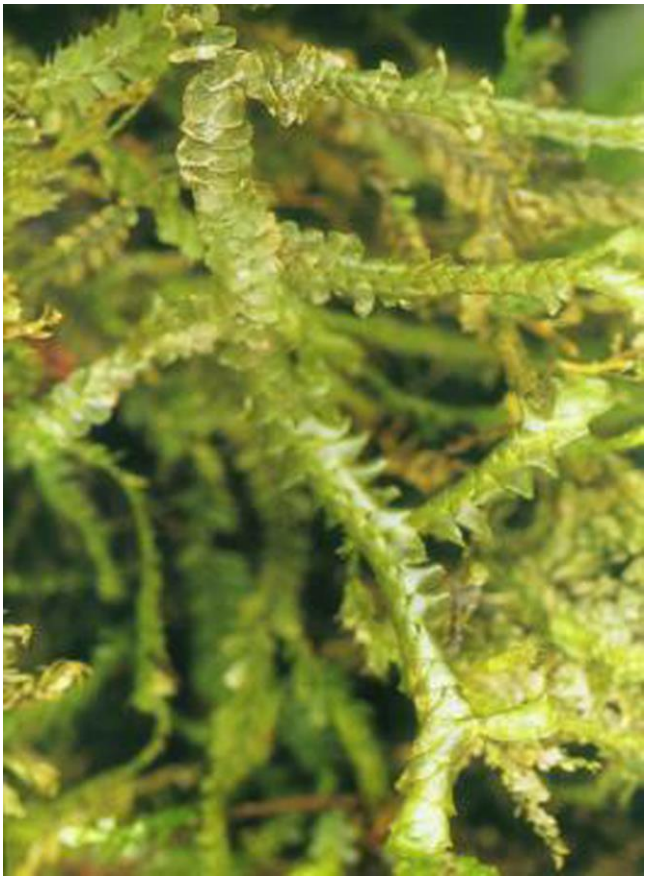

Figure 88. ***Porella japonica***, a species that produces compounds that combat carcinoma of the nasopharynx. Photo from Taiwan mosses color illustrations, through Creative Commons.

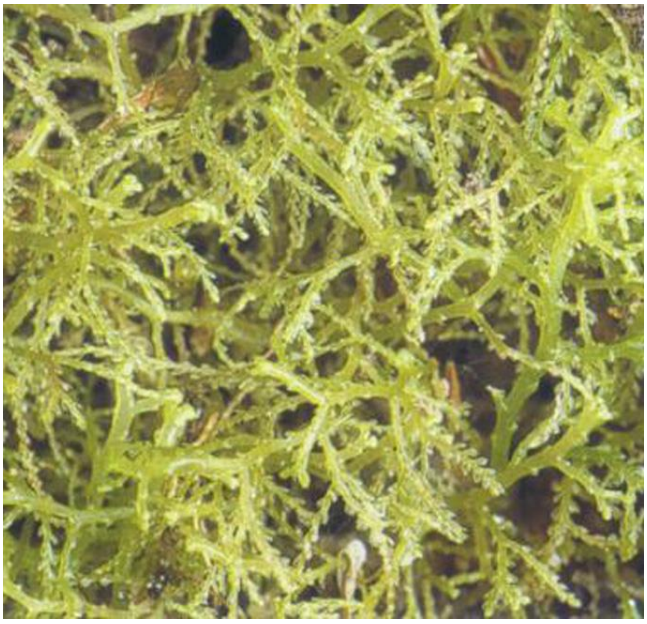

Figure 89. ***Lepidozia vitrea***, a species that produces compounds that combat carcinoma of the nasopharynx. Photo by Lin Shanxiong, through Creative Commons.

Figure 90. ***Plagiochila semidecurrens***, a species that produces compounds that combat carcinoma of the nasopharynx. Photo by Martin Hutten, with permission.

When the National Cancer Institute became interested, Spjut and his coworkers (1986) tested 184 species of mosses and 23 species of liverworts for antitumor activity. Of these, 43 species contained active substances, while those of 75 species were toxic to tested mice. The most activity was found in **Brachytheciaceae** (Figure 91), **Dicranaceae** (Figure 16), **Grimmiaceae** (Figure 92), **Hypnaceae** (Figure 61), **Mniaceae** (Figure 18, Figure 31), **Neckeraceae** (Figure 2), **Polytrichaceae** (Figure 19, Figure 80), and **Thuidiaceae** (Figure 93). However, in 1988, doubt was cast on the role of the moss when this team reported that the antitumor activity of the moss ***Claopodium crispifolium*** (Figure 94) was greatest in samples contaminated with the Cyanobacterium ***Nostoc*** cf. ***microscopicum*** (Figure 95), suggesting that ***Nostoc*** could be the direct source of the activity or a necessary partner for interaction between the species (Spjut *et al.* 1988). Interaction could result from the transfer of a precursor from the ***Nostoc*** to the moss, which could then transform it into an active substance. Alternatively, the moss might produce the substance as an allelopathic response to the ***Nostoc***. In any event, this raises important and intriguing questions, both medically and ecologically.

Figure 91. ***Brachythecium salebrosum*** (**Brachytheciaceae**). Some members of this family exhibit high antitumor activity. Photo by Hermann Schachner, through Creative Commons.

Figure 92. ***Grimmia nutans*** (**Grimmiaceae**). Some members of this family exhibit high antitumor activity. Photo by Michael Lüth, with permission.

Figure 93. ***Thuidium tamariscinum*** (**Thuidiaceae**). Some members of this family exhibit high antitumor activity. Photo by Malcolm Storey (DiscoverLife.com), with online permission.

Figure 94. ***Claopodium crispifolium***, a moss that provides habitat for ***Nostoc***, which in turn has anti-tumor properties. Photo from Botany Website, UBC, with permission.

Figure 95. ***Nostoc*** sp., a moss contaminant that can increase anti-tumor activity. Photo from Retina, through Creative Commons.

For some reason, much of the biochemical work has concentrated on the liverworts. Similar studies on activities of moss compounds are sparse and there may be good reason to presume a greater medical treasure chest among the liverworts. Since these compounds generally benefit the bryophytes by discouraging their would-be herbivores, it is the tiny, slow-growing liverworts that stand to benefit most. Where other, larger plants have spent their evolutionary history developing a diversity of structure, it would seem that small size has afforded these plants only the benefits of diversity of biochemistry as a means of combating the hungry herbivores.

Burgess *et al*. (2000) found that the leafy liverwort ***Bazzania novae-zelandiae*** (Figure 96) produces a sesquiterpene caffeate that has selective activity against certain human tumor cells. The active compound has been identified as the new compound naviculyl caffeate.

Figure 96. ***Bazzania novae-zealandiae***, a species that is active against human tumor cells. Photo by Shirley Kerr, with permission.

Even breast cancer sufferers might benefit from bryophytes. Huang *et al*. (2010) found that **marchantin A** produced by ***Marchantia emarginata*** subsp. ***tosana*** (Figure 33) induced apoptosis in MCF-7 breast cancer

cells. This compound demonstrates strong antioxidant activity, scavenging free radicals.

The leafy liverwort ***Scapania verrucosa*** and its endophytic fungal inhabitant ***Chaetomium fusiforme*** (see Figure 78) produce several compounds that act as antitumor agents (Guo *et al*. 2008).

The thallose liverwort ***Dumortiera hirsuta*** (Figure 15) produces **riccardin D**, a macrocyclic bisbibenzyl compound that induces apoptosis of human leukemia cells (Xue *et al*. 2012). Xue and coworkers verified anticancer activity by riccardin D against human non-small cell lung cancer. In mice it produced a 44.5% inhibition of cancer growth with no apparent toxicity.

Pharmaceutical Production

Welcome to Greenovation! Moss for a healthy future. So began the website <http://www.greenovation.com/> of an upstart company that is growing the tiny ***Physcomitrella patens*** (Figure 71, Figure 72) for medicinal purposes. Yes, bryophytes have indeed finally penetrated the forefront of modern medicine!

Physcomitrella patens (Figure 71, Figure 72) is able to accept transferred human genes and express them to produce human antibodies in a liquid culture, making the antibodies easy to harvest (ETH Zurich 2009). So far, this is not possible when the genes are transplanted into "higher' organisms. One advantage of ***Physcomitrella patens*** is its ability to grow in a "bioreactor" (Figure 97; Decker & Reski 2004), a fermenter in which only water and minerals are needed to nourish the moss, of course in the presence of light and CO_2 (Greenovation). These tiny plants are actually superior (and cheaper) production systems for many complex recombinant pharmaceuticals (Bauer *et al*. 2005; Decker & Reski 2007, 2012; Gitzinger *et al*. 2009). Contrary to many mammalian systems that have been used to produce pharmaceuticals but that cause serious immune responses, those produced by ***Physcomitrella patens*** are non-immunogenic, a huge advantage for the patient, and making them superior to currently used mammalian cell lines for producing antibodies.

Among its many assets, ***Physcomitrella patens*** (Figure 71, Figure 72) is able to produce human proteins (Hohe *et al*. 2002; Decker *et al*. 2003) and is the only plant being used to produce the blood-clotting factor IX for pharmaceutical use. This discovery, patented by Prof. Reski of the Institute of Biotechnology of Plants at the University of Freiburg in Germany, led to the founding of the Greenovation Company in 1999. By 2002, the company was already employing 30 people to produce this valuable blood factor (Frahm, Bryonet discussion 2002).

Bryophytes offer the researchers, and the company, a number of advantages over "higher" plants. They can be grown without antibiotics, hence avoiding the danger of contamination of the final product. The moss is quite small and thus is cultured only in the lab with little danger of the transgenic plants escaping into the environment. But the real advantage comes from the dominant gametophytic generation of mosses as opposed to the dominant sporophyte of the tracheophytes. As a result, mosses are the only plants known to have a high frequency of homologous recombination. The result – stable integration of inserted genes into the genome. Furthermore, the highly complex moss system, compared to bacteria and fungi, permits a much wider array of expression than is possible in those systems. Thus, mosses are extremely useful as production systems for targeted substances that can be produced through gene manipulation.

Figure 97. This type of bioreactor is used to grow ***Physcomitrella patens*** for human proteins and human blood-clotting factor IX. Photo by Ralf Reski.

Unfortunately, most biologically active substances so far obtained have not proved economical for use, at least in part due to the slow-growing nature and difficulty of culturing bryophytes. And, while their pharmaceutical use seems promising, we lack any understanding of their potential harmful side effects.

In the words of Ma *et al*. (2003), "Imagine a world in which any protein, either naturally occurring or designed by man, could be produced safely, inexpensively and in almost unlimited quantities using only simple nutrients, water and sunlight. This could one day become reality as we learn to harness the power of plants for the production of recombinant proteins on an agricultural scale. Molecular farming in plants has already proven to be a successful way of producing a range of technical proteins. The first plant-derived recombinant pharmaceutical proteins are now approaching commercial approval, and many more are expected to follow."

Medical Dangers

Caution is in order in exercising any medicinal use of bryophytes, particularly liverworts, because of their potential for causing allergic reactions (Mitchell *et al.* 1969, 1970; Benezra *et al*. 1985, Asakawa 2012). Often the very compounds that have these medical potentials can cause allergic reactions. For example, it is a sesquiterpene lactone (Asakawa 1981) that gives the common epiphyte ***Frullania*** (Figure 98-Figure 99) its ability to cause contact dermatitis, especially to forest workers (Mitchell *et al.* 1969). Now there is a patch test with a sesquiterpene lactone mix to determine sensitivity to ***Frullania*** (Quirce *et al*. 1994).

Yet sesquiterpene lactones are well known for their antimicrobial activity. In southern Europe, ***Frullania tamarisci*** (Figure 43, Figure 98) imparts an allergic reaction to olive pickers, yet is listed as one of the medicinal species (J. Curnow, pers. comm.). D. H. Wagner (pers. comm.) reports an allergy to ***Chiloscyphus polyanthos*** (Figure 81), especially when he squeezes it to remove excess water. By 1981, 24 liverwort species were known to have potential allergenic sesquiterpene lactones (Asakawa 1981). These compounds undoubtedly endow the same advantage to bryophytes that they do to flowering plants – discouraging consumption by hungry herbivores.

Figure 98. ***Frullania tamarisci***, showing underside of branch with lobules by which the genus may be determined. Photo by Michael Lüth, with permission.

Frullania tamarisci (Figure 43, Figure 98) grows on trees and can cause skin irritations for loggers and even for their wives who handle their clothes. Allergic reactions to ***Frullania nisquallensis*** (Figure 99) occurred in patch tests on seven forest workers who had contact dermatitis (Mitchell *et al*. 1969). These forest workers exhibited the dermatitis only when they were working on forest areas. The problem was worse in wet weather and appeared within 1-2 days of starting work. The condition persisted for 2-4 weeks after leaving work in forested areas where the liverwort grew. The condition is often known locally in British Columbia, Canada, as cedar poisoning, but in fact it is caused by the liverworts that commonly grow on the cedars (*Thuja*; Figure 100).

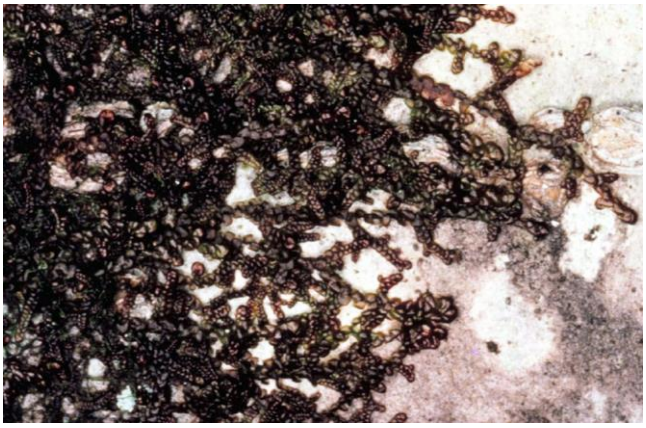

Figure 99. ***Frullania nisquallensis***, a leafy liverwort epiphyte that causes allergic reactions among forest workers. Photo by Dale Vitt, with permission.

Figure 100. ***Thuja plicata***, host plant for ***Frullania*** species. Photo from <www.nwplants.com>, through Creative Commons.

Summary

Bryophytes, especially liverworts, often have distinct odors, suggesting aromatic compounds such as phenols. However, few bryophytes have been linked to actual curative properties and identifiable associated compounds.

One danger in using bryophytes is that the same compounds that may have antibiotic properties may also be toxic or allergenic, or be associated with such compounds. Furthermore, peatland mosses may have associated fungi that cause **sporotrichosis**.

Many antibiotics have been isolated from bryophytes, but few have been developed for medical use, despite their demonstrated effectiveness. In Germany, ***Ceratodon purpureus*** and ***Bryum argenteum*** are used to cure fungal infections of horses. Several medical uses seem promising, such as antileukemic properties and anticancer agents.

The most promising uses of bryophytes in medicine seem to lie in genetic engineering. Bryophytes are being used already to produce human blood-clotting proteins, while others are known to reduce thrombin activity.

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