Examination of the antibacterial properties of sphagnum moss (*Sphagnum* spp.) and its significance with turf burning in Ireland

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Traditional herbal cures and remedies have played an important historical role in the treatment of various illnesses and diseases in Ireland for the past 300 years. Recently, their use and diversity in Northern Ireland has been reviewed in the publication by Dr Linda Ballard from the Ulster Folk and Transport Museum at Cultra, Co. Down,[1] which details the local plants used and for what purpose. From this publication, the employment of moss and peat is documented as a cure for sprains and for erysipelas, respectively, where, in the former, moss is simply rubbed onto the sprain. In the latter, one source cites a poultice of black peat from a bog hole, which should have no contact with air and be boiled with churned buttermilk. In addition, there is historical evidence[2] that peat bogs were used to preserve foodstuffs, particularly fats, commonly known as 'bog butter', where the low pH of the surrounding milieu arrested growth of food spoilage organisms and hence preserved the product for an extended period of time.

Moss and peat are derivatives of a plant within the genus *Sphagnum* spp. For generations, Irish families and households have been using Irish turf for several important domestic tasks (e.g., cooking, light and heating). Historically, poor architectural planning of Irish houses has resulted in the possible residual contamination of households with smoke distillates and volatile components emitted from the turf sods being burned. One hypothesis proposed here is that continued inhalation of turf smoke in the Irish domestic setting historically may have had a residual antibacterial effect, due to the composition of its consistuents and mode of delivery through an inhalational route.

Unfortunately, although these plants have strong associations with the local historical evidence base, no formal publications in the medical/scientific evidence base exist that examine the scientific background and clinical efficacy of sphagnum moss and its derivatives, including turf, as an antibacterial agent.

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Since the discovery and exploitation of antibiotic agents in the 20th century, the targeted selective toxicity of such agents has ensured their widespread and largely effective use to combat infection; however, their use has resulted in the emergence and dissemination of multidrug-resistant pathogens. Antimicrobial resistance in medicine and agriculture is now recognised by the World Health Organization (WHO), along with other national authorities, as a major emerging problem of public health importance. It represents a significant challenge of global dimensions to human and veterinary medicine, with the prospect of therapeutic failure for life-saving treatments now a reality.

In order to minimise the potential development of further antimicrobial resistance 'The Copenhagen Recommendations: Report from the Invitational EU Conference on The Microbial Threat' (www.im.dk/publikationer/micro98/index.htm) outlined the need for the development of "novel principles for treating or preventing infections in humans and animals". Such an approach may be to examine the antimicrobial properties of *Sphagnum* plants used in herbal medicine, as a novel source of such agents, as well as the employment of such compounds, thus limiting the use of conventional antibiotics to cases of severe and life-threatening infections (e.g., patients with cystic fibrosis [CF] suffering from chronic respiratory infections).

The aim of this small study is to examine the antimicrobial properties of sphagnum moss native to Northern Ireland, as well as one of its derivatives, namely turf.

Sphagnum moss (10 kg) was collected by hand from the Ballynahone bog in the Sperrin Mountains of Northern Ireland in January 2011. This bog is 243.24 hectares in area (latitude 54 49 25 N, longitude 06 39 40 W [grid reference H860980]) and is one of the two largest intact active bogs in Northern Ireland with hummock and hollow pool complexes. It represents one of the best examples of this habitat in the British Isles. As a result of this, the bog has been assigned an Area of Special Scientific Interest (ASSI) by the Environment & Heritage Service, Department of the Environment (NI).

Sphagnum moss which was saturated with water was collected by hand from the northern region of the bog (GPS 54°49′27.42″ N; 6°40′22.34″ W) and transported to the laboratory at ambient temperature and subsequently stored at 4°C. In addition, bog water (100 mL) was collected aseptically for quantitative microbiological examination. Sphagnum moss was examined for the presence of endogenous bacteria with antibacterial properties employing 16S rRNA polymerase chain reaction (PCR), gene sequencing and BLAST analysis, as described previously by this group.

Turf tar distillate (TTD, 20 g) was obtained from the open chimney breast of Campbell House within the Ulster American Folk Park in Omagh, Co. Tyrone, Northern Ireland (GPS 54°39131.73 N; 7°19′55.11″W; www.nmni.com). Turf had been burnt for decades in an open fire in the kitchen of this property, and turf tar distillate had accumulated in large amounts. A representative sample of this substance was collected in a sterile container and transported to the laboratory for subsequent analysis.

The following extracts were prepared and subsequently tested for their antibacterial properties:

i) *Aqueous extract of sphagnum moss*: For the extraction of aqueous components from the sphagnum moss, a recorded

fresh weight of sample (500 g) was dried in an Edwards Supermodulyo freeze drier at –40°C for a minimum of 48 h, producing a final weight of 71 g. For assay purposes, freeze-dried powder was homogenised with a pestle and mortar for 5 min prior to the addition of chilled (2°C) sterile 0.1% (w/v) peptone saline (CM0733, Oxoid, Basingstoke, UK) to give a known concentration. The resulting supernatant was filter-sterilised through a 0.22 µm syringe filter (Millipore, USA) before microbiological challenge, as detailed below.

ii) *Organic extract of sphagnum moss smoke distillate (SMSD)*: Volatile organic compounds were extracted from sphagnum moss smoke in accordance with the method of Sunesson *et al*.[3] Briefly, freeze-dried sphagnum moss (350 g) was placed in a glass round-bottomed flask and burned using a single-use butane flame torch burner for 30 min. Subsequently, the apparatus was heated for a further 30 mins with an electric heating mantle. During this entire time, the resulting smoke was collected and bubbled through an adsorbant solution (activated charcoal [1 g] and porosil [1 g] in toluene, isopropanol and water [10:1:1]) in a single U-tube trap. The resulting trap collection was decanted and washed (x3) using the same adsorbant solution into a rotary flask and concentrated to dryness. The resulting material was dissolved in 500 µL dimethylsulphoxide (DMSO; Sigma, USA) and stored at 4°C until used.

iii) *Aqueous extract of TTD*: Turf tar distillate (0.3 g) was dissolved in phosphate-buffered saline (PBS; 5 mL) to give a 6% (w/v) TTD solution. This was stored at 4°C prior to use.

iv) *Organic extract of TTD*: Turf tar distillate (1.0g) was dissolved in DMSO (5 mL) to give a 20% (w/v) TTD solution. This was stored at 4°C prior to use.

Twelve bacterial pathogens (Table 1), including six Gram-positive and six Gram-negative organisms, were examined in this study. In order to prepare the inocula for challenge, all organisms were cultured on Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood, and incubated for 24 h at 37°C. Under aseptic conditions, serial dilutions of each isolate were prepared individually in 0.1% (w/v) peptone saline (PS; Oxoid CM0733), equating to a 0.5 McFarland standard (approximately 10^8 colony-forming units [cfu] per mL), which was inoculated on fresh Mueller-Hinton agar (Oxoid CM0337) by means of a sterile cotton swab. To this, fresh extracts (10 µL) were added and the inoculum allowed to dry prior to incubation, as detailed above. Plates were examined visually and any inhibition zones were noted and their diameters measured and recorded. Sterile PBS and antibiotic susceptibility discs containing 5 µg ciprofloxacin (Mast Diagnostics, Bootle, Merseyside, UK) were employed as negative and positive controls, respectively.

Table 1. Description of bacterial organisms employed in the study.

Microorganism	Source
Gram-positive	
Bacillus cereus	NCTC 7464
Bacillus subtilis	NCTC 10400
Enterococcus faecalis	NCTC 775
Listeria innocua	NCTC 11288
Listeria monocytogenes	NCTC 11994
Staphylococcus epidermidis	NCTC 11047
Gram-negative	
Burkholderia cenocepacia	Wild-type clinical isolate
Escherichia coli	NCTC 9001
Escherichia coli O157:H7 (non-verocytotoxigenic strain)	NCTC 12900
Klebsiella aerogenes	NCTC 9528
Pseudomonas aeruginosa	Wild-type clinical isolate
Salmonella nottingham	NCTC 7832

NCTC: National Collection of Type Cultures

No antibacterial activity was observed with any bacterium tested in relation to extracts i, ii and iv, as detailed above. However, inhibition was observed with the sphagnum moss smoke distillate against *Enterococcus faecalis* NCTC 775, *Listeria innocua* NCTC 11288 and *Staphylococcus epidermidis* NCTC 11047, which had inhibition zone diameters of 15 mm, 13 mm and 16 mm, respectively. Comparison of inhibition patterns between SMSD and lack of activity with TTD suggests that the antimicrobial components of SMSD have a low boiling point, indicating that these antimicrobials in the SMSD may be volatile organic compounds (VOC).

Bog water showed a mean count of 960 cfu/mL. Sphagnum moss contained one predominant bacterial organism, *Chromobacterium subtsugae*, which was highly pigmented with a dark purple colouration. The 16S rRNA gene sequence for this organism has been deposited in GenBank with the accession number JF433933. Further examination of the spent broth culture from the *C. subtsugae* did not show any inhibitory effects with any of the organisms listed in Table 1.

In these experiments, the authors attempted to examine any antibacterial activity for sphagnum moss, SMSD and TTD. Phylogenetically, the genus *Sphagnum* shows great biodiversity and is composed of approximately 350 species. Thus, the present study aimed i) to examine the value of sphagnum moss as an antimicrobial agent, as has been suggested anecdotally as a dressing during the First World War, and ii) to examine the potential antibacterial effects of chronic inhalation of smoke from open turf fires in Ireland in an *in vitro* setting and the subsequent consequence of this on acute and chronic respiratory infection.

The medical application of sphagnum moss was discovered during the First World War. Sphagnum moss (*Sphagnum affine*) is a plant that grows in peat bogs and other muddy wet places. It was discovered that this moss could absorb three times as much fluid as standard cotton gauze and, as it was acidic, it slowed the growth of bacteria. Hence, it had all the desired properties of a bandage for dressing wounds caused by trauma from gunshot or shell shrapnel. In addition, the use of sphagnum moss was popular as it was cheaper than Lumière's gauze dressing.

From the current study, it appears that any antimicrobial activity of sphagnum moss bandages was due to the ability of the moss to absorb available moisture, thereby lowering available water (A_w) below the threshold for bacterial proliferation and thus make the proliferation of contaminating bacteria difficult, rather than having any true potent antibacterial activity.

The antibacterial and preserving effects of sphagnum moss

peat bogs has been well documented locally in Ireland. Clonycavan Man was discovered largely intact in Clonycavan, Co. Meath, Ireland, in March 2003. Old Croghan Man is the name given to a well-preserved Iron Age bog body found in an Irish bog in June 2003. The remains are named after Croghan Hill, north of Daingean, Co. Offaly, near where the body was found. The find is on display in the National Museum of Ireland in Dublin. Old Croghan Man is believed to have died between 362 BC and 175 BC, making the body over 2000 years old. The preservation of human remains in such environments may be attributed to the relatively low pH of bog water (usually pH 3.2–4.0).

Although turf has been used in Ireland for the past several hundred years for a variety of purposes, very few published papers have defined its benefits or risk to human health. One such risk is that of turf (peat) fire cancer, due to chronic exposure of legs and arms to continual heat from open turf fires and its association with erythema ab igne.[4] Previously, the authors attempted to examine the antimicrobial properties of various plants mentioned in traditional Ulster cures and remedies,[5] and the data would suggest that traditional cures and remedies solely reliant on the antimicrobial properties of aqueous extracts of these plants would have little or no microbiocidal activity.

Furthermore, there is little mention in the local historical evidence base of the efficacious nature of turf smoke associated with its healing properties of respiratory infections. Therefore, the authors postulated that the creation of VOCs through the burning of sphagnum peat turf may have some antibacterial properties.

Use of smoke inhalational as a means of drug delivery is not new. In North America, the Menominees smoked the pulverised dried root for respiratory complaints, while the Forest Potawatomis, the Mohegans and the Penobscots smoked the dried leaves to relieve asthma (http://aihc1998.tripod.com/medicine.html). In India, the fruits of *Solanum xanthocarpum* Burm. f. *Solanaceae* (Kandan-kathiri) are burned in an open fire and the smoke inhaled through the mouth to treat toothache.[6] In contrast, there has been little exploration or discussion to date of the medicinal properties and effects of turf burning in Ireland.

In conclusion, these data would suggest that the smoke of sphagnum moss contains components which are antimicrobial to certain bacteria. As yet, the exact chemical composition of these inhibitory agents is unclear. Further work is therefore justified to characterise these components and exploit such antibacterial properties against clinically significant pathogens. □

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