Journal of Ethnopharmacology 203 (2017) 171–181

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Antibacterial activity of medicinal plants from The Physicians of Myddvai, a 14th century Welsh medical manuscript

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ARTICLE INFO

Chemical compounds studied in this article:
Alliin PubChem CID: 87310
MF: C6H11NO3S
MW: 177.221 g/mol
Sabinene PubChem CID: 18818
MF: C10H16
MW: 136.234 g/mol

Keywords:
Traditional use
ethnobotany
medicinal plants
microbial infections
antibiotics

ABSTRACT

Ethnopharmacological relevance: Antimicrobial drug resistance is a growing threat to global public health. Historical records and herbal texts relating to traditional Celtic medicine indicate an extensive pharmacopeia of plants for treating infections likely caused by microbes. However, a major barrier for successful integration of these remedies into mainstream practice is the current lack of accurate interpretation and scientific validation.
Materials and methods: We investigated the flora of the Isle of Arran, Scotland, via *in situ* targeted screening of 83 out of 138 plants identified in *Meddygion Myddvai* (a 14th century Welsh manuscript) to treat conditions related to microbial infections, and an additional 18 plants from modern ethnobotanical knowledge on the island (Scottish School of Herbal Medicine). In a follow-up proof-of-concept study, bioassay-guided fractionation was performed to identify bioactive constituents from two high scoring hits that inhibited *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacterial growth.
Results: 67 historical plants (80.7%) and 14 modern plants (77.8%) were found to have detectable levels of antimicrobial activity when tested using Mobile Discovery kits, with human saliva as a source of bacteria for screening. Sabinene, a natural bicyclic monoterpene from juniper "berries" (*Juniperus communis* L.) and alliin, a natural sulfoxide from garlic cloves (*Allium sativum* L.), were isolated and confirmed as primary antibacterial leads.
Conclusion: Using historical medical sources such as those associated with traditional Celtic medicine to guide rigorous, evidence-based scientific investigation, provides additional leads for new and alternative bioactive molecules for combating bacterial diseases.

1. Introduction

Microbial infections date back to the dawn of humankind and are responsible for high mortality rates and a shorter life expectancy in medieval societies – especially among children, the malnourished, and wounded individuals. Diseases such as tuberculosis, typhus, diphtheria, typhoid, cholera, dysentery, and pneumonia took a large toll on the early medieval population of the British Isles, co-inhabited by ethnolinguistic Celts (Britton, Pict, and Gaelic tribes) and Anglo-Saxons (Germanic tribes) that arrived in the 5th century AD. Excavations of cemeteries from that time suggested a life expectancy into the mid to late thirties, but demonstrated a peak mortality rate in the teens and early twenties for those individuals who survived childhood (Fleming, 2010). From the 9th century AD, Celtic tribes became confined mostly to the west (Wales and Cornwall) and north (Scotland and the Western Isles). Despite their geographical divisions, the Celts of the British Isles shared in common the ancient healing traditions, handed down by word of mouth first by the druids and subsequently by leeches (from Gaelic *lighiche,* "physician") skilled in medical craft.

Similar to other ancient and medieval cultures, the Celts believed strongly that in nature there is somewhere and somehow a sovereign remedy for the management and treatment of diseases (Whittet, 1964). Historical records and herbal texts relating to traditional Celtic medicine indicated an extensive pharmacopeia of plants for treating

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http://dx.doi.org/10.1016/j.jep.2017.03.039
Received 22 December 2016; Received in revised form 22 March 2017; Accepted 23 March 2017
Available online 27 March 2017
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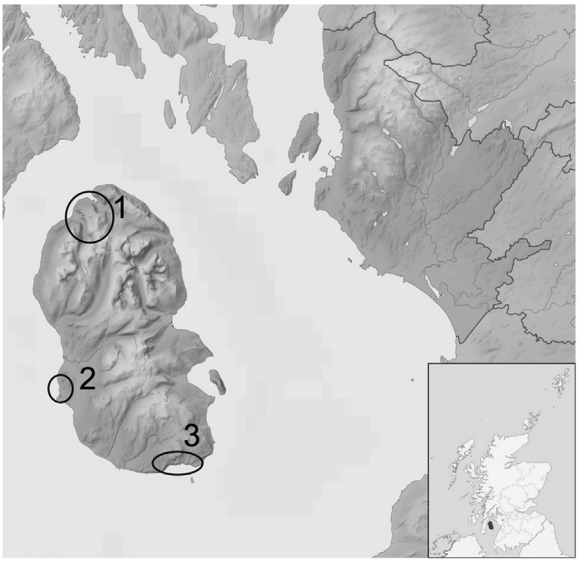

Fig. 1. Study sites on the Island of Arran, Scotland. (1) Northern site near Lochranza village, (2) Western site between Blackwaterfoot and Tormore villages, and (3) Southern site between Shannochie and Kildonan villages. Small fresh plant samples were tested *in situ* for antibacterial activity using Mobile Discovery approach.

infections likely caused by microbes (Martin, 1703; Pughe., trans., 1861; Henderson, 1994; Beith, 1995; Dobson and Robertson, 2009). One of the most thorough and concrete historical texts concerning traditional Celtic plant medicines was recorded by the Physicians of Myddvai sometime before 1233 AD (*Meddygion Myddvai*, Carmarthenshire, Wales), and is most often held as a part of the Red Book of Hergest manuscript (Llyfr Coch Hergest, c. 1382). The text of the physicians fixed insular Celtic tradition of medicinal plants in a series of some 800 remedies that featured mostly native species and manifested the older, oral knowledge, and local apothecary from the 14th century and earlier, likely dating back to the pre-Christian era (Pughe., trans., 1861). This knowledge was widely used by the hereditary scholarly physicians of the Western Isles (i.e. Macleans in Skye, O'Conachers in Argyll, Beatons in Islay and Mull). Many members of the medical families continued to practice traditional Celtic medicine in the Western Isles until the last century (Anonymous, 1906).

The Isle of Arran, the seventh largest Scottish island, is located in a mild oceanic climate zone (Fig. 1). Arran's highest peaks may have been nunataks (rocky protrusions above the ice sheet) during the Pleistocene glaciations and provided protected places for plant life to survive. This feature may explain a relatively high level of biodiversity (as compared to other parts of the British Isles) and presence of tree species endemic to the area (McKirdy et al., 2007). With a long history of traditional use, the medicinal plants of Arran presented a unique opportunity for focused screening and validation of Celtic plant-based healing traditions.

Due to the rapid emergence of antibiotic-resistant bacteria (Tommasi et al., 2015) and the apparent lack of interest from the pharmaceutical industry in antibiotic research (Payne et al., 2007), we applied a new assay, coined “Mobile Discovery approach,” for *in situ* targeted screening of plants identified in the Physicians of Myddvai manuscript to treat conditions related to microbial infections. The purpose of this new assay is to identify novel leads for further ethnopharmacological studies. As proof of concept for the assay, two high scoring hits were further characterized for their ability to inhibit *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) bacterial growth. The antimicrobial constituents that they produce were also isolated and tested to establish preclinical MICs.

2. Materials and methods

2.1. Study area

The Isle of Arran belongs to the group of islands in the Firth of Clyde, separated from the Western Isles (also called the Inner and Outer Hebrides) by the Kintyre peninsula (Fig. 1). Arran is divided into highland and lowland areas by the Highland Boundary Fault aligned southwest to northeast between Blackwaterfoot and Brodick villages (Barrow, 1912). Three collection sites were designed to capture geographical and biological diversity of the island, including (1) Northern site near Lochranza village, limited by Glen Catacol on the west, Gleann Easan Biorach on the east, and Beinn Bhreac on the south; (2) Western site between Blackwaterfoot and Tormore villages; and (3) Southern site between Shannochie and Kildonan villages. Collections occurred during May–July 2015, with the average daily temperature of 12.2 °C (6.6–17.1 °C) and the average of 10–12 precipitation days per month. Due to the nature of the Mobile Discovery approach (see below), only small samples (50–100 mg) of the fresh plants were collected and assayed daily to ensure a non-destructive method of testing. Plant identities were confirmed by the professional medical herbalists (M.R., K.R.) from the Scottish School of Herbal Medicine. Digital voucher specimens were recorded with a Motorolla Droid Mini 10 MP camera and deposited for reference in the Mobile Discovery plant collection of the Plants for Human Health Institute, NC State University, Kannapolis, NC (voucher numbers #KNC-CW001-101). When warranted by the ethnobotanical knowledge (and availability), separate samples were collected from different parts of the same plant (stem, leaf, root, bulb, seed, bark, fruit, resin, or flower).

2.2. Ethnobotanical survey of the Physicians of Myddvai manuscript

John Pughe’s 1861 English translation of the Physicians of Myddvai text was used to determine which plants to survey and screen *in situ*. The online version of this book is also available (https://archive.org/details/b2475769x). This translation included a separate index of Welsh and Latin names of each plant mentioned in the text. Since the Myddvai herbals were recorded in Welsh, translated into pre-Linnaean Latin, and then into English (post Linnaeus), we performed additional checks to ensure proper plant name identification. Those included data from (1) historical Welsh dictionary (Thomas et al. 2002), (2) Gaelic language sources concerning similar ailments, (3) common names listed in the English edition, (4) consultations with local herbalists, and (5) modern plant systematics methodology including biogeographic origin of species in question. The modern glossary of Nicholas Culpeper’s 17th century AD herbal text was consulted to aid in the understanding of various terminologies pertaining to diseases (Culpepper, 1975). Even though the Welsh were unaware of microbes at the time, the text provided clear and appropriate descriptions that are recognizable as microbial infections or related conditions (abscess, ague (fever), bites (snake, spider, dog and/or “mad” dog), colds (catarrh), cough (including whooping cough), diarrhea, erysipelas, eye problems (cloudiness, opacity, redness), fetid breath, fevers (such as typhoid), leprosy, mouth sores, plague, pneumonia, proud flesh (excessive granulation), scabs (and scabies), scrofula, skin eruptions, sore throat, stomach sores (including ulcers and wounds)). On many occasions, these plants were listed as ingredients of a more complex mixture that contained upwards of 10 components, including animal and mineral additives. A list of 138 plants was compiled based on these indications (Table 1). From this list, we successfully located, identified, and tested 83 plants (103 samples) for their antimicrobial properties.

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