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Antibacterial activity of medicinal plants from *The Physicians of Myddvai*, a 13th century Welsh medical manuscript

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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 applied Mobile Discovery approach to the Isle of Arran, Scotland, for *in situ* targeted screening of 83 out of 138 plants identified in *Meddygion Myddvai* (a 13th century Welsh manuscript) to treat conditions related to microbial infections, and an additional 18 plants from modern ethnobotanical knowledge on the island. 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*) and alliin, a natural sulfoxide from garlic cloves (*Allium sativum*), 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 and infectious diseases.

Keywords: Traditional use; ethnobotany; medicinal plants; microbial infections; antibiotics

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 infections likely caused by microbes (Beith, 1995; Dobson and Robertson, 2009; Henderson, 1994; Martin, 1703; Pughe, 1861). One of the most thorough and concrete historical texts concerning traditional Celtic plant medicines was recorded by the Physicians of Myddvai (*Meddygion Myddvai*, Carmarthenshire, Wales), 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 500 remedies that featured mostly native species and manifested the older, oral knowledge, and local apothecary from the 13th century AD. 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 modern days (Anonymous, 1906).

The Isle of Arran, the seventh largest Scottish island, is located in a mild oceanic climate zone (Figure 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 increased biodiversity 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 Mobile Discovery approach for *in situ* targeted screening of plants identified in the Physicians of Myddvai manuscript to treat conditions related to microbial infections. 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 (Figure 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 ^{o}C (6.6-17.1 ^{o}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. 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*. 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 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 (Culpeper, 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.

2.3. Incorporation of modern ethnobothanical knowledge

An additional 18 plants (27 samples) not listed in the Physicians of Myddvai text were added to the study for comparison purposes. These included remedies listed in "Healing Threads" (Beith, 1995), a popular modern compilation of the Gaelic medical tradition (Table 2), and plants that grow on Arran and are routinely used to treat microbial infections or related conditions by the modern herbalists from the Scottish School of Herbal Medicine (Table 3).

2.4. Mobile Discovery assay for antibacterial activity

The *in situ* antibacterial activity of the small samples (50-100 mg) of the fresh plants was determined by the Mobile Discovery kits against human saliva (C.W.) as a source of bacteria for screening. Plants samples were collected in the morning hours before 13:00, transported in the plastic pouches, and tested within 1 h of collection. The Mobile Discovery approach ensured an ethical, non-destructive, and safe (no external bacterial cultures were used or shipped) method to test samples from the local environment for their antimicrobial properties without associated biosafety risks to local biodiversity, population, or environment. Mobile Discovery kits were requested through the program website (http://MobileDiscovery.org, follow the Mobile Discovery Kit link), where they are available for scientists and the general public interested in antimicrobial discovery research. The antibacterial activity was determined according to the instructions from the Mobile Discovery manual shipped with the kit and visually scored against untreated and saliva-treated controls as "0" (no bacterial colonies, high score), "1" (few bacterial colonies, medium score), "2" (some bacterial colonies, low score), and "3" (many bacterial colonies, low score).

2.5. Extraction of high scoring plant materials

In a follow-up proof-of-concept study, juniper "berries" (*Juniperus communis*) and garlic cloves (*Allium sativum*), two of the high scoring hits recorded in the Mobile Discovery tests, were obtained from Frontier Co-Op (Norway, IA), freeze dried (Labconco Freezone 18, Kansas City, MO), ground to powder (IKA A11 mill, Wilmington, NC) and subjected to sequential hexane, ethyl acetate, butanol, and aqueous fractionation (60 g of plant powder, 360 ml of solvent, repeated twice, batches were combined and evaporated to dryness using Buchi Rotavapor R210, Flawil, Switzerland). Fractions and respective bioactive compounds were then tested in the laboratory settings for their ability to inhibit Gram-positive and Gram-negative bacterial growth. All chemicals and solvents (anhydrous and ACS grade) were purchased from Sigma-Aldrich (Saint Louis, MO), unless specified otherwise.

2.6. Bacterial strains and culture conditions

Gram-positive *Staphylococcus aureus* ATCC 25923 (Manassas, VA) and Gram-negative *Escherichia coli* DH5a (Promega, Madison, WI) were incubated under aerobic conditions on the LB medium at 37 ^{o}C, with or without shaking (100 rpm, New Brunswick Innova 43 shaker, Enfield, CT).

2.7. Disk diffusion

Disk diffusion was used to determine the antibacterial activities of high scoring plants in the laboratory setting. For each 100 mm Petri/LB agar plate surface spread with the appropriate bacterial culture, 6 filter paper disks (6 mm) were imprinted with 10 µl of vehicle (DMSO, negative control) or three concentrations of the test articles (10, 3, and 1 µg), tested in duplicate. Antibiotics tetracycline and ampicillin (5 µg) were used as reference drugs for Gram-positive and

Gram-negative bacteria, respectively. After an overnight incubation at 37 °C, the diameter of the inhibition zones was measured with a digital caliper in mm.

2.8. Minimum inhibitory concentration (MIC)

Different doses of the test articles (50 mg/ml stocks in DMSO) or reference drugs (5 mg/ml in DMSO) were serially 3x diluted and 20 µl of each dilution were added in triplicate to a 96 well plate containing 180 µl of diluted bacterial culture in the LB medium. The plates were incubated overnight at 37 °C, and bacterial growth was measured by absorbance read at 600 nm on the Synergy H1 microplate spectrophotometer (BioTek, Sunnyvale, CA). The MIC was determined as the lowest concentration inhibiting at least 80% of the growth.

2.9. Minimum bactericidal concentration (MBC)

MBC was determined by serial sub-cultivation of 100 µl of each dilution onto plates containing LB agar and further incubation overnight at 37 °C. The lowest concentrations without visible growth were defined as MBCs.

2.10. Cell culture

Human HT-29 cells of epithelial origin (ATCC HTB-38, Manassas, VA) were routinely passaged every 3-4 days and maintained in high glucose DMEM containing 10% fetal bovine serum FBS (Life Technologies, Carlsbad, CA), and 1% penicillin-streptomycin (Fisher Scientific, Pittsburg, PA) at 37 °C and 5% CO_2. Cell viability was estimated using the 96 well MTT assay (Mosmann, 1983) by absorbance read at 570 nm on a Synergy H1 microplate spectrophotometer (BioTek, Sunnyvale, CA).

2.11. Statistical analysis

Statistical analyses were performed using Prism 6.0 (GraphPad Software, San Diego, CA). Data were analyzed by one-way ANOVA with treatment as a factor. Post hoc analyses of differences between individual experimental groups were made using the Dunnett's multiple comparison tests. Significance was set at $p < 0.05$. Values are reported as means ± SEM.

3. Results

3.1. Diversity of plants from the Physicians of Myddvai manuscript

The ethnobotanical survey of some 500 remedies described in the Physicians of Myddvai text was focused on plants indicated for treating diseases recognizable as microbial infections or related conditions. A total of 138 plant species that fit this description were classified into 51 families and 125 genera (Table 1). The most used families were Apiaceae, Asteraceae, Lamiaceae, and Rosaceae that contributed 55 medicinal plant species (40%) with putative antimicrobial properties (Figure 2A). Important medicinal genera were *Allium* and *Artemisia* (3 species each). Several genera represented non-native plants that were either imported to the region or cultivated for their medicinal properties, for example *Crocus*, *Aloe, Piper*, *Citrus, Afromomum, and Cinnamomum*. Myddvai herbalists used different plant parts or their combinations, depending on the target plant species, however the distinction was made clear only for root, bark, juice, and fruit/seed parts of the plant. When not specified, we collected and analyzed aerial parts of the plant (leaf, stem, and/or flower) available at the time of collection.

Among 20 health conditions possibly related to microbial infections, wounds, fevers, eye problems, and pneumonia were most prominent (Figure 2B).

3.2. Modern ethnobothanical knowledge in the region

Review of Beith's "Healing Threads" and consultations with modern herbalists from the Scottish School of Herbal Medicine (Arran) resulted in the inclusion of an additional 9 genera from the former (Table 2) and 9 genera from the latter source (Table 3). Two species of lichens and an endemic species, the Arran whitebeam (*Sorbus arranensis*), were reported to be used as antimicrobial remedies in the modern traditional practice on the island. At least 26 medicinal plant species recognized as antimicrobial in the Physicians of Myddvai text were listed for the similar indications in Beith's herbal. In addition to common health conditions like cough, fevers, and wounds, modern ethnobotanical knowledge recognized and directly specified microbial infections as a direct target for traditional plant use.

3.3. Antimicrobial activity of the tested plants

About 52 (50.5%) of the plants tested were single parts, predominantly leaves (34.6%), although roots, stems, seeds and other organs were also sampled when indicated and available (Figure 3A). In many instances, a combination of plant parts was used, with an aerial sample (leaf and stem) being the most frequent. Mobile Discovery assay using human saliva as a source of bacteria for screening confirmed high (score of 0) antimicrobial activity of 37 Myddvai plants tested (44.6%). Additionally, 13 plants (15.7%) were measured as medium (score of 1) and further 17 plants (20.5%) were noted to have low (score of 2) antimicrobial activity. Altogether, out of 83 Myddvai plants tested, 67 plants (80.7%) showed any level of measurable antimicrobial properties (Table 1). We also noted the evidence of an increased level of modern ethnobotanical knowledge regarding the anti-infective plants of the region, as 14 modern leads (77.8%) showed any level of antimicrobial activity, with 10 (55.6%) plants measured as high scores (Tables 2-3). Notable exceptions were 3 plants from the order Lamiales which showed no antimicrobial activity even though indicated in the Beith's herbal (Table 2). Traditional knowledge from the modern Scottish School of Herbal Medicine (Arran) was confirmed in 8 out of 9 species tested, with majority of the plants producing high or medium scores (Table 3).

Original health indication had a major impact on confirmation of the antibacterial activity. Plants indicated for bites, pneumonia, and erysipelas were confirmed as antimicrobial in >75% tests. Bioactivity of remedies targeting sores, scrofula, eye problems, and wounds was confirmed in >65% of the cases (Figure 4A). Notable exceptions were diarrhea (0/2, 0%), scabs (1/3, 33%), and leprosy (3/7, 43%). Plants indicated for bites also showed the highest antibacterial scores (0.31±0.24), three times above the average for other health indications (1.01±0.42, $p<0.05$) (Figure 4B).

3.4. Bioactivity-guided fractionation of the high scoring plants

In a follow-up proof-of-concept study, antibacterial activity of juniper "berries" (*Juniperus communis* L.) and garlic cloves (*Allium sativum* L.) fractions were measured by disk diffusion method in the dose range of 1-10 µg. Gram-positive *S. aureus* showed dose-dependent susceptibility to the hexane fraction from juniper (10-13 mm inhibition zone diameter) and the water fraction from garlic (8-25 mm inhibition zone diameter). Gram-negative *E. coli* showed dose-dependent susceptibility from the hexane fraction of juniper (7-12 mm inhibition zone diameter) and the water fraction from garlic (6-18 mm inhibition zone diameter) (Figure 5).

The antimicrobial activity was attributed to sabinene and alliin, the major bioactive compounds found in these fractions. MICs were determined for extracts and respective bioactive compounds after 24 h and ranged from 215 µg/ml for juniper to 54 µg/ml for sabinene against *S. aureus*; and from 108 µg/ml for garlic to 27 µg/ml for alliin against *E. coli* (Figure 6). MBCs were lower for *S. aureus* (sabinene, 215 µg/ml) than for *E. coli* (alliin, 108 µg/ml).

4. Discussion

The spread of antibiotic-resistant pathogens, combined with decades of little success in discovering new antibiotics, has become an increasing problem for modern medical interventions and community health (Lewis, 2012). The current portfolio of new antimicrobial drugs in clinical trials consisted largely of already known chemical classes for which resistance has already emerged. High throughput screening of individual molecular targets (genes and proteins) against synthetic chemical libraries has had very limited success as well (Payne et al., 2007). Therefore, there is still a great need for discovering new antimicrobial compounds, but the strategy of antibiotic discovery needs to be modified to capture unexploited biodiversity and identify new natural lead compounds for further antimicrobial development (Harvey, 2000).

Traditional knowledge, such as that captured in Physicians of Myddvai, provided an initial source of screening targets containing biologically relevant chemical spaces and pharmacophores, all with a recorded history of prior human use. Mobile Discovery approach, offered a simple, low-cost, safe, and effective method to identify and validate antimicrobial hits with no geographical, technological, or ethical constraints. It relied on bacteria naturally present in human saliva, thus allowing for phenotypical, non-targeted, whole cell antibacterial screens.

Bacterial flora of the human saliva contains both Gram-positive and Gram-negative facultative cocci and rods (Socransky and Manganiello, 1971), therefore Mobile Discovery assay interrogated all targets in their physiological context simultaneously. The assay used fresh, whole plant parts rather than extracts or other preparations, thus capturing *in situ* chemical diversity and bioactivity of natural products, which is often lost during collection, storage, or separation of synergistic interactions.

Of some 500 remedies described in the Myddvai text, the largest number were purely herbal and are still in use (Beith, 1995). Many of these plants were indicated for treating diseases recognizable as microbial infections or related conditions (Figure 2). Overall, high antibacterial activity was demonstrated in 37 Myddvai plants (44.5%), while any level of antibacterial activity was detected in 67 species (80.7%). Such high hit rates are explained by extensive Celtic ethnobotanical knowledge of local plants and centuries of continuous practical use. Several plants showed high antimicrobial scores in the majority of the parts tested, including juniper (*Juniperus communis*), wild garlic (*Allium ursinum*) and its cultivated relative (*Allium sativum*), hemp-agrimony (*Eupatorium cannabinum*), and herb Robert (*Geranium robertianum*). In other cases, the antimicrobial activity was clearly restricted to one major part of the plant tested, for example, the root of dandelion (*Taraxacum officinale*) or the seed of caraway (*Carum carvi*). High antimicrobial scores were also found in certain plants that are of particular importance to traditional Celtic medicine of the Western Isles, including bog myrtle (*Myrica gale*), wood sage (*Teucrium scorodonia*), and white oxe eye daisy (*Leucanthemum vulgare*). Widespread medicinal plants traditionally used to fight infections were well represented among the high scoring hits (Figure 3), including wormwoods (*Artemisia spp*.), garlic and onion (*Allium spp*.), and sage (*Salvia officinalis*). Herbs or spices traditionally used to prepare meals and home remedies, such as cumin, fennel, saffron, dittany, rosemary, thyme, and bay laurel, were also confirmed as high scoring antimicrobial hits, thus validating the *in situ* Mobile Discovery approach (Table 1).

Many of the Myddvai plants were investigated for antibacterial properties previously, and the bioactive constituents responsible for the antibacterial effects were isolated and identified in some cases. For example, antimicrobial effects were attributed to terpenoids such as eucalyptol, thujones, camphor, borneol, and myrtenol present in the essential oil of feverfew (*Tanacetum vulgare)* (Judzanentiene and Mockute, 2005; Muresan, 2015), caffeic acid derivative plantamajoside from the leaves of plantain (*Plantago major*) (Stanisavljevic et al., 2008), terpenoids such as patchoulol, pinene, humulene, valerenic acid, and camphene in the essential oil of valerian roots (*Valeriana officinalis*) (Letchamo et al., 2004), and alkaloid berberine in barberry (*Berberis vulgaris*) (Dashti et al., 2014). However, the review of literature suggested that in many cases, while the antimicrobial properties of the plant or plant extract were reported, the specific bioactive constituents responsible for these effects were not identified, for example in case of beets (*Beta vulgaris*) (Bucur et al., 2015) and other plants.

Wounds, including bites and sores, were very common at the time, and Celtic knowledge of plants effective for treating these conditions seems to be very relevant today. Myddvai plants indicated for treating bites were confirmed to contain antibacterial activity in more than 75% of remedies, with highest antibacterial potency three times above the average for other health indications analyzed in this study (Figure 4). This could be possibly explained by the diverse and complex polymicrobial nature of the bite wound infections (Abrahamian and Goldstein, 2011). Pneumonia, skin infections (erysipelas), halitosis (fetid breath), and sores of various origins were other indications that resulted in high rates of antimicrobial discovery. On the other hand, plants indicated for health conditions of mixed etiology, for example, a combination of bacterial infection and acute nonmicrobial inflammation due to direct injury (wounds) or eye disorders, while producing some high scoring hits such as thyme (*Thymus serpyllum*), resulted in lower overall antibacterial hit rates. In other instances, however, when the eye disease is described in sufficient details to recognize a microbial infection, the laboratory follow-up confirmed the antimicrobial properties of the traditional medicine. This is the case with sty remedy from the Anglo-Saxon text, *Bald's Leechbook,* from the 10th century AD, that included a garlic (*Allium sativum*), onion (*Allium cepa*), and leek (*Allium ampeloprasum*) decoction in wine (Harrison, 2015). This makes a very interesting parallel between medicinal traditions of Celts and Anglo-Saxons, that co-inhabited the British Isles at that time and likely shared knowledge of local medicinal plants and their traditional uses.

Focusing on novel and diverse chemical structures – and not targets – that show good preclinical antibacterial activity, seems to be the most attractive approach to improve antibacterial discovery (Payne et al., 2007). Plants, due to their intrinsic chemical complexity, synergistic, multi-target, and environmental flexibility, are poised to provide such diversity. For example, an endemic Arran whitebeam (*Sorbus arranensis*) was measured as a high scoring hit in this study, however its phytochemical and bioactivity profiles remain unknown, likely due to rarity and environmental isolation of this species. In a follow-up proof-of-concept study, juniper "berries" (*Juniperus communis*) and garlic cloves (*Allium sativum*), two of the high scoring hits recorded in the Mobile Discovery assay, were further fractionated and confirmed to contain compounds with high antimicrobial activity, sabinene and alliin (Choi et al., 2007; Pepeljinjak et al., 2005). Although not novel, these leads proved a high applicability of Mobile Discovery *in situ* approach to the early screening stage. Alliin also showed excellent *in vitro* safety margin (no human cell toxicity up to 108 μg/ml, Figure 6). However, the parent structure does not follow Lipinski's rule of five (Lipinski et al., 2001) and is hydrolyzed to bioactive allicin that has stability concerns (Lawson and Gardner, 2005). When leads like this are identified, it may still take years and synthesis of numerous derivatives to ensure an antibacterial molecule with desirable pharmaceutical profile, broad spectrum activity, stability, acceptable safety at high blood levels, and a competitive dosing regimen. This is not going to happen until more antibiotic discovery work is done in academic, industrial, and citizen science settings.

5. Conclusions

Despite the historical successes of traditional medicines and early drug discovery efforts based on plants, it is likely that the vast majority of plant species have not been investigated and their bioactive constituents have not been determined. The knowledge of medicine possessed by the Celts of the British Isles was fixed in the Physicians of Myddvai manuscript in the form of a culture-specific, detailed knowledge of local medicinal plants and their traditional uses. Our study demonstrated that most of the Myddvai medicinal plants indicated for treating diseases recognizable as microbial infections showed an antibacterial effect *in vitro*, and justified at least in part their use in traditional medicine. These results encourage further investigations to extract and identify the active chemical compounds responsible for the antibacterial effects.

Author contributions

CW, JG, and SK designed the study and drafted the manuscript, CW carried out field and laboratory assays, MR and KR participated in field assays, MW participated in laboratory assays, JG and SK conceived and coordinated the study, SK developed and funded the Mobile Discovery assay. All authors read and approved the final manuscript.

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Table 1. ***The Physicians of Myddvai*** **plants indicated for treating diseases recognizable as microbial infections and their antibacterial activity measured with the Mobile Discovery approach.**

Family	Genus	*Species*	Common name	Plant part(s)	Score	Traditional use
Apiaceae	*Angelica*	*sp.*	Angelica	Leaf and stem	3	Fetid breath
	Apium	*graveolens*	Celery	Leaf and stem	2	Ague, eye problems
				Stem	2	
	Conium	*maculatum*	Hemlock	--	--	Abscess, erysipelas, pneumonia
	Carum	*carvi*	Caraway	Seed	0	Fevers, pneumonia
				Leaf and stem	2	
	Conopodium	*majus*	Pignut, kippernut	--	--	Fevers
	Coriandrum	*sativum*	Coriander	Leaf and stem	2	Ague
	Cuminum	*cyminum*	Cumin	Seed	0	Cough, wounds
	Foeniculum	*vulgare*	Fennel	Leaf and stem	0	Bites, cough, erysipelas, eye problems, fevers, pneumonia
	Petroselinum	*marinum*	Parsley	Leaf	3	Ague, eye problems, wounds
	Pimpinella	*anisum*	Anise	--	--	Cough, fevers, pneumonia
Aquifoliaceae	*Ilex*	*sp.*	Holly	Leaf and stem	3	Wounds
				Bark	3	
Amaryllidaceae	*Allium*	*sativum*	Cultivated garlic	Bulb	0	Abscess, bites, cough, proud flesh, plague, sore throat, wounds
	Allium	*ampeloprasum*	Leek	Bulb	1	Bites, cough, pneumonia
	Allium	*cepa*	Onion	Bulb	1	Cough, eye problems, pneumonia, skin eruptions, wounds (also Beith)
	Hyacinthoides	*non-scripta*	Bluebell, wild hyacinth	Leaf and stem	2	Leprosy
	Ruscus	*aculeatus*	Butcher's broom	--	--	Fevers
Iridaceae	*Crocus*	*sativus*	Saffron	Stigma (dry)	0	Ague
	Hyacinthoides	*non-scripta*	Bluebell, wild hyacinth	Fruit	2	Leprosy
	Iris	*pseudocorus*	Yellow flag	Root	3	Ague, erysipelas, skin eruptions, sore throat (also Beith)
				Leaf and stem	2	
Xanthorrhoeaceae	*Aloe*	*sp.*	Aloe	Leaf	3	Eye problems
Asteraceae	*Achillea*	*millefolium*	Yarrow, milfoil	Leaf and stem	3	Eye problems, fevers, scrofula, wounds (also Beith)
	Arctium	*lappa*	Burdock	Root	0	Fevers, skin eruptions
				Leaf	1	
	Artemisia	*vulgaris*	Mugwort	Leaf and stem	0	Abscess, ague, bites, erysipelas, fevers, pneumonia, scrofula, skin eruptions
	Artemisia	*absinthium*	Wormwood	Leaf and stem	0	Ague, fevers, pnuemonia

	Artemisia	*abrotanum*	Southernwood	--	--	Fevers
	Bellis	*perennis*	Bruisewort	Leaf and stem	3	Wounds
				Root	2	
				Flower	1	
	Calendula	*officinalis*	Marigold	Leaf and flower	2	Fevers
	Centaurea	*sp.*	Knapweed	Leaf, stem and flower	0	Bites, eye problems, fevers, scrofula, skin eruptions
	Chrysanthemum	*leucanthemum*	Common daisy	Leaf, stem and flower	3	Eye problems, fevers, pneumonia, scrofula, skin eruptions, wounds (also Beith)
	Eupatorium	*cannibinum*	Hemp-agrimony	Leaf and stem	0	Cough, wounds
				Root	0	
	Inula	*helenium*	Elecampane, elfwort	Leaf	0	Cough, leprosy, scabs, scrofula, skin eruptions, sore throat (also Beith)
	Leucanthemum	*vulgare*	White oxe eye daisy	Leaf, stem and flower	0	Cough, plague, wounds (also Beith)
	Matricaria	*chamomilla*	Chamomile	Leaf, stem and flower	0	Abscess, scrofula, wounds
	Tanacetum	*parthenium*	Feverfew	Leaf and stem	0	Bites, fetid breath, skin eruptions, sore throat, wounds
	Tanacetum	*vulgare*	Tansy	Leaf and stem	0	Eye problems, pneumonia, wounds
	Taraxacum	*officinale*	Dandelion	Leaf and stem	3	Fevers
				Flower	3	
				Root	1	
	Tragopogon	*dubius*	Yellow goat's beard	--	--	Fevers, pneumonia, proud flesh
Boraginaceae	*Borago*	*officinalis*	Borage	Leaf and flower	3	Fevers, wounds
	Cerinthe	*major*	Honeywort	--	--	Wounds
	Cynoglossum	*officinale*	Hound's tongue	--	--	Wounds
	Symphytum	*officinale*	Comfrey	Leaf	3	Abscess
Brassicaeae	*Brassica*	*oleracea var. rubra*	Red cabbage	Leaf	1	Eye problems, fevers, wounds
	Brassica	*sp.*	Mustard	--	--	Bites, cough, wounds
	Cardamine	*pratensis*	Lady's smock	--	--	Wounds
	Raphanus	*sativus*	Radish	Leaf and stem	3	Bites
				Root	2	
	Cochlearia	*officinalis*	Scurvygrass	Leaf, stem and flower	2	Wounds
Amaranthaceae	*Beta*	*vulgaris maritimus*	Sea beet	Leaf and stem	0	Pneumonia
				Root	0	
	Agrostemma	*githago*	Corn cockle	--	--	Pneumonia
Polygonaceae	*Persicaria*	*amphibian*	Amphibious persicaria	--	--	Fevers
	Rumex	*obtusifolia*	Common dock	Leaf	3	Abscess, leprosy, pneumonia, skin eruptions, wounds
	Rumex	*crispus*	Yellow dock	--	--	Abscess, leprosy, pneumonia, skin eruptions, wounds

Cucurbitaceae	*Byronia*	*alba*	White bryony	--	--	Wounds
Adoxaceae	*Sambucus*	*nigra*	Elder	Stem and Flower	0	Colds, fevers, mouth sore, sore throat, wounds
	Sambucus	*ebulus*	Dwarf elder	--	--	Fevers, mouth sore, sore throat, wounds
Caprifoliaceae	*Knautia*	*arvensis*	Field scabious	--	--	Bites, eye problems
	Lonicera	*sp.*	Honeysuckle	Leaf	0	Eye problems, leprosy, sore throat, wounds (also Beith)
	Succisa	*pratensis*	Devil's bit	--	--	Skin eruptions, wounds
	Valeriana	*officinalis*	Valerian	Root	0	Abscess, wounds
				Leaf, stem and flower	1	
Ericaceae	*Calluna*	*vulgaris*	Heather	Leaf, stem and flower	3	Fevers, pneumonia (also Beith)
	Empertrum	*nigrum*	Crowberry, crake berry	--	--	Fevers, pneumonia
Primulaceae	*Anagallis*	*arvensis*	Pimpernel	--	--	Abscess, fevers, wounds
	Samolus	*sp.*	Brook weed	--	--	Wounds
Caesalpiniaceae	*Caesalpinia*	*bonduc*	Bonduc bean, nickernut	--	--	Diarrhea
Fabaceae	*Cytisus*	*scoparius*	Broom	Leaf, stem and flower	1	Ague, leprosy, wounds
	Glycyrrhiza	*glabra*	Liquorice	--	--	Cough
Fagaceae	*Quercus*	*robur*	English oak	Bark	2	Fevers, sore throat, wounds (also Beith)
				Leaf	1	
Gentiaceae	*Centaurium*	*erythraea*	Centaury	--	--	Eye problems, fevers, wounds
Geraniaceae	*Geranium*	*robertanium*	Herb Robert	Leaf	0	Erysipelas, pneumonia, wounds (also Beith)
				Flower	0	
Rubiaceae	*Galium*	*aparine*	Cleavers, goosegrass	Leaf and stem	2	Colds, fevers, leprosy, scabs, scrofula, skin eruptions, wounds
	Galium	*odoratum*	Woodruff	--	--	Fevers, pneumonia, wounds (also Beith)
Lamiaceae	*Ajuga*	*reptans*	Bugle	--	--	Leprosy, skin eruptions, wounds
	Glechoma	*hederacea*	Ground ivy	--	--	Ague, bites, cough, eye problems, fevers, skin eruptions
	Hyssopus	*officinalis*	Hyssop	--	--	Sore throat, wounds
	Lamium	*purpeum*	Red dead nettle	--	--	Fevers, plague, scrofula, wounds
	Marrubium	*vulgare*	Horehound	Leaf, stem and flower	0	Cough, pneumonia (also Beith)
	Melittis	*melissophylum*	Bastard balm	--	--	Fevers
	Mentha	*sp.*	Mint (local)	Leaf and stem	2	Fevers
	Origanum	*dictamnus*	Dittany	Leaf and stem	0	Bites
	Rosmarinus	*officinalis*	Rosemary	Leaf and flower	0	Colds, fetid breath, fivers, mouth sores, sore throat
	Salvia	*officinalis*	Sage	Leaf, stem and flower	0	Ague, bites, cough, fetid breath, fevers, mouth sores, plague, sore throat, wounds
	Salvia	*sclarea*	Clary sage, clear eye	Leaf and stem	3	Wounds
	Stachys	*officinalis*	Wood betony	Leaf, stem and flower	0	Ague, bites, eye problems, fevers, wounds (also Beith)

	Teucrium	*scorodonia*	Wood sage	Leaf and stem	0	Abscess, colds, fevers, proud flesh
	Thymus	*officinalis*	Thyme	Leaf, stem and flower	0	Eye problems
	Thymus	*serpyllum*	Creeping thyme	Leaf, stem and flower	0	Colds, eye problems
	Vitex	*agnus-castus*	Chastetree, vitex	Bark	3	Wounds
				Leaf	1	
Oleaceae	*Ligustrum*	*vulgare*	Privet	--	--	Fevers, wounds
	Olea	*europea*	Olive	--	--	Bites, scrofula, wounds
Plantaginaceae	*Digitalis*	*purpurea*	Foxglove	Leaf and flower	2	Abscess (also Beith)
	Plantago	*major*	Greater plantain	Leaf	0	Ague, bites, erysipelas, eye problems, scrofula, skin eruptions, wounds (also Beith)
	Plantago	*lanceolata*	Ribwort plantain	--	--	Ague, bites, erysipelas, eye problems, scrofula, skin eruptions, wounds (also Beith)
	Veronica	*chamaedrys*	Germander speedwell	--	--	Abscess, wounds
Verbenaceae	*Verbena*	*officinalis*	Vervain	Leaf	0	Eye problems, scrofula, wounds
Lauraceae	*Cinnamomum*	*verum*	Cinnamon	Bark (dry)	2	Cough
	Laurus	*nobilis*	Bay laurel	Leaf and stem	0	Plague, scrofula, wounds
Hypericaceae	*Hypericum*	*perforatum*	St. John's wort	Leaf and stem	3	Abscess, fevers, wounds (also Beith)
	Hypericum	*androsaemum*	Tutsan	--	--	Fevers
Salicaceae	*Salix*	*sp.*	Willow	Bark	1	Eye problems
Violaceae	*Viola*	*sp.*	Violet	--	--	Scrofula, wounds
Malvaceae	*Althaea*	*officinalis*	Marshmallow	Leaf and stem	2	Cough, erysipelas, fevers, mouth sores, scrofula, sore throat, wounds
	Tilia	*europaea*	Lime, linden	--	--	Wounds
Oxalidaceae	*Oxalis*	*acetosella*	Wood sorrel	Leaf	2	Ague, colds, fevers, scabs, skin eruptions, sore throat, wounds (also Beith)
Cupressaceae	*Juniperus*	*communis*	Juniper	Leaf and stem	0	Bites, stomach sores, wounds (also Beith)
				Fruit	0	
Aristolochiaceae	*Aristolochia*	*rotunda*	Round birthwort	--	--	Bites, fevers, pneumonia
	Asarum	*europaeum*	Asarabacca	--	--	Pneumonia
Piperaceae	*Piper*	*nigrum*	Black pepper	Fruit (dry)	3	Ague, cough, erysipelas, eye problems, proud flesh, wounds
Cyperaceae	*Cyperus*	*longus*	Galingale	--	--	Cough
Poaceae	*Avena*	*sativa*	Oats	--	--	Abscess, wounds
Typhaceae	*Sparganium*	*sp.*	Burr reed	--	--	Eye problems, wounds
Berberidaceae	*Berberis*	*vulgaris*	Barberry	Leaf and stem	0	Wounds
				Bark	3	

Ranunculaceae	*Ranunculus*	*ficaria*	Lesser celandine	--	--	Eye problems, scabs, scrofula, skin eruptions
Cannabaceae	*Cannabis*	*sativa*	Hemp	--	--	Fevers, wounds
Moraceae	*Ficus*	*carica*	Fig	Leaf and stem	2	Cough
Rosaceae	*Agrimonia*	*eupitoria*	Agrimony	Leaf and stem	0	Bites, fevers, pneumonia, scrofula, wounds
	Argentina	*anserina*	Silverweed	Leaf and stem	2	Diarrhea, fevers, plague (also Beith)
	Crataegus	*monogyna*	Hawthorne	Leaf, stem and flower	3	Eye problems, sore throat (also Beith)
	Filipendula	*ulmaria*	Meadowsweet	Leaf	1	Pneumonia
	Fragaria	*sp.*	Wild strawberry	Leaf	0	Eye problems, wounds
	Geum	*rivale*	Water avens	--	--	Erysipelas, fevers, leprosy, pneumonia, wounds
	Potentilla	*erecta*	Tormentil	Leaf and stem	3	Diarrhea, fevers, plague (also Beith)
				Flower	2	
	Potentilla	*reptans*	European cinquefoil	--	--	Diarrhea, fevers, plague (also Beith)
	Prunus	*spinosa*	Blackthorn, sloe	--	--	Diarrhea
	Rosa	*rubiginosa*	Sweetbrier	--	--	Ague, fevers, mouth sores
	Rubus	*idaeus*	Raspberry	Leaf	0	Ague, eye problems, fevers, mouth sores, wounds
	Rubus	*fruticosus*	Blackberry, bramble	Leaf and stem	1	Ague, erysipelas, eye problems, fevers, mouth sores, wounds (also Beith)
	Sorbus	*aucuparia*	Rowan, mountain ash	--	--	Cough, fevers (also Beith)
Ulmaceae	*Ulmus*	*sp.*	Elm	--	--	Erysipelas
Urticaceae	*Urtica*	*dioica*	Common nettle	Leaf	3	Sore throat, wounds (also Beith)
Anacardiaceae	*Pistacia*	*lentiscus*	Mastic	--	--	Wounds
Burseraceae	*Boswellia*	*sacra*	Frankincense	Resin	1	Erysipelas, mouth sores, scrofula, wounds
	Commiphora	*myrrh*	Myrrh	--	--	Fetid breath, wounds
Rutaceae	*Citrus*	*sp.*	Orange	Fruit (juice)	3	Mouth sores
				Fruit (peel)	1	
	Ruta	*graveolens*	Rue	--	--	Abscess, bites, cough, fetid breath, fevers, mouth sores, plague, wounds
Crassulaceae	*Sedum*	*telephium*	Orpine	--	--	Fetid breath, fevers, scrofula
	Sempervivum	*sp.*	Houseleek	--	--	Erysipelas, eye problems, wounds
Solanaceae	*Solanum*	*dulcamara*	Bitter nightshade	--	--	Bites, skin eruptions
Sphagnaceae	*Sphagnum*	*cymbifolium*	Moss	All	0	Fevers, wounds (also Beith)
Zingiberaceae	*Aframomum*	*melegueta*	Grains of paradise	Seed (dry)	3	Ague, cough

Table 2. Plants indicated for treating diseases recognizable as microbial infections in Beith's Herbal (1995).

Family	Genus	*Species*	Common name	Plant part(s)	Score	Traditional use
Asteraceae	*Solidago*	*virgaurea*	Goldenrod	Leaf and stem	1	Wounds
Brassicaeae	*Capsella*	*bursa-pastoris*	Shepherd's purse	Leaf, stem and flower	1	Wounds
	Sisymbrium	*officinale*	Hedge mustard	Flower	0	Colds
				Leaf	1	
Caraphyllaceae	*Stellaria*	*media*	Chickweed	Leaf, stem and flower	0	Abscess
Myricaceae	*Myrica*	*gale*	Bog myrtle	Leaf	0	Parasites
				Bud	0	
Lamiaceae	*Prunella*	*vulgaris*	All heal	Leaf, stem and flower	3	Wounds
Orobanchaceae	*Euphrasia*	*officinalis*	Eyebright	Leaf, stem and flower	3	Eye problems
				Stem and root	3	
Scrophulariaceae	*Scrophularia*	*nodosa*	Figwort	Leaf	3	Scrofula
				Flower	3	
Rosaceae	*Alchemilla*	*vulgaris*	Lady's mantle	Leaf, stem and flower	0	Mouth sores, wounds

Table 3. Modern plants used to treat bacterial infections on the Island of Arran (2015

Family	Genus	*Species*	Common name	Plant part(s)	Score	Traditional use
Apiaceae	*Smyrnium*	*olusatrum*	Alexanders	Leaf, stem and flower	0	Infections
Amaryllidaceae	*Allium*	*ursinum*	Wild garlic	Leaf	1	Wounds
				Flower	1	
				Bulb	0	
				Fruit	0	
				Stem	3	
Ericaceae	*Vaccinium*	*myrtillus*	Blaeberry, bilberry	Leaf	1	Diarrhea
Lamiaceae	*Origanum*	*vulgare*	Oregano	Leaf and stem	0	Infections, wounds
		majorana	Marjoram	Leaf, stem and flower (dry)	3	Infections, wounds
Cladoniaceae	*Cladonia*	*sp.*	Lichen	All	0	Wounds
Parmeliaceae	*Usnea*	*sp.*	Lichen	All	0	Infections, wounds
Rosaceae	*Sorbus*	*arranensis*	Arran whitebeam	Leaf	1	Cough, fevers
				Fruit	1	
Grossulariaceae	*Ribes*	*nigrum*	Black currant	Leaf and fruit	0	Infections

Figure 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.

Figure 2. **Botanical profile of *The Physicians of Myddvai* plants indicated for treating diseases recognizable as microbial infections.** (A) Distribution of plant species across taxonomic plant families. (B) Frequency of health indications attributed to Celtic traditional use of these plants.

Figure 3. Plant parts analyzed for antibacterial activity. (A) Distribution of single and combinational plant parts used in this study. (B) Antibacterial activity scores of Myddvai plants quantified using Mobile Discovery approach against bacteria naturally present in human saliva.

Figure 4. Confirmed antibacterial activity of Myddavi plants. (A) Rates of successful confirmation of antibacterial activity of plants according to the original health indication for their traditional use, including high scoring hits juniper (α) and garlic (β). (B) Average antibacterial scores of plants indicated for a particular health condition. Plants indicated for treating bites showed the highest antibacterial potency, 3 times above the average for other health indications (* $P < 0.05$ by one-way ANOVA).

Figure 5. Dose-dependent quantification of antibacterial activity of two high scoring Myddvai plants using disk diffusion method. *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) bacteria were surface spread on agar plates and filter paper disks imprinted with three concentrations of juniper (hexane) and garlic (ethyl acetate) fractions were tested in duplicate.

Figure 6. Direct comparison of antibacterial activity and human cell toxicity of bioactive constituents from juniper (sabinene) and garlic (alliin). MIC curves of (A) sabinene against *S. aureus* (A) and (B) alliin against *E. coli* relative to the reference antibiotics. Human HT-29 cells of epithelial origin were treated with vehicle (0.1% DMSO), toxic control (10% DMSO), and different doses of (C) sabinene and (D) alliin, then cell viability was measured as a function of redox potential using MTT dye. Data are the mean ± SEM (n=3) normalized to vehicle control (* P<0.05, **P<0.01 by Dunnett's test subsequent to one-way ANOVA).

Graphical abstract

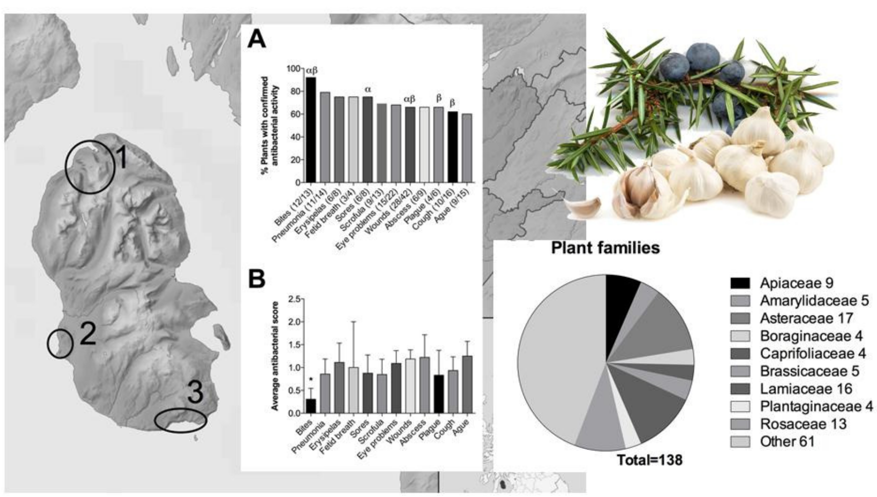


Figure 1

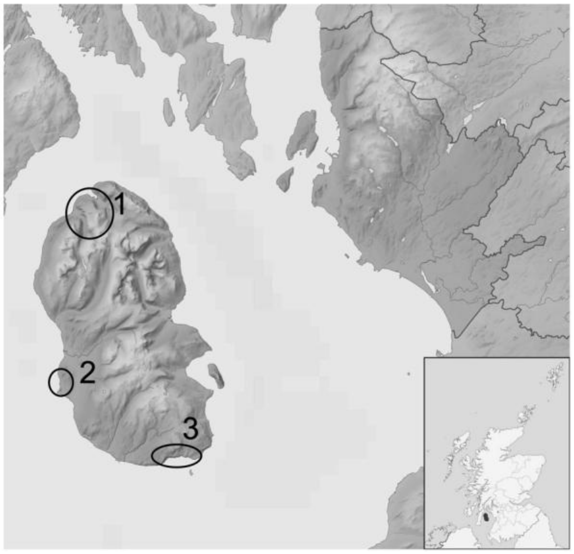


Figure 2

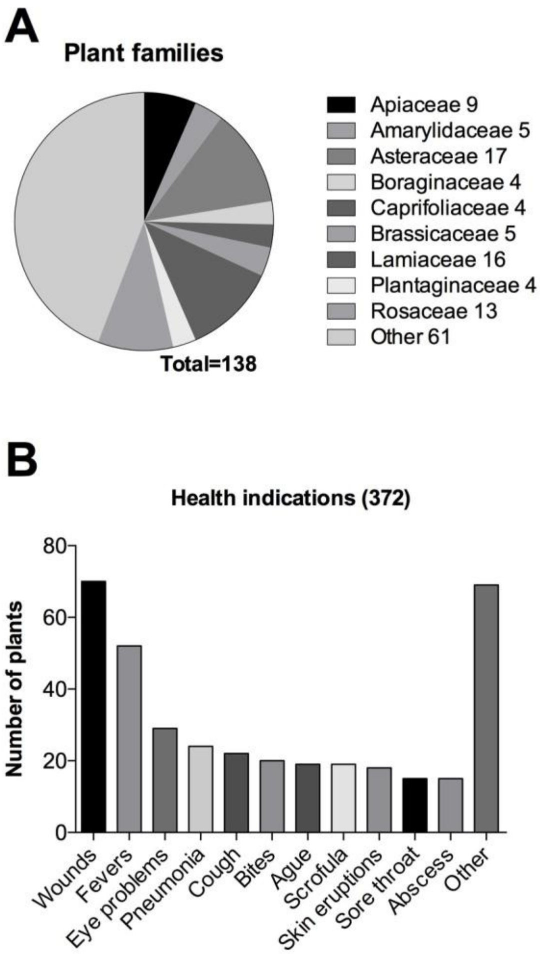

A
Plant families
Apiaceae 9
Amarylidaceae 5
Asteraceae 17
Boraginaceae 4
Caprifoliaceae 4
Brassicaceae 5
Lamiaceae 16
Plantaginaceae 4
Rosaceae 13
Other 61
Total=138
B
Health indications (372)
Number of plants
80
60
40
20
0
Wounds
Fevers
Eye problems
Pneumonia
Cough
Bites
Ague
Scrofula
Skin eruptions
Sore throat
Abscess
Other

Figure 3

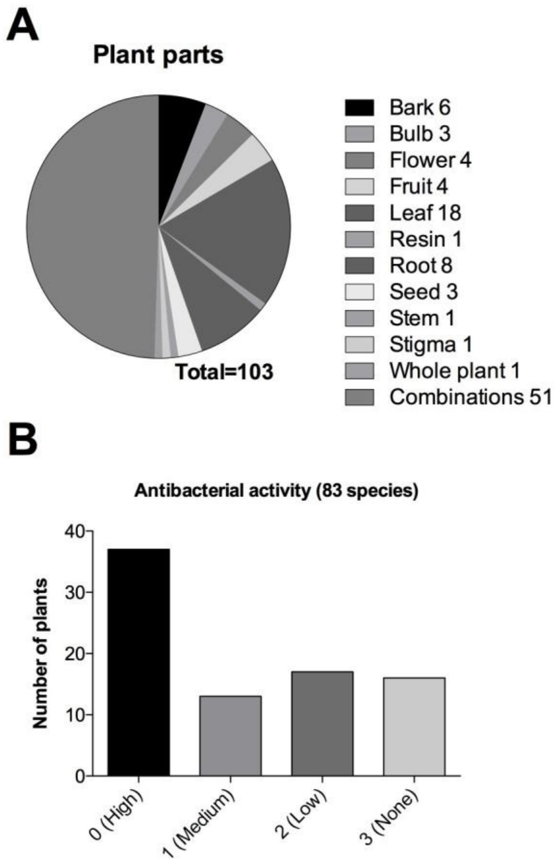

A
Plant parts
Bark 6
Bulb 3
Flower 4
Fruit 4
Leaf 18
Resin 1
Root 8
Seed 3
Stem 1
Stigma 1
Whole plant 1
Combinations 51
Total=103
B
Antibacterial activity (83 species)
Number of plants
0
10
20
30
40
0 (High)
1 (Medium)
2 (Low)
3 (None)

Figure 4

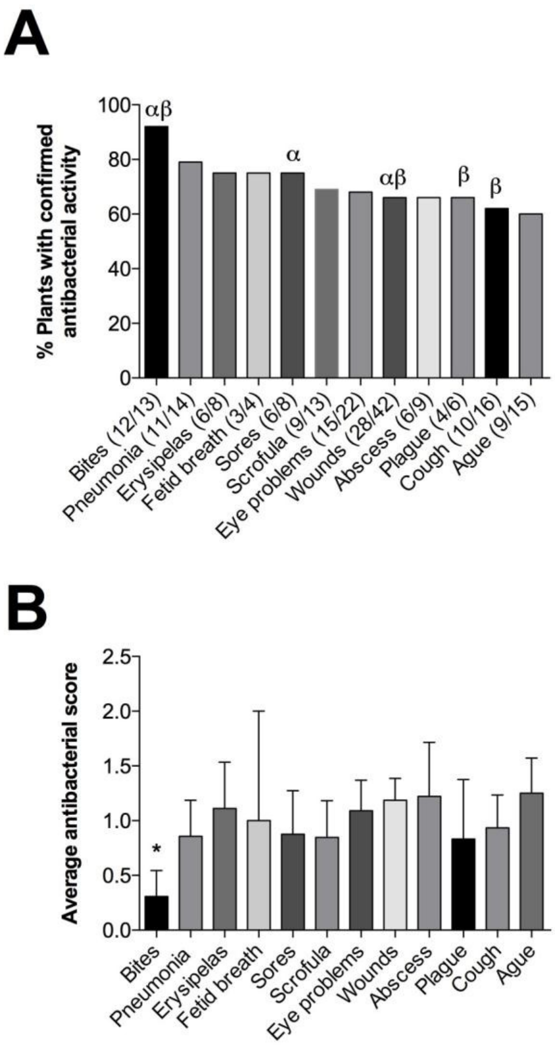

A
100
80
60
40
20
0
% Plants with confirmed antibacterial activity
αβ
α
αβ
β
β
Bites (12/13)
Pneumonia (11/14)
Erysipelas (6/8)
Fetid breath (3/4)
Sores (6/8)
Scrofula (9/13)
Eye problems (15/22)
Wounds (28/42)
Abscess (6/9)
Plague (4/6)
Cough (10/16)
Ague (9/15)
B
2.5
2.0
1.5
1.0
0.5
0.0
Average antibacterial score
*
Bites
Pneumonia
Erysipelas
Fetid breath
Sores
Scrofula
Eye problems
Wounds
Abscess
Plague
Cough
Ague

Figure 5

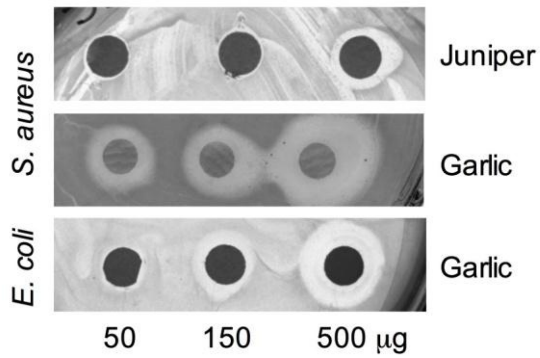
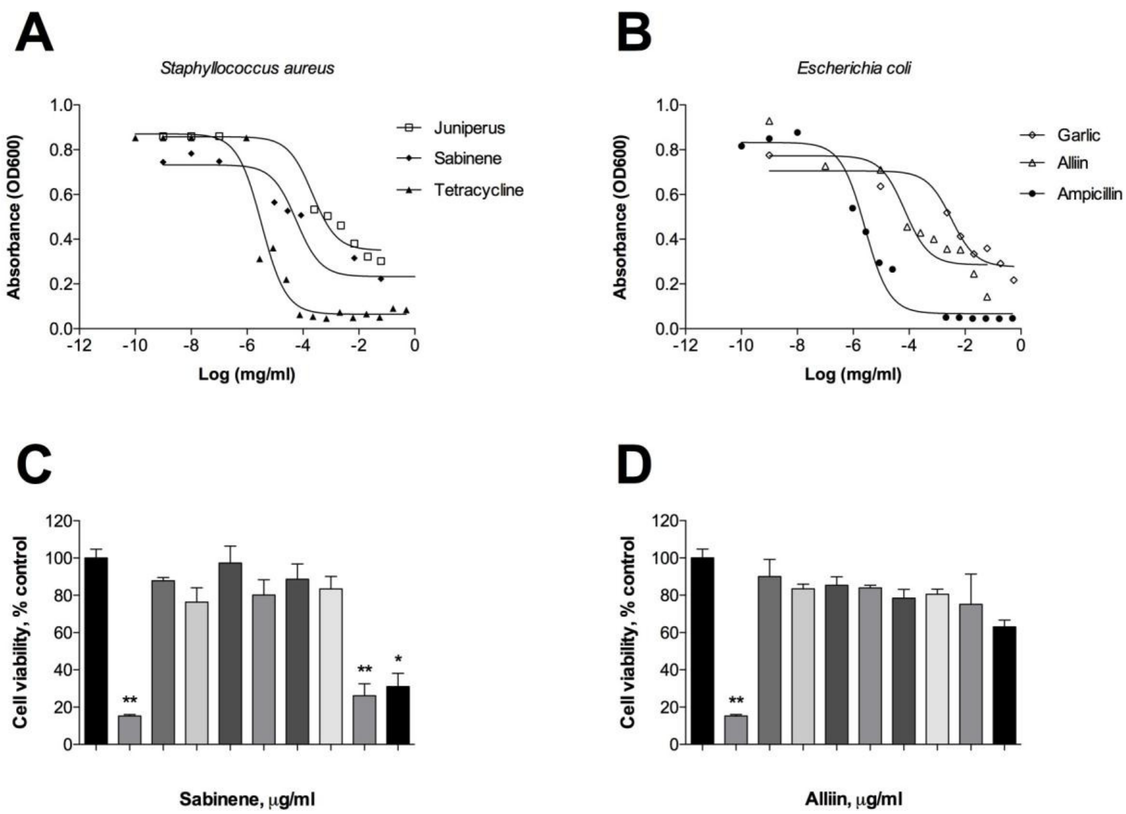


Figure 6


A
Staphyllococcus aureus
Absorbance (OD600)
1.0
0.8
0.6
0.4
0.2
0.0
-12
-10
-8
-6
-4
-2
0
Log (mg/ml)
Juniperus
Sabinene
Tetracycline
B
Escherichia coli
Absorbance (OD600)
Log (mg/ml)
Garlic
Alliin
Ampicillin
C
Cell viability, % control
120
100
80
60
40
20
0
**
**
*
Sabinene, µg/ml
D
Cell viability, % control
**
Alliin, µg/ml