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Paper

An examination of antibacterial and antifungal properties of constituents described in traditional Ulster cures and remedies

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ABSTRACT

Traditional herbal cures and remedies have played an important historical role in the treatment of a variety of illnesses and diseases in Northern Ireland for the last three hundred years. Recently, these have been reviewed in the publication by Linda Ballard from the Ulster Folk and Transport Museum at Cultra, Co. Down, which details the variety of local plants used and for what purpose. From this publication and another related publication, we note the description of several plant species that consistently appear in traditional cures and remedies, particularly used to treat infections and infectious diseases. Unfortunately, although these plants have strong associations with the local historical evidence base, there are very limited and mainly no formal publications in the medical/scientific evidence base, examining their scientific background and clinical efficacy.

INTRODUCTION

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 it has paradoxically resulted in the emergence and dissemination of multi drug resistant pathogens. Antimicrobial resistance in both medicine and agriculture is now recognized by the World Health Organisation (WHO), along with other various 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*" were published (http://www.im.dk/publikationer/micro98/index.htm), which outlined the need for the development of "*Novel principles for treating or preventing infections in humans and animals.*" Such an approach may thus be to examine the antimicrobial properties of native plants used in herbal medicine, as a novel source of such agents, as well as the employment of such novel compounds, and thus limit the use of conventional antibiotics to cases of severe and life-threatening infections, thus minimising the development of resistance to such agents. Although several traditional plant extracts have historically been known to have antimicrobial activity, to date, there has been relatively little or in some cases, no reports examining the activity against several medically important bacterial and fungal pathogens.

The aim of this small study was to scientifically examine the antimicrobial properties of seven plant species, all native to N. Ireland and which have been associated as the principal consitituents in several local traditional cures and remedies.

METHODS

Seven plant species were selected from previous literature[1,2] and included (i) cloves of garlic *(Allium sativum)*, (ii) onion *(Allium cepa),* (iii) Yarrow leaf (*Achillea millefolium)*, (iv) Meadow sweet leaf (*Filipendula ulmaria*), (v) Confrey leaf (*Symphytum officinale)*, (vi) Ragwort (*Senecio jacobaea*) and (vii) Dandelion leaf and roots (*Taraxacum officinale).* These plants were identified botanically and approximately 100g fresh weight of each were collected from the grounds of the Ulster Folk & Transport Museum, Cultra, Co. Down [54°39'05.23"N; 5°47'50.73"W] in June 2008. The harvested plants were divided into subsamples comprising leaf, stem and root tissues only. For the extraction of aqueous components from each plant, a recorded fresh weight of each subsample type was added to three times that weight (volume) of sterile distilled water in a Braun Food Processor and homogenised. This pureed sample was transferred to a suitably sized Schott bottle, capped and incubated overnight at ambient temperature on an orbital shaker at 150 rpm. Extracts were then centrifuged at 9000xg for 10 minutes using an Heraeus Biofuge Primo R centrifuge. Following this, the supernatants were carefully decanted to fresh containers and placed in an Edwards Supermodulyo Freeze drier, at -40°C for a minimum of 48 hours or until complete dryness. For assay purposes, a recorded weight of freeze dried powder was reconstituted

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with an equal weight/volume of sterile 0.1% (w/v) peptone saline (CM0733, Oxoid Ltd., Basingstoke, UK) to give a known concentration for each extract solution, as detailed in Table I. For the onion and garlic extracts, fresh produce was peeled, finely chopped and stomached at ambient temperature employing a Stomacher 400 (Fisher Scientific Ltd., UK) for 15mins, prior to recovery of supernatant, which was subsequently filter-sterilised through a 0.22µ syringe filter (Millipore Inc., USA), before microbiological challenge.

Thirty four microorganisms, including 24 bacteria and 10 fungi were challenged in this study to ascertain the antimicrobial properties of the eight plant extracts as detailed above. Of the bacterial isolates selected, 15 were Gram-negative organisms, which included seven genera, as well as nine Gram-positive organisms from four genera. Of the fungi examined, five were yeasts, with the remaining five being filamentous fungi, from five genera overall. These organisms and their origins are detailed in Table I. In order to prepare the inocula for challenge, all organisms were cultured

Table 1:

Results of antibacterial and antifungal activity of eight different aqueous plant extracts challenged with 34 pathogenic bacterial and fungal isolates.

Blank = no inhibition; *, fresh undiluted extracts; NCTC = National Collection of Type Cultures; NCIMB = National Collection of Industrial Food and Marine Bacteria; MRSA=methicillin-resistant *Staphylococcus aureus*; MSSA=methicillin-sensitive *Staphylococcus aureus*; QC=quality control isolate; ATCC=American Type Culture Collection.

	Diameter of zone of inhibition (mm)								
Organism/concentration (mg/ml)	Garlic (*Allium sativum*) *	Onion (*Allium cepa*) *	Yarrow leaf (*Achillea millefolium*) [100mg/ml]	Meadow sweet leaf (*Filipendula ulmaria*) [150mg/ml]	Confrey leaf (*Symphytum officinale*) [50mg/ml]	Ragwort (*Senecio jacobaea*) [150mg/ml]	Dandelion leaf (*Taraxacum officinale*) [130mg/ml]	Dandelion root (*Taraxacum officinale*) [200mg/ml]	Ciprofloxacin [5µg disk]
Bacteria									
Bacillus cereus NCTC 7464									24
Bacillus subtilis NCTC 10400 (NCIMB 8054)	19								33
Bacillus pumilus (wildtype hand isolate)	15								26
Cupriavidus sp.									27
E. coli NCTC 25922	16								29
E. coli NCTC 9001									27
E.coli 0157 NCTC 12900	15								27
E.coli DH5									11
Enterobacter/Klebsiella sp.									34
Enterococcus faecalis NCTC 775									15
Klebsiella aerogenes NCTC 9528									24
Klebsiella pneumoniae 700603									23
Listeria monocytogenes NCTC 11994									28
Pseudomonas aeruginosa NCTC 1662									25
Pseudomonas aeruginosa NCTC 27853	6								28
Pseudomonas sp									20
Pseudomonas sp 20									18
Salmonella poona NCTC 4840									26
Serratia marcescens									26
Serratia/Rahnella sp.									40
Staphylococcus aureus (MRSA) 43300	25								19
Staphylococcus aureus. NCTC 6571	30								27
Staphylococcus aureus (MSSA) 25923	25								25
Staphylococcus epidermidis NCTC 14990	25								28
Fungi									
Aspergillus flavus QC 6658									
Aspergillus fumigatus 27.5									
Aspergillus niger 27.5									
Candida albicans									
Candida glabrata ATCC 2001									
Candida krusei ATCC 6258 27.5									
Candida parapsilosis ATCC 22019	30								
Exophiala (*Wangiella*) *dermatitidis* QC 7895									
Penicillium sp. QC 743275									
Scedosporium apiospermum QC 7870									

on Columbia Blood Agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood and incubated for 24h at 37°C (for bacterial and yeast organisms) and for 1 week (for filamentous fungi). 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 10^6 colony forming units (cfu) per ml) which was inoculated on to fresh Mueller-Hinton Agar (Oxoid CM0337), by means of a sterile cotton swab. To this, fresh extracts (10µl) were added and the inoculum allowed to dry prior to incubation, as detailed above. Following this, plates were examined visually and any inhibition noted and its diameter measured and recorded. Sterile PS and antibiotic susceptibility disks containing 5µg ciprofloxacin (Mast Diagnostics Ltd., Bootle, Merseyside, UK) were employed as a negative and positive control, respectively.

RESULTS

The antimicrobial activity of the eight plant extracts against the 34 microorganisms tested is shown in Table I. No antimicrobial activity was observed with any bacterial or fungal pathogen, for onion *(Allium cepa),* Yarrow leaf (*Achillea millefolium)*, Meadow sweet leaf (*Filipendula ulmaria*), Confrey leaf (*Symphytum officinale)*, Ragwort (*Senecio jacobaea*) or for Dandelion leaf and roots (*Taraxacum officinale).* Only the aqueous extract from cloves of garlic showed inhibition against nine bacterial isolates (range: 6-30mm zone of inhibition; mean = 19.5mm) and only the *Candida parapsilosis* isolate was inhibited by the garlic extract (30mm). There was complete microbial confluence at the site of inoculation of the negative control,(0.1% PS) and all organisms gave a clear zone of inhibition, ranging from 11-40mm diameter zone of inhibition, with a mean zone of inhibition of 23mm,when tested against the positive control (ciprofloxacin).

DISCUSSION

Historically, Yarrow leaf (*Achillea millefolium),* named after Achilles, who used extracts of this plant to treat wounds, has been used with anti-inflammatory, spasmolytic, haemostatic and digestive effects[3]. Although the essential oil[3] and total acid[4] of two other species of this genus, namely A. *clavennae* and *A. alpina*, respectively, have been reported in the literature, as displaying antibacterial properties, we did not observe any antimicrobial activity during this study, with the species *A. millefolium*, which we tested. Likewise, we were not able to demonstrate any activity with Meadowsweet leaves (*Filipendula ulmaria*) or ragwort (*Senecio jacobaea*), although other species within these genera, other than those tested, have been shown to exhibit some antimicrobial activity. Although these plants may not be able to exert a direct physiological antimicrobial effect, it may be that their clinical efficacy lies in their associated activity in the stimulation of other systems, such as macrophages or nitric oxide. For example, Kim *et al.*[5] suggested that there was an activation of inducible nitric oxide synthase by *Taraxacum officinale* in mouse peritoneal macrophages. These results suggest that the capacity of *Taraxacum officinale* to increase NO production from rIFN-gamma-primed mouse peritoneal macrophages is the result of TO-induced TNF-alpha secretion. Likewise, Dolganiuc *et al.*[6] examined the effect of *in vivo* stimulation with an aqueous extract obtained from roots of *Symphytum officinale* on mouse peritoneal macrophages and showed that *Symphytum officinale* initially activated the respiratory burst of the cells and later inhibited it, activating the synthesis of catalase, SOD etc. Hence, such plants materials may exert a physiological effect through an alternative modality to antimicrobial inhibition.

Previously, Ballard demonstrated that garlic has been used in traditional Ulster cures to treat asthma, epilepsy, measles and whooping cough[1]. More recently, a further reference to garlic as an ingredient in a cure for measles has been found, which like the previous one came from north Co Londonderry. However, the sources of this information do not specify whether *Allium sativum or Allium ursinum* is indicated. Our data demonstrated that the aqueous extracts of garlic were the most potent plant material examined against bacterial and fungal pathogens, which is in general agreement with the published literature, where garlic is known as a potent antimicrobial. Garlic contains organosulphur groups, that can act as metal chelators, powerful nucleophiles or electrophiles and hence confer antimicrobial properties on this compound.

CONCLUSION

Our data has qualitatively shown that cloves of garlic had a limited antibacterial activity against 9/24 isolates tested and exhibited some antifungal properties against 1/10 fungal isolates examined. These 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.

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