



Research paper

A laboratory investigation of the antimicrobial activity of a selection of western phytomedicinal tinctures

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ABSTRACT

Introduction: Following the observed success of a herbal cocktail in controlling and eliminating acute respiratory infections in a group of approximately sixty, 1–3 year old equines, it was decided to test ten of the herbs individually for antibacterial activity, using in vitro methodology.

Methods: Hydroethanolic extracts of defined concentration for each of the ten herbs were sourced from a licensed supplier in the UK. Positive and negative controls were included in the study.

A quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics according to British Standard BS EN 1276:2009 was used to assess the anti-microbial activity of ten neat herbal tinctures.

Results: Bactericidal activity is described as the capability of a product to produce at least a 5 log₁₀ reduction in specified test organisms within 5 minutes when the disinfectant is tested at its intended use dilution(s). The performance of each neat herbal tincture in this study was assessed only under moderate to heavy (dirty) soiling conditions.

Commiphora molmol, *Inula helenium*, and *Thymus vulgaris* showed antimicrobial activity against all three test strains of bacteria, and over the entire dilution range (10⁻¹ to 10⁻⁷).

Baptisia tinctoria, *Echinacea pupurea*, *Marrubium vulgare*, and *Salvia officinalis*, showed maximum antimicrobial activity only to two of the three test organisms.

The tinctures of *Galium aperine* and *Glechoma hederacea* showed zero to very low antimicrobial activity against the three test organisms.

Conclusion: A herbal formulation containing a blend of the ten tested herbs constitutes a powerful antimicrobial cocktail.

1. Introduction

Across the world, traditional medicine (TM) is either the mainstay of health care delivery or serves as a complement to it. In some countries, traditional medicine or non-conventional medicine may be termed complementary or alternative medicine (CAM). A recent report by the World Health Organization (WHO) clearly sets out the course for traditional, complementary and alternative medicine (TCAM) over the next decade [1]. In many parts of the world, expenditure on TCAM is not only significant, but growing rapidly. In many developed countries, popular use of TCAM is fueled by concern about the adverse effects of chemical drugs, questioning of the approaches and assumptions of

allopathic medicine and greater public access to health information [2]. Herbal medicine is one of the oldest and most used form of TCAM throughout many cultures across the world, being the primary modality of medical treatment for at least 80% of the world's population [3]. The scientific validation of herbal medicine has been well described in the literature [4]. Due to consumer demands for natural therapies over the last twenty years, the global herbal supplements and remedies market is forecast to reach US\$115 billion by the year 2020 [5]. Herbs can be classified according to origin, habitat, their various actions, or according to their use. Western Herbal Medicine (WHM), sometimes referred to as Phytotherapy, is a term used to distinguish herbalism based on Anglo-American traditional herbal medicine from other systems of

Abbreviations: AIT, Athlone Institute of Technology; CAM, complementary & alternative medicine; ESCOP, European Society Co-operative on Phytotherapy; NaCl, sodium chloride; RCSI, Royal College of Surgeons in Ireland; TCAM, traditional, complementary, & alternative medicine; TM, traditional medicine; WHM, western herbal medicine; WHO, World Health Organization; w/v, weight/volume

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herbal medicine such as Ayurveda (Indian) or Traditional Chinese Medicine (TCM). WHM is practiced in Australia, Canada, New Zealand, the United States of America, and Western Europe [6].

It is well known that multiple drug resistance has developed due to the indiscriminate use of commercially available antibiotics [7–9]. Furthermore, these anti-infective drugs have, on occasions, been associated with adverse effects on patients, including stroke, heart attacks, heart rate irregularities, liver toxicities, seizures, psychoses, allergic reactions, immune suppression and death [10,11]. Because of these factors, there is a constant need for the development of new antimicrobial compounds that are therapeutically more effective with minimal toxicity. Many studies have shown that herbal preparations may offer a potential source of alternative compounds for the treatment of a wide range of infectious diseases [7,12–14]. However, the conventional medical professions have not been very forthcoming in prescribing unlicensed herbal medicines. Scientific and medico-legal reasons can be cited as to why many doctors and veterinary surgeons have been cautious about not recommending herbals to their patients or animal owners.

From a scientific aspect, the individualization of prescriptions containing multiple herbal and other ingredients poses a major problem in translating traditional herbal practices into conventional western medicine. The lack of studies guaranteeing the quality and efficacy of the vast range of herbal products on the world markets is of major concern to the medical profession. This is further complicated by the existence of very different regulatory requirements governing the production, preparation, and standardization of herbals throughout the many different herbal growing regions of the world.

Medico-legal reasons against the recommendation of herbals by medics are also well understood. Doctors have to be cautious against recommending herbals for patients being prescribed drugs because of possible serious drug-herbal interactions, complicated by the fact that there can often be poor patient compliance. Efforts have been made to assist doctors in this regard through the provision of commercially available clinical toxicology and related databases.

The purpose of this specific investigation was to assess the *in vitro* antibacterial activity of a range of individual high quality western herbal tinctures, sourced from a licensed wholesaler in the UK. These particular herbs were observed to be very effective in addressing acute respiratory infections in livestock when administered as a cocktail and part of a nutritional program for a two to three week period during the winter months, while the animals were stabled in Ireland. The clinical benefits of the herbal cocktail were noted over a three year period, and in a small population of approximately 60 high performance equines. It was of interest to us to initially validate the antibacterial effectiveness of the individual herbal preparations, considering that frequently viral respiratory infections may be complicated by bacterial infections.

2. Materials & Methods

All herbal tinctures were sourced from Proline Botanicals Ltd, Carmella House, 3–4 Grove Terrace, Walsall, West Midlands, WS1 2NE, United Kingdom.

Ten herbal tinctures were tested for anti-microbial activity: these included *Baptisia tinctoria*, *Commiphora molmol*, *Echinacea pupurea*, *Galium aperine*, *Glechoma hederacea*, *Inula helenium*, *Marrubium vulgare*, *Salvia officinalis*, *Thuja occidentalis* and *Thymus vulgaris*, respectively. The tinctures were obtained by maceration of the plant in an ethanol-water solution. The ratio of herb to solvent, and the specific concentration of the solvent in the case of each herbal extract is displayed in Table 1.

A quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics according to British Standard BS EN 1276:2009 was used to assess the anti-microbial activity of the herbal tinctures. The method was carried out under moderate to heavy dirty conditions. Three test bacterial cultures were used,

Table 1
List of Herbal tinctures & their composition.

Herbal Tincture	Composition
<i>Commiphora molmol</i>	1:5 in 90% alcohol
<i>Inula helenium</i>	1:3 in 45% alcohol
<i>Thymus vulgaris</i>	1:3 in 45% alcohol
<i>Baptisia tinctoria</i>	1:5 in 60% alcohol
<i>Echinacea pupurea</i>	1:3 in 45% alcohol
<i>Marrubium vulgare</i>	1:3 in 45% alcohol
<i>Salvia officinalis</i>	1:3 in 45% alcohol
<i>Thuja occidentalis</i>	1:3 in 60% alcohol
<i>Galium aperine</i>	1:3 in 45% alcohol
<i>Glechoma hederacea</i>	1:3 in 45% alcohol

including *Enterococcus hirae* ATCC 10541, *Escherichia coli* ATCC 10536 and *Staphylococcus aureus*, ATCC 6538, as recommended in the British Standard protocol. Positive and negative controls, as specified in the standard methodology, were used in the study.

The bacterial cultures were grown on nutrient agar and stored at 4 °C. The bacteria were sub-cultured every 2–3 days on to fresh nutrient agar. Each of the bacterial cultures was reactivated preceding anti-microbial activity testing by transferring them into nutrient broth and incubating at 37 °C for 24 h. 1 ml of test bacteria was aseptically transferred into a sterile universal container containing 1 ml of sterile interfering substance (3% w/v bovine serum albumin). The mixture was gently agitated and allowed to stand for two minutes at 20 °C. To this mixture was added 8 ml of the herbal tincture being tested and allowed to stand for five minutes at 20 °C. (The herbal tincture was replaced by an equal volume of sterile hard water in the case of the control sample). 1 ml of this mixture was then added to a sterile universal tube containing 8 ml of sterile aqueous neutralizing solution (3% w/v Tween-80, 3% w/v Saponin, 0.1% w/v Histidine, & 0.1% w/v cysteine) and 1 ml of sterile hard water (0.3% w/v calcium carbonate). This mixture was gently agitated and allowed to stand for five minutes at 20 °C, after which serial dilutions (up to 10⁻⁷) in sterile peptone water (1.5% w/v) were prepared. 1 ml of each of the serial dilutions, in duplicate, was aseptically transferred into separate petri dishes to which 13 ml of sterile molten nutrient agar was then poured. After mixing, the plates were allowed to set, then inverted and placed in an incubator at 37 °C for 24 h. The plates were then examined for bacterial growth and scored by counting the number of colony forming units on each plate, ensuring to count dilution plates that gave between 30 and 300 CFU's. The appropriate dilution would then have been used to determine the CFU/ml and therefore the level of kill for the test herbal tincture.

3. Results

This British Standard BS EN 1276:2009 describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have a bactericidal activity in the fields described in clause number one. Bactericidal activity is described as the capability of a product to produce at least a 5 log₁₀ reduction in specified test organisms within 5 min when the disinfectant is tested at its intended use dilution(s). The performance of each neat herbal tincture in this study was assessed only under moderate to heavy (dirty) soiling conditions.

A summary overview of the findings regarding the testing of the ten herbal tinctures is shown in Table 2.

Of the tinctures that were tested, *Commiphora molmol*, *Inula helenium*, and *Thymus vulgaris* showed antimicrobial activity against all three test strains of bacteria, and over the entire dilution range (10⁻¹ to 10⁻⁷).

Baptisia tinctoria, and *Echinacea pupurea* showed antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus* over the entire dilution range. However, *Enterococcus hirae* was resistant to these two tinctures.

Table 2

Herbal tinctures were placed in contact with a bacterial suspension under standard conditions. Data represents the logarithmic reduction in viable cell numbers. The initial cell population was 1×10^8 CFU/ml. (n = 2).

Herbal Tincture	<i>Escherichia coli</i> ATCC 10536	<i>Staphylococcus aureus</i> ATCC 6358	<i>Enterococcus hirae</i> ATCC 10541
<i>Commiphora molmol</i>	$> 10^{-7}$	$> 10^{-7}$	$> 10^{-7}$
<i>Inula helenium</i>	$> 10^{-7}$	$> 10^{-7}$	$> 10^{-7}$
<i>Thymus vulgaris</i>	$> 10^{-7}$	$> 10^{-7}$	$> 10^{-7}$
<i>Baptisia tinctoria</i>	$> 10^{-7}$	$> 10^{-7}$	0
<i>Echinacea pupurea</i>	$> 10^{-7}$	$> 10^{-7}$	0
<i>Marrubium vulgare</i>	$> 10^{-7}$	0	$> 10^{-7}$
<i>Salvia officinalis</i>	$> 10^{-7}$	$> 10^{-6}$	$> 10^{-7}$
<i>Thuja occidentalis</i>	0	$> 10^{-7}$	$> 10^{-7}$
<i>Galium aperine</i>	$> 10^{-5}$	$> 10^{-5}$	0
<i>Glechoma hederacea</i>	0	$> 10^{-3}$	0

Marrubium vulgare and *Salvia officinalis* showed antimicrobial activity against *Escherichia coli* and *Enterococcus hirae* over the entire dilution range. However, *Staphylococcus aureus*, although sensitive to the activity of *Salvia officinalis*, was resistant to *Marubium vulgare*.

Thuja occidentalis showed antimicrobial activity against *Enterococcus hirae* and *Staphylococcus aureus* over the entire dilution range. However, *Escherichia coli* was resistant to this particular herbal tincture.

The tinctures of *Galium aperine* and *Glechoma hederacea* showed zero to very low antimicrobial activity against the three test organisms.

4. Discussion

The study was conducted with the objective of determining which western medicinal plants in a nutritional cocktail of ten botanicals had antibacterial activity, using gold standard micro-biological testing methodology. The herbs had been selected for feeding to livestock based on their multicomponent botanical therapeutic properties, as described in the literature [15,16]. Because it is well known that the phytochemical composition of plants varies not alone with geographical location [17] but can also be altitude dependent, only extracts of plants from a reputable supplier were used in this study. Furthermore, to enhance the possibility of identifying an active ingredient, hydro-ethanolic extracts of large amounts of each of the ten medicinal herbs were used as test samples to screen for anti-bacterial activity.

The most susceptible microorganisms to the bactericidal effects of the herbal tinctures were found to be the gram negative bacteria of *Escherichia coli* while the least susceptible were the gram positive bacteria of *Enterococcus hirae*. The gram positive bacteria of *Staphylococcus aureus* were found to lie in the intermediate zone of sensitivity. The resistance of enterococci to extremes of temperature (5–65 °C), pH (4.5–10.0), high NaCl concentrations, and a wide range of chemicals, including known antiseptics and disinfectants, is well known, and enables them to colonize a wide range of niches [18–20]. It is therefore not surprising to find this microbe resistant to four of the ten test herbal tinctures, while six of the ten herbal tinctures were effective against this organism.

The three plants showing maximum potency and the broadest spectrum of antibacterial activity included *Commiphora molmol*, *Inula helenium* and *Thymus vulgaris*.

Commiphora molmol, also known as myrrh, is an oleo gum resin, botanically related to Frankincense. It has been known for its antibacterial, antifungal, anti-parasitic, potent antioxidant, anticancer and calming activities. The properties of myrrh have been attributed to its content of terpenoids and sesquiterpenes [21].

Inula helenium is known to have choleric, expectorant, fungicidal,

bactericidal and antiviral actions. Confirming our observations, earlier studies by O'Shea et al. [22] have shown that both sensitive and antibiotic resistant strains of *Staphylococcus aureus* are vulnerable to the bactericidal actions of this herb. Its antibiotic action has been linked to alantolactone while its antiviral properties are thought to be due to isosalantolactone.

Thymus vulgaris is one of the most well-known labiates used in food. It has been documented as having choleric, adrenergic, antibiotic, antiviral and antifungal actions [16,23]. Its numerous properties have for the most part been linked to its high concentration of thymol and carvacrol. The essential oil of this plant has been shown to contain up to 40% thymol.

The fame of *Salvia officinalis* (sage) as a medicinal herb goes back to Greek antiquity. Sage has been cited as having multitude of activities, including as an antiseptic, antifungal, antihelminthic, emmenagogic, hypoglycemic, choleric, antisudorific, antidepressant, angiolytic and oestrogenic uses [16,24]. Despite these broad range of reported activities, sage while antimicrobial to two of the test germs, it was found to have a comparatively reduced bactericidal activity against *Staphylococcus aureus*. This highlights the deficiency of the non-precise terminology (such as antiseptic, antibiotic) sometimes used in the literature to describe the actions of herbals or indeed certain drugs.

While known mainly for its expectorant [25], antispasmodic, and antinociceptive activities [26], *Marrubium vulgare* (white horehound) was found to have significant antimicrobial activity against two of the test organisms. This is in-keeping with the in vitro antibacterial observations of this medicinal plant's essential oil, which were reported by Zarai et al. [27].

Thuja occidentalis, also known as northern white cedar, is mainly used today in homeopathy as a mother tincture or in dilution. While we have found this plant to have significant in vitro antibacterial activity, this is in confirmation with the findings of others, who have noted that when *Thuja occidentalis* is used in combination with other immunomodulating plants, it has been found useful in the treatment of acute and chronic infections of the upper respiratory tract, and as an adjuvant to antibiotics [28].

While the extracts of *Galium aperine* and *Glechoma hederacea*, respectively, showed the least antibacterial activity of the ten herbal preparations, this does not necessarily mean that either, or both, of these botanicals are devoid of significant quantities of the active principal(s) responsible for antimicrobial activity. Research work by others have shown that the choice of solvent and extraction techniques can affect the yield of the active principal from a plant [29]. Alternatively, it may be that these two herbs act in an indirect way. Either they may contain precursor molecule(s) which require bio-activation in the body or they may assist the antimicrobial activities of the other herbs, in the nutritional cocktail, by some as yet unknown mechanism.

In order to understand the antimicrobial mechanisms of action of herbal tinctures, a deeper knowledge of these complex matrices is required. Much of the earlier information on the composition of herbals, which has been reported in the literature, is based only on phytochemical analysis [16] where tinctures have been characterized in terms of their content of gums and mucilages, alkaloids, steroids, saponins, flavonoids, simple phenols, tannins, coumarins, anthraquinones, cardiac glycosides, cyanogenic glycosides, mustard glycosides, resins, and volatile oils. A more rigorous set of tools and methodologies are necessary to guide the standardization of phytomedicines, to determine which specific components of a botanical preparation are responsible for any specific bioactivity, to predict the biological activities of a particular herbal composition, to determine the relatedness of herbal compositions, and for the development of improved herbal therapeutics. PhytomicsQC [30], an experimental platform incorporating the use of LC-MS for chemical characterization and chemical fingerprinting, differential cellular gene expression for bio-response fingerprinting, and animal pharmacology for in vivo validation, may be able to provide the solutions to many of these

unanswered questions over the coming years, as the technology is applied to the screening of a whole range of phytomedicinal preparations, including the ten test tinctures used in this study. The Herbalome project [31] is another example of a relatively recent initiative where new technologies and concepts are being used to help create a comprehensive resource library for Chinese herbal medicines, with the ultimate objective of improving the quality and globalization of TCM products.

In the preliminary studies reported in this paper, no attempt was made to assess the effect of pre-dilution of the botanical extracts prior to testing. It is proposed that further antibacterial studies be carried out to assess the relative potency of those neat tinctures showing the greatest antimicrobial activity. Those phytomedicinals showing significant potency will be chemically and biologically fingerprinted using one of the described experimental platforms.

Ethics & Funding

The research work described in this publication was carried out in the Microbiology Laboratories at Athlone Institute of Technology (AIT), Athlone, Co. Westmeath, Ireland. The authors gratefully acknowledge the financial support received for this research from AIT.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.eujim.2018.02.008>.

References

- [1] W.H. Organization, WHO Traditional Medicine Strategy: 2014–2023, World Health Organization, 2013.
- [2] W.H. Organization, WHO Global Atlas of Traditional, Complementary and Alternative Medicine, WHO Centre for Health Development, 2005.
- [3] M. Ekor, The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, *Front. Pharmacol.* 4 (2014) 177.
- [4] D. Mowrey, *The Scientific Validation of Herbal Medicine*, 1st ed., McGraw-Hill Education, 1986.
- [5] Global Industry Analysts, *The Global Herbal Supplements and Remedies Market: Trends, Drivers, & Projections*, (2015) USA.
- [6] K. Niemeyer, L.R. Bell, M. Koithan, Traditional knowledge of western herbal medicine and complex systems science, *J. Herb. Med.* 3 (2013) 112–119.
- [7] S.H. Buhner, *Herbal Antibiotics: Natural Alternatives for Treating Drug-resistant Bacteria*, Storey Pub., 2012.
- [8] H. Gelband, M. Miller-Petrie, S. Pant, S. Gandra, J. Levinson, D. Barter, et al., *Antibiotic Resistance in 2015. The State of the Worlds Antibiotics*, (2015), pp. 14–24 Washington D.C., USA.
- [9] P. Goyal, P. Kaushik, In vitro evaluation of antibacterial activity of various crude leaf extracts of Indian sacred plant, *Ocimum sanctum*, *Br. Microbiol. Res. J.* 1 (2011) 70–78.
- [10] J. McKenna, *Antibiotics – Are They Curing Us or Killing Us? The Catastrophic Impact of the Over-prescription of Antibiotics on Our Health*, Gill Books, 2014.
- [11] O. Pelkonen, P. Duez, P.M. Vuorela, H. Vuorela, *Toxicology of Herbal Products*, Springer International Publishing, 2017.
- [12] S. Al-Daihan, M. Al-Faham, N. Al-shawi, et al., Antibacterial activity and phytochemical screening of some medicinal plants commonly used in Saudi Arabia against selected pathogenic microorganisms, *J. King Saud Univ. Sci.* 25 (2013) 115–120.
- [13] S.H. Buhner, *Herbal Antivirals: Natural Remedies for Emerging & Resistant Viral Infections*, Storey Publishing, LLC, 2013.
- [14] L. Chaitow, *Natural Alternatives to Antibiotics: How You can Supercharge Your Immune System and Fight Infection*, HarperCollins Publishers, 2016.
- [15] J. McKenna, *Alternatives to Antibiotics*, Gill & Macmillan+, Dublin, Ireland, 1996.
- [16] S.Y. Mills, *The Essential Book of Herbal Medicine*, Arkana Penguin Books, England, U.K., 1993.
- [17] H.D. Mai, H.T. Van-Dufat, S. Michel, F. Tillequin, D. Bastien, T. Sevenet, A new diprenyl coumarin and alkaloids from the bark of *Zanthoxylum dimorphophyllum* (Rutaceae), *Zeitschrift fur Naturforschung C/J. Biosci.* 56 (2001) 492–494.
- [18] K. Fisher, C. Phillips, The ecology, epidemiology and virulence of *Enterococcus Microbiology*, *Microbiology (Reading, England)* 155 (2009) 1749–1757.
- [19] A. Garrido, A. Gálvez, R.P. Pulido, Antimicrobial resistance in Enterococci, *J. Infect. Dis. Ther.* 2 (2014) 1–7.
- [20] P. Renner, J. Peters, Resistance of enterococci to heat and chemical agents, *Zentralblatt fur Hygiene und Umweltmedizin/Int. J. Hyg. Environ. Med.* 202 (1999) 41–50.
- [21] M. Chadwick, H. Trewin, F. Gawthrop, C. Wagstaff, Sesquiterpenoids lactones: benefits to plants and people, *Int. J. Mol. Sci.* 14 (2013) 12780–12805.
- [22] S. O'Shea, B. Lucey, L. Cotter, In vitro activity of *Inula helenium* against clinical *Staphylococcus aureus* strains including MRSA, *Br. J. Biomed. Sci.* 66 (2009) 186–189.
- [23] O. Borugá, C. Jianu, C. Mișcă, I. Goleț, A.T. Gruia, F.G. Horhat, *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity, *J. Med. Life.* 7 (2014) 56–60.
- [24] M. Beheshti-Rouy, M. Azarsina, L. Rezaie-Soufi, M.Y. Alikhani, G. Roshanaie, S. Komaki, The antibacterial effect of sage extract (*Salvia officinalis*) mouthwash against *Streptococcus mutans* in dental plaque: a randomized clinical trial, *Iran. J. Microbiol.* 7 (2015) 173–177.
- [25] H. Said-Al Ahli, A. Gendy, A. Mahmoud, H. Mohamed, Essential oil composition of *Marrubium vulgare* cultivated in Egypt, *Int. J. Plant Sci. Ecol.* 1 (2015) 138–141.
- [26] R.A. De Jesus, V. Cechinel-Filho, A.E. Oliveira, V. Schlemper, Analysis of the antinociceptive properties of marrubiin isolated from *Marrubium vulgare*, *Phytomed. Int. J. Phytother. Phytopharmacol.* 7 (2000) 111–115.
- [27] Z. Zarai, A. Kadri, I. Ben Chobba, R. Ben Mansour, A. Bekir, H. Mejdoub, et al., The in-vitro evaluation of antibacterial, antifungal and cytotoxic properties of *Marrubium vulgare* L. essential oil grown in Tunisia, *Lipids Health Dis.* 10 (2011) 161.
- [28] B. Naser, C. Bodinet, M. Tegtmeyer, U. Lindequist, *Thuja occidentalis* (Arbor vitae): a review of its pharmaceutical, pharmacological and clinical properties, *Evid. Based Complement. Altern. Med.* 2 (2005) 69–78.
- [29] D. Olila, J. Opuda-Asibo, J. Olwa-Odyek, Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, *Ugandan medicinal plants, Afr. Health Sci.* 1 (2001) 66–72.
- [30] R. Tilton, A.A. Paiva, J.Q. Guan, R. Marathe, Z. Jiang, W. van Eyndhoven, et al., A comprehensive platform for quality control of botanical drugs (PhytomicsQC): a case study of Huangqin Tang (HQT) and PHY906, *Chin. Med.* 5 (2010) 30.
- [31] Y. Lao, X. Wang, N. Xu, H. Zhang, H. Xu, Application of proteomics to determine the mechanism of action of traditional Chinese medicine remedies, *J. Ethnopharmacol.* 155 (2014) 1–8.