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Research Article

Quality Control of Botanical Tinctures: Endogenous Bacterial Flora Present in Botanical Extractions

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Abstract

Botanical-based therapeutics are often prepared as either water, ethanol or glycerin-based extractions of the harvested plant material. The raw botanical material is not grown in a sterile environment and as such, may contain a variety of microbial flora. This research evaluated the level of microbial flora present in common botanical extractions prepared under different methodologies and did basic characterization to determine if these microbial populations may include potential human pathogens. The results indicated that significant bacterial flora is commonly present in botanical extracts, including potential human pathogens and that the extraction process utilized will alter the level of microbes present. This research is encouraged help physicians be aware of potential microbial contaminants present in herbal preparations and to use proper care and follow-up when treating patients.

Keywords: Botanical; Endogenous bacteria; Quality control

Introduction

From traditional herbalism to pharmaceutical medicine, plants consistently provide therapeutic sources for the treatment of disease and our ability to sustain health. Estimates suggest that approximately 25% of modern medicine is directly or indirectly derived from plant sources [1]. While the pharmaceutical branch of medicine often uses purified and isolated phytochemical constituents, Complementary and Alternative Medicine (CAM), frequently uses total herbal preparations ina modality known as botanical medicine. Despite lacking extensive research and regulation, botanical medicine's safe use is often justified by the historical applications of herbalism over centuries and the relative limited frequency of adverse effects experienced when using most botanical preparations [1].

As worldwide demand for botanical medicine rises, it becomes increasingly paramount to understand the aspects of quality control used during the production of herbal therapeutics [2]. Oftentimes the only processing done during the agricultural phase of medicinal plant production is the drying of the raw plant material after harvest [3]. After desiccation, the herbal components are typically added to an extraction agent, such as hot water, alcohol or glycerin depending on whether the medicine is to become a tea, tincture or glycerite respectively [3,4]. Tinctures are defined as simple grain alcohol infusion [4,5]. Depending on the type of active constituent that is being extracted from the plant material, optimal ethanol by volume percentages of tinctures can typically range from 20%-70%. Glycerites are a glycerol (70-75%) infusion that is most often used when the active constituent is a water-soluble compound [5].

Although multiple studies have demonstrated the therapeutic value of many botanical preparations and the active constituents extracted [5-9], only limited research has profiled the microbiological populations present in the raw plant preparations. Some previous research has addressed concerns of fungal contamination in raw plant materials used for medicinal extractions [10,11], however potential bacterial contaminants remain largely uninvestigated.

Research exposing the presence of molds and fungi in medicinal plant preparations highlights the need for further investigation of other microbial contaminants in order to maintain therapeutic safety. It therefore becomes necessary to determine the likelihood and abundance of bacterial contaminants in medicinal plants and whether these contaminants could be found in the end products that are administered to patients. The goal of this study was to explore the safety of botanical supplements, specifically teas, tinctures and glycerites, by examining the abundance of bacteria in commonly used medicinal plants with different extraction agents. This data may provide a better understanding and awareness of which extraction process may be effective in reducing bacterial populations and whether or not patients could receive a therapeutic contaminated with potentially pathogenic bacteria. The outcomes of this research may help physicians to take appropriate precautions when treating patients with herbal preparations.

Methods

Botanical sources

Several botanical species were used in this study and were selected based on their frequent use in herbal medicine, the portion of the plant commonly used and the typical extraction process. The following plant species were used in this study: flowering parts of St John's Wort (*Hypericum perforatum)* originating from Bulgaria; leaves of garden sage *(Salvia officinalis)* originating from Egypt; whole purple coneflower (*Echinacea purpurea)* originating from the United States; leaves of lemon balm (*Melissa officinalis)* originating from Bulgaria; flowering parts of elderberry *(Sambucus nigra)* originating from Croatia; roots of the *Astragalus* plant (*Astragulus membranaceus)* originating from China; Siberian ginseng root (*Eleutherococcus senticosus)* originating from China; barberry root (*Berberis vulgaris)* originating from India; leaves of Greek oregano (*Origanum vulgare*) originating from Turkey; and the flowering parts of Lavender

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(*Lavandula angustifolia)* originating from France. The organic herb supplier, Starwest Botanicals Inc sourced all of the aforementioned herbs used in this experiment.

Extraction preparation

Ten grams of each desiccated herb was ground to a fine powder under sterile conditions. Two-hundred mls of the appropriate sterile extraction solution (water, 70% glycerin or 15%, 30% or 65% ethanol) was then added to the botanical powder and the herb-liquid mixture was mixed well for 5 minutes. For the hot water extraction, the herb-liquid mixture was heated to 90°C for 20 minutes. All extractions were subsequently incubated at room temperature without light for 7 days. After 7 days, the mixture was filtered through sterile, unbleached paper filters and the effluent used in the described assays.

Bacterial assays

The undiluted botanical extraction or a 1:100 diluted preparation (in water), was plated onto the following media: Trypticase Soy Agar (TSA), blood agar, Eosin Methylene Blue (EMB) agar, *Salmonella-Shigella* agar (SS), Mannitol Salt Agar (MSA) agar, HardyCHROM™ *Staphylococcus aureus* agar, and KF *Streptococcus* agar. The agar plates were incubated for 18 hours at 37°C. Colony numbers, morphology and color reactions were recorded. Colonies present on selective/differential media (Eosin Methylene Blue (EMB) agar, *Salmonella-Shigella* agar (SS), Mannitol Salt Agar (MSA) agar, HardyCHROM™ *Staphylococcus aureus* agar, and KF *Streptococcus* agar) were further analyzed by gram stain and microscopy.

Results

Total bacterial concentration related to extraction process

The goal of this study was to determine the level of endogenous bacteria present in dried plant material which was subsequently released during various extraction process. Since the botanical material used in these studies was not sterilized, it was not surprising to find substantial endogenous bacteria present in all the botanical species tested. However, the methods of extraction did alter the amount of bacteria present. The extraction solution that contained the most bacteria across all samples was the cold water extraction. This was expected and the results from this extraction process were used as a control indicating the maximal bacterial population level present on each herb. In these studies, 1ml of extraction liquid was used per 50mg of solid botanical. In order to assess the total concentration of bacteria in the extracts (Colony Forming Units (CFU)/ml), the extracts were serially diluted and plated on non-selective Tryptic Soy agar. Figure 1 shows that the total number of CFU/ml in four of the samples (*B vulgaris*, *S nigra*, *M officinalis*, *O vulgare*) when using a water extraction ranged from $1x10^6$-$1x10^7$ CFU/ml of extraction solution. Five of the samples (*E purpurea*, *L angustifolia*, *S officinalis*, *E senticosus*, *H perforatum*) contained $1x10^5$-$1x10^6$ CFU/ml following the water extraction (Figure 1, Tryptic Soy agar). *A membranaceus* contained the lowest bacterial population level at approximately $5x10^3$ CFU/ml.

Botanicals used in medicinal teas often go through minimal processing and therefore may contain endogenous bacteria contaminants. A hot water extraction was used to simulate making of a botanical tea and to test if bacterial populations are reduced in the process. When comparing the hot water extractions to the cold water extraction, there was a substantial reduction in the total

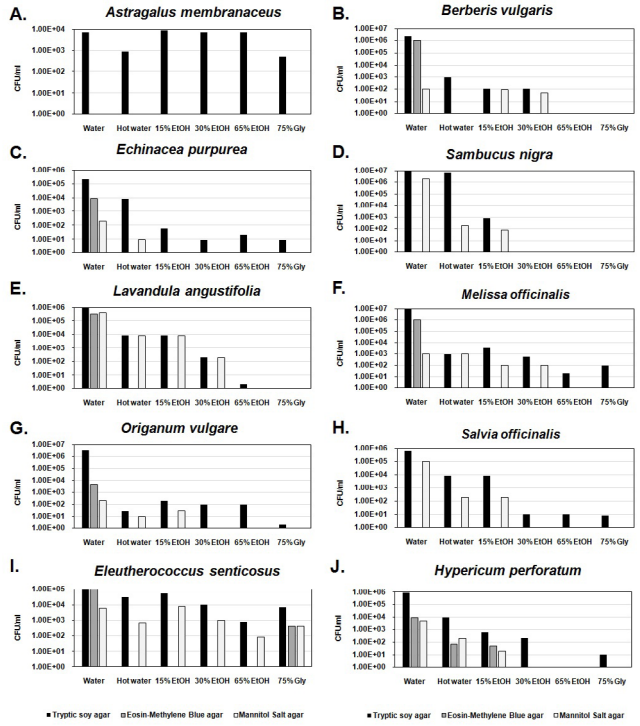

Figure 1: Quantification of bacteria in botanical extracts.

Each graph illustrates the number of Colony Forming Units (CFU) per ml in each of the six different extraction methods (water, hot water, 15% EtOH, 30% EtOH, 65% EtOH and 70% glycerin). Each botanical extraction was serially-diluted and grown on Tryptic Soy agar (non-selective media), eosin-methylene blue agar and mannitol-salt agar.

bacterial concentration in the majority of botanical samples with the exception being *E senticosus* (Figure 1, Tryptic Soy agar). There was an approximate 100-fold reduction in the number of CFU/ml in the hot water extractions of *E purpurea*, *L angustifolia* and *H perforatum* when compared with their water controls (Figure 1, Tryptic Soy agar). An approximate 1,000-fold reduction of CFU/ml was seen in the hot water extraction of *S officinalis* and *S nigra* when compared with their water controls (Figure 1, Tryptic Soy agar). In two of the ten hot water extracts (*B vulgaris and M officinalis*), an approximate 10,000-fold reduction in the number of CFU/ml was observed when compared to their water controls (Figure 1, Tryptic Soy agar). The botanical with the greatest reduction in CFU/ml (approximately 100,000-fold) from hot water extraction when compared to its control was *O vulgare* (Figure 1, Tryptic Soy agar). These results suggest that either the bacterial populations between the different herbs vary in sensitivity to heat or that the heating process may release different components from the herbs, some of which may have direct anti-bacterial properties.

Ethanol is known to have antibacterial properties. While not the most common extraction solution, 15% ethanol is used in some tinctures to extract certain constituents, however, because of the low alcohol content, anti-bacterial properties of the ethanol may be minimal. As shown, the majority of 15% ethanol extractions contained less CFU/ml than the coldwater samples and were typically similar to or lower than the hot water extraction (Figure 1, Tryptic Soy agar). In the 15% ethanol extractions of *E senticosus* and *A membranaceus,* no reduction in the number of CFU/ml was observed (Figure 1, Tryptic Soy agar). An approximate 100-fold reduction in the number

of CFU/ml in *L angustifolia* and a 1,000-fold reduction in the number of CFU/ml in *S officinalis*, *H perforatum* and *M officinalis* were observed compared to their respective controls (Figure 1, Tryptic Soy agar). The largest reduction was an approximate10,000 fold reduction seen in the *B vulgaris*, *E purpurea*, *S nigra* and *O vulgare* samples when compared to their respective controls (Figure 1, Tryptic Soy agar). Due to the low ethanol concentrations in these extracts, the reductions in bacterial titers may be related to the extraction of antibacterial constituents from the herbs, especially since similar trends were observed with hot water and 15% ethanol for several herbs.

The most common extraction solution for tinctures is 30-40% alcohol since many active constituents can be extracted at in this concentration range [5]. In six of the samples (*A membranaceus*, *B vulgaris*, *E purpurea*, *O vulgare*, *E senticosus* and *H perforatum*) there was very little difference in the concentration of CFU/ml in the 30% ethanol and 15% ethanol extractions (Figure 1, Tryptic Soy agar). In *M officinalis*, *S officinalis* and *L angustifolia* there was an approximate 10-fold reduction in the concentration of CFU/ml from the 30% and 15% ethanol extractions (Figure 1, Tryptic Soy agar). Whereas in *S nigra* there was an approximate 100-fold reduction in the number of CFU/ml between the 30% and 15% ethanol extractions (Figure 1, Tryptic Soy agar). Overall, the 30% ethanol extraction reduced the average number of bacteria for all the herbs approximately 1,000-fold compared to the cold water extraction. This supports that the increase in ethanol to 30% likely produced a safer therapeutic comparatively, but one that still contained a substantial amount of bacterial contaminants.

Tinctures containing higher ethanol content are not uncommon but are typically focused on the extraction of specific components, including resins and oleoresins [5]. When compared to the hot water, 15% ethanol and 30% ethanol, 65% ethanol extraction had the greatest reduction in the number of CFU/ml when compared with the water control. Three of the ten botanicals contained no detectable bacteria (*B vulgaris*, *S nigra* and *H perforatum*). Two botanicals (*L angustifolia* and *S officinalis*) contained very low ($<1x10^1$) levels of bacteria (Figure 1, Tryptic Soy agar). This resulted in a 100,000 to 1,000,000-fold reduction in the number of CFU/ml in these samples. In four of the samples (*A membranaceus*, *E purpurea, O vulgare*, *S officinalis*) there was no significant reduction of the CFU/ml between the 30% and 65% ethanol extractions (Figure 1, Tryptic Soy agar). This result may suggest variations in ethanol sensitivity between endogenous bacteria or the extraction of different antimicrobial constituents at the higher ethanol concentration in some, but not all botanicals tested.

Tinctures containing glycerin have grown in popularity in recent years because of their palatability as well as their ability to extract water-soluble constituents from botanicals. The 70% glycerin extraction solution was very effective at reducing the number of CFU/ml when compared with the other extraction solutions. In two of the samples (*B vulgaris* and *S nigra*), both the 70% glycerin extraction and 65% ethanol extractions reduced the number of the bacteria to non-detectable levels in the sample (Figure 1, Tryptic Soy agar). This resulted in a 1,000,000-fold reduction of CFU/ml when compared to the control. *O vulgare* also produced an approximate 1,000,000-fold reduction in the number of CFU/ml (Figure 1, Tryptic Soy agar). In three samples (*L angustifolia*, *M officinalis* and *S officinalis*), 70% glycerin reduced the number of CFU/ml by an approximate 100,000-fold when compared to their respective cold water controls. There was an approximate 10,000-fold reduction in the CFU/ ml in both the *H perforatum* and *E purpurea* samples (Figure 1, Tryptic Soy agar). Compared to 65% ethanol, there was an approximate 10-fold reduction in the CFU/ml in the *A membranaceus*. Across all samples, both the 70% glycerin and 65% ethanol were the most effective at reducing the number of CFU/ml when compared to the water control and other extraction solutions.

Partial identification of bacteria present in botanical extractions

Selective media are often used to partially identify potentially pathogenic organisms by selective and differential components present in the media. In addition to assessing total bacterial concentrations present in the extracts (by assessing growth on Tryptic Soy agar), figure 1 shows the concentrations of bacteria present which are capable of replicating on selective media, including Eosin-Methylene Blue (EMB) and Mannitol Salt (MSA) agars. EMB preferentially inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose. MSA is selective for Gram-positive bacterium *Staphylococci* and *Micrococcaceae* and is differential for mannitol fermentation. In almost all samples there was growth on both MSA and EMB agars with the water extraction. These results may suggest the presence of potentially pathogenic microbes on many dried, unprocessed botanicals.

As shown in figure 1, ethanol or glycerin extractions from several herbs had no or minimal bacterial growth on EMB and MSA media. However, bacterial growth on EMB could still be detected with *E senticosus* extracted with 70% glycerin or *H perforatum* extracted with hot water or 15% ethanol. Growth on MSA was detected in most herbs extracted with hot water or 15% ethanol at levels up to $4x10^3$ CFU/mL. For some herbs, including *B vulgaris, L angustifolia, M officinalis* and *E senticosus*, growth on MSA was observed even at 30% ethanol extractions. Sixty-five percent ethanol or glycerin extraction reduced MSA growth to non-detectable levels for all herbs, except *E senticosus.* These results suggest that, although typically minimal, potential human pathogens may be occasionally present in herbal extraction preparations.

To further evaluate these potentially pathogenic bacteria, additional selective/differential media (including *Salmonella-Shigella* agar, hardy-chrom *Staph aureus* agar, KF *Streptococcus* agar) and Gram-staining procedures were utilized. Since hot water or ethanol extractions often had no detectable bacteria on EMB or MSA, these additional characterizations were done with cold water extracted botanicals only. Based on the growth characteristics (growth detected and colony color) and Gram-staining results, potential genera of the bacteria could be determined and the results are summarized in table 1. Although typically at minimal or low levels (1-10 CFU/ml of extract), potentially pathogenic bacteria were present in many of the botanical samples tested. Although definitive identification of these microbes was beyond the scope of this study, potential genera of these microbes including *Escherichia, Proteus, Salmonella, Shigella, Staphylococcus, Streptococcus* and *Enterococci* as shown in table 1, *Streptococcus spp* and *Enterococcus spp* were present in 10% and 20% of the herbs tested, respectfully. Similarly, potential Gram-negative bacteria including *Escherichia spp* (30% of herbs), *Proteus spp* (10% of herbs), *Salmonella spp* (30% of herbs) and *Shigella spp* (10% of herbs). Although the detection of potentially pathogenic bacteria was reasonably uncommon and when detected, was at

	H perforatum	*S officinalis*	*E purpurea*	*M officinalis*	*S nigra*	*A membranaceus*	*E senticosus*	*B vulgaris*	*O vulgare*	*L angustifolia*
Possible Genus (Gram -)										
Escherichia	+	-	-	-	-	-	+	-	+	-
Proteus	+	-	-	-	-	-	-	-	-	-
Salmonella	-	-	+	+	-	-	-	+	-	-
Shigella	-	-	-	-	-	-	+	-	-	-
Possible Genus (Gram +)										
Staphylococcus	-	-	+	+	+	-	+	+	-	+
Streptococcus	-	-	-	-	-	-	+	-	-	-
Enterococcus	-	-	-	+	+	-	-	-	-	-

Table1: Identification of potential pathogenic bacteria in botanical extracts.

Cold water extracted botanicals (identified above each column) were serially diluted and grown on selective and differential media (Eosin-Methylene Blue agar (EMB), Salmonella-Shigella agar (SS), Mannitol Salt Agar (MSA) agar, HardyCHROM™ Staphylococcus aureus agar, and KF Streptococcus agar). Colonies present were subsequently analyzed by Gram stain. Potential genera of the bacteria were identified based on colony growth and color, Gram stain reaction, and cell shape. (-) indicated no detectable bacterial growth. (+) indicates 1-10 CFU/ml of extract.

minimal levels, these results do support the need for care when preparing and administering therapeutic extractions.

Discussion

Previous research evaluating endogenous microorganisms in raw medicinal plants has focused on fungi with limited regard to bacteria. In 1998, a study concluded that a significant percentage of botanical preparations were contaminated with fungal genera such as *Aspergillus, Penicillium, Mucor, Rhizopus, Absidia, Alternaria, Cladosporium, Trichoderma* and *Aspergillus flavus* [10]. In 2014, a similar study indicated not only the presence of fungi in most medicinal plants sampled, but also detected mycotoxins including aflatoxin and Ochratoxin A [11].

Expanding upon the safety concerns raised by fungal contaminants, this study sought to investigate the level of bacterial contaminants present in raw medicinal plant extractions and how extraction methods may alter the level of these microbial contaminants. The goal of this study was not meant to identify specific bacteria present in specific botanicals since this could and will likely vary between different geographical sources and seasons. Instead, the overall goal was to give a screening of several medicinal herbs and different extraction processes and measure the level of total and potentially pathogenic bacterial contaminants present.

The overall results verified that medicinal plant material is by no means sterile and agrees with previous findings regarding microbial contaminants [10,11]. It should be noted that there seemed to be no correlation between the part of the plant harvested (roots, leaves and flowers) and the concentration of endogenous bacteria present. This is important to consider since roots and subterranean portions are often washed after harvest while aerial parts are harvested and dried without significant processing other than to separate out usable/desirable parts of the herb by removing all impurities and adulterations.

For medicinal use, botanicals are extracted in various solutions, such as hot water, alcohol or glycerin, depending on whether the medicine is to become a tea, tincture or glycerite, respectively. Most tinctures are prepared using ethanol by volume percentages typically ranging from 30-60% [5]. This concentration range is often optimal for extraction of the active constituents, but is often assumed to provide bactericidal activity as well. This is of concern since literature has established a threshold of 60-90% ethanol by volume for optimal bactericidal activity, also noting that bactericidal activity sharply declines at concentrations less than 50% [12,13]. Interestingly with most herbs tested, the bacterial populations decreased considerably when extracted using 15% ethanol and even further with 30% ethanol. These results may suggest that anti-microbial constituents are likely being extracted from the plant material at these low ethanolic extractions leading to the reduction in bacterial cell numbers. In support of this, a 2010 study established the antiseptic qualities of multiple phytochemicals present in the elderflower and illustrated the ability of *S nigra* elderflowers to inhibit a wide range of bacteria [9]. The absence of bacteria in the 35% ethanolic extraction of *S nigra* is consistent with previous research and supports the extraction of bactericidal active constituents in low ethanolic concentrations.

Like ethanol and glycerol has been shown to possess bactericidal activity [12,13]. A previous study examined the use of glycerol and demonstrated increased mortality against Gram-negative bacteria compared to Gram-positive bacteria [14]. In our glycerol-based tinctures (70% glycerol), bacterial titers were greatly reduced in 80% of the herbs tested (~10,000 fold) and substantially reduced in the other herbs 10-100 fold. Even if non-pathogenic, the substantial number of bacteria in many of the extraction processes may lead to additional and possibly undesirable effects when treating patients, including immunological, inflammatory or even toxin-related effects.

This study also sought to partially identify the presence of potential human pathogens in herbal preparations. Although specific and definitive identification of bacteria was not done, the presence of bacteria belonging to potentially pathogenic genera was determined. Although these genera were relatively uncommon, and when present, were at low to minimal levels, the mere presence of these organisms warrants concern as levels may vary between crops, seasons and geographical locations. Ultimately, without the sterilization of botanical medicines, administration of a botanical-based therapy, either topically or orally to a patient will likely introduce bacteria, including potentially pathogenic bacteria which may lead to deleterious responses. Currently, many nutraceutical companies that manufacture tinctures and herbal products do not take extra

precautions for sterilization of their products. This practice is not mandated, regulated or a common standard of procedure in the field. Every treatment has a degree of risk to the patient and it falls to the discretion of the practitioner to determine if the risks outweigh the benefits. In many cases with botanicals, the therapeutic value has been established making them an essential part of treatment therapy. Previous studies have demonstrated a significant presence of fungal contaminants in herbal preparations and this study supports the presence of additional microbial contaminants, including bacteria. This cumulative data is not meant to discourage therapeutic botanical use but rather to educate prudence amongst those who use botanicals. In summary, when using raw plant materials or unsterilized extracts, special care and observation should be taken to limit possible secondary infections especially during the treatment of immunocompromised patients, or other unexpected responses, including inflammation, in other patients.

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References

1. Calixto JB (2000) Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). Braz J Med Biol Res 33: 179-189.

2. Fu PP, Chiang HM, Xia Q, Chen T, Chen BH, et al. (2009) Quality assurance and safety of herbal dietary supplements. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27: 91-119.

3. Falk CL, van Voorthuizen H, Wall MM, Kleitz KM, Guldan SJ, et al. (1999) Costs and returns of growing selected medicinal herbs in New Mexico indicate positive return to land and risk likely. HortTechnology 9: 681-686.

4. Brevoort P (1998) The booming US botanical market: a new overview, Herbal Gram. The Journal of the American Botanical Council 44: 33- 48.

5. Bone K (2003) A clinical guide to blending liquid herbs: Herbal Formulations for the Individual Patient. Churchill Livingstone, Edinburgh, UK.

6. Denzler KL, Waters R, Jacobs BL, Rochon Y, Langland JO (2010) Regulation of inflammatory gene expression in PBMCs by immunostimulatory botanicals. PLoS One 5: 12561.

7. Block KI, Mead MN (2003) Immune system effects of echinacea, ginseng, and astragalus: a review. Integr Cancer Ther 2: 247-267.

8. Tan BK, Vanitha J (2004) Immunomodulatory and antimicrobial effects of some traditional chinese medicinal herbs: a review. Curr Med Chem 11: 1423-1430.

9. Hearst C, McCollum G, Nelson D, Ballard LM, Millar BC, et al. (2010) Antibacterial activity of elder (*Sambucus nigra* L) flower or berry against hospital pathogens. J Med Plant Res 4: 1805-1809.

10. Halt M (1998) Moulds and mycotoxins in herb tea and medicinal plants. Eur J Epidemiol 14: 269-274.

11. Ahmad B, Ashiq S, Hussain A, Bashir S, Hussain M (2014) Evaluation of mycotoxins, mycobiota and toxigenic fungi in selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. Fungal Biol 118: 776-784.

12. Morton He (1950) The relationship of concentration and germicidal efficiency of ethyl alcohol. Ann N Y Acad Sci 53: 191-196.

13. Rutala WA, Weber DJ, Healthcare Infection Control Practices Advisory Committee (HICPAC) (2008) Guideline for disinfection and sterilization in healthcare facilities. Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

14. Saegeman VS, Ectors NL, Lismont D, Verduyckt B, Verhaegen J (2008) Short- and long-term bacterial inhibiting effect of high concentrations of glycerol used in the preservation of skin allografts. Burns 34: 205-211.