

Antibacterial Activity of Homoeopathic Tinctures on Bacterial Strains of *Streptococcus mutans* and *Enterococcus faecalis*: An In vitro Study

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ABSTRACT

Introduction: In view of increasing resistance to existing antimicrobial agents herbal drugs are being looked as very important source for discovery of new agents for treating various ailments related to bacterial infections. Hypericum perforatum, Arnica Montana, Echinacea Angustifolia and Calendula Officinalis are well known homoeopathic tinctures which possess a wide range of pharmacological activities.

Aim: The present study was carried out to investigate the antibacterial activity of Hypericum perforatum, Arnica Montana, Echinacea angustifolia and Calendula officinalis against two strains of bacteria namely *Streptococcus mutans*, *Enterococcus faecalis*.

Materials and Methods: The antibacterial susceptibility of the Mother tinctures was determined by disc diffusion method and minimum inhibitory concentration. Two bacterial strains *S. mutans* (ATCC No 25175) and *E. faecalis* (ATCC No 35550) were obtained from department of microbiology Maratha Mandal's Nathajirao G Halgekar Institute of Dental Sciences and Research Centre Belgaum. For disc diffusion testing, blood agar and brain heart infusion agar were used. After inoculum preparation, the agar plates were incubated for 24 hours at 37 degree Celsius. For minimum inhibitory concentration

9 dilutions of each drug were done with brain heart infusion broth. Then serial dilution was repeated upto 10⁻⁹ dilutions for each drug and then the above culture containing the strains of microorganisms used were added and the tubes were incubated for 24 hours at 37°C.

Results: For agar diffusion among the tinctures largest zone of inhibition for *S. mutans* was seen with Hypericum perforatum and for *E. faecalis* largest zone of inhibition was seen with Echinacea angustifolia. Arnica Montana also exhibited good antibacterial activity with both organisms whereas Calendula officinalis demonstrated lower inhibitory activity with *E. faecalis* and no activity with *S. mutans*. The Minimum Inhibitory Concentration (MIC) for all the tinctures for *S. mutans* was 62.5 mg/mL and for *E. faecalis* the MIC for Hypericum perforatum was 1 mg/mL for Arnica Montana and Echinacea angustifolia 4 mg/mL and for Calendula officinalis 16 mg/mL.

Conclusion: So, it was concluded that all the homoeopathic medicines used in the form of tinctures exhibited good antibacterial activity against both strains of bacteria, except for Calendula officinalis for disc diffusion against *S. mutans* but with Hypericum perforatum the highest activity was observed. The use of homoeopathic tinctures is safe and may provide a good alternative for use as antibacterial agents.

Keywords: Disc diffusion, Inhibitory activity, Minimum inhibitory concentration, Mother tincture

INTRODUCTION

As the over enthusiastic use of antibiotics by conventional medicine, the diseases especially viral, remain resistant to traditional antibiotics, so the mainstream medicine is increasingly in favour of the use of antimicrobial and other drugs derived from plants [1]. New antimicrobial agents are needed to treat diseases in humans and animals caused by drug resistant microorganisms [2]. Because of the increased microbial resistance to antibiotics, toxic and harmful effects of few common antimicrobial agents, there is a continuous need for alternative therapies which are affordable, non-toxic and effective [3]. Homoeopathy a mode of therapy was established in the late 18th century by German physician Samuel Hahnemann. During his experiments he prepared medicines from a wide variety of natural products [4]. Homoeopathy is a set of procedures using highly diluted medicines made from animal, mineral and vegetable sources. These remedies are used as substitutes for antibiotics and pain medication. It is a natural system of medicines that stimulates the body to heal itself [5]. The inclusion of homeopathy in dentistry is specifically useful from simple mild pain due to carious tooth to chronic and complex conditions that have been shown to be difficult to treat; for instance, dental anxiety, atypical facial pain, burning mouth syndrome and post

extraction ostietis [6]. In dentistry Homoeopathic prescriptions are combinations of constitutional and pathological prescriptions. Also, it may be suitable for many conditions for which the conventional medicine falls short [7]. Hypericum perforatum commonly known as ST John's Wort. Hypericum contains flavonoids, hyperforins and the degradation product of hypericin. The highly lipophilic hyperforin possesses antimicrobial, anti-inflammatory and antioxidant effects [8]. Echinacea species popular as immune stimulant were used as a treatment for throat infection, wounds and pain by north American indigenous people. Bioactive compounds present in Echinacea are phenolics, alkylamides and polysaccharides [9]. Calendula officinalis is a medicinal plant belonging to Asteraceae family. The chemical compounds identified in methanol extract of Calendula officinalis include phenolic acids, flavonoid glycoside [10]. In homoeopathic system of medicine, Arnica Montana is used since centuries. It has proved its worth as anti-inflammatory agent. Arnica Montana also exhibits antimicrobial activity against *S. mutans*. The thymol derivatives present have been reported to have bactericidal property [11]. Generally facultative anaerobic bacteria are less susceptible to antimicrobial activities than anaerobes and therefore can be expected to persist more frequently [12]. Bacteria and their by-products are the major cause of pulpal and periapical diseases [13]. The most frequent bacterial genera isolated from necrotic

pulps are *Peptostreptococcus*, *Prevotella*, *Porphyromonas*, *Fusobacterium*, facultative *Streptococcus* [14]. On the other hand recent work investigating the microbial findings of teeth with failure of endodontic treatment have reported a very limited assortment of microorganisms with predominantly facultative anaerobic gram positive species especially *E.faecalis* [15]. Thus considering the importance of microorganisms in pathogenic processes the purpose of this study was to evaluate the effectiveness of homoeopathic tinctures to eliminate the infectious process and susceptibility pattern of microorganisms to antimicrobial drugs.

MATERIALS AND METHODS

An in vitro study was done in Maratha Mandal's Nathajirao G Halegekar Institute of dental Centre sciences and Research Belgaum, Department of Microbiology and Immunology from July 2017-Sept 2017 to evaluate the antimicrobial effect of four homoeopathic tinctures namely *Hypericum perforatum*, *Arnica Montana*, *Echinacea angustifolia* and *Calendula officinalis* against two strains of bacteria *S. mutans* and *E.faecalis*. A mother tincture is the original tincture prepared with the aid of alcohol (ethyl alcohol with 50%-70% v/v) directly from the crude drugs secured from any source. The homoeopathic tinctures of *Hypericum perforatum* (50% to 80% v/v) (SCHWABE), *Arnica montana* (60% to 70% v/v) (SCHWABE), *Echinacea angustifolia* (70% v/v) (SCHWABE) and *Calendula officinalis* (40% to 60% v/v) (SCHWABE) were purchased from commercial sources. Ethical clearance was obtained from the Ethical Committee (Ref.No.MM/BDS/MOS/2017-18/25).

Microorganisms and Growth Conditions

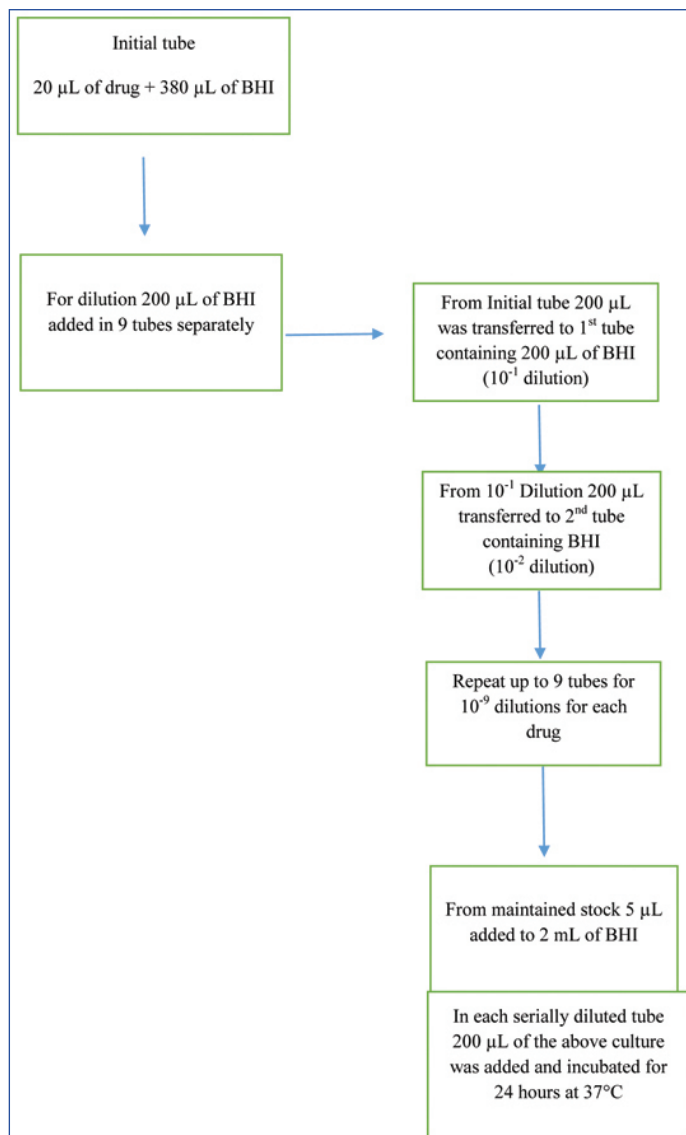
Two bacterial strains *S.mutans* (ATCC No 25175) and *E. faecalis* (ATCC No 35550) were obtained from department of microbiology. All strains were maintained in 15% glycerol and stored at -80 degree Celsius until use.

Determination of antibacterial susceptibility testing by agar diffusion [16]: The disc diffusion method for antimicrobial susceptibility testing was carried out according to standard method as described. Homoeopathic tinctures were screened for antibacterial activity against two species of bacteria *S.mutans* and *E.faecalis*. Blood agar and Brain heart infusion agar was used for inoculation. The agar plates were brought to room temperature before use.

Inoculum preparation: The colonies were transferred to the plates using a loop or swab and the turbidity was adjusted visually with equal to standard 0.5 McFarland turbidity that has been vortexed. The suspension was standardised with a photometric device.

Inoculation of agar plate: Within 15 minutes of adjusting the inoculum to a 0.5 McFarland turbidity standard, a sterile cotton was dipped into the inoculum and rotated against the wall of tube above to remove excess inoculum [16]. The entire surface of the agar plate was swabbed 3 times rotating the plates approximately 60 degrees between streaking to ensure even distribution. The inoculated plates were allowed to stand for atleast 3 minutes but no longer than 15 minutes before making wells. Then a 5mm diameter hollow tube was taken, heated and then pressed on the above inoculated agar plate thereby making a well in the plate and then immediately removed. Equal quantity of homoeopathic tincture was added into the respective wells on each plate. The plates were incubated within 15 minutes of compound application and inverted and stacked no more than five high. The plates were then incubated for 24 hours at 37 degree Celsius in incubator. Zones of inhibition were measured in mm after 24 hours of growth by a measuring device.

Determination of Minimum Inhibitory Concentration (MIC) [16]: To determine the minimum inhibitory concentration 9 dilutions of each drug were made with Brain Heart Infusion (BHI) broth [Table/Fig-1].



[Table/Fig-1]: Flowchart for minimum inhibitory concentration technique.

RESULTS

Agar Diffusion

The two bacteria used were sensitive to homoeopathic tinctures [Table/Fig-2]. Among the tinctures *Hypericum perforatum*, *Arnica montana*, *Echinacea angustifolia* demonstrated relatively strong antibacterial activity with prominent zones of inhibition against *S.mutans* and *E. faecalis*. *Calendula officinalis* demonstrated lower inhibitory activity against *E.faecalis* as compared with the other tinctures and no inhibitory activity against *S.mutans*. Of the four tinctures, *Echinacea angustifolia* showed the highest activity against *E. faecalis* strains and *Hypericum perforatum* showing the highest activity against *S.mutans*. *Calendula officinalis* was not active against *S. mutans* and showed the lowest activity against *E. faecalis*. Tinctures of *Arnica montana* and *Hypericum perforatum* showed equal activity against *E. faecalis* strains. Also tinctures of *Echinacea angustifolia* and *Arnica montana* showed equal amount of activity against *S. mutans* strains.

	<i>Hypericumperforatum</i>	<i>Arnica montana</i>	<i>Echinacea angustifolia</i>	<i>Calendula officinalis</i>
Zones of inhibition in mm				
<i>E. faecalis</i>	16	15	18	10
<i>S. mutans</i>	24	16	16	Nil

[Table/Fig-2]: Antibacterial activity of tinctures by agar diffusion.

Minimum Inhibitory Concentration

It was expressed as the lowest concentration of an agent that will

inhibit the growth of microorganisms. The extracts were diluted to 1/500 level. The minimum inhibitory concentration of the tinctures for all the four drugs showed antibacterial activity [Table/Fig-3]. The *E. faecalis* strain (ATCC No- 35550) was more sensitive to all the tinctures than *S.mutans* (ATCC No-25175) strain. All the tinctures exhibited equal inhibition towards *S. mutans* upto a level of 62.5 mg/mL. But the tinctures showed varied levels of inhibition towards *E.faecalis*. *Hypericum perforatum* exhibited the highest inhibition against *E. faecalis* with dilution upto 1 mg/mL. *Arnica montana* and *Echinacea angustifolia* showed slightly low inhibition with dilution upto 4 mg/mL. *Calendula officinalis* showed the lowest inhibition against *E. faecalis* with dilution upto 16 mg/mL.

S. mutans	500	250	125	62.5	31.5	16	8	4	2	1
<i>Hypericum perforatum</i>	S	S	S	S	R	R	R	R	R	R
<i>Arnica montana</i>	S	S	S	S	R	R	R	R	R	R
<i>Echinacea angustifolia</i>	S	S	S	S	R	R	R	R	R	R
<i>Calendula officinalis</i>	S	S	S	S	R	R	R	R	R	R
E. faecalis										
<i>Hypericum perforatum</i>	S	S	S	S	S	S	S	S	S	S
<i>Arnica montana</i>	S	S	S	S	S	S	S	S	R	R
<i>Echinacea angustifolia</i>	S	S	S	S	S	S	S	S	R	R
<i>Calendula officinalis</i>	S	S	S	S	S	S	R	R	R	R

[Table/Fig-3]: Minimum inhibitory concentration in mg/ml (units of measurement).

*S: Susceptible *R: Resistant

DISCUSSION

The type of biological activity revealed by any plant material depends on multiple factors including the geographical source, plant part, moisture content, and the conditions in which it is stored. The homoeopathic medicines chosen for this study were commonly used for treating infectious diseases and are known to produce a wide range of bioactive compounds including antimicrobials. Among these *Hypericum perforatum*, *Arnica montana*, *Echinacea angustifolia* exhibited strong inhibitory activities towards both organisms tested while *Calendula officinalis* exhibited a lower inhibitory activity towards only one organism. The potentially effective antimicrobial agents from *Hypericum perforatum*, *Arnica montana*, *Echinacea angustifolia*, *Calendula officinalis* were confirmed by demonstrated bioactivity in agar diffusion and broth dilution. In this study *Hypericum perforatum* demonstrated a strong inhibitory activity against both the organisms. *Hypericum perforatum* L is a well-known medicinal plant that has been in use for a decade (Gluten O, Neslihan B 2017) [17]. This plant is a representative of Hypericaceae family with curative effects on burns, bruises, swelling and anxiety. It is also a mild to moderate antidepressant, antiviral, helps in wound healing, analgesic, hepatoprotective, antioxidant and has antimicrobial activity [17]. *H. perforatum* is well-characterised chemically with many secondary metabolites for e.g., naphthodianthrones (hypericin), flavonoid glycosides (hyperoside), biflavones, phloroglucinols (hyperforin) and anthocyanidins [18]. It was postulated that hypothetical substances hypericin 1 and hypericin 2 as active principles. However investigation was done for antimicrobial activity of hyperforin and it was found effective against multiresistant strains [19]. In homoeopathy, *Arnica montana* has been used with great level of tolerance for acute trauma such as strains, contusions, and bruises [20]. Its major constituents are essential oil, fatty acids, thymol, pseudoguaianolide, sesquiterpene lactones and flavonoid glycosides [21]. The most active components of the plant are sesquiterpene lactones which are known to reduce inflammation. *Arnica* is known to stimulate blood circulation. It has strong antibacterial and anti-inflammatory properties that can reduce pain and swelling [22]. In the present study, *Arnica montana* tincture showed good antibacterial activity against both the organisms with prominent zones of inhibition with agar diffusion and broth dilution. The polyacetylenes from the root

have demonstrated the antimicrobial effect of *Arnica montana* against various pathogenic fungi and bacteria [23]. Also, *Echinacea angustifolia* tincture was effective in inhibiting the organisms tested in this study. Earlier studies indicated that different species of *Echinacea* have been used traditionally for the treatment of various symptoms of cold, flu, for treatment of candidiasis, lung diseases and wound healing as well [24]. In this study, *Echinacea* was used against two organisms and showed good antibacterial activity with prominent zones of inhibition. Studies with different commercial preparations of *Echinacea* indicated a wide variety of responses by various human pathogenic bacteria [25]. *Echinacea* belongs to the Asteraceae, a family important to commerce for its many medicinal and culinary herbs. *Echinacea* species chemistry is well known. Also, the caffeic acid derivatives, flavonoids, pyrrolizidine alkaloids, alkamides, polysaccharides polyacetylenes, and glycoproteins have been isolated [26]. The echinacoside and caffeic acid content of *Echinacea* inhibits the bacterial growth. *Echinacea* components which are held responsible for various bioactivities namely alkylamides polysaccharides are not responsible individually for direct bactericidal activities but some of the phenolic compounds could also contribute conceivably to these activities [24]. In the present study the tincture of *Calendula officinalis* did not show any significant bioactivity against *S.mutans* in agar diffusion but showed a low inhibitory activity against *E. faecalis*. However it showed significant inhibitory concentration against both the organisms in broth dilution. Studies done by Lauk L et al., showed the antibacterial activity of *Calendula officinalis* against anaerobic and facultative aerobic bacteria [23]. In a study conducted by Shrinidhi MS et al., *calendula officinalis* showed significant antimicrobial activity against *S. mutans* [27]. Also, in a study by Çetin B et al., the extracts of *calendula officinalis* showed various antimicrobial activities against the test bacteria which also included *E. faecalis* [10]. *Calendula* has been widely used as tincture for healing wounds and burns in homoeopathy extensively. *Calendula officinalis* has a long history of usage as it is of rich medicinal value and reported to possess anti inflammatory, antioxidant, antibacterial and antiseptic properties [28]. Therapeutic active substances include flavonoids, saponins, triterpenoids, carotenoids, sterol, cholesterol, vitamin A, organic acid, mucilage, gum and albumin [29]. Terpene lactones and terpene alcohols of the essential oil exhibit antimicrobial, antifungal and antiviral activities as concluded in a study [30].

LIMITATION

The limitations of the study were that it was an in vitro study. Also, since the study has been tested only for two gram positive bacteria, the homoeopathic medicines against other bacterial infections stands a big question mark since majority of gram positive and gram negative organisms are becoming multidrug resistant.

FUTURE RECOMMENDATIONS

Due to the presence of various phytochemicals with significant antibacterial potential experimental studies are needed to determine the quality and extent of effect of the above mentioned tinctures on other microorganisms. The use of homoeopathic tinctures is safe and may provide a good alternative for use as antibacterial agents. Further in vivo studies can also be conducted.

CONCLUSION

A number of studies have mentioned the necessity of developing alternative antimicrobial drugs. Homoeopathic medicines would appear to be an excellent choice. In this study, all the homoeopathic medicines used in the form of tinctures exhibited good antibacterial effects against both *S.mutans* and *E.faecalis* and may offer prospective new alternative for bacterial infections. As search for new antimicrobial agents intensifies homoeopathic medicines may provide attractive alternate sources for consideration.

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