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Comparative Analysis of Antibacterial Activity of Ciprofloxacin and Homeopathic Mother Tincture

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Author`s Contribution
All the authors contributed significantly to the research that resulted in the submitted manuscript.

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ABSTRACT

Objective: The aim of this study was to evaluate and compare *in vitro* antibacterial effects of ciprofloxacin and homeopathic mother tinctures.

Methods: All the experimental homeopathic mother tinctures were donated by Masood Homeopathic Pharmaceuticals, Pakistan. Among tested bacteria, *Escherichia coli* (014) and *Bacillus subtilis* (143) were taken in the form of standard culture agar from the First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences (IAGS), the University of Punjab, Lahore and *Pseudomonas aeruginosa* (ATCC 9027) and *Staphylococcus aureus* (ATCC 6538) were taken from American Type Culture Collection (ATCC). Ciprofloxacin (standard antibiotic) 1mg/ml was taken as positive control and 70% alcohol was taken as negative control. Homeopathic mother tinctures were checked for their antibacterial activity against four bacterial strains (*Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*) through agar well diffusion method.

Results: Ciprofloxacin expressed maximum activity against *Pseudomonas aeruginosa* among all tested bacteria. All tested mother tinctures expressed more or less antibacterial activity against *Staphylococcus aureus* with maximum activity of *Eucalyptus globulus* (zone of inhibition 21.7 mm). Eight out of ten mother tinctures expressed activity against *Escherichia coli* with maximum activity of *Eucalyptus globulus* (zone of inhibition 17 mm). Nine out of ten homeopathic mother tinctures expressed significant antibacterial activity against *Pseudomonas aeruginosa* with maximum activity of *Hydrastis canadensis* (zone of inhibition 28 mm). Similarly, nine out of ten homeopathic mother tinctures expressed significant antibacterial activity against *Bacillus subtilis* with maximum activity of *Hydrastis canadensis* (zone of inhibition 26 mm).

Conclusion: Hence, concluded that homeopathic mother tinctures showed antibacterial potential.

Keywords: Antibacterial activity, comparison, ciprofloxacin, mother tincture.

INTRODUCTION

Human being has the right to choose the best system of medicine according to their opinion. After the arrival of antibiotics where there is treatment of lethal diseases is appreciated, multiple complications due to its adverse effects are also raised. This situation seeks the attention of cure seeking people towards the complementary and alternative system of

medicine (CAM) Human being has the right to choose the best system of medicine according to their opinion. After the arrival of antibiotics where there is treatment of lethal diseases is appreciated, multiple complications due to its adverse effects are also raised. This situation seeks the attention of cure seeking people towards the complementary and alternative system of medicine (CAM) [1].

Homeopathy is the system of medicine which encouraged the body immune system to fight against the acute, chronic, infectious and non infectious diseases by using diluting form of natural substances [2]. Homeopathic medicine has spread in use throughout the world baecause of their effectiveness, safety, increase cure rate and cost-effective [3].

Homeopathic mother tinctures are in liquid form, prepared by the use of suitable concentration of alcohol treated with raw material (which may be fresh or dried). Homeopathic mother tinctures of plant origin carry the hydro-alcoholic extracts of medicinal plants [4].

Microbes cannot be flourished in the presence of homeopathic mother tinctures. White blood cells and epithelial tissues are not abolished by the use of homeopathic mother tinctures which are the body's first defensive line against infection. Clinically it is observed that during the process of healing of the wound, homeopathic mother tinctures exhibit the early and rapid curative action in comparison with allopathic system of medicine [5].

The following homoeopathic plant mother tinctures were selected for the antibacterial testing

Baptisia tinctoria is an herbaceous plant related to Leguminoseae family. It gives good results in septic condition of the blood. It produces antibodies in the body when it is used in low potencies. It robust the body immune system [6]. *Baptisia tinctoria* is also effective when tropically applied especially in condition of infection of nipples and skin [7]. *Baptisia tinctoria* expressed reasonable antibacterial activity in a work against *Staphylococcus aureus* [8].

Berberis aquifolium is scientifically known as *Mohania aquifolium*, have its place in Berberidaceae family [9]. Due to presence of isoquinoline alkaloids, the plant possesses antibacterial, antifungal, antipsoriatic as well as antioxidant properties. It shows valuable effects in the treatment of various dry scaly skin ailments such as psoriasis vulgaris, atopic dermatitis and fungal infections [10-12].

Echinacea angustifolia is plant of Compositae family. It is a remarkable medicine as a corrector of blood dyspraxia. Homeopathically it is used in cases of acute auto-infection, blood poisoning, septic conditions such as diarrhea in typhoid, gonorrhea, boils, erysipelas, foul ulcers, gangrene with exophthalmic symptoms, cerebrospinal meningitis and puerperal infections (Vaid, 2002c). It became famed for cleaning and healing supportive wounds [13].

Eucalyptus globulous is a plant of Myrtaceae family. *Eucalyptus* species contain a variety of antioxidant and antimicrobial agents. Multiple studies were conducted to verify its antibacterial properties against a number of microorganism [14]. It is powerful antiseptic and destructive to low forms of life, a stimulating expectorant and an efficient diaphoretic [6]. A study shows antioxidant and antimicrobial effects *of Eucalyptus globulus* against *Bacillus subtilis* [15].

Hydrangea arborescens belongs to the family of Saxifragaceae [6]. A study show the moderate antibacterial activity against *B. subtilis* [16]. In another study, powder form of hydrangea's flower shows antibacterial activity against *S. aureus* [17].

Hydrastis Canadensis is a small perennial, medicinal plant of Ranunculaceae family. Its rhizomes and roots have antimicrobial agents, use to treat genitourinary infections. It is also used as vasoconstrictor and uterine hemostatic [17]. In a study, extract from *Hydrastis canadensis* showed significant antibacterial activity against *H. pylori* [18]. A Research Study also expressed the valuable antioxidant capacity of *Hydrastis* [19].

Hypericum perforatum is perennial herb belongs to the Hypericaceae family. The plants contain multiple phytochemicals such as naphthodianthrones, flavonoids, prenylated phloroglucinols, tannins, and volatile oil. It has confirmed antiseptic, antidepressant, wound-healing, anti-inflammatory, antiviral, hepato protective, antioxidant and antidiabetic properties. Antibacterial and antifungal activities has been discovered in different research studies [20].

Kreosotum is a phenolic mixture especially contain guaiacol, cresol, methylcresol and phenol [6]. *Kreosote* possessed the bactericidal and antioxidant property against various microorganism [21].

Pulsatilla nigrican is representative of Ranunculaceae family. Therapeutically it is well-known remedy for the treatment of anxiety, melancholy, mild restlessness and mental disturbance. Ovaritis, ovaralgia, pain due to acute inflammation, epididymitis, orchitis, uterine affections, indigestion, coryza, otitis, rhinitis, conjunctivitis, coughs, cutaneous affections and acute meningitis are treated by *P. nigricans*. Its roots are reported as antibacterial, antiamoebic, antitumor, blood-cooling and detoxifier [22].

Thuja occidentalis is illustrative of Cupressaceae family. It prevents from surgical intervention in cases of papilloma, epithelioma, polyps, pustules, ulcers and skin eruptions [7]. In different research studies, *Thuja occidentalis* has been proved as antimicrobial against multiple pathogens [23].

METHODOLOGY

The present study was conducted in the Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur. All the selected homoeopathic mother tinctures were purchased by Masood Homoeopathic Pharmaceuticals, Pakistan. Ciprofloxacin®, batch A460358 was used as positive control while 70% ethanol was used as negative control in each test.

Preparation of Inoculum

Primary culture plate of the three sleeted bacteria was prepared by streaking the swab of the KWIK-STICK containing the respective bacterial strain on the fresh purified nutrient agar plate. A few colonies from the overnight primary culture plate of Streptococcus pyogenes, Staphylococcus aureus, and Pseudomonas aeruginosa were suspended in 10 ml sterile nutrient broth separately for three bacteria, and the solution was adjusted to the 0.5 McFarland equivalence turbidity standards.

Antibacterial Susceptibility Test

For testing the antibacterial activity, the disk diffusion method was used. Nutrient agar plates were made by pouring (15 ml) sterilized nutrient agar medium and allowed to solidify for a few minutes in aseptic condition. A volume of 60 µl of prepared inoculum was poured into each plate. With glass rod of L shape, the poured inoculum is spread on the whole surface of the agar uniformly. Filter paper discs of 6 mm diameter loaded with 10 µl of one selected mother tincture, ciprofloxacin and 70% ethanol separately were placed on the surface of the bacteria seeded nutrient agar medium in the respective labeled area. All the plates were incubated in an inverted position 37°C for 24 hours. After incubation, the clear zone surrounding each disc (zone of inhibition) was measured with the help of Vernier caliper. The work performed in triplicate for each mother tincture against each bacterial strain.

Statistical Analysis

Analysis of variance was applied for statistical analysis of results. $P < 0.05$ was considered to be statistically significant.

RESULTS

Antibacterial activity of various homeopathic mother tinctures against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* are shown in Figures **1-4**, Table **1**.

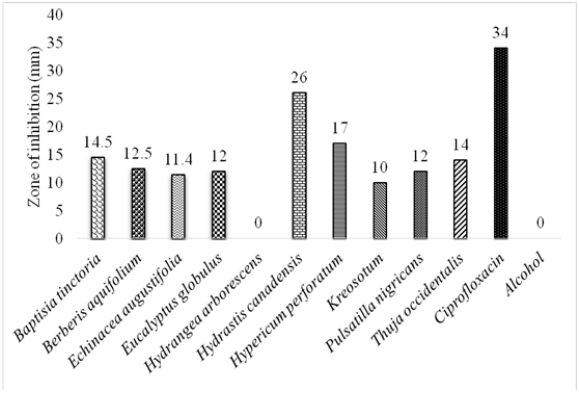

Figure 1. Graphical presentation of antibacterial activity of various homeopathic mother tinctures against *Bacillus subtilis*.

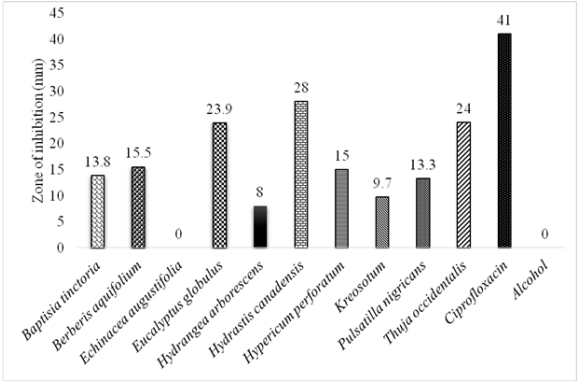

Figure 2. Graphical presentation of antibacterial activity of various homeopathic mother tinctures against *Pseudomonas aeruginosa*.

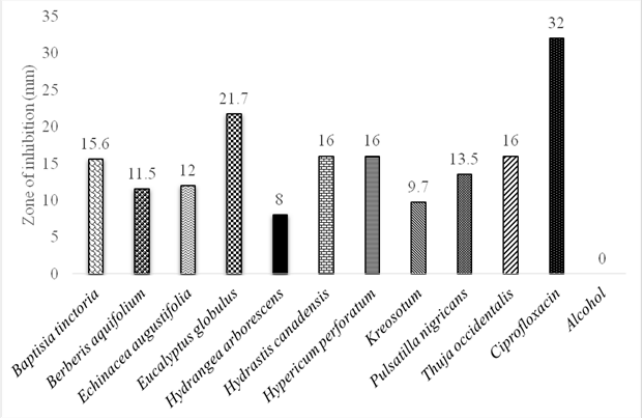

Figure 3. Graphical presentation of antibacterial activity of various homeopathic mother tinctures against *Staphylococcus aureus*.

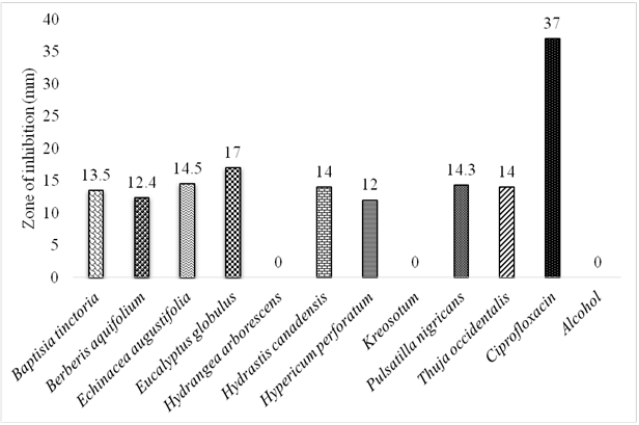

Figure 4. Graphical presentation of antibacterial activity of various homeopathic mother tinctures against *Escherichia coli*.

DISCUSSION

In current study, ten homeopathic mother tinctures were investigated for their antibacterial potential. Antibacterial activity was tested against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). *Hydrastis canadensis* exhibited the maximum zone of inhibition among different homeopathic mother tinctures against *Pseudomonas aeruginosa.* It might be due to the presence of alkaloid content as a previous study reported the presence of various alkaloids (hydrastine, palmatine, berberine, oxycathine berbamine, jatrorrhizine) in root extract of *Hydrastis canadensis* [24]. *Eucalyptus globulus* was the second plant in order of antibacterial potential in current study. It has marked antibacterial activity as compared to other tested mother tinctures especially against *Staphylococcus aureus.* Eucalyptus globulus mother tincture with zone of inhibition of 21.7 ± 0.6 and 17 ± 0.2 mm against *Staphylococcus aureus* and *Escherichia coli*, respectively are also comparable with another previous study [25]. *Thuja occidentalis* showed moderate antibacterial activity with zones of inhibition of 14-24 mm against all tested bacterial strains. While testing the Baptisia these results are also comparable to findings of current study in which zone of inhibition are 15.6 and 13.8 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa,* respectively. *Berberis aquifolium* showed antibacterial activity through disk diffusion method

Table 1. Results of antibacterial activity of various homeopathic mother tinctures, alcohol and ciprofloxacin against four bacteria.

Tested material	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
	Zone of inhibition (mm) Mean ± S.E.M*			
Ciprofloxacin	32±0.5	37±0.5	41±0.5	34±0.5
Alcohol	Nil	Nil	Nil	Nil
Baptisia tinctoria	15.6±0.3	13.5±0.2	13.8±0.6	14.5±0.2
Berberis aquifolium	11.5±0.2	12.4±0.2	15.5±0.2	12.5±0.2
Echinacea augustifolia	12±0.5	14.5±0.2	Nil	11.4±0.2
Eucalyptus globulus	21.7±0.6	17±0.2	23.9±0.5	12±0.2
Hydrangea arborescens	8±0.5	Nil	8±0.5	Nil
Hydrastis canadensis	16±0.5	14±0.5	28±0.5	26±0.5
Hypericum perforatum	16±0.5	12±0.5	15±0.5	17±0.5
Kreosotum	9.7±0.3	Nil	9.7±0.3	10±0.5
Pulsatilla nigricans	13.5±0.2	14.3±0.3	13.3±0.3	12±0.5
Thuja occidentalis	16±0.5	14±0.5	14±0.5	14±0.5

* Results were expressed as mean ± S.E.M (n =3).
Nil - No inhibition.

against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with zone of inhibitions of 11.5 and 15.5 mm, respectively [7], (Table **1**).

The current study showed similar zone of inhibition (11.5 mm) against *Staphylococcus aureus* however, superior in its antibacterial potential against *Pseudomonas aeruginosa* with double zone of inhibition (15.5 mm). Antibacterial activity of *Berberis aquifolium* might be due to the presence of alkaloids such as berberine and jatrorrhizine [26] *Echinacea augustifolia* showed moderate zones of inhibition against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus,* however showed no zone of inhibition against *Pseudomonas aeruginosa. Pulsatilla nigricans* showed moderate antibacterial activity with zone of inhibition of 12–14.3 mm. Antibacterial activity of *Pulsatilla nigricans* may be due to the presence of flavonoids and tri terpenoids as flavonoids and tri terpenoids have marked antimicrobial activity [27]. *Kreosotum* may be due to presence of phenols like *m*-cresol. *Hydrangea arborescens* showed minimum zones of inhibitions among all tested mother tinctures. *Hydrangea arborescens* showed no zone of inhibition against *Escherichia coli* and *Bacillus subtilis.* An old study also showed similar results with no antibacterial activity against *Escherichia coli* [16] The present study showed 8 mm zone of inhibition against *Staphylococcus aureus* by mother tincture of *Hydrangea arborescens* roots and rhizomes. The reason of greater zone of inhibition may lie in the difference in quantity of chemical constituents of roots and flowers. Major constituents of roots of *Hydrangea arborescens* are hydrangin, hydrangenol and flavonoids like kaempferol, quercetin and rutin [28].

CONCLUSION

It is concluded from present study that ten homeopathic mother tinctures showed antibacterial potential. Among the tested mother tinctures, *Hydrastis canadensis* and *Eucalyptus globulus* possess strongest antibacterial potential while *Hydrangea arborescens* and *Kreosotum* showed weakest antibacterial activity against all the tested bacterial strains. *Baptisia tinctoria, Berberis aquifolium, Echinacea augustifolia, Hypericum perforatum, Pulsatilla nigricans* and *Thuja occidentalis* have moderate zone of inhibitions against tested bacterial strains.

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