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ORIGNAL ARTICLE

Screening of Antibacterial Potential of Some Selected Homoeopathic Mother Tinctures against Common Uropathogens: Research Paper

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Authors' Contributions
1Data Collection & Processing, Data Analysis and/or Interpretation.
2Conception & Study Design, Data Analysis and/or Interpretation.
3Critical Review.
4Drafting of Manuscript.
5Drafting of Manuscript.
6Drafting of Manuscript.

Article info.
Received: September 30, 2022
Accepted: November 04, 2022

Funding Source: *Nil*
Conflict of Interest: *Nil*

Cite this article: *Nawaz H, Sumreen L, Asif HM, Qamar S, Tanveer R, Shaheen G, Shamim T. Screening of Antibacterial Potential of Some Selected Homoeopathic Mother Tinctures against Common Uropathogens: Research Paper. RADS J Pharm Pharm Sci. 2022; 10(2):55-60.*

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ABSTRACT

Objective: Medicinal plants have a number of therapeutic properties to cure variety of diseases. Medicinal plants are also employed for treatment purpose from centuries because of fewer side effects, lesser cost with better outcome. In homeopathic system of medicine, mother tinctures prepared from medicinal plants are employed for the treatment of various diseases. This study was designed to evaluate the antibacterial potential of seven homeopathic mother tinctures against some uropathogenic bacteria.

Methods: Selected mother tinctures were tested against three clinically important human uropathogenic bacteria namely *Escherichia coli, Staphylococcus aureus* and *Klebsiella pneumonia* by disc diffusion and well diffusion method. *Ciprofloxacin* was used as standard drug in concentration of 200mg/100ml whereas 70% alcohol was taken as negative control.

Results: Homeopathic mother tinctures showed good results against *Escherichia coli* and the *Staphylococcus aureus*, but these remedies showed minimum results against *Klebsiella*. Mother tincture of *Sepia officianalis* exhibited maximum antibacterial activity (30mm) against *Escherichia coli* among all tested mother tinctures.

Conclusion: This study showed that above tested plant based mother tinctures have good antibacterial potential against the particular microorganisms, especially uropathogens.

Keywords: *Anti-bacterial activity, Escherichia coli, Mother tincture, Phytomedicine, Uropathogens.*

INTRODUCTION

Phytomedicine is the system in which the diseases are cured by the medicines that are prepared by plants. [1] Homeopathy, Ayurveda, Unani, Traditional Chinese Medicine and Kampo are worldwide Alternative medical systems based on natural plant products. Almost 70 % of homeopathic medications are acquired from plants. [2] Homeopathic tinctures can be prepared from any part of the plant like seed, stem, bark, leaf, flower, fruit leaf and roots (Homoeopathic Pharmacopoeia.[3] The World Health Organization evaluates that overall 80% of individuals worldwide depends on plant based medicines for the basic health care essentials. [4] For the preparation of homoeopathic medicines, only small quantity of the

original drug material is sufficient. Basic sources of homeopathic medicines are nearly the identical as of other drug systems.[5] Plants have different properties to cure different diseases such as heart diseases, asthma, gastrointestinal diseases, liver and skin disorder. The plants extracts contains secondary metabolites such as glycosides, polyphenols, flavonoids, alkaloids and various others. They can produce antioxidant, antibacterial, antihypertensive, anti-inflammatory and other various effects.[6]

In homeopathy mother tincture prepared from plant extract are used to cure different types of infections such as wound infections, skin infections and ulceration.[7] Some homeopathic mother tinctures have antioxidant potential that helps in treating the deteriorating diseases [8,9]. By the passage of time after getting advance treatment methods, the bacterial agents are becoming more resistance. Different methods are used to evaluate the antibacterial activities of the plants. Commonly agar well diffusion and disc diffusion methods are used to assess the antibacterial activities.[10]

All the selected homeopathic mother tinctures are used to cure different infections. However, the *Allium cepa* belongs to Alliaceae family and is rich in containing Sulphur. This medicine is used in urinary tract infection if the symptoms are due to weakness of the bladder and urethra.[11] *Lycopodium clavatum* is also known as from club moss. *Lycopodium* is good in kidney disease when there is red sand in the urine, the pain moves in renal region and its symptoms get worse before urination.[12] *Thuja occidentalis* medicine is prepared by American Arbor Vitae, a spiry evergreen.[13] Its urinary complains come with inflamed swollen urethra, burning trickling sensation in urethra and urge to urinate along with serve cutting pain.[14] *Azadirachta indica* medicine is made from neem tree. It is also reported for best results in anti-inflammatory, anti-bacterial activities.[15] *Echinacea* is prepared from purple cone-flower. This medicine is also known as a “corrector of blood dyscrasia”.In practice it is given in blood poisoning, and septic condition. Urinary complains are frequent, scanty and involuntary urination. *Sepia* is used mainly for women especially in pregnancy and in lactating mothers. The urinary tract symptoms are that urine color is reddish clay with sand in it. Burning in the urine, offensive urine must be removed from the room. Slow micturition with bearing down sensation above pelvis. [16] *Cantharis* medicine extract contain phosphate, uric acid and Cantharidin. Cantharidin is a strong poison that mainly affects the urinary tract and also causes the skin blistering. [17]

The aim of this study is to check the antibacterial activities of some homeopathic medicines on the different bacteria that responsible for urinary tract infection. For this purpose we have utilized *Lycopodium clavatum, Azadirachta indica, Allium cepa, Thuja occidentalis, Echinacea purpurea, Sepia officianlis and Cantharis* tincture against three different strains of bacteria.

MATERIAL AND METHODS

Material

The plant tinctures named as *Allium cepa (*12015731Q*), Echinacea purpurea (*MT 8531*), Lycopodium clavatum (*MT 6992*), Thuja occidentalis (*MT 7362),*Azadirachta indica (*MT 6937*), Cantharis (*0870919) *and Sepia officinalis* (CH 19055) of Masood Homeopathic Pharmaceutical Company were used.

Bacterial Strains

Three bacteria, *Escherichia coli (*ATCC 25922*), Klebsiella pneumonia (*ATCC 13883), and *Staphylococcus aureus* (ATCC 25922) were selected. *Klebsiella pneumonia* and *Staphylococcus aureus* were obtained from the culture bank of Quaid-e-Azam medical college and the *Escherichia coli* was taken from the Microbiology Laboratory of The Islamia University of Bahawalpur. This research was conducted at Microbiology Laboratory of The Islamia University of Bahawalpur.

Preparation of Inoculums

The bacterial inoculums were checked by McFarland standard. To obtain 0.5 McFarland standards, we mixed 99.5 ml of sulfuric acid and 0.5 ml of 1.175 % barium chloride. Here we got the McFarland standard. Optical density of this solution was checked at 625nm range that showed absorbance at 0.08 to 0.10 nm. Now the bacterial inoculums were prepared from the 24 hours old culture. In 5 ml saline solution, few colonies of bacteria were mixed to make the bacteria suspension. Then suspension density was checked that was $1.5x10^8$CFU/ml under the UV lights.

Preparation of Agar Plates

28g of nutrient agar was taken by measuring on weight scale and mixed it with distilled water. It was stirred on the hot plate for 5 minutes and conical flask

was placed in autoclave for 15 minutes at the temperature 121°C. After 15 minutes, mixture was allowed to cool on room temperature.

Antibacterial Assay

First, all the instruments were sterilized in the autoclave for 15 minutes at temperature of 121°C. Laminar flow was sterilized with ethanol and all the instruments were placed in the laminar flow. 20 ml of nutrient agar was poured in each petri dish and allowed to solidify at room temperature. Mouth of test tube containing bacteria was sterilized by passing it from the burner and sterilized swab was dipped into the test tube. Extra bacterial culture was gently removed by touching the inner wall of the test tube and spread on each petri dish vertically and horizontally. For each bacterium, duplicate of 100 and 200 µl of two petri plates were employed. Petri dishes were covered with the lid and swab was discarded. Afterwards petri dishes were covered with aluminum foil and placed in the incubator for 24 hours so that bacteria could grow on each petri dish.

Disc Diffusion Method

For disc diffusion method, disc of 6mm of Whatmann filter paper were prepared. The discs were dipped into the medicine and allowed them to cool in the laminar flow. Each disc was placed in the petri dish, covered by lid and aluminum foil and were placed in the incubator.

Well Diffusion Method

In well diffusion method, make 8mm of well were made by using mirco-pepitte needles. The medicines were poured into the wells with the help of pipette. Petri dishes were covered by the lid and aluminum foil and placed into the incubator for overnight at the temperature of 37°C.

Statistical analysis

The results were examined by SPSS (IBM statistical package of social science version 20.0). The mean value and standard deviation was calculated by SPSS software. Significant level was set at $P< 0.05$

RESULTS

The seven different mother tinctures known for their medicinal properties of Masood Homeopathic Pharmaceutical Company, (Pvt) LTD were used in this study. The study showed the effects of each medicine against different bacteria with two different methods. The *Ciprofloxacin* was taken as a positive control. The result of ciprofloxacin was good with maximum zone of inhibition. The result against *Escherichia coli* was 32.33±1.45mm in maximum respectively.

Antibacterial Activity through Disc Diffusion Method

Homeopathic mother tincture *Allium cepa*, *Sepia officianalis* and *Thuja* showed maximum % of inhibition against the *Escherichia coli* as 19.33±1.45 mm; 28.33±0.89 mm and 7.66±0.81mm respectively. *Azadirachta indica* and *Lycopodium* against *Staphylococcus* gave 23.66±2.09 mm and 19.66±1.45mm zone of inhibition.

Significant results were observed in all seven mother tinctures in comparison to the positive control against *E. coli*, *Klebsiella* and *Staphylococcus.* From the obtained results it was observed that each of the mother tincture had varying inhibition potential on the tested organisms (Fig **1**).

Table 1. Result of Antibacterial Activity by Disc Diffusion Method.

Medicines		Bacteria		
No		***Escherichia coli (mm)***	***Klebsiella (mm)***	***Staphylococcus (mm)***
1	*Ciprofloxacin*	32.33±1.453	30±1.15	30.33±0.89
2	*Sepia officianalis*	28.33±0.881	0	18.66±1.45
3	*Allium cepa*	19.33±1.45	9.33±0.89	15.33±1.09
4	*Cantharis*	15.33±1.20	4±5.11	11.33±2.45
5	*Azadirachta indica*	12.33±0.881	10±5.77	23.66±2.02
6	*Lycopodium*	13±1.15	13±1.73	19.66±1.45
7	*Echinacea*	9±1.05	15.1±1.15	15±1.73
8	*Thuja*	7.66±0.81	5.33±0.81	1.66±1.20

Results were shown as mean±SEM (n=3) SEM standard deviation.

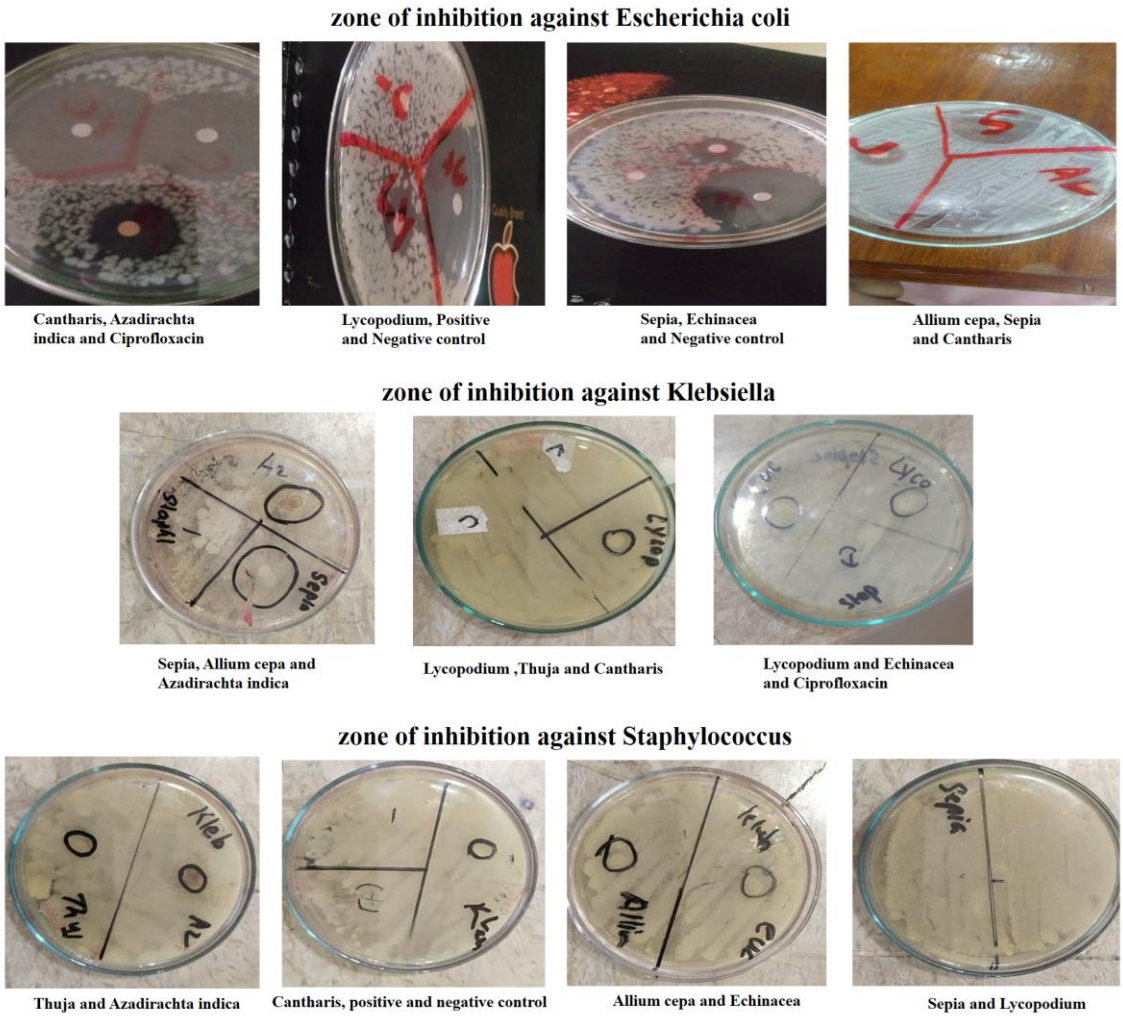

Figure 1. Antibacterial Activity of Homoeopathic Mother Tinctures.

Table 2. Result of Antibacterial Activity by Well Diffusion Method.

S.No.	Medicines	*Escherichia (mm)*	*Klebsiella (mm)*	*Staphylococcus (mm)*
1	Ciprofloxacin	35±1.52	29.33±1.20	27.66±1.45
2	*Sepia officianalis*	23.66±2.02	0	15±1.15
3	*Allium cepa*	22.66±2.18	8.66±1.45	9±1.7
4	*Cantharis*	17.66±1.45	6.33±1.20	5.66±1.76
5	*Azadirachta indica*	15.66±1.85	6.04±1.89	17.66±1.89
6	*Lycopodium*	13.33±1.45	5.33±1.45	13±1.53
7	*Echinacea*	8.66±0.89	7.33±1.78	5.33±0.86
8	*Thuja*	5.33±1.45	0	4.66±1.64

Results were shown as mean±SEM (n=3). SEM standard deviation

Antibacterial Activity by Well Diffusion Method

In well diffusion method the medicines showed result against different bacteria as shown below in Table **2**. The *Sepia officianalis* medicine showed maximum result against *Escherichia* as 23.66±2.02 mm and *Allium cepa* against *Klebsiella*as 8.66±1.45 mm in Table **2**.

DISCUSSION

Urinary tract infection is the third most common infections proficient by human after respiratory and gastrointestinal infection. UTIs commonly occur in any age especially in women of child-bearing mothers. One out of two women suffered from UTIs during their life. Urinary tract can be infected by various bacteria but *Escherichia coli* are the most common cause of urinary tract infection. Various studied showed that increase use of antibiotics develop resistance to uropathogens.[18] High recurrence rates and aggregate antimicrobial resistance threaten the economic burden of these infections. Therefore, it is essential to discover the role of homeopathic medicine to treat urinary tract infection. [19] Maximum number of clinical, vitro and animal studies are reported for the effectiveness of Homeopathic medicines in treatment of urinary tract infection. The drug produces the similar symptoms in the patient as the bacteria produce in the body so the defense mechanism become active and kills the bacteria. [20] Homeopathic drugs are always better than the conventional antibiotics as they are more injurious. Bacteria have become more resistant against the antibiotics drugs. Some people have allergic reactions against these antibiotics such as swelling of face, rashes on the skin meanwhile these antibiotics show side effects that lead to complications such as yeast infections, kidney failure and liver damage. So, further studies are required to systematize their pharmacokinetics and pharmacodynamics. [21]

Efficiency of homoeopathic remedies in different types of urological problems such as in renal calculi,[22] UTI [23] and hypertrophy of prostate.[24] The evidence for *Allium cepa* medicine gave its antibacterial activity against the *Escherichia coli, Staphylococcus, Klebsiella, Pseudomonas, Proteus* and *Salmonella* which was 13, 11, 10, 08, 05, 10 and 09 respectively [25] in support of our findings. Same like *Sepia* against *Aeromonas hydrophila* was 1.716±0.12. [26]

In primary health care system homoeopathic remedies and tincture are performing a significant role in reducing the burden of diseases.[27] As known well that homeopathic mother tinctures are being utilized for antimicrobial purpose. Now a day the homeopathic remedies are demonstrating quick and positive response to cure as compared to other treatments.[28]

CONCLUSION

This study showed the efficiency of Homeopathic medicines especially *Allium cepa, Thuja occidentalis, Echinacea purpurea, Lycopodium clavatum, Azadirachta indica, Cantharis* and *Sepia* in its mother tincture form confine the bacterial growth. In many studies, it showed that homeopathic medicines cure many types of bacterial infections. Homeopathic medicines can control the UTIs infections and also helps in reducing the use of antibiotics and their complications. It is shown that alternative medicines are the best system that kill the microbes and protect the immune system of the body. However, there is further need of research, to know the pharmacokinetics and pharmacodynamics of these homeopathic medicines.

ETHICAL APPROVAL

Not applicable

FUNDING SOURCE

None.

CONFLICT OF INTEREST

There is no conflict of interest

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