

Antibacterial Activity of Homeopathic Mother Tincture *Aesculus hippocastanum*

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ABSTRACT

Objectives: Various antimicrobial are used widely in health practice to rheostat the growth of microorganism on living tissues. They are vital parts of infection control practices, especially in wound treatment and assistance in the prevention of infections. The current research was done to evaluate and compare the antibacterial effects of ciprofloxacin and *Aesculus hippocastanum* homeopathic mother tinctures via well diffusion method. The objective of this study was to estimate *in vitro* antibacterial effects of homeopathic mother tincture.

Materials and Methods: The experimental homeopathic mother tincture was donated by Masood Homeopathic Pharmaceuticals, Pakistan. *Pseudomonas aeruginosa* (ATCC 9027) and *Salmonella typhus* were taken from American Type Culture Collection (ATCC). Ciprofloxacin (standard antibiotic) 1 mg/ml was taken as positive control and alcohol 70% was taken as negative control group.

Results: Ciprofloxacin expressed maximum activity against *Pseudomonas aeruginosa* among all tested bacteria. *Aesculus* showed zone of inhibition 9 mm for *Pseudomonas aeruginosa* and 11 mm against *Salmonella typhus*.

Conclusion: *Aesculus hippocastanum* showed higher effective results against *Pseudomonas aeruginosa* and *Salmonella typhus*.

Keywords: *Aesculus*, antibacterial, mother tincture.

Authors' Contributions

1,6 Conception & Study Design, Data Collection, Critical Review.

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INTRODUCTION

The drugs derived from plant origin play a valuable role to reduce or prevent serious diseases in developing countries. In world 60-80 % population use long established medicines for the treatment of common diseases [1, 2]. In past natural products are used for the ailment of human diseases. Natural plant products field collections of which they have yield extract with antimicrobial, antifungal and antiviral activities [3, 4]. The plants have variety of chemical

compounds which are present in their roots, leaves and flowers, in all over the world. They are used for the treatment of thousands of human diseases. A lot of plants have been used by Turkey nationals for the treatment of various disorders, including microbial infections for emetic and strengthening effects and for increasing urine output and reducing stress [5, 6].

The branch of medical science deals with the diagnosis and treatment of infectious agents is called microbiology. That agent that kills microbes or stops their growth is called antimicrobial agents.

Microorganisms produce antibiotics partially or fully prepared by chemical synthesis [7-9]. In minute concentration these antibiotics inhibit the growth of microorganisms. The microbial origin antibiotics are purely synthetic and semi-synthetic [10, 11].

In the last three decades, there are a number of new antibiotics and are becoming resistance to these drugs by microorganisms has increase. The bacteria have power to transmit and also have resistance against the drugs, which are utilized as therapeutic agent. As the cause is concerned, in the hospital the number of patients have suppressed immunity due to bacteria stain which is multi resistance. Bacteria develop resistance against microorganisms, due to this resistance multiple infectious diseases are produces. Moreover, the antibiotics produce bad effects on patients. Homeopathic system based upon "law of similar" which means likes cure likes. Homeopathic cures take place because use of the high potency of similar remedy in small [12-14]. In homeopathic system selected similar remedy to the disease, which produce similar artificial disease in patient, the medicinal disease requires life force to remove similar artificial disease and return the organism to complete recovery. The artificial disease show in the foam of sign and symptoms both mental and physical, than physician should selected one similar remedy according the signs and symptoms which is the curative remedy [15].

Aesculus hippocastanum belongs to a family Sapindaceae is a species of flowering plant. It is a tall tree known as Horse-chestnut or Conker tree. Horse-chestnut trees are found in Albania, Bulgaria, 1. Romania and Serbia, and also grow worldwide. 2. Horse-chestnut is not edible sweet chestnut. The raw form of chestnut have poisonous effects on the body, but after removal of toxic effect it is use for treatment [16, 17].

Chestnut used to treat the backache, hemorrhoids, whooping cough, rheumatism, gynecological bleeding and arthritis. Different parts of the chestnut plant are used for treatment which includes seeds, bark and leaves. The seeds of chestnut used as analgesics, anti-pyretic, toxic and vasoconstrictor. The bark is use for induce sneezing. The flowers of chestnut use for constriction of skin cells and other body tissues [18, 19].

Antibacterial drugs are useful in killing bacteria and other microorganisms, it also stop the multiplication of bacteria [20-22].

METHODOLOGY

The antibacterial activity was performed in microbiology lab of Khawaja Freed campus The Islamia university of Bahawalpur, Pakistan. All the selected mother tincture was purchased by Masood Homeopathic Pharmaceutical Private Limited Lahore Pakistan. To perform work in the lab it is necessary to select the chemicals and instruments for the search work. While considering these points, the following chemicals (as shown in Table 1) were selected to perform antibacterial activity of homeopathic mother tincture of *Aesculus hippocastanum*. *Pseudomonas aeruginosa* were provided by the Institute of Agriculture Sciences, the University of Punjab, Lahore. *Salmonella typhus* was provided by the Frist Fungal Culture Bank of Pakistan (FCBP) [23, 24].

Table 1. Drugs and batch numbers of medicines.

Drugs	Company	Batch Number
<i>Aesculus hippocastanum</i>	Masood Pharmaceutical	41597
Ciprofloxacin	SAMI Pharmaceutical (Pvt.) Ltd. F-95, S.I.T.E, Karachi, Pakistan	P000388

Media

For conducting the antibacterial activity following media were used.

Nutrient Broth Media.

Nutrient Agar Media.

Preparation of Broth Media

The sterilization was done at 121°C at 15 pound per square inch for 15 minutes by autoclave. Dissolve 13 g nutrient broth in 1 L distilled water.

Preparation of Agar Media

After sterilization at 121°C for 15 minutes at 15 pounds per square inch in autoclave dissolve 28 g nutrient agar in 1 L distilled water.

Preparation of Inoculums

After preparing McFarland by mixing of 0.5 ml of 1.175% w/v barium chloride and 85 ml of 1% v/v sulfuric acid. Optical density of this solution was checked at 625 nm and absorbance should be within

the range of 0.08-0.10. From 24 hours, old culture all inoculum were prepared. Then take few colonies and introduce it into 5 ml normal sterile solution and adjust the turbidity to 0.5 McFarland turbidity standard equivalents to cell density of $1-5 \times 10^8$ CFU/ml.

Agar Well Diffusion Method

The procedure of well diffusion method was performed by using pour plate technique.

Pour Plate Technique

The test was performed in triplicate, sterilized petri plates (10 cm) and 0.5 ml inoculated broth media was poured into each petri dish and then 15 ml of nutrient agar were poured in each of them in laminar air flow to avoid contamination. The petri dishes were then placed at room temperature to solidify agar media. After solidification petri plates were placed in invert position to prevent from condensation.

Preparation of Well

After solidification, sterile end cork borer (6 mm diameter) was used to bore a well in the medium in each petri plate. All the petri dishes were labeled with the name of bacteria strains which were being inoculated.

Spread of Bacteria Through Streak Method

Take cotton bud and dip it into selected bacteria and spread bacteria on all petri dished by streak method in three dimensions.

Pouring in Well

With the help of micro-pipette, the wells were filled with selected mother tincture and ciprofloxacin (as positive control). Then plates were inoculated for 24 hours at 37°C.

Measurement of Antibacterial Activity

After 24 hours of incubation at 37°C, all the petri plates were observed for zone of inhibition and the diameters of zones were measured in millimeters with a ruler. Result was expressed by using S.E.M (Standard Error of Mean) and SPSS was used for analysis of significance.

Statistical Analysis

The results were statistically analyzed by using SPSS software using version 20.

Determination of Antibacterial Activity of Mother Tincture of *Aesculus hippocastanum*

The present study was concern with the antibacterial activity medicinal plant mother tincture of *Aesculus hippocastanum*. The activity was performed by using well diffusion method as explained before. After 24 hours of incubation the petri dishes were examined for the zone of inhibition of selected mother tincture and ciprofloxacin as positive control against selected bacteria (*Pseudomonas aeruginosa* and *Salmonella typhus*) in millimeter with the help of scale.

Maximum inhibitory concentrations of different drugs against the tested organisms are given below in Table 2.

Table 2. Antimicrobial activity of test drugs against microorganisms.

S. No.	Sample tested	Zone of inhibition against P.A (mm)	Zone of inhibition against S.T (mm)
1	<i>Aesculus hippocastanum</i>	9 mm	11 mm
2	Ciprofloxacin	15 mm	19 mm

RESULTS

Table 2 shows the broad antibacterial activity of *Aesculus hippocastanum* against two bacteria *Pseudomonas aeruginosa* and *Salmonella typhus* ranging 15-19 mm. Ciprofloxacin had a maximum activity against *Pseudomonas aeruginosa* measured 15 mm and against *Salmonella typhus* measured 19 mm.

In case of *Aesculus hippocastanum*, the mother tincture inhibited the growth of bacteria *Pseudomonas aeruginosa* and *Salmonella typhus* used in study. *Aesculus hippocastanum* had a maximum activity against *Pseudomonas aeruginosa* measured 9 mm and higher activity 11 mm measured against *Salmonella typhus*. The above results showed that the homeopathic mother tinctures are potent enough to inhibit the growth of bacteria.



Figure 1. Antibacterial activity of *Aesculus hippocastanum* and ciprofloxacin against *Salmonella typhus*.

AHG = *Aesculus hippocastanum* (Germany),
CIPRO = ciprofloxacin, **ST** = *Salmonella typhus*.

DISCUSSION

It is a miracle that the homeopathic drugs are inhibiting the growth of bacteria because homeopathic drugs are potent enough. The homeopathic mother tincture of *Aesculus hippocastanum* show moderate zone of inhibition against all the bacteria are tested.

Overall studies made a conclusion that the homeopathic drugs not act against the bacteria. The homeopathic drugs are symbiotic (similar to bacteria) [25]. The homeopathic drugs produce similar symptoms in the patient as produce by the bacteria so the defense mechanism of the patient become active and kill the bacteria. In this study the prepared mother tincture of *Aesculus hippocastanum* of two different brands were used. The entire mother tincture showed activity against all selected bacteria. We have compared mother tincture of *Aesculus hippocastanum* of two different brands both were show activity. Then we compared mother tincture with allopathic drug ciprofloxacin for the positive control [26].

CONCLUSION

The ciprofloxacin show more results but homeopathic mother tincture also show considerable results [27]. When all the results are compared *Aesculus hippocastanum* show higher effective result against *Pseudomonas aeruginosa* and *Salmonella typhus* as shown Figure 1. So the present studies justified that plant origin homeopathic remedies treat various infectious diseases caused by gram negative and gram positive bacteria [28]. Thus in homeopathy the remedies have potential to strong the person's immune system and help the person to fight with

bacterial and viral infections. The homeopathic drugs are always preferable over the conventional antibiotics. Conventional antibiotics are more harmful. Over use of antibiotics increase the bacterial infection because bacteria develop resistance to the antibacterial drugs. Some people develop allergic reaction against antibiotics, some reaction include swelling on face, rashes and difficulty in breathing. Some allergic reactions of antibiotics are very serious and sometime become fetal to the peoples. The overuse of antibiotics killed the normal flora of our body. Over use of antibiotics sometimes lead to complications like yeast infection. Severe side effects of antibiotics include damage to the kidney, liver, bone marrow and other tissues [29, 30]. So, further studies are required to standardize their pharmacokinetics and pharmacodynamics profile.

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