

ANTIBACTERIAL ACTIVITY OF VARIOUS HOMEOPATHIC MEDICINES AGAINST DIFFERENT BACTERIAL STRAINS



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Science in Zoology

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CERTIFICATE

This is to certify that the project report entitled “**ANTIBACTERIAL ACTIVITY OF VARIOUS HOMEOPATHIC MEDICINES AGAINST DIFFERENT BACTERIAL STRAINS**” submitted by Ms. Anna Silfa Sebastian, Reg No: AB20ZOO002 in partial fulfilment of the requirement of Bachelor of Science degree of Mahatma Gandhi University, Kottayam, is a bonafide work under my guidance and supervision and to my best knowledge, this is her original effort.

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EXAMINERS

1)

2)

DECLARATION

I, hereby declare that this project work entitled “ANTIBACTERIAL ACTIVITY OF VARIOUS HOMEOPATHIC MEDICINES AGAINST DIFFERENT BACTERIAL STRAINS” is submitted to St. Teresa’s College (Autonomous), Ernakulam affiliated to Mahatma Gandhi University, Kottayam in partial fulfilment of the requirement of Bachelor of Science degree in Zoology. This work has not been undertaken or submitted elsewhere in connection with any other academic course and the opinions furnished in this report are entirely my own.

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ABSTRACT

Homeopathy is a therapeutic method, first developed by Samuel Hahnemann (1755–1843) and is now practiced throughout the world. Though controversies exist regarding the efficacy of homeopathic substances; however, these remedies are used in many countries for the treatment of various pathological conditions. The purpose of this study was to evaluate the in vitro antibacterial activity of six homeopathic tinctures (Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200 and Bryonia alba 200) against different bacterial strains inhabiting respiratory tract (*Klebsiella*, *Staphylococcus aureus*, *Streptococcus iniae*) and intestinal tract (*Escherichia coli*, *Vibrio parahaemolyticus*, *Enterococcus*). Antibacterial properties of the homeopathic drugs were evaluated by Kirby Bauer disc diffusion method. Homeopathic medicines exhibited inhibition zones ranging from 0.2 cm to 2.5cm. The highest zone of inhibition was exhibited by Natrum sulphuricum 200 (2.5cm) against *E. coli*. The lowest zone of inhibition was exhibited by Nux vomica against *St. aureus* (0.2cm). The bacterial strains *E. coli*, *Klebsiella*, and *Streptococcus iniae* were sensitive to all the medicines with significant zones of inhibition. *Vibrio parahaemolyticus* was found to be the most resistant bacteria since no zone of inhibition was created by any of the medicines. *Enterococcus* was resistant to all the medicines except phosphorus 200.

INTRODUCTION

The system of homeopathic medicine introduced by Samuel Hahnemann (1755–1843) is based on the Law of Similars, which suggests that any substance having a capacity of producing disease in its crude form also has the capacity to treat a similar disease if administered in a very small dose. The functioning of the homeopathic system of medicine is comparable in some respects with hormesis and vaccination (though they differ in their modes of application), and also it involves sourcing the drugs from biological materials, including live and inactivated organisms their isolates, or diseased materials. (Renuka et al., 2022).

Many homeopathic drugs are now in use against common bacterial diseases. Although, mechanism of action of these medicines is not yet identified, they are claimed to be potent antibiotic or similibiotic (Soyoda et al., 2018). The purpose of this study was to evaluate the in vitro antibacterial activity of six homeopathic medicines (Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200 and Bryonia alba 200) against six different bacterial strains (*Escherichia coli*, *Klebsiella*, *Vibrio parahaemolyticus*, *Enterococcus*, *Staphylococcus aureus* and *Streptococcus iniae*). All the selected drugs are conventionally used for the treatment of various types of diseases including infections in respiratory tract and digestive tract. Homeopathic medicines are made from a variety of plant, mineral or animal substances (Istiak et al., 2021). Of the six homeopathic drugs tested, Nux vomica 200, Lycopodium 200 and Pulsatilla 200 were the medicines commonly prescribed for intestinal disorders. Nux vomica is an effective homeopathic remedy which is made from the strychnine containing seeds of Nux vomica plant (poison nut). It is said to have a wide range of action in gastric troubles and stomach infections. Lycopodium 200 is a widely used medicine for the treatment of liver, urinary and digestive disorders. It is derived from a fungus *Lycopodium clavatum*. Lycopodium 200 is believed to have its main action on

respiratory tract, mucous membrane, genitourinary tract, kidney, liver & digestive tract. Pulsatilla 200 is a homeopathic remedy used to treat a wide range of health problems such as gastric disorders, insomnia, conjunctivitis, abdominal cramps etc. It is prepared from a plant, *Pulsatilla vulgaris*, it contains a substance called ranunculin, which is converted to anemonin when the plant is dried.

The remaining 3 tinctures were the drugs commonly prescribed for respiratory ailments. Natrum sulphuricum 200 is an effective homeopathic remedy against chronic asthma and other respiratory diseases. It is also a liver remedy. It is made of Sulphate of Sodium (Glauber's salt). Phosphorus 200 is a homeopathic drug used for the treatment of respiratory disorders such as excessive cough, bronchitis and asthma. The main ingredient of this dilution is phosphorus. It relieves congestion in chest and helps in improving breathing. Phosphorus 200 is helpful in treating hepatitis, pneumonia etc. Bryonia alba 200 is a drug that is prepared from Bryonia plant. The root is the part of the plant used in homeopathic healing. It is commonly used for cold and flu-like illnesses, spasmodic cough, and rheumatic pains, as well as to treat acute abdominal situations like gastroenteritis, diarrhoea, nausea, and vomiting.

REVIEW OF LITERATURE

Homoeopathy was developed in the late 1700s by Samuel Hahnemann (1755–1843), a German physician and chemist. Hahnemann argued that it was possible to restore health by stimulating the body to regain its balance. He said that it could be done by administering substances that provoked the same signs and symptoms as the disease. He called this the “similia principle” or “like cures like” (Michael McCarthy, 2005). The word homoeopathy is derived from the Greek words homoios and pathos which means ‘similar illness’ that refers to the use of drugs to treat illnesses that can induce similar effects in healthy patients when ingested. Homeopathic medicines are made from a variety of plant, mineral or animal substances and were developed as an alternative medicine (Istiak et al., 2021).

Today the world is facing a threat of losing battle to micro-organisms in the wake of growing indiscriminate use of Antibiotics. Antibiotics are targeted at killing microorganism without any attempt to support and guide the immune system. But homeopathic literatures show that infective diseases are very well handled with homeopathic drugs (Nisanth et al., 2017). Earlier it was presumed that the homeopathic medicines would induce a therapeutic immune response only, if administered to the host. Research by other scientists has shown antimicrobial effects against malaria (Bagai et al., 2014) and *H. pylori* (Gosavia et al., 2012)).

Homeopathic medicines are widely used over the world for different disease conditions. Approximately 70% are derived from plants. (Prajapati et al., 2019). Homeopathic mother tinctures of plant origin are the hydroalcoholic extracts of medicinal plants, with some difference of ratio of alcohol and medicinal plants. The

extracts of plant source contain secondary metabolites including glycosides, polyphenols, flavonoids, saponins, alkaloids and various others. They can significantly produce antioxidant, antimicrobial, anti-diabetic, anti-inflammatory, antipyretic and various other therapeutic effects (Tayyebba et al., 2021).

Resistance to antibiotics is a major public health concern worldwide and often lead to the quest of alternative medicine to microbial diseases. Antibiotic resistance results in reduced efficacy of antibacterial drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality (Aslam B et al., 2018). Many homeopathic drugs are now in use against common bacterial diseases (Soyoda et al., 2018). This study evaluates the efficacy of six commonly prescribed homeopathic medicines against some bacteria residing in the respiratory tract and intestinal tract.

METHODOLOGY

MATERIALS REQUIRED

Nutrient agar-agar, nutrient broth, distilled water, filter paper, petri plates, conical flasks, test tubes, forceps, cotton plug, sterile swab, autoclave, weighing machine, measuring cylinder, nichrome loop, alcohol, newspaper, ruler, paper, pen etc.

HOMEOPATHIC MEDICINES:

Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200, Bryonia alba 200

BACTERIAL STRAINS:

Staphylococcus aureus, *Escherichia coli*, *Klebsiella*, *Streptococcus iniae*, *Enterococcus*, *Vibrio parahaemolyticus*

NUTRIENT BROTH CULTURE

1.3 g of nutrient broth was weighed. It was added to 100 ml distilled water and mixed well. The broth was prepared in 100 ml conical flask, and it was sterilized by autoclaving for 15 minutes and cooled to room temperature. The broth was then poured into sterilized test tubes (each test tube containing 5 ml broth) and closed using a cotton plug.

INOCULATING THE BROTH

The nutrient broth was inoculated. The cotton plug of both the stock culture to be inoculated was loosened, and then the inoculating loop was flamed to red hot and cooled by waving for few seconds. The cotton plug from the stock culture tube was removed and the mouth was flamed. The cooled sterilized loop was inserted into the culture tube carefully without touching the sides to prevent contamination. A visible amount of the culture was scrapped and removed using the loop and mouth of the tube was plugged back carefully after flaming. By the same procedure, the cap was introduced into the broth using the loop. Tube mouth was flamed and recapped after the loop has been withdrawn. The inoculating loop was then resterilized and the broth culture was gently rotated for the proper mixing of its content. The contents in each test tube were labelled with names of respective microbes and the date was noted. For sufficient bacterial growth, the inoculum was kept for 2-6 hours of incubation.

PREPARATION OF NUTRIENT AGAR (CULTURE MEDIA)

The medium was prepared using 1.3g of nutrient broth and 2 g of nutrient agar. At first, both nutrient broth and agar was weighed out and was made upto 300 ml using distilled water. It was poured into a conical flask and sterilized for 15 minutes in an autoclave at 15 psi. the medium was allowed to cool to an ear bearing heat -15 degree celsius. Cooled agar was poured into petri dishes and waited till it got set. It was then kept upside down. These petri dishes were used for the study.

PREPARATION OF FILTER PAPER DISC

Filter paper disc was prepared using a punching machine and sterilized using autoclave. The disc was then soaked in the extracts for specific time and was used for anti microbial sensitivity tests.

METHOD

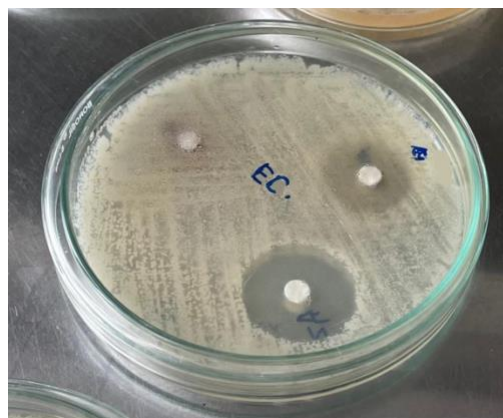
The method used for antibacterial sensitivity was Kirby Bauer disc diffusion method. A lawn culture of each bacterium was prepared using sterilized cotton swabs. A sterilized swab was dipped into the bacterial suspension and moved side to side from top to bottom leaving no space uncovered. The plate is rotated to 90 degree and the same procedure was repeated so that entire plate was coated with bacteria. This procedure was followed for plating all the six different strains of bacteria. Once the lawn had been prepared, the sterilized filter paper impregnated with the medicines to be tested was placed on the plate. This plate was incubated at 37 degree C for 48 hrs. Name of the bacteria was labelled on each plate and was examined for sensitivity (zone of inhibition). The radius of each zone was measured using a standard ruler in centimeters. If the compound is effective against bacteria at certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition, which is a measure of the compound effectiveness, the larger the clear area around the filter paper, the more effective the compound

KILLING OR DISPOSING:

After the experiment, the bacteria are destroyed by autoclaving the plate for 20 minutes. All the glasswares used for the experiment were also autoclaved to remove any bacteria if present.

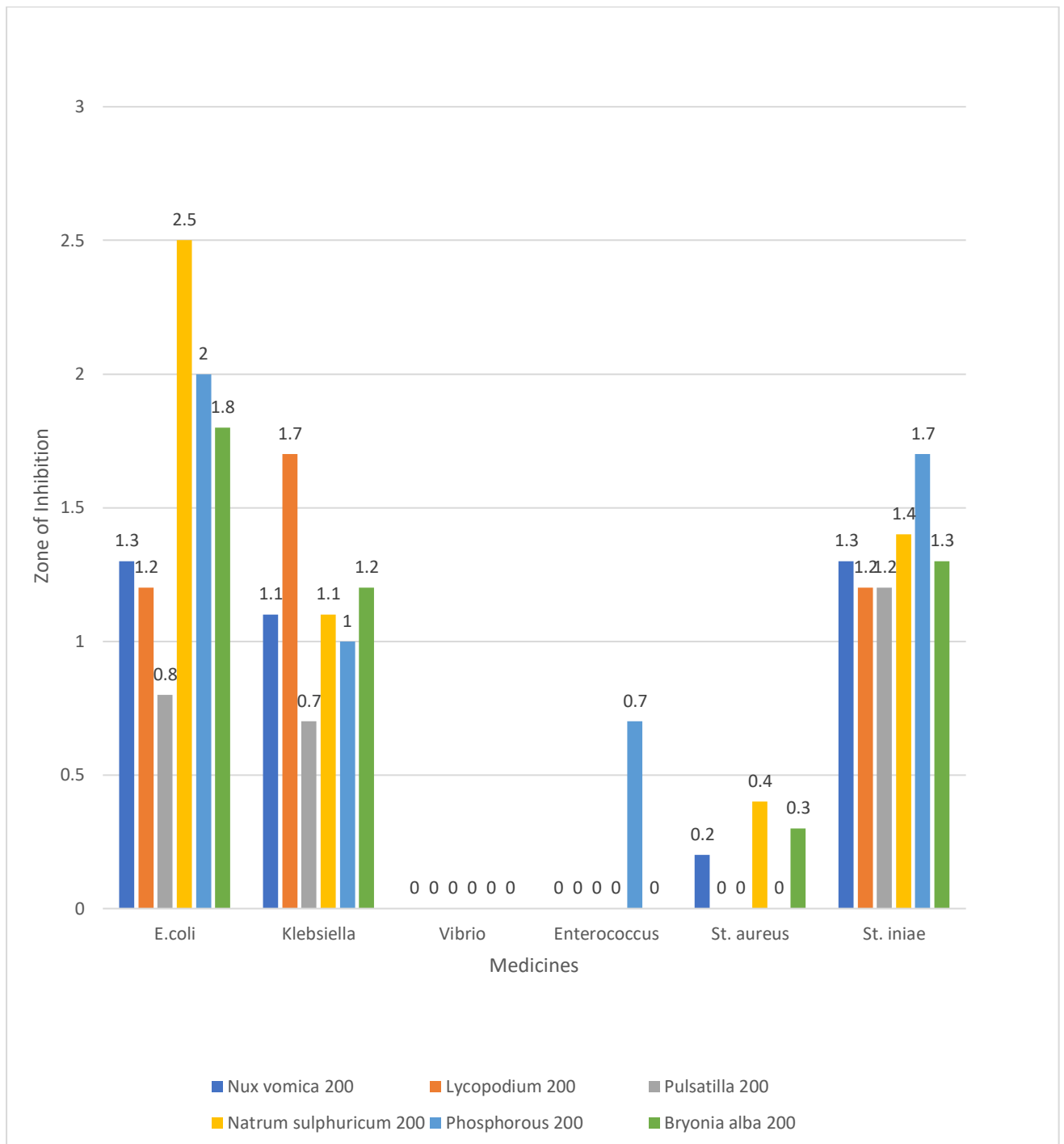
Medicines	Zone of inhibition (diameter in cm)					
	Gram negative bacteria			Gram positive bacteria		
	<i>Escherichia coli</i>	<i>Klebsiella</i>	<i>Vibrio parahaemolyticus</i>	<i>Enterococcus</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus iniae</i>
Nux vomica 200	1.3	1.1	0	0	0.2	1.3
Lycopodium 200	1.2	1.7	0	0	0	1.2
Pulsattilla 200	0.8	0.7	0	0	0	1.2
Natrum sulphuricum 200	2.5	1.1	0	0	0.4	1.4
Phosphorus 200	2	1	0	0.7	0	1.7
Bryonia alba 200	1.8	1.2	0	0	0.3	1.3

OBSERVATION

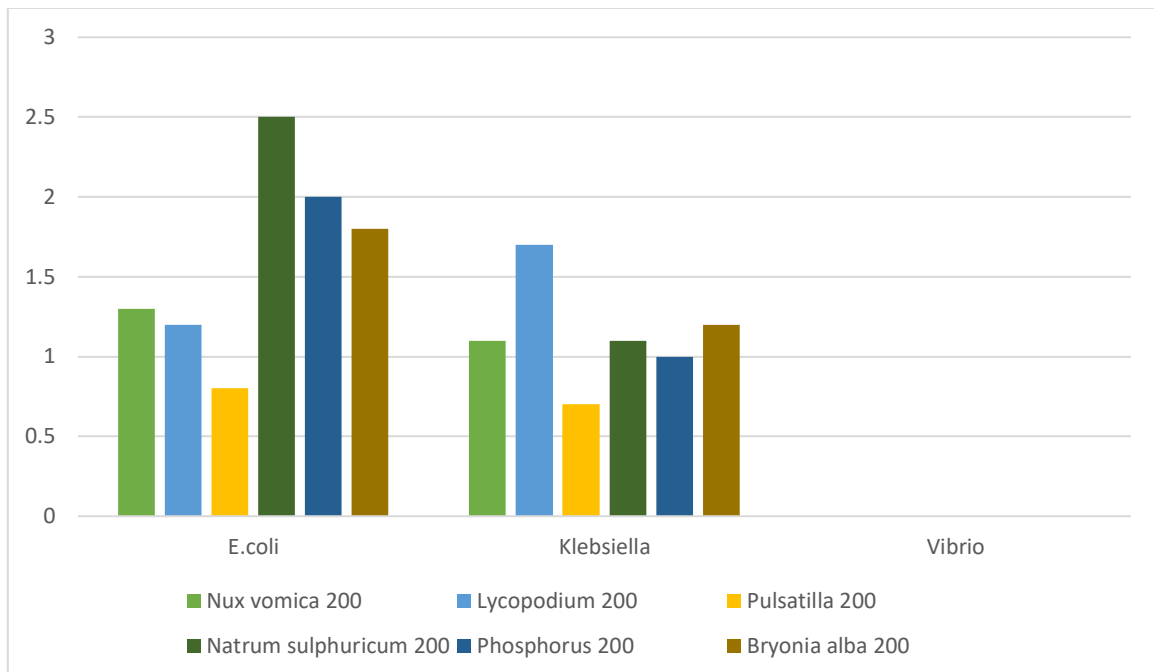


Zone of inhibition shown by different medicines

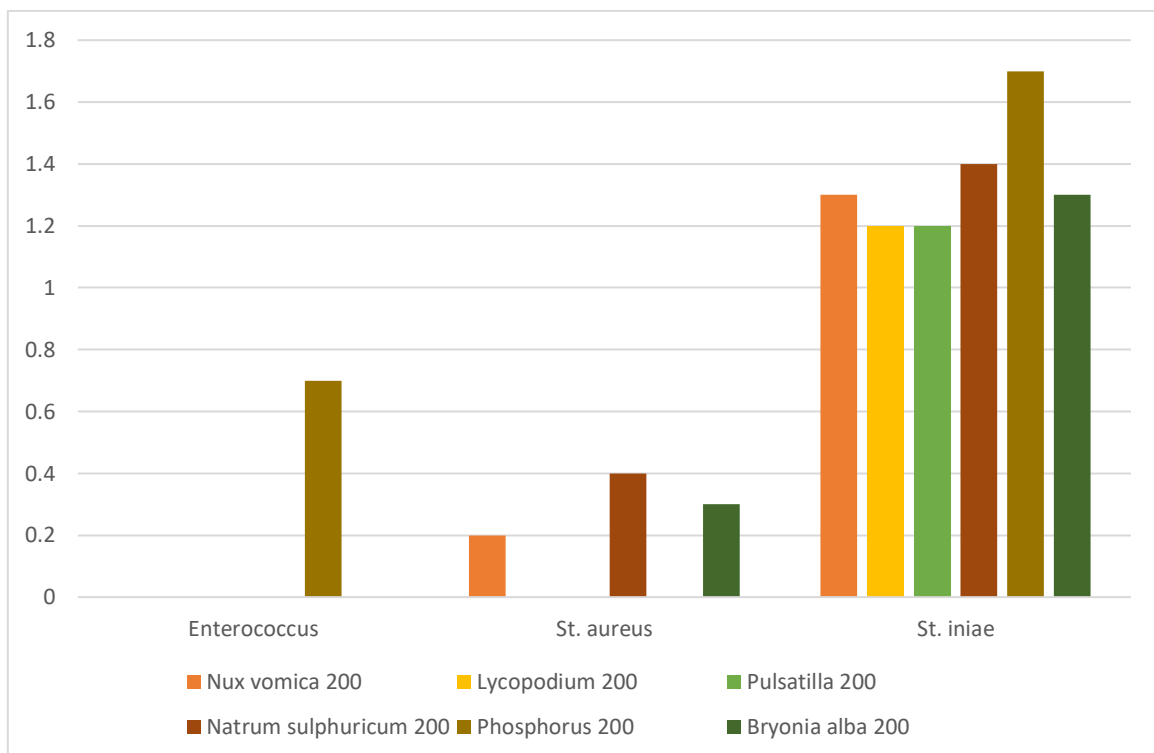
ZONE OF INHIBITION SHOWN BY HOMEOPATHIC MEDICINES AGAINST DIFFERENT BACTERIAL STRAINS



ZONE OF INHIBITION SHOWN BY HOMEOPATHIC MEDICINES AGAINST GRAM-NEGATIVE BACTERIA



ZONE OF INHIBITION SHOWN BY HOMEOPATHIC MEDICINES AGAINST GRAM-POSITIVE BACTERIA



RESULT

The objective of this study was to evaluate the antibacterial activity of different homeopathic medicines (Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200 and Bryonia alba 200) against 6 different bacterial strains (*Escherichia coli*, *Klebsiella*, *Vibrio parahaemolyticus*, *Enterococcus*, *Staphylococcus aureus* and *Streptococcus iniae*). Homeopathic medicines exhibited inhibition zones ranging from 0.2 to 2.5cm.

Among the intestinal bacteria tested, *Vibrio parahaemolyticus* was resistant to all the medicines including the medicines used for intestinal infections. But in case of *Enterococcus*, Phosphorus 200 inhibited the growth even though it is a respiratory remedy. In contrast to the other two intestinal bacteria, *E. coli* was sensitive to all the medicines irrespective of whether it is a medicine for respiratory or intestinal infection. Natrum sulphuricum, which is an effective remedy for respiratory infections, exhibited highest zone of inhibition against *E. coli*. Another respiratory remedy, Phosphorus 200 also exhibited highest activity against *E. coli*.

In case of bacteria causing respiratory infections, *Streptococcus iniae* and *Klebsiella* were sensitive to all the medicines. But another respiratory bacterium, *Staphylococcus aureus* was sensitive only to three medicines of which two were respiratory remedies. Nux vomica, which is a drug used for digestive disorders also exhibited an inhibition zone of 0.2cm against this respiratory bacterium. Nux vomica was found to be effective against all the three tested bacteria that are causing respiratory infections. It showed similar zones of inhibition (1.3cm) against *E. coli* and *Streptococcus iniae*. Lycopodium 200 also exhibited similar effects (1.2cm) against these bacterial strains.

An inhibition zone of 1.1 cm was created by both Nux vomica and Natrum sulphuricum against *Klebsiella*. Also, Lycopodium 200 and Pulsatilla 200 exhibited similar zones of inhibition against *St. iniae* (1.2 cm).

Of the homeopathic medicines used for the study, the highest significant zone of inhibition was exhibited by Natrum sulphuricum 200 (2.5cm) followed by Phosphorus 200 (2cm) against *E. coli*. The lowest zone of inhibition was exhibited by Nux vomica against *St. aureus* (0.2cm). The bacterial strains *E. coli*, *Klebsiella*, and *Streptococcus iniae* were sensitive to all the medicines with significant zones of inhibition. But in case of *Staphylococcus aureus*, most of the medicines showed comparatively lesser effects. *Vibrio parahaemolyticus* was found to be the most resistant bacteria since no zone of inhibition was created by any of the medicines. *Enterococcus* was resistant to all the medicines except phosphorus 200. *E. coli* was the most sensitive bacteria.

DISCUSSION

In the present study, six homeopathic drugs (Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200, Bryonia alba 200) were tested against different bacterial strains inhabiting intestinal tract (*E. coli*, *Vibrio parahaemolyticus*, *Enterococcus*) and digestive tract (*Klebsiella*, *St. aureus*, *St. iniae*)

Homeopathy is based on two main principles. According to the 'like cures like' principle, patients with particular signs and symptoms can be helped by a homeopathic remedy that produces these signs and symptoms in healthy individuals. According to the second principle, homeopathic remedies retain biological activity after repeated dilution and succussion even when diluted beyond Avogadro's number. (Ernest., 2002).

Nux vomica which is an effective remedy for digestive disorders exhibited action against *E. coli*, *Klebsiella*, *St. aureus* and *St. iniae* which was in accordance with a previous study (Louds et al., 2015). Among the 3 intestinal bacteria tested, Nux vomica was effective only against *E. coli*. In an earlier study by Gnanavel et al., (2012), Nux vomica inhibited only the growth of gram-positive bacteria. But in our study, it exhibited zones of inhibition against both gram-positive and gram-negative bacteria, which was in accordance with the study conducted by Loud et al., 2015. The medicinal properties of Nux vomica are substantially due to the abundance of alkaloids strychnine and brucine (Maji et al., 2017).

In case of *Klebsiella* which is a causative organism of urinary tract infections, the highest zone of inhibition (1.7cm) was exhibited by Lycopodium 200. It is a widely used homeopathic medicine for the treatment of liver, urinary and digestive disorders. It is derived from the Fungus, *Lycopodium clavatum*. Lycopodium 200 was found to be the most appropriate homeopathic medicine against urinary tract infections in a previous study (Deepen et al., 2020). It is rich in alkaloids

with high toxicity (Ayer W., 1991). This contributes to the antimicrobial activity of lycopodium extracts (Ainge et al., 2002).

Of the six homeopathic tinctures tested, Natrum sulphuricum exhibited the highest zone of inhibition against *E. coli* (2.5cm) which is an intestinal bacteria. It is a homeopathic drug against chronic asthma and other respiratory diseases. Natrum sulphuricum is called the water eliminating tissue salt. This tissue salt is essential for the proper digestion of sugars and starches and for the effective withdrawal of water from the cells. (Shah HK et al., 2020).

Another respiratory remedy, Phosphorus 200 also exhibited highest activity against *E. coli*. It is a homeopathic composition used for the treatment of a wide range of issues such as anxiety, Alzheimer's disease and respiratory disorders such as bronchitis and asthma. The main ingredient of this dilution is phosphorus. Organic salts of phosphorus are diluted numerous times until it reaches the desired dynamization (Cassiane et al., 2018). It relieves congestion in the chest and helps in improving breathing.

Pulsatilla 200 which is a homeopathic medicine used to treat a wide range of health problems such as gastric disorders, conjunctivitis, abdominal cramps etc. was more effective against *St. iniae*. It is prepared from a plant *Pulsatilla nigricans*. It contains a substance called ranunculin, which is converted to anemonin when the plant is dried. This is the medicinally active component. It is comprised of flavonoids, tannins, carbohydrates, glucoside pulsatoside, triterpene, saponins and steroids (Goyal et al., 2011). Antibacterial activity of *Pulsatilla nigricans* may be due to the presence of flavonoids and triterpenoids. A previous study showed that protoanemonin from *Pulsatilla nigricans* is the main constituent responsible for its antimicrobial activity (Holden et al., 1947).

Bryonia alba 200 is an effective remedy commonly used for the treatment of cold and flu-like illnesses, spasmodic cough, and rheumatic pains

as well as to treat acute abdominal conditions like gastroenteritis, diarrhoea, nausea and vomiting. It showed highest activity against *E. coli*. In a previous study conducted by Ajmed et al., (2017) Bryonia alba extracts showed highest activity against *E. coli* which is in accordance with our study. In this study the assumption is that a mixture of active components in the extract contributes to the antimicrobial effects.

CONCLUSION

Antibacterial activity of six commonly prescribed homeopathic medicines (Nux vomica 200, Lycopodium 200, Pulsatilla 200, Natrum sulphuricum 200, Phosphorus 200, Bryonia alba 200) were tested against different gram negative (*E. coli*, *Klebsiella*, *Vibrio parahaemolyticus*) and gram positive (*Enterococcus*, *St. aureus*, *St. iniae*) bacteria using Kerby Bauer disc diffusion method. Homeopathic medicines exhibited considerable activity against the tested bacterial strains. They showed zones of inhibition ranging from 0.2 cm to 2.5 cm. The bacterial strains *E.coli*, *Klebsiella* and *Streptococcus iniae* were sensitive to all the medicines with significant zones of inhibition. But *Vibrio parahaemolyticus* was resistant to all the medicines.

From the present study we can conclude that homeopathic medicines are effective in treating bacterial infections. These medicines have the ability to boost the immune system as well. Hence it can be used as an effective alternative against antibiotics.

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