SVOA Microbiology

ISSN: 2634-534X

ScienceVolks Open Access

Research Article

Determination of the Inhibitory Effects of Commercially Available Homeopathic Drugs on Pathogenic Bacterial Growth

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***Received:** April 06, 2021 **Published:** April 16, 2021*

Abstract:

Antibiotic resistance is a significant public health issue around the world. New treatment options are needed to combat the situation and homeopathy is thought to be an option. Present study was conducted to evaluate the efficacy of homeopathic drugs as a suitable alternative to antibiotics in the elimination of bacterial infections. In this regard, four diluted homeopathic drug samples such as Apis mellifica, Graphites, Arsenicum album and Pulsatilla used against different diseases at the potency of 30C and 200C, were collected. The samples were tested for antibacterial potential by agar well diffusion method and Minimal Inhibitory Concentration (MIC) assay against previously isolated clinical bacterial isolates. Through the agar well diffusion method, no noticeable antimicrobial activity was found by the homeopathic drug samples. Only a trace quantity of inhibitory effect was found against *Staphylococcus* spp. by all the homeopathic drugs with highest zone of inhibition of 8.7±1.15 mm. The findings from the MIC assay revealed that relatively higher concentrations of the samples were needed to retard the growth of the pathogenic bacteria in most of the cases. The lowest MIC of 128 µL of three homeopathic drugs was found against *Staphylococcus* spp. However, in the majority of the cases, the MIC was counted as 512 µL and 1024 µL of the homeopathic drugs. The present study could not able to provide profound evidence to claim tested homeopathic drugs as alternatives to antibiotics.

***Keywords:** Antibacterial activity, homeopathic drugs, pathogenic bacteria, minimal Inhibitory Concentration*

Introduction

The use of antibiotics to cure diseases has increased drastically (1). Several medically important bacterial species have been shown to have resistant genes, resulting in the emergence and spread of multi-resistant microorganisms (1-4). Excessive and indiscriminate use of these drugs in human and veterinary medicine has lead to the dissemination of antibiotic resistance, putting their efficacy in doubt (2, 5, 6). Researchers are looking for alternative approaches, such as herbal remedies and natural resources, in treating both antibiotic-resistant and -susceptible infections, owing to the rise in antibiotic resistance and the harmful side effects associated with traditional therapies (1, 7).

Homeopathy is another possible treatment alternative that may lead to a decline in the use of antibiotics, with an increasing number of scientific research, a solid safety record, and evidence of cost-effectiveness (2, 8). Homeopathic medicines are made from a variety of plant, mineral, or animal substances and were developed as an alternative medicine method in 1796 by Samuel Hahnemann (9-11). The word homeopathy comes from the Greek words *homoion pathos*, which means "similar illness" that refers to the use of drugs to treat illnesses that can induce similar effects in healthy patients when injested (10, 12). Homeopathic medications are used to help the body's own healing processes in the fight against disease (13, 14). When the body's immune system is unable to adequately defend itself against the pathogenic effects of bacteria or some other microorganism, an infection occurs. The body develops several symptoms during infection in an effort to combat the microorganism, the most well-known example being fever. Homeopaths claim that a properly selected homeopathic drug will aid the body's own battle against a microbe, allowing it to defeat it and improve natural immunity resulting in reduced frequency of infectious diseases (13-15).

Homeopathy remains one of the most controversial traditional medicine treatments, despite the fact that it has been around for over 200 years. Many scientists question the medicinal efficacy of homeopathic medications because they are so dilute due to potentialization that a single molecule of the active compound is hardly found in the final preparation in many cases (2, 10). There is another argument among the scientists that all therapeutic effects in homeopathic preparations are just placebo effects or a misinterpretation of regular healing that happens spontaneously when time passes. However, experiments concerning the use of homeopathic medicine have shown mixed, conflicting findings, leaving us with no way of resolving the conundrum (10). Despite a lengthy period of scientific debate, homeopathy has proven to be adaptive, is widely used around the globe, and is an established feature of many countries' medicinal systems (2, 16-18). The popularity of homeopathic medicines is due to the less side effects for the use of extreme dilution and cost-effectiveness (19).

To fully comprehend the mode of action of homeopathic drugs, one must first establish a convincing hypothesis with experimental support that describes how a heavily diluted drug would influence a biological system (12). Many scientific papers on the antibacterial efficacy of homoeopathic drugs have recently been published (9, 19). However, a shortfall of evidence to claim the antimicrobial effectiveness of homeopathic medicines still exist. Considering all these facts, the present study was carried out to investigate the inhibitory potential of homeopathic drugs in commonly prescribed diluted forms against pathogenic bacterial isolates by agar well diffusion and multiple tube dilution methods.

Materials & Methods

Sample collection

The study was carried out in the Microbiology laboratory of Stamford University Bangladesh. Homeopathic drugs such as Apis mellifica, Graphites, Arsenicum album and Pulsatilla, which have multi-organ application to treat several ailments, were collected in diluted concentrations of 30C and 200C of mother tincture from the different homeopathic drug store in Dhaka city, Bangladesh. The samples were immediately transported to the laboratory following the standard protocol (9, 20-22). Expiry dates of the samples were checked and storage criteria were maintained until analyzed.

Test microorganisms

The bacterial isolates tested for the antimicrobial assay in this study were previously isolated and biochemically identified from various clinical specimens (wound swab, pus, urine and stool) in the laboratory of the Department of Microbiology, Stamford University Bangladesh. The selected clinical bacterial isolates include *E. coli, Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Salmonella* spp., and *Vibrio* spp. Selected bacterial isolates were preserved at -20 °C and before be used in the current study, subcultures were made on Nutrient agar (HiMedia Laboratories, Mumbai, India).

Determination of the inhibitory effect of the homeopathic drug samples

For the determination of antimicrobial activity, modified agar well diffusion method was applied using the Mueller-Hinton agar plate (20, 23-27). Suspension of each of the clinically isolated bacteria was prepared using normal saline, consisting of 10^6 cfu/mL bacteria with turbidity equivalent to that of the 0.5 mL McFarland standard, and each suspension was then subjected to the lawn on the Muller-Hinton agar (MHA) (Oxoid Ltd., Basingstoke, Hampshire, England). The wells were dug (8 mm^3) on the inoculated Muller Hinton agar medium and 100 µl of each of the samples were introduced into the well. Normal saline was used as negative controls whereas a commercial disc of Gentamycin (GEN, 10 µg) was used as the positive control. The plates were incubated at 37 ^{O}C overnight and examined for the zone of inhibition. The diameter of the inhibition zone was measured in mm using slide callipers.

Determination of Minimal Inhibitory Concentration (MIC) of the tested homeopathic drugs

The assessment of minimal inhibitory concentration (MIC) was performed by multiple tube dilution method to determine the lowest concentration of homeopathic drugs which could able to reduce the extent of the viability of the test bacteria (20, 23, 25-27). An aliquot of 100 µL of the overnight (~12 hours) culture of each of the selected clinical bacterial isolate (*Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., *Staphylococcus* spp., and *Vibrio* spp.) was inoculated into the appropriately labelled sterile tubes containing Mueller Hinton (MH) broth (Oxoid Ltd, England) at the turbidity adjusted to 0.5 McFarland standard. Different volumes of each of the 30C homeopathic medicines (16 µL, 32 µL, 64 µL, 128 µL, 256 µL, 512 µL, 1024 µL and 2048 µL) were introduced onward to make a total volume of 3 mL. All the tubes were incubated at 37 ˚C for 24 hours. The least concentration of the sample which could retard the multiplication of the tested bacteria, as judged visually by lack of turbidity in the tube comparable to the McFarland standard, was recorded and considered as the MIC value.

Statistical analysis

SPSS statistics version 20.0 (IBM, Georgia, USA) and Microsoft Office Excel Professional Plus 2016 (Microsoft Corporation, Redmond, Washington, USA) software packages were used to statistically validate the data found in this report.

The standard deviations (SD) and mean values were measured. One-way ANOVA was used to interpret the data, and mean values were separated by the posthoc statistic of Tukey's HSD (honest significant difference). The significant differences in mean results were scored at $P < 0.05$.

Results & Discussions

Over the past few decades, concerns about the uses of traditional and alternative medicines have been increased worldwide due to the emergence of antibiotic resistance (10). Among which homeopathy offers a low-cost, comprehensive solution or substitute for the management of a wide range of infections (2). Homeopathic drugs have been shown to be successful in the treatment of chronic diseases in many clinical trials (10). Therefore, the study was designed to evaluate the antimicrobial potential of selected homeopathic drugs against clinically isolated pathogens.

In the present study, all the diluted homeopathic drug samples were failed to impart any significant inhibitory effect against the pathogenic bacterial isolates as revealed from agar well diffusion method (Table 1). The data was found to be statistically significant ($P < 0.05$). All the samples irrespective of their potency showed antimicrobial activity against *Staphylococcus* spp. with the mean zone of inhibition peak at 8.7±1.15 mm. Graphites also had antibacterial activity against *Salmonella* spp. (30C and 200C), *Vibrio* spp. (30C) and *Pseudomonas* spp. (30C and 200C) with the higher mean zone of inhibition of 7.7±0.58 (Table 1). Apis mellifica 30C showed inhibitory potential against *Pseudomonas* spp. and Pulsatilla 30C retarded the growth of *Pseudomonas* spp. and *Salmonella* spp. beside *Staphylococcus* spp. However, the antibacterial effect was not substantial in any of the cases. *E. coli* and *Klebsiella* spp. were remained to be unaffected by the diluted homeopathic drug samples.

Table 1: Antimicrobial activity of the diluted homeopathic drug samples

Homeopathic Drugs (n)	Potency	*Zone of inhibition (mm) against tested microorganisms					
		E. coli	*Klebsiella* spp.	*Pseudomonas* spp.	*Staphylococcus* spp.	*Vibrio* spp.	*Salmonella* spp.
Apis mellifica (3)	30C	0±0	0±0	5.7±0.58	8.7±1.15	0±0	0±0
	200C	0±0	0±0	0±0	6.7±0.58	0±0	0±0
Graphites (3)	30C	0±0	0±0	5.3±1.15	7.7±0.58	5.3±0.58	7.3±0.58
	200C	0±0	0±0	5.0±1.73	7.3±0.58	0±0	6.3±1.15
Arsenicum album (3)	30C	0±0	0±0	0±0	7.0±1.00	0±0	0±0
	200C	0±0	0±0	0±0	6.7±1.15	0±0	0±0
Pulsatilla (3)	30C	0±0	0±0	6.3±0.58	8.7±0.58	0±0	5.7±0.58
	200C	0±0	0±0	0±0	8.0±1.00	0±0	0±0
Gentamicin (Positive control)	10 µg	20.3±0.58	22.3±0.58	19.0±1.73	21.7±1.15	20.7±0.58	21.3±1.15

*The experiments were carried out three times. Mean ± SD values have been shown here. The differences observed in mean results were significant ($P < 0.05$).
Normal saline was used as negative control and showed no effect.

Similar to the current study, Mokarroma and Shammi (9) reported insignificant bacterial inhibition by four tested homeopathic drugs including Acconite 30, Arsenicum album 30, Mercuris corrosives 30 and Mercurius solution 200. Hossen et al. (10) also found the resistance of tested bacteria against different dilutions of Podophyllum, China, Nux vomica and Mercurius solution in their study. Nambison et al. (28) found different pathogenic bacteria to be weakly sensitive to different homeopathic drugs including Apis mellifica, Arsenicum album, Pulsatilla, Capsicum, Cantharis, Mercurius solibiliris, Medorrhinum and Lycopodium at potencies of Q, 30, 200, 1M, 10M and CM. Similar kinds of results were also evident by the experiment of Pareek and Jadhav (19), Ott and Morris (1) and Bonhoft et al. (29). On contrary, Almaguer-Flores and Gonzalez-Alva (30) reported strong antimicrobial activity in decimal (1dH) and centesimal (1cH) dilutions of Arsenicum album and Lycopodium clavatum against periodontal bacteria. Sarkar et al. (31) found significant inhibitory activity against *Staphylococcus epidermidis* by different potencies (6C, 12C, 30C, 200C, 1M) of homeopathic drug Sulphanilamide. Some other reports claimed homeopathic drugs to be effective against bacterial diseases (32-34).

On the other hand, the results of MIC assay portray that higher concentrations of the homeopathic drug samples were required to inhibit the growth of most of the pathogenic bacteria (Table 2). In cohort with the result of the agar well diffusion method, the lowest MIC of 128 µL was scored against *Staphylococcus* spp. by Graphites, Arsenicum album and Pulsatilla.

A concentration of 256 µL of Apis mellifica and Graphites was required to kill *Staphylococcus* spp. and *Pseudomonas* spp., respectively. In other cases, rather higher concentrations from 512 µL to 1024 µL were required for the elimination of bacteria (Table 2). Mokarroma and Shammi (9) found MIC of four homeopathic drugs (Acconite 30, Arsenicum album 30, Mercuris corrosives 30 and Mercurius solution 200) between 256 µl/3ml to 1024 µl/3ml. Hossen et al. (10) reported MIC of selected drugs (Podophyllum, China, Nux vomica and Mercurius solution) against clinically isolated microorganisms was within 201µL/mL to 300µL/mL. The reason for the insufficient antimicrobial potential of the homeopathic drugs may be that the samples were in diluted form, the active ingredient in the tested medicine might not be present in sufficient quantity to kill the disease causing microorganisms. Nevertheless, the effect of homoeopathic dilution has been a point of controversy for many years (19).

Table 2: Minimal inhibitory concentration of the diluted homeopathic medicine (30C) samples

Homeopathic Drug (30C)	Minimal Inhibitory Concentrations (µL)					
	E. coli	***Klebsiella* spp.**	***Pseudomonas* spp.**	***Staphylococcus* spp.**	***Vibrio* spp.**	***Salmonella* spp.**
Apis Mellifica	1024	1024	512	256	1024	512
Graphites	512	512	256	128	1024	512
Arsenicum album	512	1024	1024	128	512	1024
Pulsatilla	512	1024	1024	128	1024	1024

Conclusion:

In the present study, no significant inhibitory effect of the diluted homeopathic drug samples was found against the pathogens through the agar well diffusion method. The MIC assay revealed the antimicrobial activity of the homeopathic drugs at relatively higher concentration in most of the cases. Hence, the finding of the current study could not draw any supportive evidence for the *in vitro* antibacterial potential of the homeopathic drug samples. The outcomes also portray the need for further thorough *in vitro* investigation of homeopathic drugs in different diluted forms or conducting several *in vivo* trials in model organisms to claim the effectiveness of homeopathic drugs as a suitable therapeutic agent against pathogens.

Conflict of Interest

The authors declare no conflict of interest.

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Citation: Md. Istiak Kabir, Asif Ikram, Md. Bahadur Sikder, Md. Hafizul Haq Tushar and Saurab Kishore Munshi. "Determination of the Inhibitory Effects of Commercially Available Homeopathic Drugs on Pathogenic Bacterial Growth". SVOA Microbiology 2:1 (2021) Pages 01-06.