



Biological Evaluation of Different Mother Tinctures Available in the Market

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Abstract

In recent research, commercial homoeopathic mother tinctures were tested for biological activity. Masood homoeo pharmaceuticals supplied all mother tinctures. Homoeopathic mother tinctures were hydroalcohol. Today, strong antimicrobial medicines are needed to fight fatal and life-threatening infections caused by antibiotic-resistant bacteria. Scientists are developing more effective chemicals by chemically modifying antibacterial drugs to battle resistant microorganisms. This study comprises 11 homoeopathic mother tinctures for *Proteus*, *Shagilla*, *kebsiela*, *Cornybacterium baccilus*, *Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. All homoeopathic mother tinctures were bactericidal in 96-well microplates. Against *Salmonella typhi*, all mother tinctures showed significant age inhibition, except *millefolium* ($29.03 \pm 2.84\%$). Two DPPH radical scavenging assays assessed antioxidant activity. Strong antioxidant action in homoeopathic mother tinctures *Cinchona officinalis* contains 84.61 ± 3.98 , *Berberis* 69.09 ± 3.35 , *Hmamelis* 68.10 ± 3.66 , *Rhustox* 68.10 ± 3.66 , *Millefolium* 77.81 ± 3.98 , *Calendula* 83 ± 0.72 , *Chamomilla* 71.17 ± 2.80 , *Thuja* 82.34 ± 2.08 , and ascorbic acid *Calendula* Maximum antioxidant activity. Antifungal activity against *Fumigatus niger* was measured by agar well diffusion. All homoeopathic mother tinctures had minor antifungal activity compared to Fluconazol.

Introduction

The integration of traditional healing systems, such as homeopathy, with modern technological advancements has the potential to reshape the landscape of healthcare. Homeopathy, founded by Dr. Samuel Hahnemann in the late 18th century, has long

been recognized for its holistic approach to treating patients, emphasizing the "like cures like" principle and the use of minimal doses to stimulate the body's natural healing abilities [1-2]. Despite its widespread use in alternative medicine, there has been a growing need for scientific validation of its therapeutic efficacy,

More Information

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particularly in the context of antimicrobial, antioxidant, and antifungal activities.

Recent advancements in biotechnology, artificial intelligence, digital twins, and e-healthcare are offering new tools to bridge the gap between traditional medicine and modern healthcare practices [3-4]. Biotechnology, particularly in the field of plant-based therapies, has enabled the extraction and analysis of bioactive compounds from various herbal sources, including homoeopathic mother tinctures, to assess their pharmacological properties. By leveraging cutting-edge techniques, such as molecular biology and high-throughput screening, researchers are uncovering the underlying mechanisms that govern the biological effects of these tinctures, thus enabling their potential integration into modern treatment regimens [5-7].

Dr Christian Frederick Samuel Hahnemann (1790) is founder of Homoeopathic system of medicine. "Similia Similibus Curantur" (Latine) i.e. "like cures like is the basic natural principle of Homoeopathy. This system has its own dosage form for various diseases [8]. Homoeopathy term comes from the Greek roots homoios and pathos. Following are research gaps:

- Disease with organic and irreversible structural changes, such as lungs cavitation, and foreign body presence.
- Large obstruction in the system, such as gall stone, renal stone, and thrombus in the circulatory system.
- Increase size of abnormal growth in the tissues, such as tumour, cyst, etc.
- Abnormalities both congenital and acquired, such as overriding of aorta, ventricular septal defect, etc.

When homoeopathic medicine is proved on animals is called animal drug proving. Animal drug proving gives only objective symptoms, not the subjective symptoms. Subjective Symptoms are those symptoms which are collected from the individual himself. Objective symptoms are those symptoms which are collected from friends, relatives and physician observation. When drug is proved on human is called human drug proving. Human drug proving gives us both subjective and objective symptoms. Human drug proving gives complete picture of disease. Drug proving on plants is called plant drug proving. Plant drug proving gives only objective symptoms. Confusion between homeopathic and herbal system is 2000 homeopathic medicines are prepared from plants [9]. Folk medicine consists knowledge that developed over generations within many societies before the era of modern medicine.

Therefore, urgent need to monitoring antibacterial resistance by improved antibiotic use and reduce infection in hospital, but the development of new antibiotics such as natural must be continued to maintain effectiveness of antibacterial [10]. Different

parts of plants, including fruits and leaves have a wide range of terpenoids, phenolic compounds, vitamins and some metabolites, which are rich in antioxidant activity. Himalayan plants are main source of herbal pharmaceutical industry both of India and Pakistan [11]. Bacterial growth rate depends on several factors including temperature, medium and pH. A generation period under optimal conditions is specified for each type of bacteria which mean the shortest time it takes for the bacteria to duplicate. Growth of bacteria can be studied by measuring optical density (OD). Optical density is a way of counting the number of bacteria in a sample. Bacteria are grown in batch samples, which mean that they are in a controlled environment.

The second phase is known as log phase, is the inclining slope of the graph. It is symbolized by exponential or logarithmic growth of number of bacteria but also maturing of cells. Log phase begins with accelerating growth phase, where, speed increases exponentially or logarithmic. Log phase begins with accelerating growth phase which greatly increase cell division from previous lag phase. Decelerating phase where slope on the growth curve flattens out and connects to following stationary growth phase is end of the log phase. In stationary phase bacterial growth is constant. There can also be inhibitory metabolites/end products or limitations of space are preventing bacterial further growth. Bacterial secondary metabolites protect it from various hazards such as vermin or oxidation and dehydration. Death phase occurs when death of bacteria is greater than that of division of bacteria. Due to lacking of survival conditions, total amounts of bacteria are decreasing because due to accumulation of nutrients or toxic products [12]. This research investigates the biological activity of various commercially available homoeopathic mother tinctures, focusing on their antimicrobial, antioxidant, and antifungal properties. The following are the key contributions of this study:

- In this research, 11 homoeopathic mother tinctures were tested against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Most tinctures demonstrated significant antibacterial efficacy, with *Cinchona officinalis* and *Thuja occidentalis* inhibiting *Salmonella typhi* and other gram-negative bacteria notably well.
- The mother tinctures' antioxidant capacity was measured using the DPPH radical scavenging test. *Cinchona officinalis* and *Calendula officinalis* have the highest antioxidant activity, making them suitable medicinal and cosmetic antioxidant sources.
- The study evaluated these tinctures against *Fusarium solani* and *Aspergillus niger* for antifungal activity. These drugs had weaker antifungal activity



than fluconazole, suggesting they need additional development.

- Alpha-Glucosidase Inhibition: Nux vomica and Belladonna tinctures showed strong alpha-glucosidase inhibitory activity, which could help diabetics manage their blood glucose levels.
- The study compared the tinctures' biological effects to show their variability. Some tinctures had strong antibacterial and antioxidant properties, while others were weaker, helping to comprehend their therapeutic applications.
- The study used microplate methods, agar diffusion assays, and DPPH assays to evaluate homoeopathic mother tinctures' biological qualities. This method can be used to study different herbal medications' medicinal potential.

Material and Method

Chemicals

Solvents Ethanol used were all of HPLC, Sabroud Dextrose agar, Nutrient broth and Nutrient agar were purchased from Oxoid Ltd. Stanadard antibiotic (ciprofloxacin) was gifted by Pharmadica. 2,2-diphenyle-1-picryl-hydrazyle DPPH, Ascorbic acid

Drugs

Homoeopathic mother tinctures of Massod company available in the market were used such as Allium Sativum, Atropa belladonna, Berberis vulgaris, Matricaria chamomilla, Cinchona officinalis(China), Hamamelis virginica, Archillia millefolim, Nux vomica, Pulsatilla and thuja occidentalis.

Instruments

Absorbance of samples and media was measured with Spectrophotometer (Irmeco UV-Vis U2020) and 96-wells plate reader (Biotek Synergy HT, USA). Digital electronic balance (AUW 220D, Shimadzu, Japan) was used for weighing. The glass apparatus (flasks, beakers, test tubes) purchased from Pyrex, Japan was sterilized by using hot air oven (Mettler) at 137°C for 20-30 minutes. Flasks and Test tubes used for the preparation of slants and media, respectively, were plugged with cotton before sterilization. Autoclave (EMMAY Enterprises) was used for autoclaving microbial growth media. Reciprocating dry shaker and hot incubator used were of General Laboratory Corporation, Pakistan.

Microbial strains

The bacteria used were obtained from The Islamia University of Bahawalpur. Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Bacillus subtilis, Staphylococcus aureus was included in the study. The organisms were maintained on stock culture agar. Fungal strains Fumigatus Niger was included in the study.

Antibacterial assay

Nutrient broth was prepared at a concentration of 13g/L by dissolving it in distilled water and autoclaved

at 121°C for 15 minutes. Fresh culture was prepared by mixing 50µl of stock culture bacteria into 50ml of nutrient broth contained in the flask. The flaks were kept on horizontal shaker at 200rpm for overnight at 37°C. The turbidity of the overnight culture was measured according to McFarland standards 0.12-0.19. Nutrient agar medium was prepared at a concentration of 28g/L by dissolving it in distilled water sand autoclaved at 121°C for 15 minutes. Commercial homoeopathic mother tinctures were used.

96-Wells Microplate Method

After 12 hrs incubation, absorbance of culture was measured at 540nm by using spectrophotometer. The absorbance of culture was adjusted in the range of 0.12-0.19 according to Macfarland standard by diluting it with nutrient broth. Two wells containing 200µl of diluted culture were marked as control.180µl of this culture was pipetted into each well of 96 well microplate and then 20µl of each test compound solution was added to make the final volume 200µl in each well. For 2nd Dilution 190µl of this culture was pipetted into each well of 96 well microplate and then 10µl of each test compound solution was added to make the final volume 200µl in each well and for 3rd dilution 195µl of this culture was pipetted into each well of 96 well microplate and then 5µl of each test compound solution was added to make the final volume 200µl in each well. Ciprofloxacin was used as standard drug for comparison. Microplates incubated at 37°C for 18-24 hours. Absorbance of the microplate wells noted after 24 hours of incubation. The % inhibition was measured using the following formula.

$$\text{Inhibition (\%)} = \frac{100(X-Y)}{X} \quad (1)$$

where, X is absorbance of wells with bacterial culture, Y represents the absorbance of test sample. Results are mean of triplicate (n = 3, ±S.E.M). To find out the percentage inhibition dilutions of the test samples were made. Absorbance of the each well with different concentration was noted at 540nm.

Agar well Diffusion Method

The antifungal activity was performed with slight method modification. 22ml of agar was poured in petri dishes and solidified in aseptic environment. 5ml of diluted fungal culture was spread. 6mm holes were made with the help of sterile borer in each petri dish. 4µl of commercially available homoeopathic mother tinctures were poured in each hole and kept in petri dishes at 27°C 24-36 hrs. After 24-36 hrs zone of inhibition was calculated.

Enzymatic Assay

Simple technique used to measure antioxidant activity of different test samples by 2,2-Diphenyle-1-picrylhydrazyl (DPPH). Different volume of each 70% hydroalcoholic (70% ethanol and 30% water) plant



extract was taken, ranged from 10 μ l, 5 μ l, 2.5 μ l and 90 μ l, 95 μ l and 97.5 μ l of 5 μ M of DPPH was added to make the final volume upto 100 μ l in 96 well plate. Samples were shaken and incubated for 30 minutes at 37 °C . Optical density was measured at 517 nm. Ascorbic acid was used as a standard. 70% hydroalcoholic solution was used as negative control. Negative control used as an OD of control % age DPPH free radical scavenging activity = $100 - (A/B \times 100)$. To find out the minimum inhibitory concentration of 10 μ l, 5 μ l and 2.5 μ l of mother tinctures available in the market. Absorbance of the each well was noted at 517 nm.

Results

Antibacterial Results

In worldwide, there are several systems of medicines such as homeopathic medicine system, Allopathic

medicine system, Ayurvedic medicine system, modern medicine etc. But homeopathy is system of medicine in diagnosis and treating medical problems harmlessly. Skin aging is of two types one is chronological aging which is age related and second is premature aging due to environmental stress. The clinical signs of premature aging sallowness, atrophy, premalignant lesions and including irregular dryness, dark or light pigmentation and leathery. In Table 1, the % inhibition of homeopathic mother tinctures against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* is shown. Table 2 represents the % Inhibition of homeopathic mother tinctures against *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella*.

Table 1. % Inhibition of Homeopathic Mother Tinctures

Codes	%INHIBITION				
	<i>Salmonella typhi</i>	<i>E. coli</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
China	89.23 $\pm$ 0.22 77.34 $\pm$ 1.45 57.78 $\pm$ 2.98	85.29 $\pm$ 0.51 67.39 $\pm$ 1.39 45.89 $\pm$ 0.89	75.33 $\pm$ 1.34 60.65 $\pm$ 2.98 44.76 $\pm$ 1.68	80.79 $\pm$ 1.69 65.03 $\pm$ 2.76 49.42 $\pm$ 1.78	71.73 $\pm$ 2.49 59.67 $\pm$ 3.21 42.39 $\pm$ 0.03
Berberis	60.75 $\pm$ 2.14 47.07 $\pm$ 1.61 -----	87.83 $\pm$ 0.12 67.90 $\pm$ 2.78 50.30 $\pm$ 1.56	69.69 $\pm$ 2.04 51.30 $\pm$ 1.09 -----	71.14 $\pm$ 0.09 54.65 $\pm$ 62 -----	64.69 $\pm$ 0.09 49.78 $\pm$ 0.04 -----
Allium stivum	88.49 $\pm$ 0.52 66.43 $\pm$ 3.39 51.42 $\pm$ 1.20	83.29 $\pm$ 0.18 60.36 $\pm$ 2.57 48.29 $\pm$ 0.39	84.68 $\pm$ 0.12 59.92 $\pm$ 2.05 43.49 $\pm$ 1.04	86.90 $\pm$ 1.66 68.35 $\pm$ 1.42 51.38 $\pm$ 2.01	81.14 $\pm$ 2.20 56.38 $\pm$ 0.45 39.92 $\pm$ 2.09
Nuxvomica	88.34 $\pm$ 1.40 71.60 $\pm$ 3.11 55.56 $\pm$ 2.70	71.41 $\pm$ 1.20 51.03 $\pm$ 2.46 36.09 $\pm$ 0.41	85.95 $\pm$ 1.40 59.09 $\pm$ 1.21 49.04 $\pm$ 3.02	83.20 $\pm$ 0.55 65.90 $\pm$ 0.31 50.30 $\pm$ 3.11	88.90 $\pm$ 0.90 72.98 $\pm$ 0.72 56.39 $\pm$ 0.09
Pulsatilla	78.85 $\pm$ 2.96 58.90 $\pm$ 3.02 45.90 $\pm$ 2.49	39.37 $\pm$ 3.71 -----	78.52 $\pm$ 0.84 59.39 $\pm$ 1.90 47.08 $\pm$ 4.30	67.92 $\pm$ 1.10 50.20 $\pm$ 0.34 28.30 $\pm$ 2.06	80.94 $\pm$ 0.91 58.90 $\pm$ 4.01 41.80 $\pm$ 3.95
Belladonna	85.99 $\pm$ 0.22 65.41 $\pm$ 2.37 50.01 $\pm$ 0.13	74.21 $\pm$ 2.56 56.98 $\pm$ 1.05 42.35 $\pm$ 2.76	81.02 $\pm$ 0.38 63.98 $\pm$ 3.09 48.29 $\pm$ 1.22	69.03 $\pm$ 1.55 54.83 $\pm$ 1.24 38.43 $\pm$ 0.35	81.49 $\pm$ 2.25 60.29 $\pm$ 0.09 43.27 $\pm$ 0.04
Hamamelis	80.64 $\pm$ 0.89 61.90 $\pm$ 0.34 53.54 $\pm$ 0.07	12.99 $\pm$ 2.85 -----	79.28 $\pm$ 0.59 56.45 $\pm$ 1.45 42.39 $\pm$ 0.87	58.49 $\pm$ 2.66 40.03 $\pm$ 1.34 -----	82.28 $\pm$ 2.96 64.60 $\pm$ 2.83 37.70 $\pm$ 1.30
Millefolium	29.03 $\pm$ 2.84 -----	59.50 $\pm$ 0.88 46.05 $\pm$ 1.42 -----	65.73 $\pm$ 0.08 47.54 $\pm$ 3.45 -----	68.03 $\pm$ 1.86 46.39 $\pm$ 1.21 -----	74.85 $\pm$ 1.24 56.20 $\pm$ 3.57 35.85 $\pm$ 0.16
Calendula	85.77 $\pm$ 1.25 67.32 $\pm$ 1.35 43.51 $\pm$ 2.10	77.45 $\pm$ 1.38 56.21 $\pm$ 1.54 42.31 $\pm$ 0.42	76.61 $\pm$ 0.63 52.04 $\pm$ 0.41 34.09 $\pm$ 1.52	74.25 $\pm$ 1.55 59.94 $\pm$ 0.49 40.08 $\pm$ 0.38	79.80 $\pm$ 1.32 55.46 $\pm$ 0.54 38.94 $\pm$ 3.48
Chamomilla	85.46 $\pm$ 2.24 64.29 $\pm$ 2.19 50.37 $\pm$ 2.67	76.91 $\pm$ 3.40 60.20 $\pm$ 3.84 47.51 $\pm$ 0.14	77.80 $\pm$ 1.60 55.41 $\pm$ 0.99 37.50 $\pm$ 2.99	74.58 $\pm$ 0.04 60.76 $\pm$ 3.81 44.72 $\pm$ 1.29	79.18 $\pm$ 2.27 54.90 $\pm$ 1.54 27.50 $\pm$ 0.79
Thuja	62.62 $\pm$ 1.33 44.70 $\pm$ 0.89	62.75 $\pm$ 1.15 49.54 $\pm$ 2.74	43.88 $\pm$ 1.15 -----	14.09 $\pm$ 0.44 -----	37.25 $\pm$ 0.49 -----
Ciprofloxacin	92.87 $\pm$ 0.91	92.27 $\pm$ 0.64	91.63 $\pm$ 0.05	90.57 $\pm$ 0.35	92.34 $\pm$ 0.35

Note: All compounds are soluble in ethanol*. The mean difference is significant at the 0.05 level.



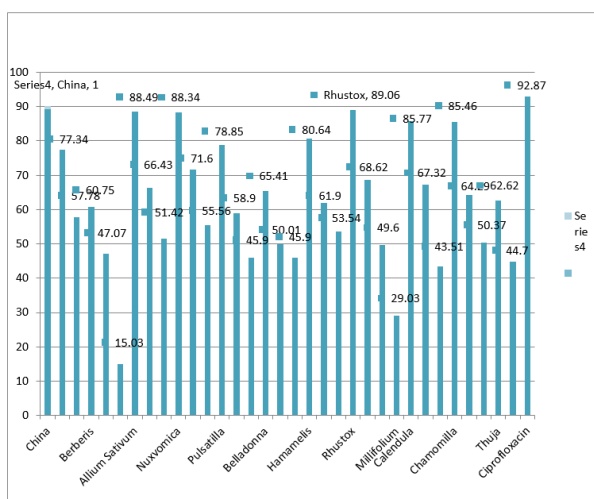
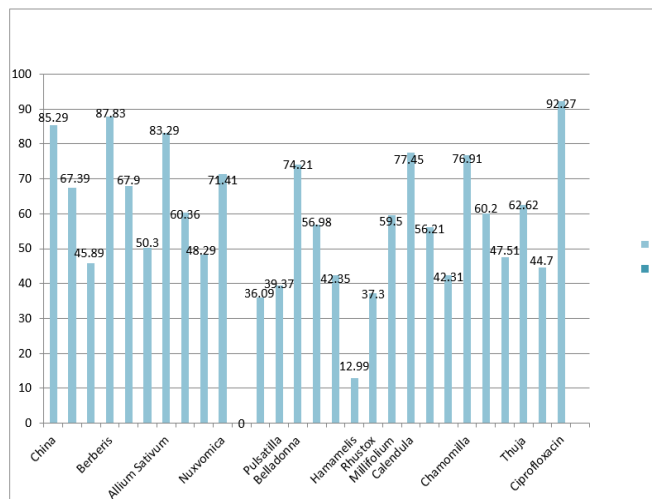
Table 2. % Against Proteus, Klebsiella, Corynebacterium Bacillus and Shigella

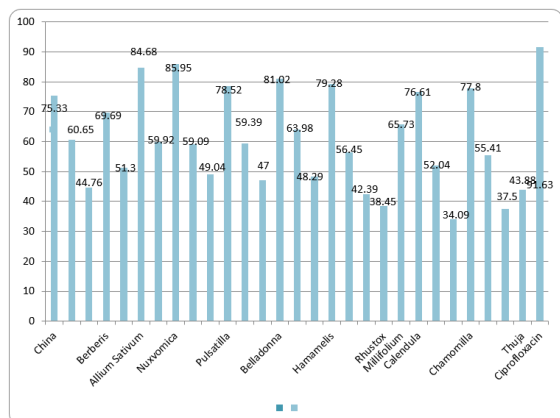
Codes	Proteus	Klebsiella	Corynebacterium bacillus	shigilla
China	58.23 ±0.22	53.29±0.51	77.33±1.34	71.79±1.69
Berberis	60.75±2.14	65.83±0.12	73.69±2.04	53.14±0.09
Allium stivum	54.49±0.52	57.29±0.18	55.68±0.12	45.90±1.66
Nux vomica	45.34±1.40	60.41±1.20	47.95±1.40	53.20±0.55
Pulsatilla	68.85±2.96	57.37±3.71	87.52±0.84	67.92±1.10
Belladonna	35.99±0.22	44.21±2.56	19.02±0.38	48.03±1.55
Hamamelis	50.64±0.89	42.99±2.85	56.28±0.59	58.49±2.66
Millifolium	59.03±2.84	50.50±0.88	59.73±0.08	68.03±1.86
Calendula	85.77±1.25	57.45±1.38	82.61±0.63	74.25±1.55
Ciprofoxacin	92.87±0.91	92.27±0.64	91.63±0.05	90.57±0.35

Note: All compounds are soluble in ethanol. “*” the mean difference is significant at the 0.05 level.

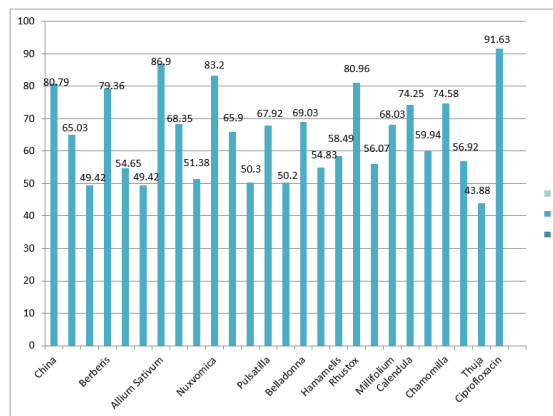
Thuja orientalis was very effective in inhibiting the growth of Salmonella mutans (MIC less than or equal to 2.0-7.8 mg/ml) [13]. Thuja extract showed significant ($P \leq 0.05$) inhibitory activity against both gram negative and gram positive organisms. It was active against Bacillus subtilis, Staphylococcus aureus, E.coli, and Agrobacterium tumefaciens. MICs of E1 extract ranged from 0.40 to 0.85 mg/ml and E2 extract 0.55 to 1.15 mg/ml. Homoeopathic mother tincture Thuja occidentalis showed %age inhibition 62.62 ± 1.33 , 62.75 ± 1.15 , 43.88 ± 1.15 , 14.09 ± 0.44 , 37.25 ± 0.49 against Salmonella typhi, E.coli, bacillus subtilis, Staphylococcus aerus and Pseudomonas aeruginosa respectively. About 100, 150, 200, 250 & 300 µg ethanol fraction of extract of aerial part of Thuja occidentalis inhibited the FeSO₄ induced lipid peroxidation in a dose dependent manner and showed IC₅₀ value 195.60 µg/ml [14]. Homoeopathic mother tincture Thuja showed excellent %age inhibition by DPPH

method which is 82.34 ± 2.08 , 63.00 ± 2.08 , 49.78 ± 4.81 by pipetting of 10, 5 and 2.5 µl respectively. The antifungal activity of Thuja was performed against six fungi, viz., Saccharomyces cerevisiae, Fusarium solani, Candida albicans, Aspergillus parasiticus, Trichophyton rubrum and Macrophomina. The extract showed significant results against different fungal strains [15]. Homoeopathic mother tincture of Thuja showed negative results against Fumigatus niger. Thuja mother tinctures showed poor results against alpha glucosidase inhibitory assay. The essential oils exhibit different degrees of antimicrobial activities depending on the doses concentration [16]. Figures 1 and 2 represent the % inhibition of Salmonella typhi, E.coli, Bacillus subtilis, Staphylococcus aerus, and Pseudomonas aeruginosa by homoeopathic mother tinctures and % inhibition of proteus, klebsiella, corynebacterium bacillus, and shigilla by homoeopathic mother tinctures, respectively.

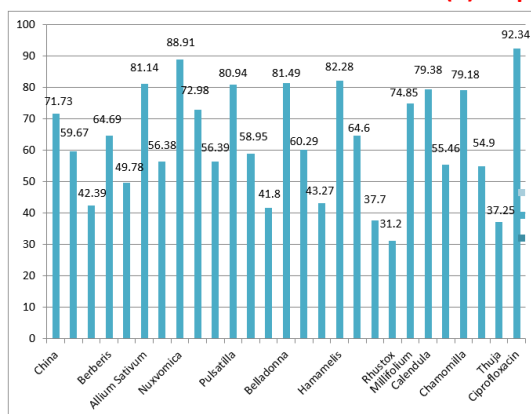
**(a) Salmonella typhi****(b) E. coli**



(c) *Bacillus subtilis*

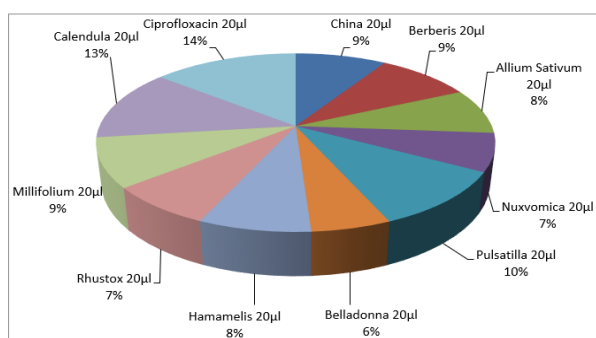


(d) *Staphylococcus aerus*

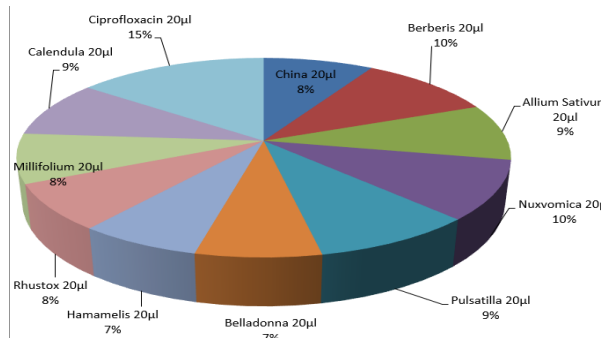


(e) *Pseudomonas aeruginosa*

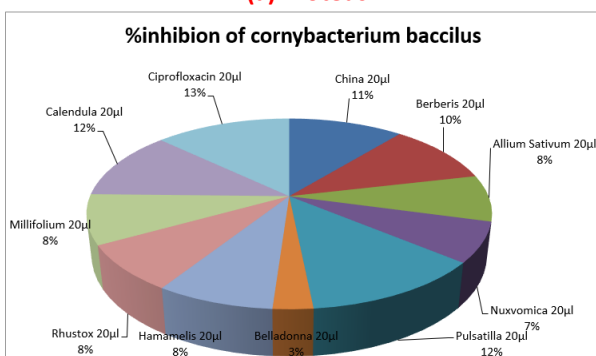
Figure 1. % Inhibition of *Salmonella typhi*, *E.coli*, *Bacillus subtilis*, *Staphylococcus aerus*, and *Pseudomonas aeruginosa* by Homoeopathic Mother Tinctures



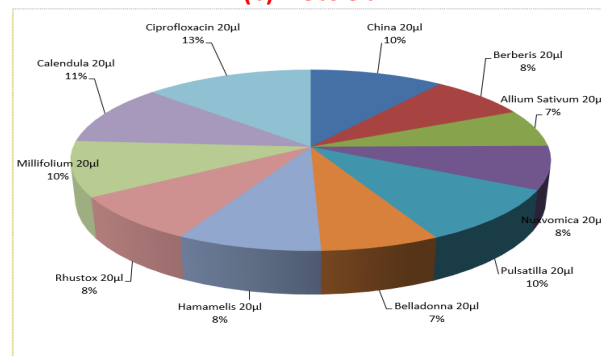
(a) *Proteus*



(b) *Klebsiella*



(c) *Corynebacterium bacillus*



(d) *Shigella*

Figure 2. % Inhibition of *Proteus*, *Klebsiella*, *Corynebacterium bacillus*, and *Shigella* by Homoeopathic Mother Tinctures



It can be seen in Figures 1 and 2, the homoeopathic mother tincture Chamomilla showed percentage inhibition against Salmonella typhi, E. coli, Bacillus subtilis, Staphylococcus aerus and Pseudomonas is 85.46 ± 2.24 , 76.91 ± 3.40 , 77.80 ± 1.60 , 74.58 ± 0.04 and 79.18 ± 2.27 respectively. Chamomilla showed highest percentage inhibition against salmonella typhi. Chamomilla showed medium percentage inhibition against E.coli, bacillus subtilis, Staph. Aerus and

Pseudomonas aeruginosa. The essential oil IC₅₀ values were 3.19 ± 0.13 and 5.63 ± 0.20 mg/ml for A. dracunculus and M. chamomilla, respectively. M. chamomilla essential oil were able to decrease the oxidation rate of soybean oil under accelerated conditions at 60 °C [17]. Homoeopathic mother tincture Chamomilla showed antioxidant activity by DPPH method which 71.17 ± 2.80 (as in Table 3 and Figure 3).

Table 3. Antioxidant Results

No	Mother tinctures	% inhibition	% inhibition	% inhibition
		10 μ l sample	5 μ l sample	2.5 μ l sample
1	china	84.61 ± 3.98	62.70 ± 3.04	47 ± 2.99
2	Berberis	69.09 ± 3.35	53.83 ± 2.43	44.65 ± 3.98
4	Allium sativum	14.61 ± 8.65	-	-
5	Nux vomica	47.64 ± 4.49	-	-
6	pulsatilla	42.85 ± 4.34	-	-
7	Belladonna	15.02 ± 3.24	-	-
8	hamamelis	68.10 ± 3.66	57.24 ± 3.88	41.37 ± 2.46
9	Millifolium	77.81 ± 3.98	50 ± 4.23	-
10	Callendula	83 ± 0.72	64.54 ± 3.91	42.89 ± 3.12
11	chamomilla	71.17 ± 2.80	59.58 ± 2.56	39.34 ± 2.99
12	Thuja	82.34 ± 2.08	63.00 ± 2.08	49.78 ± 4.81
Positive control	Ascorbic acid	95.51 ± 0.12	95.51 ± 3.12	95.21 ± 0.19

Note: The mean difference is significant at the 0.05 level.

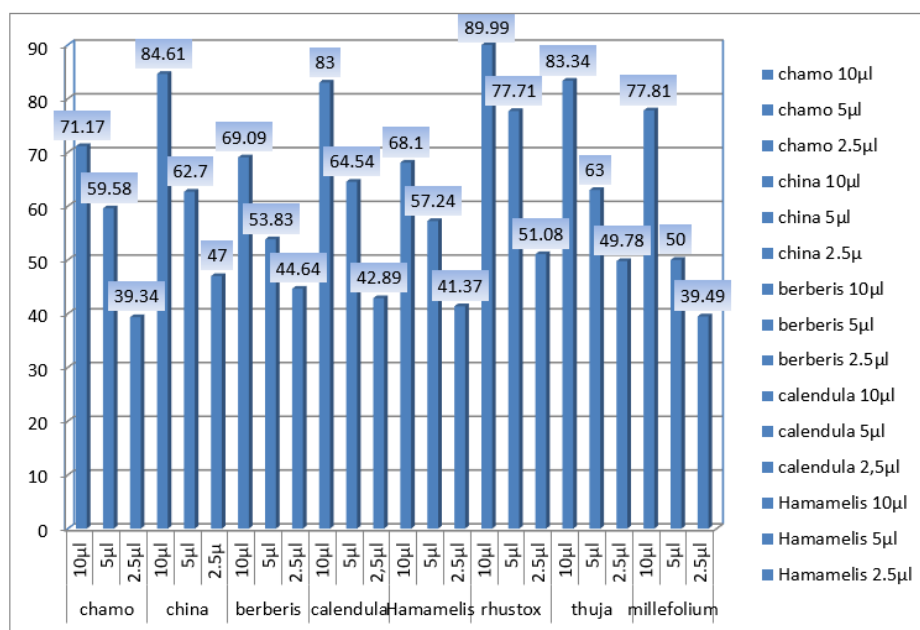


Figure 3. Antioxidant activity by homoeopathic mother tinctures

Note: * the mean difference is significant at the 0.05 level.

It can be seen in table 3 and Figure 3 that the homoeopathic mother tincture Hamamelis showed percentage inhibition against Salmonella typhi, E. coli, Bacillus subtilis, Staphylococcus aerus, Pseudomonas,

Proteus, Klebsiella, Corynebacterium bacillus and Shagilla is 80.64 ± 0.89 , 12.99 ± 2.85 , 79.28 ± 0.59 , 58.49 ± 2.66 , 82.28 ± 2.96 , 50.64 ± 0.89 , 42.99 ± 2.85 , 56.28 ± 0.59 and 58.49 ± 2.66 . Hamamelis mother



tincture showed an excellent bacterial inhibitory activity against *Salmonella typhi*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, moderate to medium activity against *Staphylococcus aureus*, *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella*. *Hamamelis* showed medium antioxidant activity which is 68.10 ± 3.66 . Polyphenolic flavonoids, anthocyanidins, auxin and cytokinin [18]. Dihydroxy-isosteviol methyl ester, the principal biological component isolated from the medicinal plant *Pulsatilla nigricans* was administered in cervical cancer.

Homoeopathic mother tincture *Pulsatilla* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella* is 80.64 ± 0.89 , 12.99 ± 2.85 , 79.28 ± 0.59 , 58.49 ± 2.66 , 82.28 ± 2.96 , 50.64 ± 0.89 , 42.99 ± 2.85 , 56.28 ± 0.59 and 58.49 ± 2.66 respectively. *Pulsatilla* showed good %age inhibition against *Salmonella typhi* and *Pseudomonas aeruginosa*, showed moderate to medium activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus*, *Corynebacterium bacillus* and *Shigella*. *Pulsatilla* showed poor activity against *E. coli* and *Klebsiella*. *Pulsatilla* showed poor antioxidant activity by DPPH method which is 42.85 ± 4.34 . *Pulsatilla* showed poor antifungal activity against *Fumigatus Niger* which has zone of inhibition 7mm while fluconazole has zone of inhibition 15mm.

Alpha glucosidase %age Inhibition

Atropa Belladonna was evaluated for the antibacterial activity against certain pathogenic bacteria namely *Bacillus subtilis* and *Staphylococcus epidermidis* antibacterial activity. Both the extracts i.e. ethanolic and methanolic extract of the therapeutic plant *Atropa belladonna* has revealed antibacterial activity against the tested bacterial species [19]. Homoeopathic mother tincture *Atropa Belladonna* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella* is 85.99 ± 0.22 , 74.21 ± 2.56 , 81.02 ± 0.38 , 69.03 ± 1.55 , 81.49 ± 2.25 , 35.99 ± 0.22 , 44.21 ± 2.56 , 19.02 ± 0.38 and 48.03 ± 1.55 , respectively. *Belladonna* has excellent %age inhibition against *Salmonella typhi*, *E. coli* and *Pseudomonas aeruginosa*, moderate activity against *E. coli* and *Staphylococcus aureus*. *Belladonna Q* has poor activity against *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella*. *Belladonna* has poor %age inhibition of free radical which is 15.02 ± 3.24 . It has good alpha glucosidase inhibitory activity which is 82.63 (Tables 4 and 5).

Table 4. Alpha Glucosidase inhibition Activity of Homoeopathic Mother Tinctures available in the market

Homoeopathic Mother Tinctures	%age inhibition of Alpha glucosidase Activity
China	5.67 ± 3.66
<i>Allium sativum</i>	11.80 ± 0.29
<i>Nuxvomica</i>	98.22 ± 0.15
<i>Pulsatilla nigricans</i>	32.95 ± 2.90
<i>Belladonna</i>	82.63 ± 0.14
<i>Hamamelis</i>	66.20 ± 0.62
<i>Rhustox</i>	68.27 ± 1.32
<i>Millefolium</i>	31.42 ± 0.44
Camphor	53.33 ± 0.62

Table 5. Antifungal Results of *Fumigatus Niger* of different Mother Tinctures available in the Market

Homoeopathic Mother Tinctures	Zone of Inhibition mm <i>Fumigatus niger</i>
<i>Belladonna</i>	3 ± 0.32
<i>Allium sativum</i>	6 ± 0.64
<i>ipecacuanha</i>	8 ± 0.62
<i>Pulsatilla</i>	7 ± 0.94
<i>Berberis</i>	4 ± 0.13
<i>Calendula</i>	5 ± 0.30
<i>Nuxvomica</i>	7 ± 0.51
<i>Rhustox</i>	6 ± 0.92
<i>Hamamelis</i>	9 ± 0.46
Fluconazole	15 ± 0.10

Note: The mean difference is significant at the 0.05 level. Homoeopathic mother tinctures showed poor zone of inhibition against fungi *Fumigatus niger*

Discussion

The antibacterial activity of the *nuxvomica* extract was performed by disc diffusion method. The extract was tested against four pathogenic bacterial strains of gram negative and gram positive bacteria. Ethyl acetate extract of *Strychnos nux-vomica* shows a significant antimicrobial activity [20]. Homoeopathic mother tincture *nuxvomica* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Corynebacterium bacillus* and *Shigella* is 88.34 ± 1.40 , 71.41 ± 1.20 , 85.95 ± 1.40 , 83.20 ± 0.55 , 88.90 ± 0.90 , 45.34 ± 1.40 , 60.41 ± 1.20 , 47.95 ± 1.40 and 53.20 ± 0.55 respectively. *Nuxvomica* has excellent %age inhibition against *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, moderate activity against *E. coli*, *Klebsiella* and *Shigella* and poor inhibition against *Proteus* and *Corynebacterium bacillus*. *Strychnine*, the major component in the alcoholic extract of the seeds of *Strychnos nux vomica*, is responsible for its antilipid



peroxidative property. Antioxidant of action of this drug is through the chelation of the free iron in the system. Because strychnine does not have any pro oxidant property, because it does not convert [21]. Nuxvomica Q has poor antioxidant activity which is 47.64 ± 4.49 . Nuxvomica has excellent %age inhibition of alpha glucosidase which is 98.22. Nuxvomica has medium inhibition against *Fumigatus niger* which has zone of inhibition 7mm while Fluconazol has zone of inhibition 15mm.

Antibacterial activity of garlic extract was apparent within 1 h of incubation and 93% killing of *Staphylococcus epidermidis* and *Salmonella typhi* was achieved within 3 h of incubation. Yeasts were totally killed in 1 hr by garlic extract. Anti candidal activity of garlic was greater than by nystatin [22]. Homoeopathic mother tincture *Allium sativum* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus*, *Pseudomonas*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and *Shagilla* is 88.49 ± 0.52 , 83.29 ± 0.18 , 84.68 ± 0.12 , 86.90 ± 1.66 , 81.14 ± 2.20 , 54.49 ± 0.52 , 57.29 ± 0.18 , 55.68 ± 0.12 and 45.90 ± 1.66 respectively. *Allium sativum* Q has excellent %age inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus* and *Pseudomonas aeruginosa*, medium inhibition against *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and poor inhibition against *Shagilla*. Radical scavenging and antioxidative effects garlic powder has proved to be an effective antioxidant when tested against at hepatic micro some stressed by ascorbic acid/Fe". Alliin has a very good hydroxyl radical. But *Allium sativum* has poor %age inhibition of antioxidant free radical scavenging which is mentioned as 14.61. *Allium sativum* has zone of inhibition as very high (41–50 m) [23]. Homoeopathic mother tincture of *Allium sativum* has zone of inhibition 6mm as compared to Fluconazol whas zone of inhibition 15mm. *Allium sativum* has poor alpha glucosidase %age inhibition activity which is 11.80.

Cinchona showed antibacterial activity against several bacteria. *Cinchona* is highly recommended for the formulation of cosmetic products to protect the skin and hair against harmful microorganisms [24]. Homoeopathic mother tincture *Cinchona officinalis* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus*, *Pseudomonas*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and *Shagilla* is 89.23 ± 0.22 , 85.29 ± 0.51 , 75.33 ± 1.34 , 80.79 ± 1.69 , 71.73 ± 2.49 , 58.23 ± 0.2 , 53.29 ± 0.51 , 77.33 ± 1.34 and 71.79 ± 1.69 respectively. China has excellent %age inhibition against *Salmonella typhi*, *E. coli* and *Staphylococcus aerus*. Medium percentage inhibition was showed by *Bacillus subtilis*, shgilla and *Corynebacterium bacillus*. Moderate inhibition was showed by *Proteus* and *Klebsiela*. Due to the presence of caffeic acid, flavonoids and the extract

itself, cinchona is useful to treat mature skin. *Cinchona* has been shown antioxidant effects (Alonso, J., 2004). Homoeopathic mother tincture *Cinchona officinalis* has excellent %age inhibition which is 84.61 ± 3.98 .

Achillea millefolium was found to be mildly active against gram positive and gram negative bacteria [25]. Homoeopathic mother tincture *Achillea millefolium* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus*, *Pseudomonas*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and *Shagilla* is 29.03 ± 2.8 , 59.50 ± 0.88 , 65.73 ± 0.08 , 68.03 ± 1.86 , 74.85 ± 1.24 , 59.03 ± 2.84 , 50.50 ± 0.88 , 59.73 ± 0.08 and 68.03 ± 1.86 respectively. *Millefolium* Q has moderate to medium %age inhibition against *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus*, *Staphylococcus aerus*, *Pseudomonas aeruginosa*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and *Shagilla*. *Achillea millefolium* possess a significant free radical scavenging activity which is due to the presence of active compounds amongst phenolic compounds [26]. The extract of *Achillea millefolium* has been investigated scavenging effects against DPPH radical. The antioxidant and total phenolic constituent levels are also positively correlated. *Millefolium* mother tincture has good antioxidant activity which is 77.81 ± 3.98 . The ethanolic and aquatic extracts of *berberis vulgaris*, at a concentration from 35 to 40 µg/ml, showed a antibacterial effect against both Gram positive and Gram negative bacteria. In particular, *Proteus vulgaris* (MIC=32 microg/ml), *Pseudomonas aeruginosa* (MIC=16 microg/ml) and *Escherichia coli* (MIC=2 microg/ml) were the most inhibited.

Homoeopathic mother tincture *Beberis vulgaris* showed percentage inhibition against *Salmonella typhi*, *E. coli*, *Bacillus subtilis*, *Staphylococcus aerus*, *Pseudomonas*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and *Shagilla* is 60.75 ± 2.14 , 87.83 ± 0.12 , 69.69 ± 2.04 , 71.14 ± 0.09 , 64.69 ± 0.09 , 60.75 ± 2.14 , 65.83 ± 0.12 , 73.69 ± 2.04 and 53.14 ± 0.09 respectively. *Berberis vulgaris* has excellent percentage inhibition against *E. coli*, Moderate to medium %age inhibition against *Salmonella typhi*, *Bacillus subtilis*, *Staphylococcus aerus*, *Pseudomonas aeruginosa*, *Proteus*, *Klebsiela*, *Corynebacterium bacillus* and shagilla. Immunomodulatory and antioxidant effects of anthocyanins are abundant in *berberis vulgaris* fruits representing that they may have beneficial effects on inflammatory bowel diseases (IBD). *Berberis vulgaris* extract was effective to protect against colitis [27]. *Berberis* Q has good antioxidant activity which is 98.43. Homoeopathic Q *calendula officinalis* has excellent Antioxidant activity which is 83 ± 0.72 . Homoeopathic mother tinctures evaluated moderate to medium and excellent antibacterial activity against all strains. Homoeopathic mother tinctures showed antioxidant activity. Antifungal activity of homoeopathic mother



tinctures was lowered as compared to extracts of that plant. Alpha glucosidase inhibitory activity of different homoeopathic mother tinctures was excellent.

Conclusion

The study successfully demonstrated that homoeopathic mother tinctures exhibit promising biological activities, including significant antibacterial and antioxidant effects, while showing limited antifungal activity. The tinctures of *Cinchona officinalis*, *Calendula officinalis*, *Thuja occidentalis*, and *Nux vomica* were among the most effective in inhibiting bacterial growth, especially against *Salmonella typhi* and other pathogens. The antioxidant potential of these tinctures further supports their application in health-related products aimed at combating oxidative stress and inflammation. However, the antifungal activity of these tinctures was relatively modest, suggesting the need for further refinement or combination with other compounds to enhance their efficacy against fungal pathogens. Additionally, the high alpha-glucosidase inhibitory activity of certain tinctures, particularly *Nux vomica* and *Belladonna*, indicates their potential for future development in managing conditions like diabetes. In addition, the study provides valuable insights into the biological properties of homoeopathic mother tinctures, highlighting their potential for therapeutic use in combating infections, managing oxidative stress, and regulating blood glucose levels. Further research is needed to optimize these tinctures for broader clinical applications, especially in combination therapies, and to explore their efficacy in real-world settings.

Future Directions

Building upon the findings of this study, several avenues for future research and development can be explored to enhance the therapeutic potential of homoeopathic mother tinctures:

Enhancement of Antifungal Activity: While the tinctures demonstrated notable antibacterial and antioxidant effects, their antifungal activity was relatively weak compared to the standard antifungal agents. Future research could focus on optimizing the extraction methods or formulating enhanced tinctures by incorporating synergistic plant compounds known for their antifungal properties. Additionally, exploring the use of adjuvants or carrier systems may help improve their antifungal effectiveness.

Combinatory Therapeutic Approaches: Investigating the potential synergistic effects of combining different homoeopathic mother tinctures could lead to more potent formulations. These combinations could be tested not only for antibacterial and antifungal activity but also for their ability to address multiple therapeutic targets simultaneously, such as inflammation and oxidative stress. Multi-target therapies could be particularly beneficial in treating chronic diseases.

Clinical Trials and In Vivo Studies: The promising results observed in vitro need further validation in clinical settings. Conducting randomized controlled trials (RCTs) or animal studies will be crucial to evaluate the real-world efficacy and safety of these tinctures. This would also help identify optimal dosages, potential side effects, and the best ways to administer them for maximum therapeutic benefit.

Exploring Mechanisms of Action: Further investigations into the mechanisms behind the antimicrobial and antioxidant activities of these tinctures are needed. Advanced techniques, such as molecular docking studies, enzyme inhibition assays, and proteomic analysis, could provide deeper insights into the molecular pathways these tinctures interact with, leading to better-targeted treatments.

Formulation Development: To enhance the bioavailability and effectiveness of homoeopathic mother tinctures, future studies could focus on their formulation into more stable, user-friendly dosage forms. For instance, developing tinctures into creams, gels, or oral supplements could make them more accessible and effective in treating specific conditions. Investigating the stability and shelf life of these formulations will also be essential for commercial viability.

Broader Spectrum of Microbial Testing: While the study focused on a limited set of bacteria and fungi, future research should include a wider range of pathogens, including antibiotic-resistant strains. This would further establish the role of homoeopathic tinctures in addressing the growing global challenge of antibiotic resistance and provide valuable data for their incorporation into modern pharmacological therapies.

Sustainability and Sourcing of Medicinal Plants: Research on sustainable farming practices and ethical sourcing of the plants used in homoeopathic tinctures is essential for ensuring that these products are produced responsibly. This could involve investigating the environmental impact of cultivation, harvesting, and processing, as well as exploring eco-friendly and sustainable methods for large-scale production.

Collaboration with Modern Medicine: Collaborative research between homeopathic and conventional medicine could lead to the integration of homoeopathic tinctures in complementary treatments. Exploring their role in integrative healthcare, especially for conditions that are difficult to treat with conventional medicine alone, may offer a new frontier in holistic health approaches.

Conflict of Interest

No conflict of interest.

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