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Review

Ultra high dilutions: A review on *in vitro* studies against pathogens

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Multiple *in vitro* studies using homeopathic medicines are conducted for their effectiveness against various microbes. The reporting of results and the methodology in many studies are a query. The present review reveals the evidence based medicinal effects of homeopathic medicines on various plant and human pathogens *in vitro* with the help of quality studies. The studies showed positive outcome for homeopathic medicines. Thus, homeopathy is an effective agent in *in vitro* studies. However, substantial evidence on these serially diluted medicines must be replicated with the help of a standardized methodology for more precise evidences and conclusion.

Key words: Homeopathy, *in vitro*, manuscript information score, review.

INTRODUCTION

Homeopathy is a bicentennial system of medicine founded by Samuel Hahnemann (1755-1843), based on principle of “Similia Similibus Curentur”, which was revealed after repeated human experimentation and is currently used by approximately 500 million consumers (Manchanda, 2018). Hahnemann put forth the theory of “vital force” which believed that the succussed medicine shows medicinal effects, even beyond the Avogadro’s constant unit, which turns homeopathy as a science of quantum mechanics following the principle of quantum field theory (Khuda-Bukhsh, 2003). Homeopathy has been always challenged for its high dilution properties, clinical methodologies and its mechanism of action (Manchanda, 2018). The similia principle and the dynamization phenomena of the homeopathic medicines, in these recent years, are implemented in preclinical studies (*in vitro* and *in vivo*), in testing the mechanism of these highly diluted medicines in various models of biological system (Bellavite et al., 2006; Clausen et al., 2011). The concentrations of these serial diluted medicines are found to be less than 1 g molecule surpassing the Avogadro’s Constant (6.024×10^{23}) which is implausible in interpreting the concept with dose-dependent model of modern pharmacology (Teixeira and Carneiro, 2017).

In vitro studies are aimed to create evidence,

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understand the mechanism and validate the role of certain medicine against a particular condition. Of late, there have been various *in vitro* experiments performed on plant, animal and human cellular models in plausibly explaining the action of these ultra-high diluted medicines (Manchanda, 2018). Despite the multiple studies, the reporting of results and the methodology are a query.

Homeopathic Materia Medica lacks in understanding the principle of “Similia Similibus Curentur” in specific species of pathogens in their preclinical experiments (Teixeira and Carneiro, 2017). The present review reveals the evidence based medicinal effects of homeopathic medicines on various pathogens *in vitro* with the help of quality studies (Tables 1 and 2). This can lead to a mean development in the field of homeopathy in form of a new preclinicalHomeopathic Materia Medica on pathogens.

IN VITRO EXPERIMENTS AND HOMEOPATHY

According to Asha et al. (2014), *Thuja occidentalis* (Q, 30 C, 1 M, 10 M and 50 M) had a significant inhibition among all the 5 fungal genera species of *Fusarium, Aspergillus flavus, Bipolaris, Exserohilum* and *Curvularia;* however, exact mechanism of action of *Thuja* is unknown and can be future perspective of research in this fungal cultures (Asha et al., 2014).

In the study of Chakraborty et al. (2015), *Aconite napellus* (6C, 30C, 200C) showed bactericidal activity against *Staphylococcus aureus* and *Escherichia coli* and also identifed the nano-sized particle through Field Emission Scanning Electron Microscope of *Aconite* (Chakraborty et al., 2015).

Damin et al., (2015) studied homeopathic medicines like *Arsenicum album, Calcarea carbonica, Kali iodatum, Phosphorus, Silicea, Staphysagria, Spodoptera frugiperda, Sulphur, and Th. occidentalis* against *Metarhizium anisopliae* (strain *UNIOESTE 22)* which showed all the treatments as compatible in their dilutions (24CH, 30CH, 100CH; 3CH, 30CH; 6CH, 30CH, 100CH; 30CH, 100CH, 200CH; 200CH, respectively)(Damin et al., 2015).

In Gupta et al. (2015) study, homeopathic medicine *Acid benzoicum, Apis mellifica, K. iodatum, Mezereum, Petroleum, Sulphur, Tellurium, Sulphur iodatum, Graphites, Sepia, Silicea* and *T. occidentalis* in 30C and 200C acted as an evidenced based medicine that conformed both *in vitro* and *in vivo* on oral candidiasis.

The Toledo et al. (2016) study revealed the fungi toxicity action of homeopathic medicine against black rot disease of tomato crops. *Sulphur* and *Staphysagria* 100CH showed suppressive activity as compared to both controls in mycelium growth, even when successed distilled water at 60CH and 100CH inhibited mycelium growth. *Propolis* 6CH, 30CH and 60CH and *Ferrum sulphuricum* 6CH and 30CH caused inhibition and differed from both controls in sporulations. Also, spores germination of the pathogen was found to be reduced by *Isotherapic* of *A. solani* in 6CH, *Isotherapic* of ash in 6CH and *Ferrum sulphuricumin* 30CH medicines (Marcia et al., 2016).

According to the study of Hanif and Dawar (2016), both *in vitro* and *in vivo* experiment showed fungicidal potentials of homeopathic medicines of *T. occidentalis* and *Arnica montana* in globules 30CH against root rot disease in non-leguminous plants (Hanif and Dawar, 2016).

In the study of Prajapati et al. (2017), homeopathic mother tincture *S. jambolanum, F. religiosa, O. sanctum, A. cepa, T. occidentalis* and *H. antidysenterica* showed inhibitory action against human pathogenic fungi *Candida albicans* (Prajapati et al., 2017).

Passeti et al., (2017) experiment proved that *Belladonna* and *bacterial nosode* in 6CH and 30CH, *Silicea* and *Hepar sulphur* in 6CH, 12CH and 30CH, and *oxacillin* showed a significant reduction ($p< 0.001$) on *Methicillin-resistant S. aureus* (Passeti et al., 2017).

Shinde et al. (2018) had conducted two *in vitro* studies on both *Pityrosporum ovale* and *C. albicans* (NCIM-3557), regarding fungal culture homeopathic medicine *Selenium, Cinchona officinalis, Azadiracta indica, Phosphorus, Acidum benzoicum, Zingiber, Sulphur, Acidum sulphuricum, Iodium,* and *Zincum metallicum* in 6CH, 12CH, 30CH, 200CH, 1M respectively that showed inhibitory effects in both fungal culture (Shinde et al., 2018).

EXPERIMENTATION PERSPECTIVE

The above mentioned studies elaborate on scope of homeopathic medicine in the era of drug resistance to various fungal and bacterial cultures. Homoeopathic treatment can be used as an alternative therapy, as cost effective, with no adverse event observed. The experimental methodologies used by the included studies were quite different from each other; needs to be standardized and must be modified by the European Committee on Anti-microbial Susceptibility Testing (EUCAST) and Clinical Laboratory Standards (CLS) guidelines (Hombach et al., 2011) in performing experiments, specially designed with ultra-high dilution, in order to get more precise and accurate results. Also, it must be replicated in *in vivo* studies. In recent times, these serial diluted medicines have been proved to show the presence of nanoparticles in size of quantum dots and should be seriously taken into consideration about their nano-pharmacological aspects (Chikramane et al., 2010). Various hypothetical models have been put forth in understanding the mechanism of action of these nano medicines and attempts have been made with various molecular studies in identifying the mechanism of action of these medicines (Khuda-Bukhsh , 2003); however, a standard protocol still remains, which is a question of development for the methodologies performed in *in vitro*

Table 1. Summary of *in vitro* studies evaluated with Manuscript Information Score (MIS) ≥ 5.

Author	Organism	Methods and assay	Control	Homeopathic medicine	Potency	MIS	Remark
Asha et al. (2014)	*Fusarium, Aspergillus flavus, Bipolaris, Exserohilum, Curvularia.*	Cello tape flag method, MFC	Sterile water, rectified spirit, ketoconazole.	*Thuja occidentalis*	Q, 30 C, 200 C, 1 M, 10 M and 50 M	7	Inhibitory activity against the fungi causing keratitis
Monalisa Chakraborty et al. (2015)	*Staphylococcus aureus, Escherichia coli.*	Antibacterial activity spread plate technique, FESEM.	Control plates of both the strains	*Aconitum napellus*	6C, 30C, 200C	9	Activity in bacteria in high dilution
Silvana Damin et.al. (2015)	*Metarhizium anisopliae* (strain UNIOESTE 22)	Insecticidal activity, CFU, Germination.	Hydroalcoholic solution (0.1%)	*Arsenicum album, Calcarea carbonica, Kali iodatum, Phosphorus,Silicea; Staphysagria ,Spodopterafrugiperda Sulphur,Thuja occidentalis*	24C; 30C; 100CH 3CH; 30CH; 6CH, 30CH, 100CH; 30CH; 100CH 200CH; 200CH resptectively.	10	All treatments Compatible in fungus *M. anisopliae*
Girish Gupta et al. (2015)	*Oral candidiasis*	Disc diffusion method.	Ketoconazole, rectified spirit, distilled water.	*Acid benzoicum, Apismellifica, Kali iodatum, Mezereum, Petroleum, Sulphur, Tellurium, Sulphur iodatum, Graphites, Sepia, Silicea and Thuja Occidentalis*	30C, 200C	7	Inhibitory activity against *Candida albicans*
Márcia Vargas Toledo et al. (2016)	*Alternaria solani*	Mycelial growth, sporulation and conidial Germination.	Distilled water and hydroalcoholic solution with dynamizations	*Sulphur, Silicea terra, Staphysagria, Phosphorus, Ferrum sulphuricumand Kali iodatum*	6CH and handled to 12, 30 and 100CH (CH:	7	Activity in control of black spot disease in tomato crops
AsmaHanif et al. (2016)	*Rhizoctoniasolani, Fusarium* spp. and *Macrophomina phaseolina*	Growth inhibition percent Paper Disc Diffusion Method	Sterilized distilled water, absolute alcohol, Globules	*Thuja occidentalis and Arnica montana*	30C	7	Activity against non-leguminous crops
Suneel Prajapati et al. (2017)	*C.albicans (MTCC No. 3017)*	Agar disc diffusion method	Ketoconazole, 90% alcohol	*Syzygiumjambolanum, Ficusreligiosa,Oscimum sanctum, Alliumcepa, Thuja occidentalis, Holarrhenaantidysenterica.*	Mother tincture (Q)	10	Antifungal activity present
TâniaAguiarPasseti et al. (2017)	*Multi-Resistant Staphylococcus aures (MRSA)*	MIC	Oxacillin	*Silicea, Hepar sulph, Belladonna and bacterial nosode*	6 CH, 12 CH and 30 CH in sterile 30% alcohol	9	Activity different in different potency of live cells
Chetan H.shinde et al. (2018)	*Pityrosporum ovale*	Anti-dandruff assay, MIC, Lysis studies	Dispensing alcohol, Zinc pyrithione	*Selenium, Cinchona officinalis, Azadiractaindica, Phosphorus, Acidumbenzoicum, Zingiber, Sulphur, Acidumsulphuricum, Iodium, Zincum metallicum,*	6C,12C,30C,200C,1M	9	Inhibitory activity against the fungus

Table 1 Contd.

Chetan H.Shinde et al. (2018)	*Candida albicans (NCIM-3557)*	Agar diffusion assay, MIC, Germ Tube Inhibition	Clotrimazole Vehicle Control (Dispensing alcohol)	*Acidum Sulphuricum, Acidum Benzoicum, Azadirachta indica, Cinchona officinalis, Iodium, Phosphorus, Selenium, Sulphur, Zincum Metallicum, Zingiber officinale*	6C, 12C, 30 C, 200 C, 1M	8	Inhibitory effect against *Candida albican*

MIC= Minimum Inhibitory Concentration; C or CH= Centesimal Scale (1:99); M=1000CH; NCIM= National collection of Industrial Micro-organism; CFU= Colony Forming Unit; FESEM= Field Emission Scanning Electron Microscopic; MFC= Minimum Fungicidal Concentration; MTCC= Microbial Type Culture Collection; UNIOESTE= Universidade do Oeste do Parana; MIS= Manuscript Information Score.

Table 2. Manuscript Information Score of the included studies.

Author	Year	Type	Description in the study					Total score
			Experimental procedure	Materials	Measuring instruments	Potentization	Controls	
Asha et al.	2014	*Fusarium, Aspergillus flavus,Bipolaris, Exserohilum, Curvularia*	2	2	1	2	1	8
Monalisa Chakraborty et al.	2015	*Staphylococcus aureus, Escherichia coli*	2	2	2	2	1	9
Silvana Damin et al.	2015	*Metarhiziumanisopliae(strain UNIOESTE 22)*	2	2	2	2	2	10
Girish Gupta et al.	2015	*Oral candidiasis*	2	2	1	1	1	7
Márcia Vargas Toledo et al.	2016	*Alternaria solani*	2	2	1	1	1	7
AsmaHanif et al.	2016	*Rhizoctoniasolani, Fusarium spp. And Macrophominaphaseolina.*	2	1	1	2	1	7
Suneel Prajapati et al.	2017	*C.albicans(MTCC No. 3017)*	2	2	2	2	2	10
TâniaAguiarPasseti et al.	2017	*Multi-Resistant Staphylococcus Aures (MRSA)*	2	2	2	2	1	9
Chetan H. Shinde et al.	2018	*Pityrosporumovale*	2	2	2	1	2	9
Chetan H. Shinde et al.	2018	*Candida albicans (NCIM- 3557)*	2	1	2	1	2	8

and *in vivo* studies in homeopathy.

CONCLUSION

This systematic review demonstrates homeopathy as an effective agent, in *in vitro* studies and can lead to a new development with the help of a new preclinical Homeopathic Materia Medica on pathogens. However, substantial evidence on these serial diluted medicines results must be replicated with a standardized methodology to provide conclusive evidence.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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