



**"AN EVIDENCE-BASED STUDY OF INHIBITORY EFFECT OF HOMOEOPATHIC REMEDIES TARAXACUM, BORAX, CALCAREA CARB, PULSATILLA ON CANDIDA ALBICANS- AN IN VITRO STUDY"**

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**ABSTRACT**

**Background-** *Candidiasis* is one of the serious health issues due to persistent rise in easy infection and transfer of candida albicans infection especially due to cross transmission. The management of *Candidiasis* is the prevention of the infection itself. Homoeopathy is a system of medicine which has a positive effect in preventing and controlling the infection and is considered safe for administration. In this study, 4 commonly used Homoeopathic medicines having positive results primarily have been considered for the in-vitro antifungal activity.

**Aim-** The aim of this study is to screen the anti-fungal activity of various homoeopathic medicines against *Candida albicans*  
**Methodology-** The medicines namely *Taraxacum officinalis*, *Borax veneta*, *Calcarea carbonica* and *Pulsatilla nigricans* in potencies from mother tincture and 30 CH, 200CH, 1M, 10M whose aqueous solution can be made are short-listed. The Antifungal Assay was performed with the Agar well diffusion method and Zone of inhibition was recorded. The experiment was done in duplicates. The MIC value of the medicines was recorded with the Spectrophotometer at 0 hour and 3 hours. The medicines showing best MIC values were shortlisted for the Germ tube Inhibition. The Germ tube Inhibition experiment was performed by the 96 well plates.

**Result-** Homoeopathic medicines namely *Taraxacum 200C*, *Borax 10M*, *Calcarea carb 30C*, *Pulsatilla 10M* showed above 50 percent of inhibition. Homoeopathic medicine *Borax 10M* showed the highest percentage of inhibition.

**Conclusion-** The present in vitro study has shown potential role of Homoeopathic medicines in inhibiting the growth of *Candida albicans*.

**KEYWORDS :** *Candida albicans*, Homoeopathy, Antifungal, germ tube inhibition, in-vitro

**INTRODUCTION:**

Father of physiology aptly said that no great cure can occur without it being similar in nature, Dr Hahnemann's thoughts found a destination while translating the Cullen's *Materia Medica*; after finding out that Malaria was cured by cinchona bark due to its bitter taste is when he got the ground for cause and cure equation. The law of similar's had seen the light in the year 1796 marking the beginning of Holistic and most natural form of treatment which came to be known as Homoeopathy. It is not a therapy based on whims but it has a solid ground planning and it's the cardinal principles<sup>[2]</sup>. The modus operandi of homoeopathic medicines is not similar to that of the modern medicine and it occurs by triggering a complex mechanism of action involved in body's natural healing ability based on the theory of 'Vital force'<sup>[3]</sup>

In fission reactions<sup>[4]</sup>, minute nucleus can destroy a whole city when broken into sub particles in chain reaction. After a series of dilution, you will know that the original molecule is not present in the dilution<sup>[5]</sup>, but every drop of dilution has been imparted with the programming that is biological like a virus or bacteria or virus is biologically programmed. Homoeopathy is like Galileo; way ahead of time. He also was tried in court after stating that Earth rotates around the Sun<sup>[6]</sup>. We also know that science is still study in about the sun as it's the nature hasn't revealed itself completely yet. There are mainly 3 major types of microbes identified for various infections namely bacteria, virus and fungus. Others are parasites, protozoans, helminths, ectoparasites, prions.

Around 5000 types of viruses, 1 nonillion bacterial variety present on Earth, 5.1 million species of fungus are known. There is a great rise in mortality and if not death but a prolonged hospitalisation. *Candidiasis* although preventable has been maintaining the fourth highest position in blood-stream pathogenic infection, about 8% of all hospital-acquired infections. Most common candida blood-stream infections are caused by the species *Candida albicans*<sup>[7]</sup>. Prevention is an important feature of management of *candidiasis* in blood-stream infection.

**Epidemiology**

United States: Overall, *Candida albicans* infections remain as the highest source of yeast infections in immune-challenged people like the Human Immuno-deficiency Virus compromised patients, over 90%<sup>[8]</sup> show a high possibility of suffering from the commonest type of candidal infection that is oropharyngeal candidiasis, whereas approximately 75%<sup>[9]</sup> of female population suffer from vulval or vaginal candidiasis or both at the same time. It is also possible that they can suffer many a times with this.

In today's world of evidence-based medicine we are in constant need of proving the studies and results in the form of logical and dependable data. Evidently there is less literature regarding supporting and backing the use of Homoeopathic medicines in treating the fungal infections. Hence, this study is a sincere effort to bring light on the evidence-based study of homoeopathic medicines to provide strong basis for the clinical practice of drugs against yeast infections.

**MATERIALS AND METHODS:****• Laboratory study:**

- i) Maintenance of pathogenic culture in agar slants.
- ii) Cultivation of culture in liquid broth (Candida full medium)
- iii) Anti - fungal activity on agar plates.
- iv) Check the activity of Homoeopathic medicines by agar well diffusion method in vitro.
- v) Minimum inhibitory concentration (MIC)
- vi) Germ tube inhibition

**• Selection of tools:**

Spectrophotometer, Autoclave, Agar plate and micropipette, Incubator, Laminar air flow, borer, ELISA plate, 96 well plates, Human pathognomic Fungus *Candida albicans* [CA-3557]

**• Media and Chemicals:**

Hi-Media Lab, Mumbai, India was the source of obtaining the chemicals and were kept at Rajiv Gandhi Institute of Biotechnology which were of AR grade.

**• Homoeopathic Medicines:**

Homoeopathic medicines such as *Calcarea Carbonica*, *Taraxacum officinalis*, *Pulsatilla nigricans* and *Borax veneta* in Q, 30C, 200C, 1M and 10M that are available in liquid potencies and are commonly used in clinical practice were bought from GMP approved standard Homoeopathic manufacturing unit (SBL)

(Table No. 1: Name of Homoeopathic medicines with Potency used.)

Sr. No.	Name of Homoeopathic medicines	Potency
01.	Calcarea carbonica	30C, 200C, 1M, 10M
02.	Taraxacum officinalis	Q, 30C, 200C, 1M, 10M
03.	Pulsatilla nigricans	Q, 30C, 200C, 1M, 10M
04.	Borax veneta	30C, 200C, 1M, 10M

**• Control:**

Ethanol (Dispensing alcohol 90%) used as vehicle control, Fluconazole as standard control. Distilled water and Broth is used as negative control.

**• Microorganism:**

*Candida albicans* [CA-3557] was used that was available at Rajiv Gandhi Institute of Information Technology and Bio technology (RGITBT) which they had procured from NCIM(Mumbai) India. It was well maintained by the institute in the optimum environment required by it. Culture required for the growth of *Candida albicans* was done in Sabouraud Dextrose Agar at the optimum pH of 7 followed by incubating it at a temperature of 37°C continuously undisturbed for a period of 24 hrs.

**• Media Preparation:**

**1.Preparation of media and correction of pH** – Mix Sabouraud dextrose broth with distilled water in the ratio of 24 gm in 1000ml. Add Agar agar type 1 in above solution in ratio of 18gm in 1000ml. During this process the pH of Sabouraud Dextrose Broth solution ranges in between 6.00 to 6.5. We set the pH at 7 by adding NaOH.

**2.Preparation of Broth-** We needed 50 ml of Sabouraud Dextrose Broth, thus we took 1.5 gm of Sabouraud Dextrose Broth in 50 ml of distilled water. Then pH was set at 7 with the help of pH meter. The tubes used were autoclaved earlier. Then the mixture was autoclaved for 15 minutes. Then broth was validated for 24 hours.

**3.Preparation of Agar-** 400 ml of Sabouraud Dextrose Broth was prepared and then 20 plates were wrapped and kept for autoclaving for 1 hour. After autoclaving, the plates were

poured with the agar and kept for 24 hours for solidification and validation.

**• Anti-fungal assay:**

This was done with the help of a method known as 'Agar Well Diffusion Assay'<sup>[9]</sup>. Thus, 100 µl of culture suspension was uniformly layer on Sabouraud dextrose agar (SDA) plates with the help of swab and then it was incubated at 25°C for 30 minutes followed by adding the medicines in different potencies of 40µl quantity into the wells with the help of a 5 mm borer. This mixture was incubated at a temperature of 37°C for 24 hours. This was followed by addition of Fluconazole in the concentration of 1mg/ml (chemical control) in the well. Dispensing alcohol was also added in the well followed by incubation at 37°C for 24 hrs. The diameter of the zone of inhibition was seen after completion of twenty-four hours.

**• Minimum Inhibitory Concentration value:**

Minimum Inhibitory Concentration<sup>[10]</sup> was determined with the value of broth dilution assay. Sabouraud Dextrose Broth (SDB)- half milli-litre, potencies of particular Homoeopathic medicines (half milli-litre) and the culture of *Candida albicans* (half milli-litre), together gave around 1.5 milli-litre of total constituents of the tube. The negative control was Sabouraud dextrose broth whereas chemical/standard control was Fluconazole. Ethanol (Dispensing alcohol 90%) was the vehicle control. Calculation of the MIC figures was done after three hours. Optical Density (OD)- 0.5 at 580 nm was kept constant as required for the experiments.

**• Germ Tube Inhibition-**

It was conducted according to modified Braymen and Wilkes as follows-

*Candida* cultures (100 mlitre) in sabouraud dextrose broth containing 0.2% human serum was added in treated 96 well flat bottom plates followed by the addition of 100 mlitre of selected medicine with and without heat treatment at 60 degrees Celsius for one hour. The plate was incubated at 37 degrees Celsius for 4 hours to induce germ tube formation. *Candida* with Saline was used as negative control. After incubation, the medium in the wells were discarded by inverting the plate and each well was washed with 70% ethanol. Then the wells were washed with 25 % SDS. Then the wells were washed with distilled water for 3 times. Then it were stained with 200 mlitre of 0.1 % violet crystal for a duration of 10 min. Then plate was washed 3 times with distilled water followed by washing with 0.25% SDS and again washed with distilled water for 3 times. Plates were dried and a mixture of 200 mu litres of isopropanol with 0.04 HCl and 50 millilitres 0.25% SDS were added to elute the crystals violet. Absorbance of the eluted dye was determined at 590 nanometres using an ELISA plate reader (Biotek India). The measurement of germ tube inhibition was done in percentage<sup>[11]</sup>.

**• STATISTICAL ANALYSIS:**

In agar well diffusion method experiment was conducted in the duplicates and mean value and standard deviation were calculated by using Graph Pad Prism Version 7.00 software.

**OBSERVATIONS AND RESULTS:****• The Anti-fungal assay –**

The Zone of inhibition starts from 0.55 up to 1.10cm. for different potencies like *Calcarea Carbonica* 30C (0.0 ; 0.0) cm, 200C(1.0 ; 1.1) cm, 1M(0.6 ; 0.5) cm, 10M(0.0 ; 0.0) cm; *Pulsatilla pratensis* Q (1.5 ; 1.5) cm, 30C (0.0 ; 0.0) cm, 200C (0.0 ; 0.0) cm, 1M (0.0 ; 0.0) cm, 10M (0.0 ; 0.0) cm; *Borax veneta* 30C (0.6 ; 0.7) cm, 200C (0.0 ; 0.0) cm, 1M (0.0 ; 0.0) cm, 10M (1.2 ; 1.1) cm; *Taraxacum officinalis* Q (0.0 ; 0.0) cm, 30C (0.9 ; 1.0) cm, 200C (0.9 ; 1.1) cm, 1M (0.3 ; 0.5) cm, 10M (0.0 ; 0.0) cm.

The average of the ZOI of the Homoeopathic medicines is given below-

(Table No. 2: Antifungal Activity of Homoeopathic medicines with mean and S.D.) The MIC of the medicines with difference

		Zone Of inhibition against Candida albicans (Diameter in cm)				
Name of Medicines	Potency	1 <sup>st</sup> reading	2 <sup>nd</sup> reading	Mean	STD Dev	Mean + Std deviation
Calcarea Carbonica	30C	1	1	1	0	1±0
	200C	1	0.9	0.95	0.07071068	0.95±0.07
	1M	0.6	0.5	0.55	0.07071068	0.95±0.07
	10M	0	0	0	0	0
Pulsatilla	Q	1	0.9	0.95	0.07071068	0.95±0.07
	30C	0	0	0	0	0
	200C	0	0	0	0	0
	1M	0	0	0	0	0
	10M	1	1	1	0	0
Taraxacum	Q	0	0	0	0	0
	30C	0.9	1	0.95	0.07071068	0.95±0.07
	200C	0.9	1.1	1	0.14142136	1±0.14
	1M	0.3	0.5	0.4	0.14142136	0.4±0.14
	10M	0	0	0	0	0
Borax	30C	0.6	0.7	0.65	0.07071068	0.65±0.07
	200C	0	0	0	0	0
	1M	0	0	0	0	0
	10M	1.2	1.1	1.15	0.07071068	1.15±0.07
Flucanazole	1mg/ml	0	0	0	0	0
Alcohol (Dispensing )	90%	0	0	0	0	0
Distilled Water	--	--	--	--	--	--

(Table No. 3: 0 hr and 3 hr MIC of the Homoeopathic medicines.)

Sr. No	Name of Medicine	Potency	Optical Density (0 hr)	Optical density (3 hr)	Difference between the readings
1	Calc Carb	30C	0.164	0.157	-0.007
2		200C	0.305	0.563	0.821
3		1M	0.206	0.418	0.212
4		10M	0.206	0.418	0.212
5	Pulsatilla	Q	1.433	1.294	-0.139
6		30C	0.08	0.387	0.307
7		200C	0.116	0.286	0.17
8		1M	0.151	0.255	0.104
9	Taraxacum	10M	0.231	0.153	0.078
10		Q	0.494	0.733	0.239
11		30C	0.131	0.247	0.116
12		200C	0.262	0.173	-0.089
13	Borax	1M	0.273	0.308	0.035
14		10M	0.333	0.269	-0.064
15		30C	0.149	0.234	0.085
16		200C	0.147	0.243	-0.116
17	Flucanazole	1M	0.141	0.107	-0.034
18		10M	0.202	0.182	-0.02
19		--	0.225	0.109	-0.116
20	Alcohol	--	0.194	0.217	0.023

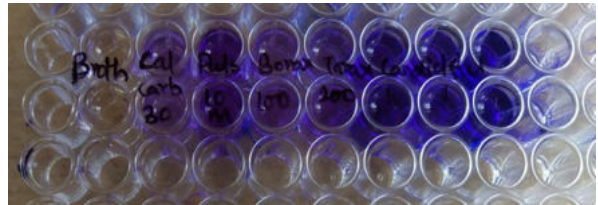
21	Blank (fungus + broth)	--	0.042	0.291	0.023
22	Broth	--	-0.12	-0.12	0

The MIC of best Homoeopathic medicine varied from Q to 10M potencies as given below-

(Table No. 4: MIC of the Homoeopathic medicines.)

Sr. No.	Homoeopathic Medicine	MIC (Homoeopathic Potency)
1	Calcarea Carbonica	30C
2	Pulsatilla	10M
3	Taraxacum	200C
4	Borax	10M

1) Germ Tube Inhibition-



[Figure no: 1- Germ tube inhibition]

Percentage of the Germ tube inhibition-

(Table No. 5: Percentage of Germ tube inhibition)

Sr. No.	Homoeopathic Medicine	Homoeopathic Potency	Percentage of the Germ tube inhibition
1	Calcarea Carbonica	30C	61.1%
2	Pulsatilla	10M	10.9%
3	Taraxacum	200C	64.3%
4	Borax	10M	82.1%
5	Fluconazole	1mg/ml	-26%

DISCUSSION:

Clinical trials have been conducted in various parts of the globe regarding candida albicans and also about different other fungal infections. However, there are very few in-vitro researches studies performed regarding Candida albicans with Homoeopathic medicines according to the conducted literature survey. The whole objective behind performing this in-vitro experiment was - ascertainment of antimycotic effect of the selected Homoeopathic remedies for inhibiting of growth Candida albicans in an in-vitro setup. This study will throw light on the concept of high dilutions and its comparative effect of remedies vs placebo effect.

Homoeopathic medicines may not produce instantaneous but it does not cause adverse reactions by far today is considered. Thus, treating candidiasis with Homoeopathic medicines will be fruitful and beneficial. Studies in near future may find a new viewpoint in treating resistant strains of candida. Homoeopathy is often claimed to have a placebo effect, but this experiment proves otherwise. The high - diluted medicines in various potencies and strength are effective in in-vitro fungal testing as an evidence-based medicine.

Borax veneta has been used for years together clinically for Candida albicans, but the potency of 10M was a prescriber's hesitation. Similarly, Taraxacum which is used in all potencies but did not give enough relief may be achieved; hence now we know if prescribed in 200 potency with adequate repetition then the results are sure as we have the ground work for it. Calcarea carb in 30 potency and Pulsatilla in 10 M potency also showed the inhibition of growth of Candida albicans.

CONCLUSION:

All the modes of treatments are now working rigorously to provide visible results for the use of simple cosmetics to the use

of major antibiotics and steroids.

The outcome of this particular study validates the postulate of 'medicine based on evidence'. This concludes that all the medicines employed under the experiment elicit defined inhibitory action in opposition to *Candida albicans*, specifically the following remedies, namely *Calcarea Carbonica*, *Pulsatilla*, *Taraxacum*, *Borax* wherein potencies showing the best inhibitory effect are *Calcarea carbonica 30 CH*, *Taraxacum 200 CH*, *Pulsatilla nigricans 10 M*, *Borax veneta 10M*. Thus, these medicines are effective against *Candida albicans* to treat candidiasis. Further study is needed in the form of field trials in order to check the anti-microbial activity against *Candida albicans* and identifying the exact mechanism of action of Homoeopathic medicines and effect on human beings.

#### Conflicts of interest

Author declare there is no any reported conflict of interest.

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