

**AN IN-VITRO STUDY
OF THE BACTERICIDAL
AND BACTERIOSTATIC
EFFECTS OF
CALENDULA
OFFICINALIS
—A HOMOEOPATHIC
MOTHER TINCTURE.**

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During the American civil war in the 16th Century, *Calendula Officinalis* Linn was found serviceable in gunshot wounds promoting healing, lessening suppuration and relieving pain. It was then used in the proportion of 2 dr. of the tincture to 1 pint of sterile normal saline solution. The application was found effective more when applied hot. Those were the preantiseptic eras, and the results of such treatment is not less encouraging even to-day.

Calendula officinalis linn is a common pot or garden marigold and is a native of France and Southern Europe. The word *Calendula* comes from "*Calendae*" meaning *Calendae* as it flowers on the first day of the month. It was introduced in homoeopathic practice by Dr. Franz in 1838.

Its sphere of use has however been narrowed by the introduction of aseptic surgery but where union has broken for any reason it is found to favour granulation and to remove unpleasant odours. It is reported that in staphylococcal infections where secretions are not pent up, the application of this drug is soothing and favours healing. (1)

Regarding the medicinal properties and uses of this plant it is reported by Nadkarni 1954, Chopra et al 1956, that—

- (i) It has an astringent and styptic property
- (ii) Its leaves are resolvent and diaphoretic.
- (iii) Its flowers are stimulant and emagogic.
- (iv) It is useful in healing wound. (2)

It contains :—
Ash = 44.9%
Calcium = 3.04%
Tannins = 6.45% (3)

Pharmacological study reveals that an aqueous extract of the plant contains both calcium and tannins, that it can exert a haemostatic effect and that fibrin formation occurs in 67 seconds. The alcoholic extract as well as tannin-free aqueous extract has been found to have little haemostatic effect. (4)

An alcoholic extract of the whole plant has been found to possess an anti-protozoal property specially in respect of *E. histolytica*. The flowers contain an amorphous bitter principle, Calendulin. (5)

Dr. Dorothy Sepherd in "Homoeopathy For the First Aider" has stated that *Calendula Officinalis* not an anti-septic in the true sense of the word, but then the germs do not thrive in its presence. This she has inferred from her own clinical observations. (6)

C. Hering has recorded that *Calendula* acts when the wound is painful and threatening to suppurate or, when there is a constitutional tendency to erysipelas, or, when sloughing has occurred, or when the ulcer is indolent, or, when a suppurative wound becomes painful. The drug has also been stated to hinder or prevent suppuration. (7)

In British pharmaceutical Codex it has been mentioned that *Calendula* preparation has the power of promoting absorption of diffused blood, and tincture has been used in amenorrhoea in doses of 3-12 decimal in 5-20 minims. (8)

From the above observations it seems obvious that we are not having concrete data with us regarding the bactericidal and bacteriostatic effects of this plant. It is thus imperative that further work should be undertaken to prove whether it has any bacterostatic and/or bacterocidal effect.

All the above clinical observations made by various workers in homoeopathy are not however supported by any data pointing to its bactericidal or bacterostatic properties, though clinically such properties of *calendula* can not be ruled out.

To make the work of our Master complete, it was decided to study in-vitro the effect of mother tincture of *Calendula officinalis* linn, on various organism producing surface lesions. Hence both qualitative and quantitative tests were undertaken.

1- Qualitative Test —

To assess the bactericidal and bacteriostatic effect of *C. Officinalis* external (having alcoholic quantity 60% by volume which is prepared from whole plant including flowers) in serial dilutions.

2. Quantitative Test :—

To assess the bactericidal and bacterostatic effect of *C. officinalis* external (having alcoholic quantity 60% by volume which is prepared from whole plant including the flower) directly by disc. diffusion test against the commonest organism.

MATERIALS AND METHODS

The culture media employed in the present work included

1. **Nutrient Agar**
(Hindusthan dehydrated media)
2. **Nutrient broth**
(DIFCO Laboratories, U.S.A.), and
3. **Brain Hearts infusion broth**
(DIFCO Laboratories, U.S.A.),

Drug - The homoeopathic mother tincture of *Calendula officinalis* (External) obtained from M/s. Hahnemann Publishing Co., (Pvt) Ltd., 165, B.B. G. Street, Cal-9.

Three solutions were prepared for the purpose of this experiment. Double distilled water was used for diluting the mother tincture to final dilutions of 1 in 10, 1 in 100 and 1 in 1000 of the solutions.

Absolute alcohol containing 99.5% by volume was fractioned down to the equivalent quantity present in the drug under trial, and was used as control.

The quantity of alcohol was standardised to strength of alcohol contained in the drug substance for obvious reasons of uniformity of result and inference.

Strains of the following organisms were procured from the Deptt. of Bacteriology, School of Tropical Medicine, Calcutta.

- Streptococcus faecalis*
- Staphylococcus aureus*
- Streptococcus haemolyticus*
- Pneumococcus*
- Klebsiella*
- Pseudomonas pyocyanea*
- Proteus*
- Escherichia coli*,

Method :

Subcultures were made from the strains obtained from STM in nutrient broth.

However, brainheart infusion broth was used for the subculture of *Str. haemolyticus* and pneumococcus.

EXPERIMENTAL DETAILS :

Serial dilution tube technique :

(a) Bacterial strains viz., : *streptococcus faecalis*, *staphylococcus aureus*, *pseudomona pyocyane*, *Klebsiella*, *proteus* and *Esch. Coli* supplied in nutrient agar were subinoculated each into 10 ml. of standard nutrient broth.

(b) Strains of pneumococcus and streptococcus haemolyticus grown in blood agar media were each subcultured in 10 ml. of standard Brain-heart infusion broth.

Following incubation for 24 hrs. at 37°C., the culture were subjected to treatment with the drug as per following schedule.

Test Series :

(with organisms grown in nutrient broth)

0.8 ml. of nutrient broth

+ 0.1 ml. of 24 hrs. culture in nutrient broth.

+ 0.1 ml. of trial drug in varying dilutions viz., 1 in 10, 1 in 100 and 1 in 1000 dilutions.

Control Series - (1)

0.8 ml. of nutrient broth.

+ 0.1 ml. of 24 hours culture in nutrient broth. + 0.1 ml. of alcohol in similar dilutions, viz., 1:10, 1:100, and 1:1000 dilutions.

Control Series - (2)

0.8 ml. of nutrient broth.

+ 0.1 ml. of 24 hrs. Culture in nutrient broth.

Similar preparations were made with organisms grown in brain heart infusion broth.

All the tubes were incubated at 37°C. for 24 hrs.

Presence or absence of turbidity was recorded for each of the eight organisms.

To study the bacteriostatic and bactericidal effects, subculture, were made from each such tube on the solidified surface of the nutrient agar plates.

After 24 hrs. incubation, the growth was studied. The result obtained is presented in the attached table.

EXPERIMENTAL DETAILS :

DISK METHOD

Disks of 6.25 mm. in diameter were punched from whatman No. 1 filter paper and were kept in batches of 100 in two sterile petri dishes.

C. Officinalis was poured into one of the plates with disks and to another absolute alcohol of 60% strength was added.

Inoculation of nutrient agar plates was made from broth culture over the whole surface of the medium by a sterilized pasteur pipette. The plates were left to dry for 1 hr. after which the surface disks were placed on the surface of nutrient agar with sterile forceps.

After 24 hrs. of incubation, the growths were studied for zone of inhibition.

Results :

The result of this test is presented in a tabular form (table 1).

Data Evaluation :

From the data obtained and presented in the tabular form (table 1), it can be seen that the result was rather fallacious in the first experiment.

However, the subsequent experiments performed on more than four occasions showed an unequivocal result with growth in drug dilutions, in alcohol and in the positive control. Similar results are also obtained in the serial tube dilution tests.

Conclusion :

The present experiments done "in-vitro" have shown the validity of Homoeopathic laws. Homoeopathy is a qualitative approach to the problem of health and disease and its resolution through the application of a dynamic force represented by a drug whose capacities of effecting a change in the state of health are fully known to clinician. Such yardsticks as employed by the modern medicine to assess the efficacy of toxicity, sensitivity etc. cannot be employed for the assessment of similar norms in Homoeopathy. Homoeopathic philosophy is based on certain fundamentals which essentially include.

i) Law of Similia Similibus Curenter.

The fundamental law of selection of the similar dynamic force which demands a total understanding of the concept of Hahnemannian totality, the ability to perceive it in the areas of disease (drug disease as well as natural) and capacity to achieve its establishment. On similar plane through the adoption of suitable techniques and the application of right clinical judgement.

RESULTS
(Disk method)

Table - 1

Dilutions and Readings	STR. FAECALIS			KLEB SIELLA			PSEUDO. PYOCY- ANEA			PROTEUS.			E. COLI			S T R. AUREUS			S T R. HAEMOL YTICUS			PNEUMO- COCCUS			
	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	Drug.	Alcohol.	Control.	
6 ml. of Alco- hol in 100 ML. of Dist. water	1.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	2.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	3.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	4.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	5.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
01 0.6 ml of Alco- hol in 100 ml. of Dist water.	1.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	2.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	3.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	4.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	5.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
0.06 ml. of Alcohol in 100 ML. of Dist. water.	1.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	2.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	3.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	4.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
	5.	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p

P = Positive

RESULT

Table - 2 (Tube Dilution Method)

Test	Str. faecalis	Kleb-siella	Pseudo-pyo.	E. Coli	St. aureus	St. haemolyticus	Proteus	Pneumo-coccus
1.	y	n	y	y	y	y	y	y
2.	y	y	n	n	y	y	y	n
3.	y	y	y	y	n	y	y	y
4.	y	y	y	y	y	y	y	y
5.	y	y	y	y	y	y	y	y
Control :								
1.	y	y	y	y	y	y	y	y
2.	y	y	y	y	y	y	y	y
3.	y	y	y	y	y	y	y	y
4.	n	y	y	y	y	y	y	y
5.	y	y	y	y	y	y	y	y

y = Positive

n = Negative

ii) Law of direction of cure. The law of verification that gives us the requisites feed back in respect of the accuracy we have achieved in relation to the correct application of the law of selection. (9)

iii) Doctrine of potentiation.

iv) Doctrine of vital force.

v) Doctrine of individualisation (10)

As Stuart Close has rightly pointed out, homoeopathy as a science; rests fundamentally upon four general principles :-

i) Similarity

ii) Contrarity

iii) Proportionality

iv) Infinitesimality (11)

The quantitative statistical numerology is not suitable for homoeopathic experimental design since the effect achieved is qualitative and not quantitative.

Susceptibility plays a major role in the production of the disease as well as in the cure of disease and throughout one Man's life this susceptibility gets modified with the sole purpose of maintaining the balance and this is a variable factor.

In conclusion, therefore, such experiments as carried out in the present work may not help the research workers. One will have to follow a qualitative approach to the problem of research in Homoeopathy.

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