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SHORT COMMUNICATION

An *In Vitro* Comparative Study of Antibacterial Activity of *Calendula officinalis* Mother Tincture and 30 Potency

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Abstract

In this research work, the antibacterial activity of *Calendula officinalis* was tested (*in vitro*) as opposed to the commonly encountered important bacterial strains, namely *Staphylococcus aureus* and *Pseudomonas aeruginosa*. For this research, the mother tincture and 30 potency of *Calendula officinalis* were used. The mother tincture and further potencies (in centesimal scale) were prepared with the help of absolute alcohol (99.6% v/v) following the guidelines given in the Homoeopathic Pharmacopoeia of India (HPI). The Minimum Inhibitory Concentration (MIC) was studied using the micro-broth dilution method, and the Zone of Inhibition (ZOI) was studied using the agar-well diffusion method. The results of the MIC and ZOI were then compared with Amoxicillin, which was used as the standard and finally the conclusions were drawn. The selected microorganisms were procured from the National Centre for Microbial Resources, Pune.

Keywords: Antibacterial, *Calendula officinalis*, Centesimal Scale, Minimum Inhibitory Concentration, Mother Tincture, Zone of Inhibition

1. Introduction

Infectious diseases contribute very significantly to the global disease burden and keep drawing the attention of common people as well as medical and laboratory professionals. The use of antibiotics is the mainstay of treatment for infectious diseases but it is associated with many other problems such as life-threatening anaphylactic reactions, haphazard usage, development of resistance and economic burden on patients, etc. Herbal extracts can be very helpful in the treatment of these types of illnesses, as the extracts have fewer side effects and are cost-effective as well. Hence, this study was conducted to investigate the antibacterial activity of *Calendula officinalis* (Figure 1).

Calendula officinalis is also called pot marigold. It is a plant belonging to the Compositae family. This family is also known as the Asteraceae family. This family consists of shrubs, trees and flowering plants like pot marigold, *Arnica montana*, poison oak, *Hypericum* and *Staphysagria*. The plants from this family are found worldwide. Some of these plants can be annuals, biennials, or perennials[1]. The extracts obtained from these plants have been used for various reasons since ancient times.

According to the Hahnemannian classification of medicinal plants, *C. officinalis* belongs to Class 1 medicines as it is a highly juicy plant[1]. *Calendula officinalis* is an annual herb and the plants are bisexual, i.e., individual flowers are either male or female but

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both flowers can be found on the same plant. The flowers are known to attract wildlife and honeybees play an important role in the pollination process. The plants are widely distributed throughout Europe and the Mediterranean region. The flowers can be yellowish or orange in colour. The flowers, leaves, and buds of this plant are used for preparing homoeopathic medicine.

The plant has a variety of bioactive compounds such as terpenoids, flavonoids, carotenoids, lutein, coumarins, quinones, volatile oils, amino acids, carbohydrates, lipids etc. It has been noticed that the terpenoids of *C. officinalis* have anti-inflammatory and anti-adenosis actions whereas the flavonoids have anti-viral and enzyme inhibition properties[2]. The extracts of *C. officinalis* can be used both internally and externally to accelerate the healing of wounds.

The carotenoids are nothing but the colouring compounds or pigments present in plants. The carotenoids of *C. officinalis* are flavoxanthin, lutein, rubixanthin, b-carotene, g–carotene and lycopene. It has been noticed that these carotenoids have anti-inflammatory, anti-oxidant and anti-proliferation actions as well[3]. Some researchers have suggested that *C. officinalis* has anti-tumour properties[4]. It has been noticed in various research studies that *C. officinalis* has antiseptic, antispasmodic, astringent, diaphoretic and stimulant actions as well[5]. Also, methanolic extracts of *C. officinalis* can inhibit the growth of *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli*[6].

2. Materials and Methods

In the present research, the antibacterial action of mother tincture and 30 potency of *C. officinalis* were studied on *S. aureus* and *P. aeruginosa*. The mother tincture was prepared by the extraction method as well as by percolation while the potentisation was done as per the guidelines given in HPI.

2.1 Collection of Plant Material

Fresh plants were collected from the local nurseries in different localities of Vadodara city during the growing season. The plant materials were authenticated by the senior botanists of the Biotrik Organization, Vadodara. The herbarium sheets were deposited at the Biotrik organisation (Certificate No. PAC-2020-002, SOP No. BTO-C/S/001, Rev No. 001).

2.2 Preparation of Mother Tincture from Fresh Plant (Traditional Method – Extraction)

For preparing the mother tincture, about 55 grams of fresh flowers and buds were collected early in the morning. The collected parts were washed with running tap water and then they were grounded to pulp. The juice was then squeezed out through a clean cotton cloth, and the quantity of juice was measured. The measured quantity of juice (5ml) was taken into a glass bottle. Then it was mixed with an equal quantity of absolute alcohol (99.6% v/v) (5ml) and preserved for 8 days, after which it was filtered through the filter paper. Then the mother tincture was collected and tested for various parameters like specific gravity, pH, viscosity, and density. The results were then cross-checked with the products available in the market.

2.3 Preparation of mother tincture from dried plant parts: (New Method – Percolation)

The selected plant parts were sun-dried for about 1 to 2 weeks. The dried parts were grounded well into a

Figure 1. An image of *Calendula officinalis* flowers.

powdered form using a porcelain mortar and pestle. The powder was then sieved to give uniform particles. The powder was then stored in airtight polythene bags before percolation. About 19 grams of powder was used for preparing the mother tincture by percolation. The percolator was assembled as per the guidelines given in Homoeopathic Pharmacopoeia of India. The fine powder was kept as the topmost layer in a percolator and absolute alcohol (99.6% v/v) was used as a percolating vehicle. The assembly was kept for 24 hours, and on the next day, the mother tincture was extracted from the percolator. The collected mother tincture was then tested for various parameters like specific gravity, pH, viscosity, and density. The results were then cross-checked with the products available on the market.

2.4 Preparation of 30 Potency from the Mother Tincture

The potencies were prepared according to the guidelines given in the HPI i.e., 2 parts of mother tincture were mixed with 98 parts of absolute alcohol (99.6% v/v), and 10 succussions were given. Similarly, 2nd potency was prepared by taking 1 part of 1st potency and mixing it with 99 parts of absolute alcohol (99.6% v/v), followed by 10 succussions. Further potencies were prepared by taking 1 part of the previous potency and 99 parts of absolute alcohol (99.6% v/v), followed by 10 succussions.

2.5 Preparation of Inoculum

The microbial stock cultures of *S. aureus* and *P. aeruginosa* were procured from NCMR Pune. The culture mediums were prepared by dissolving all the ingredients in distilled water and then sterilising them in an autoclave at 121°C for 15-20 minutes. The petri plates were washed thoroughly and sterilised in a hot air oven at 80°C for 30–45 minutes. For preparing the bacterial suspensions, McFarland nephelometer standards were used. The cultures were activated by taking a loop full of cells from the stock cultures and adding it to the test tubes of nutrient agar and Muller-Hinton agar, respectively, and then incubating them for 24 hours at 37°C to achieve the optical densities corresponding to 1.5×10^8 colony-forming units for bacteria. The cultures were then maintained on the slopes of nutrient agar and Muller-Hinton agar, respectively at 4°C.

2.6 Measurement of Minimum Inhibitory Concentration

The micro-broth dilution method has been used here for determining the MIC. For the estimation of MIC, 10 test formulations have been prepared in different concentrations by using tubes containing double-strength broth. One un-inoculated and one inoculated control was kept. The test tubes were inoculated with the suspension of bacteria and incubated at 37°C for 24 hours. MICs were recorded as the lowest concentration of the test formulations showing no visible growth of organisms in the broth media.

2.7 Measurement of Zone of Inhibition

The minimum inhibitory concentrations of the *C. officinalis* mother tincture, 30 potency and amoxicillin were tested for antibacterial activity. The activity was determined by using the agar-well diffusion method. At the beginning of the experiment, the plates of nutrient agar and Muller-Hilton agar media were prepared in distilled water and autoclaved at 121°C for 15 minutes. The nutrient agar plate was inoculated with an aliquot (0.1 ml) of the *S. aureus* bacterial suspension (105-106 CFU/ml), which was spread evenly in the plate under aseptic conditions. The Muller Hinton agar plate was inoculated with an aliquot (0.1 ml) of the *P. aeruginosa* bacterial suspension (105-106CFU/ml), which was spread evenly in the plate under aseptic conditions. Then both bacterial suspensions were allowed to dry for 20-25 minutes. Then, on each plate, three wells measuring 6 mm in diameter were made with the help of a sterile cork borer in the solid medium. The wells on nutrient agar medium were filled with 2ml of mother tincture of *C. officinalis*, 1ml of 30 potency of *C. officinalis* and 1ml of amoxicillin, whereas the wells on Muller-Hinton agar medium were filled with 2ml of mother tincture of *C. officinalis,* 0.5 ml of 30 potency of *C. officinalis* and 1ml of amoxicillin. Both plates were incubated at 37°C for 24 hours. The antibacterial activity of each test formulation was assessed by measuring the diameter of the zone of inhibition (in mm) produced around each well. To avoid bias, three replications were carried out for each test formulation against each of the test organisms.

3. Results

Table 1. MIC value of *C. officinalis a*nd Amoxicillin against *S. aureus*

MIC Value of Test Formulations Against *S. aureus*	
Sample	**Volume (ml)/ Concentration in [µg/ml]**
C. officinalis Mother Tincture	2.0ml
C. officinalis Liquid Dilution 30 potency	1.0ml
Amoxicillin (Standard)	0.20(µg/ml)

Table 2. MIC value of *C. officinalis a*nd Amoxicillin against *P. aeruginosa*

MIC Value of Test Formulations Against *P. aeruginosa*	
Sample	**Volume (ml)/ Concentration in [µg/ml]**
C. officinalis Mother Tincture	2.0ml
C. officinalis Liquid Dilution 30 potency	0.5ml
Amoxicillin (Standard)	0.20(µg/ml)

Table 3. Zone of inhibition of *C. officinalis* and Amoxicillin against *S. aureus*

Sample	**Volume [ml]/ Concentration [µg/ml]**	**Diameter [cm]**			**Average [cm]**
Calendula officinalis Mother Tincture	2ml	3.5	3.3	3.2	3.33±0.1527
Calendula officinalis Liquid dilution 30 potency	1ml	4.0	4.2	4.3	4.16±0.1527
Amoxicillin (Standard)	0.20 (µg/ml)	4.5	4.9	4.6	4.66±02018

Table 4. Zone of inhibition of *C. officinalis* and Amoxicillin against *P. aeruginosa*

Sample	**Volume [ml]/ Concentration [µg/ml]**	**Diameter [cm]**			**Average [cm]**
C. officinalis Mother Tincture	2ml	4.5	4.7	5	4.73±0.2516
C. officinalis Liquid Dilution 30 potency	0.5ml	8	7.5	7.3	7.6±0.3605
Amoxicillin (Standard)	0.20 (µg/ml)	5	5	5.3	5.1±0.1732

4. Discussion

From the results of MIC (Table 1 and Table 2), it can be concluded that *C. officinalis* mother tincture is less effective than *C. officinalis* 30 potency in arresting the growth of both the microorganisms *S. aureus* and *P. aeruginosa*.

From the results of Zone of Inhibition (Table 3 and Table 4) done by the agar well diffusion method, it can be concluded that *C. officinalis* mother tincture has a poorer bactericidal activity than *C. officinalis* 30 potency for both the microorganisms *S. aureus* and *P. aeruginosa*.

It has been noticed that *C. officinalis* mother tincture and 30 potency have poor antimicrobial properties against *S. aureus* as compared to Amoxicillin.

It has also been noticed that *C. officinalis* 30 potency has better antimicrobial properties against *P. aeruginosa* as compared to *C. officinalis* mother tincture as well as Amoxicillin.

5. Conclusion

Calendula officinalis in mother tincture form and 30 potency have different antibacterial activity against *S. aureus* and *P. aeruginosa*. The results indicated that *C. officinalis* has comparable antibacterial effects with Amoxicillin against both bacteria. Furthermore, it can be seen that *C. officinalis* mother tincture and *C. officinalis* 30 potency showed more inhibition against *P. aeruginosa* bacteria as compared to *S. aureus* bacteria.

While comparing the results with the standard formulation of Amoxicillin, it has been noticed that *C. officinalis* in 30 potency is more effective in arresting the growth of *P. aeruginosa* than Amoxicillin.

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