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In Vitro Evaluation of Antibacterial Activity of Homoeopathic Medicine Belladonna at 200c and im Potencies against Klebsiella Pneumoniae

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ABSTRACT

Background: The increasing prevalence of antibiotic-resistant bacteria has created a need to investigate potential antimicrobial agents. *Klebsiella pneumoniae* is an important bacterial pathogen associated with various infections and antimicrobial resistance. Investigating the in vitro effects of different preparations may provide preliminary information for further laboratory research.

Aim: To evaluate the in vitro antibacterial activity of Belladonna 200C and Belladonna 1M against *Klebsiella pneumoniae*.

Materials and Methods: An experimental in vitro study was conducted to assess the antibacterial activity of Belladonna 200C and Belladonna 1M against *Klebsiella pneumoniae* using the agar well diffusion method. Gentamicin was used as the positive control. Antibacterial activity was assessed by measuring the zones of inhibition in millimetres.

Results: Belladonna 200C and Belladonna 1M each produced a zone of inhibition measuring 9 mm. The positive control, gentamicin, produced a zone of inhibition measuring 21 mm. Thus, the observed inhibition zones for both Belladonna preparations were smaller than that of the positive control. No difference in the measured inhibition-zone diameters was observed between the two preparations.

Conclusion: Under the conditions of this experiment, Belladonna 200C and Belladonna 1M produced measurable inhibition zones against *Klebsiella pneumoniae*. Further controlled studies with appropriate replication, validated methods, and additional microbiological assessments are required to determine the reproducibility and significance of these observations. These findings should not be interpreted as evidence of clinical efficacy.

Keywords: Agar well diffusion, Antibacterial, Belladonna, Gentamycin.

INTRODUCTION

Klebsiellae, which are member of Enterobacterales, are widely distributed in nature, occurring both as commensals in the intestines and as saprophytes in soil and water

Three species are pathogenic to man, klebsiellla-pneumoniae, klebsiellla-ozaenae, and klebsiellla-rhinoscleromatis. Klebsiellla-oxytoca is rarely isolated from clinical infections.

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. Researchers focus on the investigation of plant extracts and microbial extracts, essential oils, pure secondary metabolites and new synthetized molecules as potential antimicrobial agents. A variety of laboratory methods can be used to evaluate or screen the in vitro antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disc/welldiffusion and broth or agar dilution methods.

Atropa belladonna, commonly known as deadly nightshade or Belladonna, is a medicinal plant belonging to the family Solanaceae. It contains biologically active alkaloids, particularly atropine, hyoscyamine and scopolamine. These compounds are known primarily for their effects on the nervous system, while the potential antimicrobial properties of Belladonna and its extracts may also be investigated experimentally. The antibacterial activity of Belladonna can be evaluated in a laboratory by testing a plant extract against selected bacterial strains and measuring the inhibition of bacterial growth.

The present study is conducted to evaluate whether Homoeopathic dialutions of Belladonna shows antibacterial activity evidently and how the variation in potency effect the bacterial growth.

MATERIALS AND METHODS :

Reagents and Materials

- Klebsiella pneumoniae (MTCC 3384)
- Belladonna 200C
- Belladonna 1M
- gentamicin
- Mueller- Hinton agar, sterile Petri plates
- sterile agar-well diffusion equipment
- micro sterile tips

Agar Well Diffusion Assay: Methodology

In this procedure, agar plates are inoculated by spreading a volume of standardized inoculum of the test microorganism over the entire agar surface. Then, a hole with a diameter of 6 to 8mm is punched aseptically with a sterile cork borer or a tip, and a known volume (20-100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. The petri dishes are incubated under suitable conditions. Generally, antimicrobial agents diffuse into the agar and inhibit germination and growth of the test microorganism and then the diameters of inhibition growth zones are measured.

Procedure

Mueller-Hinton agar medium (15-20 mL) was poured on glass petri plates of the same size and allowed to solidify. Standardized inoculum of the test organism was uniformly spread on the surface of the plates using sterile cotton swabs. Four wells with a diameter of 8mm (20 mm apart from one another) were punched aseptically with a sterile cork borer in each plate. The test samples (100µL) were added into the wells T1 and T2 directly from the sample. Gentamicin (40µl from 4mg/ml stock) was added as positive control. The plates were incubated for 24 hours at 36°C ± 1°C, under acrobic conditions. After incubation, the plates were observed and the zone of bacterial growth inhibition around the wells was measured in mm.

Composition of culture media

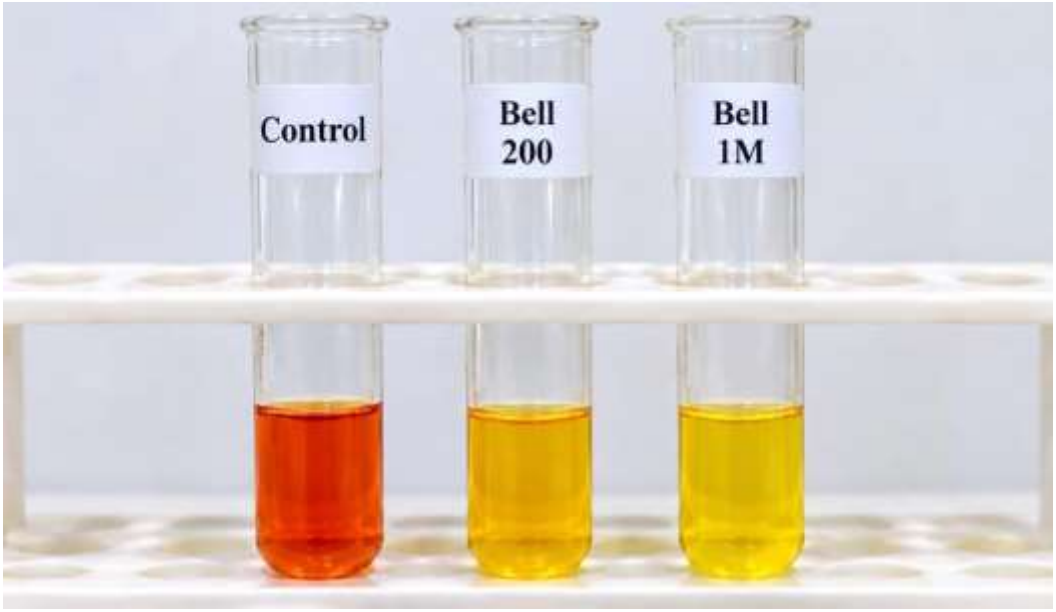
Muller Hinton Agar medium (HIMEDIA- M173) is used for determination of susceptibility of microorga-

nisms to antimicrobial agents.

Sl. No	Ingredient	Quantity
1	Beef extract	2.00g/L
2	Acid hydrolysate of casein	17.50g/dL
3	Starch	1.50g/L
4	Agar	17.00g/L
5	Distilled water	1000mL

Preparation of culture media

Preparation of culture media suspend 38grams of MHA (Himedia) in 1000 ml distilled water. Heat until it boils to dissolve the medium completely. Sterilize by



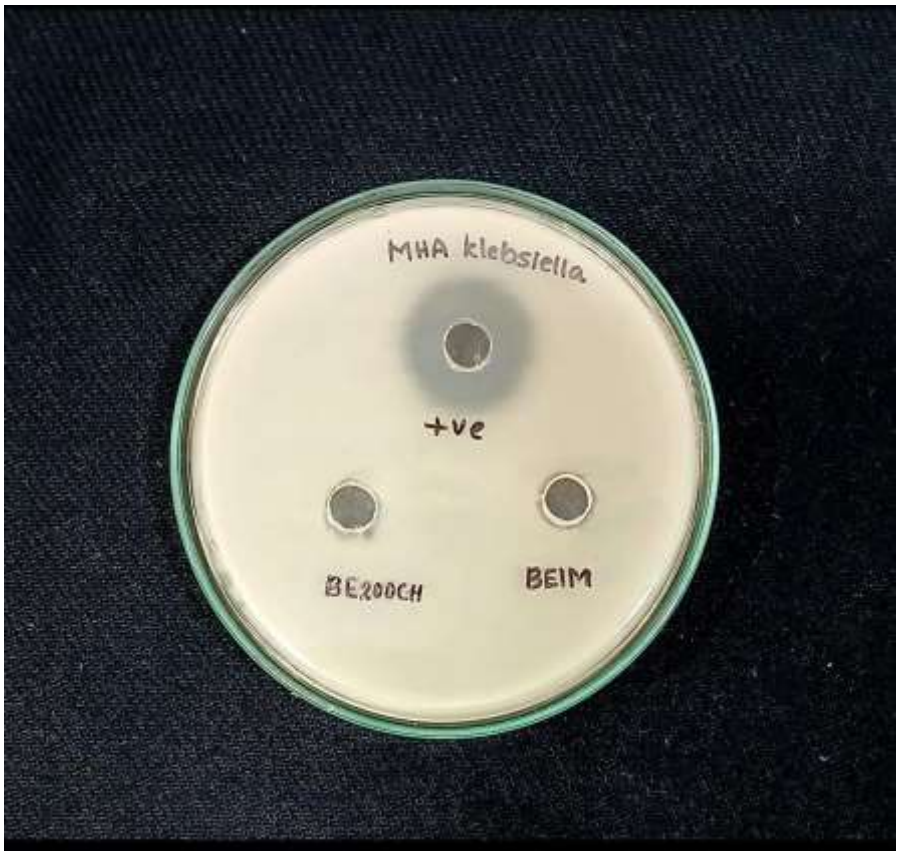
autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile petri plates.

RESULT

The results revealed that both Belladonna 200C and Belladonna 1M produced measurable zones of inhibition against Klebsiella pneumoniae, indicating antibacterial activity under the experimental conditions.

The standard antibiotic Gentamycin demonstrated the highest antibacterial activity with a zone of inhibition measuring 21 mm. This result confirms the susceptibility of the test organism and validates the reliability of the experimental procedure.

Both preparations showed the same reported zone of inhibition under the conditions of this experiment. The inhibition zones observed for the two preparations were smaller than that of the positive control.



DISCUSSION

The present study evaluated the in-vitro antibacterial activity of Belladonna 200C and Belladonna 1M against Klebsiella pneumoniae using the agar well diffusion method. The reported zone of inhibition was 9 mm for both preparations, whereas the positive control, gentamicin, showed a zone of inhibition of 21 mm.

The observed results indicate that the inhibition zones recorded for Belladonna 200C and Belladonna 1M were smaller than that of gentamicin under the experimental conditions used. Both preparations produced identical reported inhibition-zone measurements; therefore, no difference in their observed activity was demonstrated by these measurements.

However, the findings should be interpreted cautiously. The agar well diffusion method is influenced by several factors, including sample composition, diffusion through the agar, well diameter, inoculum density, incubation conditions, and measurement procedures. In addition, the presence of a measurable inhibition zone alone does not establish the mechanism of antibacterial activity.

The study has limitations, including the need for independent replicates, appropriate negative controls, and statistical analysis to assess the reproducibility of the observations. The preparation characteristics and the possibility of effects attributable to the vehicle or other experimental factors should also be considered. Further investigation using a validated experimental protocol is necessary before drawing firm conclusions about the antibacterial activity of these preparations.

Overall, the findings provide preliminary observations that may guide further investigation. They do not establish clinical efficacy or support the use of these preparations as substitutes for established antimicrobial treatment.

CONCLUSION

In this preliminary in-vitro study, Belladonna 200C and Belladonna 1M each showed a reported inhibition zone of 9 mm against Klebsiella pneumoniae, compared with 21 mm for the gentamicin positive control. The two preparations showed identical measured inhibition zones in the reported experiment.

Further studies incorporating independent replicates, appropriate negative controls, validated measurement procedures, and suitable statistical analysis are required to confirm the reproducibility and significance of these observations. No conclusion regarding clinical effectiveness can be drawn from the present findings alone.

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