



**PONDICHERRY CENTRE FOR BIOLOGICAL SCIENCE AND
EDUCATIONAL TRUST**

Reg.No.2840/B4/2016 Pondicherry

GST IN: 33AADTP1541L1ZL

SUSTAINABLE RESEARCH FOR BETTERMENT OF MANKIND

CERTIFICATE OF ANALYSIS

PCBSET/2021-2022/RN00133

Date: 30/03/2022

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Sample information: Semisolid

Sample Code: Joe 2

Analysis: Antimicrobial assay_well diffusion method

Date of sample received: 13/03/2022

Date of Analysis: 23/03/2022

Date of report: 25/03/2022

NITI Aayog



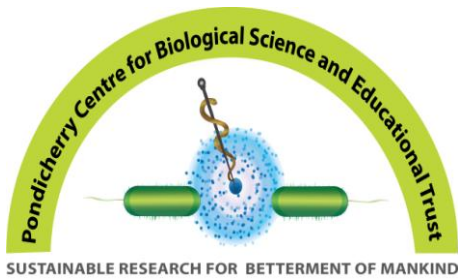
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MINISTRY OF MICRO, SMALL & MEDIUM ENTERPRISES
UDYAM REGISTRATION NUMBER - UDYAM-TN-31-0009450



Methodology:

Antimicrobial activities of test materials were determined with a well-diffusion method (Standards Institute Clinical Laboratory (CLSI) 2006; Pobiega et al., 2019). The bacterial strains were cultured on Nutrient Agar (Himedia, India) at 37 °C for 24 h. Bacterial inocula were prepared in sterile saline (0.85% NaCl) (w/v) solution with the quantity corresponding to 0.5 McFarland ($\sim 1 \times 10^8$ cfu/mL). Test pathogen was spread on Mueller-Hinton agar (MHA) plates. A well of diameter 6 mm was made using a sterile cork borer and loaded with required concentration of drug over the agar. The test plates were incubated for 24h at 37°C. Chlorhexidine was used as standard antibacterial agent respectively. The zone of inhibition (mm in diameter) were read and taken as the activity against the test pathogen.

TESTED STRAIN:

<i>E.coli</i> (ATCC 8739)

<i>E.lenta</i> (ATCC 43055)

Reference:

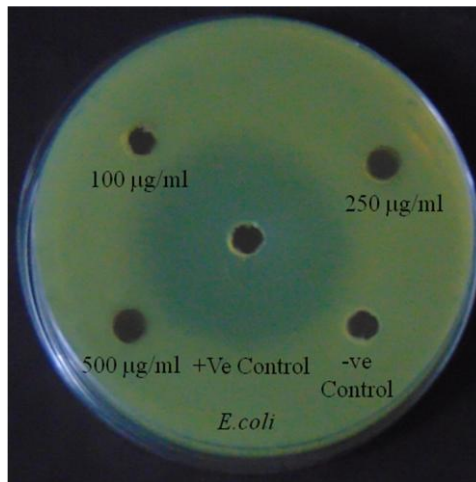
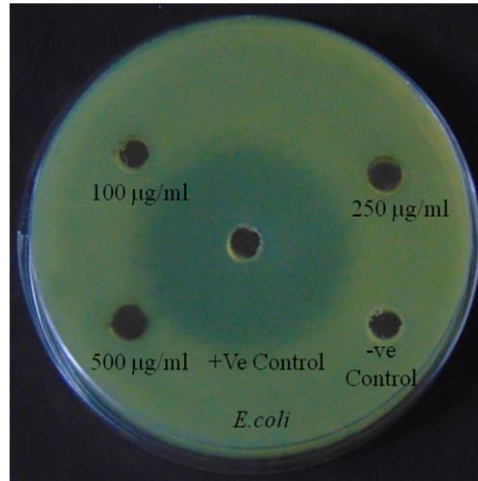
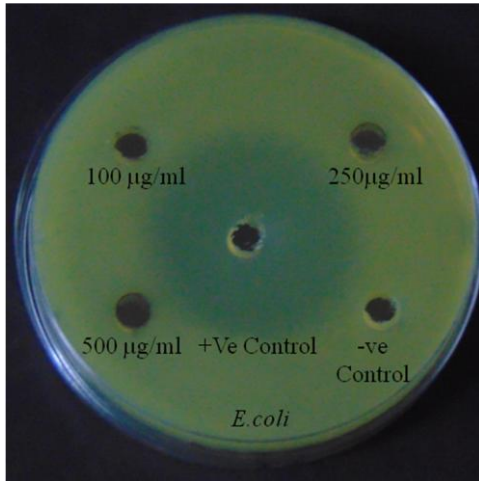
1. Standards Institute Clinical Laboratory (CLSI) (2006) Performance standards for antimicrobial disk susceptibility tests; approved standard—9th ed. CLSI document M2-A9. The Clinical & Laboratory Standards Institute, Wayne, p 26
2. Pobiega, K., Kraśniewska, K., Derewiaka, D. et al. Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. J Food Sci Technol 56, 5386–5395 (2019). <https://doi.org/10.1007/s13197-019-04009-9>

Results:

S.No	Sample Code	Strain	100 (µg/ml) (mm)	250 (µg/ml) (mm)	500 (µg/ml) (mm)	Negative Control (mm)	Positive Control (mm)
1	Joe2	<i>E.coli</i> (ATCC 8739)	-	-	-	-	35
2			-	-	-	-	36
3			-	-	-	-	36
4		<i>E.lenta</i> (ATCC 43055)	-	-	-	-	35
5			-	-	-	-	34
6			-	-	-	-	36

Sample Code: Joe 2

E.coli



Sample Code: Joe 2

E.lenta

