

**Investigation the antimicrobial activity of ethanolic extract
of green tea leaves against the Gram-negative strains
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Green tea is a popular beverage that has been consumed for centuries in many parts of the world. It is made from the leaves of the *Camellia sinensis* plant, which are rich in antioxidants. The antibacterial properties of green tea extract are mainly attributed to its high content of polyphenols, particularly catechins, which have been shown to have potent antimicrobial effects. The mechanism of antibacterial activity of catechins can be associated by the disrupting the bacterial cell membrane, leading to the leakage of cell contents and ultimately cell death. Additionally, catechins can also inhibit bacterial DNA replication and the production of bacterial virulence factors, reducing the pathogenicity of the bacteria.

The aim of the study was determined the antibacterial activity of green tea leaves ethanolic liquid extract against the Gram-negative strains.

Green tea leaves of spices Chun My were taken for the study, the raw material was collected in Anhui province (China) from March to May. 10.0 g of the grinded leaves was mixed with 200 mL of 96% ethanol. Extraction was carried out within 1 hour on water bath with a condenser, then repeated two times with a new portion of the solvent. After that the obtained extracts were filtrated and concentrated using rotary evaporator to 20 mL.

The antibacterial activity was determined by the method of wells. Preparation of microorganisms` suspensions with determined concentrations of microorganisms (optical density) was carried out by the standard of turbidity (0.5 units according to scale of McFarland) with using of equipment of Densi-La-Meter (Czech, wavelength 540nm). Colony forming unit was 10^7 microorganisms at 1 mL of growth medium and determined by standard of McFarland). On solidified agar, using a pipette under sterile conditions in Petri dishes made 1 mL of a suspension of microorganisms. After

uniform distribution of microorganisms over the entire surface of the agar, the plates were incubated at room temperature for 15-20 minutes. Next, wells with a diameter of 6 mm were made in the cups, into which solutions of the test substances were introduced. The samples incubated at 37° C for 16-24 hours. After incubation, the plates were placed upside down on a dark matte surface so that light fell on them at an angle of 45° (accounting in reflected light). The diameter of the growth retardation zones measured using a caliper. In the study following museum strains were used *Escherichia coli* ATCC 25922, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853. Chlorophyllipt spray manufactured by the State Scientific Center of Drugs (DNCLZ) with concentration 1% in 96% ethanol was used as the reference drug. The analyzed solution was 1% prepared solution of obtained ethanolic extract.

Table 1. Antimicrobial activity of green tea leaves extract and reference drug

Sample	Diameter of the growth retardation zone, mm		
	<i>Escherichia coli</i> ATCC 25922	<i>Proteus vulgaris</i> ATCC 4636	<i>Pseudomonas aeruginosa</i> ATCC 27853
Ethanolic extract	23.67± 0.50	21.33 ± 0.50	22.67 ± 0.50
Chlorophyllipt	19.33 ± 0.50	17.67 ± 0.50	17.67 ± 0.50

According to the conducted research, it was found that ethanolic extract strongly inhibited the growth of Gram-negative strains such as *Escherichia coli* (23.67±0.5 mm), *Proteus vulgaris* (21.33 ± 0.50 mm) and *Pseudomonas aeruginosa* (22.67 ± 0.5 mm). Comparing results of investigated extract and reference drug – «Chlorophyllipt» (DNCLZ) it can be pointed out that reference drug inferior of antibacterial activity.

The present work showed that the ethanolic extract of green tea leaves possess remarkable antibacterial activity against Gram-negative strains.