



Biological Properties of Escherichia coli. Symptoms and Diagnosis of Colibacillosis

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ABSTRACT

Escherichia coli is a short, polymorphic, Gram-negative, rod-shaped bacterium whose non-pathogenic strains are present in the normal intestinal flora. Escherichia is stable in the external environment and can remain viable in soil, water, and feces for up to several months. E. coli produces a variety of enzymes that decompose many carbohydrates and polyhydric alcohols (glucose, galactose, levulose, lactose, maltose, lures, rhamnose, intermittent sucrose, and dulcitol, raffinose, salicin, sorbitol, glycerin) with the formation of pyruvates, which then turn into milk, acetic and formic acids. The biochemical properties of Escherichia coli have been studied by many researchers from different countries, but no correlation has been established between their enzymatic activity and pathogenic properties. Colibacillosis is an acute, predominantly intestinal infection caused by certain serovars of the bacterium Escherichia coli. The route of transmission of E. coli is fecal-oral. This article describes in detail the morphological, tinctorial, cultural, and biochemical properties of Escherichia coli, as well as their antigenic structure. In addition, the symptomatology and diagnosis of the course of colibacillosis caused by various groups of Escherichia coli are described.

Keywords: Escherichia coli, Colibacillosis, Enteropathogenic, Enterotoxigenic, Enteroinvasive, Enterohemorrhagic.

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INTRODUCTION

Colibacillosis is an acute, mainly intestinal infection caused by some serovars of the bacterium Escherichia coli [1]. Colibacillosis manifests itself in the form of enteritis and enterocolitis and can generalize and proceed with extra-intestinal symptoms [2]. The route of transmission of E. coli is fecal-oral. More often, infection occurs when eating infected dairy and meat products. A contact-household route of

transmission of intestinal infection is also possible. The diagnosis of colibacillosis is established when Escherichia coli are detected in vomit and feces, and when an infection is generalized - in the blood [3].

Colibacillosis (coli infections) is a group of infections caused by E. coli and occurs with lesions of the gastrointestinal tract, urinary tract, respiratory tract, meninges, and bacteremia. They are more common in young children. Intestinal infections caused by Escherichia coli are the most common: they are the most common

cause of diarrhea in infants and adults [4]. Some strains and their toxins cause life-threatening lesions of internal organs [5].

Escherichia coli is a short polymorphic gram-negative rod-shaped bacteria, non-pathogenic strains of which are present in the normal intestinal flora [6]. Colibacillosis is caused by diarrheal serovars (**Table 1**).

Table 1. Groups of diarrheal serovars

Pathogen groups	Characteristics of colibacillosis
enteropathogenic	They cause colibacillosis in children, especially in the first year of life. Infection usually occurs by contact and household means
enterotoxigenic	They cause cholera-like infections, the incidence is high in countries with hot climates and low hygienic cultures. Infection occurs through food and water.
enteroinvasive	Enterocolitis is caused by the type of dysentery. Infection occurs by water and food, and the summer-autumn seasonality is noted. They are mainly distributed in developing countries.
enterohemorrhagic	Epidemiological data are insufficient for detailed characterization. In the epidemiology of colibacillosis, the main importance is played by hygienic measures, both of a general and individual nature.
enteroadhesive	Bacteria do not form cytotoxin, do not penetrate epithelial cells, and do not have plasmid adhesion factor. They got their name due to the rapid attachment to the surface of the cells.

Escherichia coli are stable in the external environment and can remain viable in soil, water, and feces for up to several months. In food products (especially in milk), they multiply, forming numerous colonies, and easily tolerate drying [7]. *Escherichia coli* are killed by boiling and the action of disinfectants.

The reservoir and source of infection are sick people or healthy carriers. Persons with colibacillosis caused by enteropathogenic and enteroinvasive *Escherichia coli* have the greatest epidemic significance in the spread of the pathogen, other groups of bacteria are less dangerous. Patients with colibacillosis caused by infection with enterotoxigenic and enterohemorrhagic *E. coli* are contagious only in

the first days of the disease, while enteroinvasive and enteropathogenic *E. coli* are isolated by patients for 1-2 (sometimes 3) weeks. Isolation of the pathogen can last quite a long time, especially in children.

The transmission mechanism of *Escherichia coli* is fecal-oral, most often the food way of infection is realized for enterotoxigenic and enteroinvasive *E. coli* and household for enteropathogenic *E. coli*. In addition, an infection can be carried out by water. In children's groups and among people who neglect the rules of hygiene, it is possible to spread *E. coli* through contact with and household through contaminated hands, objects, and toys. Infection with enterohemorrhagic *Escherichia coli* often occurs as a result of the consumption of insufficiently cooked meat, and raw unpasteurized milk [8-12].

There is a clinical classification that distinguishes gastroenteric, enterocolitic, gastroenterocolitic, and generalized forms of the disease. The generalized form can be represented by colisepsis or damage to various organs and systems by *E. coli* (meningitis, meningoencephalitis, pyelonephritis, etc.). Colibacillosis can occur in mild, moderate, and severe forms [13, 14].

Morphological, tinctorial, cultural, and biochemical properties of escherichia coli

According to the morphology, *E. coli* has the shape of a straight rod, short, relatively thick, with rounded ends, 2–3 µm long, thick 0.6–1.0 µm (**Figure 1**). They are located singly, less often in pairs, and in the body, they can take a coccus-like form [15]. *E. coli* stains negatively according to the Gram method, stains well with conventional aniline dyes, and is often bipolar, especially in tissues and exudates [16]. Under the influence of various abiotic factors, they can form L-forms and acquire the ability to pass through bacterial filters.

E. coli is unpretentious in terms of nutrient media, it can grow in the range from 15° to 55°C (temperature optimum is about 37-38°C). It is an aerobe or facultative anaerobe, pH 7.0–7.4. It grows well on ordinary nutrient media - MPA (meat peptone agar), MPB (meat peptone broth); Endo, and Levin media [17]. On MPA, after 24 hours, transparent juicy colonies with a grayish-blue tint are formed, easily merging, with smooth edges and a smooth shiny surface (S-shape) or

flat, dry with a slightly wavy edge and a rough surface (R-shape). In BCH, *E. coli* causes abundant growth with significant and intense turbidity of the medium and the presence of a grayish, easily broken precipitate when shaken, sometimes forming a film on the surface or a parietal ring. When sowing on a medium with gelatin, no liquefaction of the medium occurs. On injection, a grayish-white growth is obtained. Milk coagulates, and litmus milk quickly turns pink (formation of acid) and coagulates.

On Levin's diagnostic medium, *E. coli* forms dark purple or black colonies; on Endo's differential diagnostic medium, lactose-positive strains form raspberry-red colonies with or without a metallic sheen; on McConkey's medium - pink, red (individual strains of *Escherichia coli* may not ferment lactose and form colorless colonies on the listed media). *Escherichia coli* colonies of other serogroups are crimson red [18]. Colonies of hemolysin-forming strains are surrounded by a zone of hemolysis on blood agar. The culture is characterized by a fecal odor [19].

E. coli produces a variety of enzymes that decompose many carbohydrates and polyhydric alcohols (glucose, galactose, levulose, lactose, maltose, lures, rhamnose, intermittent sucrose, and dulcitol, raffinose, salicin, sorbitol, glycerin) with the formation of pyruvates, which then turn into milk, acetic and formic acids [20]. Pathogenic and non-pathogenic varieties of *E. coli* do not differ from each other in morphological, cultural, and enzymatic properties, which makes it difficult to identify pathogens of infectious diarrhea [21].

Escherichia coli antigenic structure

The serological classification of *Escherichia coli*, developed by F. Kaufman, is based on the analysis of O-, K-, and H-antigens. Initially, researchers identified 146 variants of O-antigen, and 88 variants of K-antigen, by the end of the 80s, 171 serological varieties of O-antigen, more than 100 varieties of K-antigen, and 60 varieties of H-antigen were identified in *Escherichia coli*. Later, 173 O-serogroups and 56 types of *E. coli* K-antigen, as well as 80 types of H-antigen were identified [22]. However, not all of these varieties of *Escherichia coli* are capable of causing intestinal infections in animals and humans.

Escherichia coli cells contain three types of antigens: O - somatic; K - sheathed and H - flagellated (**Figure 1**).

The O-antigen is thermostable (withstands heating at 100°C for 2.5 hours). It is a lipopolysaccharido-protein complex, heterogeneous, and on its basis, *Escherichia coli* are divided into groups (more than 160 O-groups) [22].

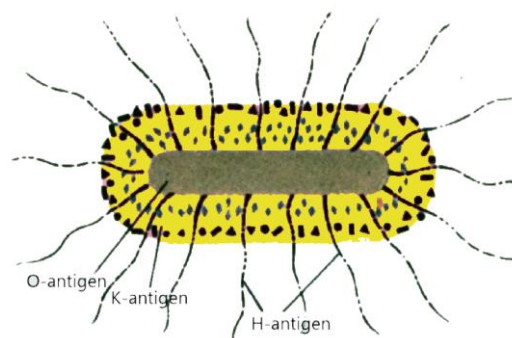


Figure 1. The structure of *Escherichia coli*

K-antigens surface, shell (acid polysaccharides, rarely proteins). According to their properties, they are divided into three types: L-antigen is thermolabile, inactivated at 100 °C for an hour; In the same conditions, the B-antigen loses its agglutination; A-antigen is thermostable, inactivated at 120°C for 2.5 hours, is present in the mucous membranes of capsule-forming *Escherichia coli* O-groups 08,09,020,0101 [23]; The H-antigen is thermolabile, inactivated at 60 °C for 1 hour, the protein is located in the fimbriae (piles) penetrating the cell wall, therefore, recently it is more often called the fimbrial antigen.

K antigens coat O antigen and render *Escherichia coli* cells innaglutinable in homologous O sera. They have adhesive properties, i.e. with their help, bacteria attach to intestinal epithelial cells. The H-antigen is flagellated, consists of a protein that is thermolabile and has several dozen varieties determined in RA. It is not essential for diagnosis. The combination of O-, K- and H-antigens characterizes the serological variant (serovar) of *Escherichia coli* [24].

Escherichia coli are resistant to environmental factors. They remain in soil from 6 to 11 months, in water - for up to 300 days, and in manure - for up to 11 months. When stored in semi-liquid agar under a layer of vaseline oil, *E. coli* retain their pathogenic properties for more than three years. *Escherichia coli* are unstable to high

temperatures. When the medium is heated to 60°C, they die within 10 min, and at 100°C, they die instantly. Many disinfectants and substances have a detrimental effect on *E. coli*: 2.5% formaldehyde solution, 2% solutions of active chlorine and sodium hydroxide, and 3% solution of iodine monochloride [25].

Symptoms and diagnosis of colibacillosis

Enteropathogenic colibacillosis usually develops in young children, the incubation period is several days, manifested mainly by vomiting, loose stools, severe intoxication, and dehydration. There is a possibility of developing a generalized septic form. Adults fall ill with enteropathogenic colibacillosis, and the disease proceeds like salmonellosis [26].

Enteroinvasive colibacillosis is a characteristic course similar to dysentery or shigellosis. The incubation period lasts from one to three days, the onset is acute, there is moderate intoxication (headache, weakness), fever ranging from subfebrile to high values, and chills. Then there are pains in the abdomen (mainly around the navel), and diarrhea (sometimes with streaks of blood, and mucus). Palpation of the abdomen notes pain along the colon. Often, colibacillosis of this type occurs in a mild and erased form, a moderate course can be noted. Usually, the duration of the disease does not exceed a few days [27].

Enterotoxigenic colibacillosis may present with clinical symptoms similar to those of salmonellosis, food poisoning, or resemble a mild form of cholera. The incubation period is 1-2 days, intoxication is mild, the temperature usually does not rise, repeated vomiting is noted, profuse enteric diarrhea, dehydration gradually increases, and oliguria is noted. There are pains in the epigastric region, which are cramping in nature [28].

This infection is often referred to as the "traveler's disease", as it often affects people who have traveled on a business trip or vacation to countries with a tropical climate. Climatic conditions contribute to the occurrence of severe fever with chills and intoxication symptoms, and intense dehydration.

Enterohemorrhagic colibacillosis develops most often in children. At the same time, intoxication is moderate, the body temperature is subfebrile. There is nausea and vomiting, and loose watery

stools. In severe cases, by 3-4 days, the disease appears severe pain in the abdomen of a cramping nature, diarrhea intensifies, and in feces that lose their fecal character, an admixture of blood and pus may be noted [29].

Most often, the disease resolves on its own in a week, but in severe cases (especially in young children) on days 7-10, after the disappearance of diarrhea, there is a possibility of developing hemolytic-uremic syndrome (a combination of hemolytic anemia, thrombocytopenia, and acute renal failure). There are frequent violations of brain regulation: cramps of the limbs, muscle rigidity, impaired consciousness up to stupor, and coma. The lethality of patients with the development of these symptoms reaches 5% [30].

Complications

Colibacillosis is usually not prone to complications. In the case of an infection provoked by the pathogen of the Enterohemorrhagic group of *Escherichia coli*, there is a possibility of complications from the urinary system, hemolytic anemia, and cerebral disorders [31].

Diagnostics

For the diagnosis of colibacillosis, the pathogen is isolated from feces and vomit, in cases of generalization - from blood, urine, bile, or cerebrospinal fluid. After that, a bacteriological examination is performed, sowing on nutrient media. Due to the antigenic similarity of the causative agents of colibacillosis with the bacteria that make up intestinal normocenosis, serological diagnosis is not very informative.

In some cases, the detection of bacterial toxins in the feces of patients can be used. With this type of colibacillosis, signs of hemolytic anemia, and an increase in the concentration of urea and creatinine can be noted in the blood test. Urinalysis usually shows proteinuria, leukocyturia, and hematuria [32].

Prevention

Colibacillosis - a disease associated with low hygienic culture. Personal prevention of these infections is to follow hygiene standards, especially when communicating with children, and washing hands, food, toys, and household items. General prevention is aimed at monitoring compliance with the sanitary and hygienic

regime in children's institutions, food industry enterprises, and medical institutions, as well as control over the flow of sewage waste and the state of water sources [33].

Patients after the transfer of colibacillosis are discharged from the hospital after clinical recovery, as well as the results of a threefold bacteriological test. Admission to the team of children who have been in contact with the patient is also carried out after bacteriological diagnosis and confirmation of the absence of isolation of the pathogen. Persons excreting pathogenic *Escherichia coli* are subject to isolation for the entire period of contagiousness. Food industry workers are subjected to regular examinations for the isolation of the pathogen, in case of a positive test, they are suspended from work [34-38].

CONCLUSION

Different groups of *Escherichia coli* cause the course of colibacillosis with different symptoms and varying degrees of severity. Enteropathogenic colibacillosis usually develops in young children, the incubation period is several days, manifested mainly by vomiting, loose stools, severe intoxication, and dehydration. Enteroinvasive colibacillosis proceeds like dysentery or shigellosis. The incubation period lasts from one to three days, the onset is acute, there is moderate intoxication (headache, weakness), fever ranging from subfebrile to high values, and chills. Then there are pains in the abdomen (mainly around the navel), and diarrhea (sometimes with streaks of blood, and mucus).

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stools that lose their fecal character, an admixture of blood and pus may be noted.

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REFERENCES

1. Da Silva GJ, Mendonça N. Association between antimicrobial resistance and virulence in *Escherichia coli*. *Virulence*. 2012;3(1):18-28. doi:10.4161/viru.3.1.18382
2. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental *Escherichia coli*: ecology and public health implications-a review. *J Appl Microbiol*. 2017;123(3):570-81. doi:10.1111/jam.13468
3. Leimbach A, Hacker J, Dobrindt U. *E. coli* as an all-rounder: the thin line between commensalism and pathogenicity. *Curr Top Microbiol Immunol*. 2013;358:3-32. doi:10.1007/82_2012_303
4. De Biase D, Lund PA. The *Escherichia coli* Acid Stress Response and Its Significance for Pathogenesis. *Adv Appl Microbiol*. 2015;92:49-88. doi:10.1016/bs.aambs.2015.03.002
5. Jayamani E, Mylonakis E. Effector triggered manipulation of host immune response elicited by different pathotypes of *Escherichia coli*. *Virulence*. 2014;5(7):733-9. doi:10.4161/viru.29948
6. Luneva AV, Lysenko YA, Gneush AN, Shantyz AY, Simonov AN, Verevkin MN, et al. Assessment of the Biosafety of Microorganisms and their Joint Composition. *Pharmacophore*. 2021;12(3):42-8. doi:10.51847/M60cnxYHxz
7. Schuldiner S. The *Escherichia coli* effluxome. *Res Microbiol*. 2018;169(7-8):357-62. doi:10.1016/j.resmic.2018.02.006

8. Mueller M, Tainter CR. Escherichia Coli. 2022 Oct 17. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2022.
9. Mirhoseini A, Amani J, Nazarian S. Review on pathogenicity mechanism of enterotoxigenic Escherichia coli and vaccines against it. *Microb Pathog.* 2018;117:162-9. doi:10.1016/j.micpath.2018.02.032
10. Gelalcha BD, Brown SM, Crocker HE, Agga GE, Kerro Dego O. Regulation Mechanisms of Virulence Genes in Enterohemorrhagic Escherichia coli. *Foodborne Pathog Dis.* 2022;19(9):598-612. doi:10.1089/fpd.2021.0103
11. Sizonenko MN, Timchenko LD, Rzhepakovskiy IV, DA SP AV, Nagdalian AA, Simonov AN, et al. The New Efficiency of the «Srmp»-Listerias Growth-Promoting Factor during Factory Cultivation". *Pharmacophore.* 2019;10(2):85-8.
12. Farfán-García AE, Ariza-Rojas SC, Vargas-Cárdenas FA, Vargas-Remolina LV. Virulence mechanisms of enteropathogenic Escherichia coli. *Rev Chilena Infectol.* 2016;33(4):438-50. [In Spanish]. doi:10.4067/S0716-10182016000400009
13. Mariotti A, Ezzraimi AE, Camoin-Jau L. Effect of antiplatelet agents on Escherichia coli sepsis mechanisms: A review. *Front Microbiol.* 2022;13:1043334. doi:10.3389/fmicb.2022.1043334
14. Vila J, Sáez-López E, Johnson JR, Römling U, Dobrindt U, Cantón R, et al. Escherichia coli: an old friend with new tidings. *FEMS Microbiol Rev.* 2016;40(4):437-63. doi:10.1093/femsre/fuw005
15. Gneush AN, Luneva AV, Machneva NL, Lysenko YA, Aniskina MV, Verevkin MN, et al. Biotechnology of Microorganisms Growing-Fundamentals for the Development of a Litter Biodestructor. *J Pharm Res Int.* 2021;33(36B):1-1. doi:10.9734/jpri/2021/v33i36B31946
16. Newell DG, La Ragione RM. Enterohaemorrhagic and other Shiga toxin-producing Escherichia coli (STEC): Where are we now regarding diagnostics and control strategies? *Transbound Emerg Dis.* 2018;65 Suppl 1:49-71. doi:10.1111/tbed.12789
17. Juarez GE, Galván EM. Role of nutrient limitation in the competition between uropathogenic strains of Klebsiella pneumoniae and Escherichia coli in mixed biofilms. *Biofouling.* 2018;34(3):287-98. doi:10.1080/08927014.2018.1434876
18. Mizuochi S, Nelson M, Baylis C, Green B, Jewell K, Monadjemi F, et al. Matrix Extension Study: Validation of the Compact Dry EC Method for Enumeration of Escherichia coli and non-E. coli Coliform Bacteria in Selected Foods. *J AOAC Int.* 2016;99(2):451-60. doi:10.5740/jaoacint.15-0268
19. Devane M, Dupont PY, Robson B, Lin S, Scholes P, Wood D, et al. Mobilization of Escherichia coli and fecal source markers from decomposing cowpats. *Sci Total Environ.* 2022;853:158509. doi:10.1016/j.scitotenv.2022.158509
20. Falcicchio P, Levisson M, Kengen SWM, Koutsopoulos S, van der Oost J. (Hyper)Thermophilic Enzymes: Production and Purification. *Methods Mol Biol.* 2021;2178:469-78. doi:10.1007/978-1-0716-0775-6_29
21. Köhler CD, Dobrindt U. What defines extraintestinal pathogenic Escherichia coli? *Int J Med Microbiol.* 2011;301(8):642-7. doi:10.1016/j.ijmm.2011.09.006
22. Yura T. Regulation of the heat shock response in Escherichia coli: history and perspectives. *Genes Genet Syst.* 2019;94(3):103-8. doi:10.1266/ggs.19-00005
23. Yang S, Xi D, Jing F, Kong D, Wu J, Feng L, et al. Genetic diversity of K-antigen gene clusters of Escherichia coli and their molecular typing using a suspension array. *Can J Microbiol.* 2018;64(4):231-41. doi:10.1139/cjm-2017-0620
24. Nakae K, Ooka T, Murakami K, Hara-Kudo Y, Imuta N, Gotoh Y, et al. Diversification of Escherichia albertii H-Antigens and Development of H-Genotyping PCR. *Front Microbiol.* 2021;12:737979. doi:10.3389/fmicb.2021.737979
25. Elmakaoui A, Bourais I, Oubihi A, Nassif A, Bezinar T, Shariati MA, et al. Chemical composition and antibacterial activity of essential oil of Lavandula multifida. *J Microbiol Biotechnol Food Sci.*

- 2022;11(6):e7559.
doi:10.55251/jmbfs.7559
26. Fukuda S, Toh H, Hase K, Oshima K, Nakanishi Y, Yoshimura K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. *Nature*. 2011;469(7331):543-7. doi:10.1038/nature09646
27. Aribam SD, Hirota J, Kusumoto M, Harada T, Shiraiwa K, Ogawa Y, et al. A rapid differentiation method for enteroinvasive *Escherichia coli*. *J Microbiol Methods*. 2014;98:64-6. doi:10.1016/j.mimet.2013.11.012
28. Zhang Y, Tan P, Zhao Y, Ma X. Enterotoxigenic *Escherichia coli*: intestinal pathogenesis mechanisms and colonization resistance by gut microbiota. *Gut Microbes*. 2022;14(1):2055943. doi:10.1080/19490976.2022.2055943
29. Goto T, Shirano M. Enterohemorrhagic *E. coli* (EHEC). *Nihon Rinsho*. 2012;70(8):1343-7. [In Japanese].
30. Nguyen Y, Sperandio V. Enterohemorrhagic *E. coli* (EHEC) pathogenesis. *Front Cell Infect Microbiol*. 2012;2:90. doi:10.3389/fcimb.2012.00090
31. Fründt T, Leuffert J, Groth S, Rösch T, Steurer S, Lohse AW, et al. Low incidence of colonic complications after severe Shiga toxin-producing *E. coli* O104:H4 infection. *Z Gastroenterol*. 2022;60(7):1104-10. [In English]. doi:10.1055/a-1545-5322
32. Lutful Kabir SM. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control, and public health concerns. *Int J Environ Res Public Health*. 2010;7(1):89-114. doi:10.3390/ijerph7010089
33. Bachinina KN, Povetkin SN, Simonov AN, Pushkin SV, Blinova AA, Sukhanova ED, et al. Effects of Selenium Preparation on Morphological and Biochemical Parameters of Quail meat. *Int Trans J Eng, Manag Appl Sci Technol*. 2021;12(13):1213. Available from: <http://TUENGR.COM/V12/12A13K.pdf> doi:10.14456/ITJEMAST.2021.263
34. Delsignore M, Siddiqui SA. Chapter 8. From waste to food: legislative insights. In *Waste to Food: Returning nutrients to the food chain 2022 Feb 15* (p. 1010). Wageningen Academic Publishers. doi:10.3920/978-90-8686-929-9_8
35. Hassoun A, Siddiqui SA, Smaoui S, Ucak İ, Arshad RN, Garcia-Oliveira P, et al. Seafood Processing, Preservation, and Analytical Techniques in the Age of Industry 4.0. *Appl Sci*. 2022;12(3):1703. doi:10.3390/app12031703
36. Akshita C, Vijay BV, Praveen D. Evaluation of phytochemical screening and antimicrobial efficacy of *Mesua ferrea* and *piper cubeba* fruit extracts against multidrug-resistant bacteria. *Pharmacophore*. 2020;11(2):15-20.
37. Mirza AS, Baig MT, Huma A, Ibrahim S, Shahid U, Jabeen A, et al. Antibacterial Activity of Methanol Extract of *Capparis Decidua* Edgew (Forssk.) Against *Staphylococcus Aureus*, *Bacillus Cereus*, *Salmonella Typhi*, and *Escherichia Coli*. *Pharmacophore*. 2020;11(4):46-50.
38. Tati S, Nurul Fatimah N, Yandri Y, Rahmat Kurniawan R, Syaiful B, Sutopo H. The anticancer, antimalarial, and antibacterial activities of moracalkon isolated from *Artocarpus kemando* Miq. *J Adv Pharm Educ Res*. 2021;11(4):105-10.