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The effect of mangosteen pericarp (*Garcinia mangostana Linn)* extract on inhibits the growth of bacteria *Escherichia Coli* ATCC 25922 and bacteria *Staphylococcus Aureus* ATCC 25923

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Abstract
The mangosteen (*Garcinia mangostana Linn)* fruit is dubbed the "queen of fruits" because of its fragrant aroma, sweet taste and slightly sour but rich in health benefits. This research aims to determine the antibacterial potential of mangosteen pericarp *to inhibit the growth of E. coli ATCC 25922 and S. Aureus ATCC 25923*. Mangosteen pericarp were extracted using maceration method and antibacterial sensitivity testing by Kirby Bauer diffusion method that was duplicated four times. The result of this study is that mangosteen pericarp extract showed inhibition against *Staphylococcus aureus* ATCC 25923 with diameter zone of inhibition of 10.23 mm, 10.95 mm, 13.45 mm, 16 mm, 19.5 mm on 10%, 30%, 50%, 70% and 100% of extract mangosteen pericarp respectively and there is no zone of inhibition on mangosteen pericarp extract against *Escherichia coli* ATCC 25922. This research needs to be done with a better methodology and technique

Keywords: mangosteen pericarp extract, escherichia coli ATCC 25922, staphylococcus aureus ATCC 25923, kirby bauer diffusion test

Introduction

Antibiotic treatment is the cornerstone of the treatment of infectious diseases. This treatment is beneficial in treating infectious diseases caused by bacteria. When Alexander Flemming received the Nobel Prize for discovering the antibiotic penicillin in 1945, he warned that bacteria would become resistant to antibiotics. Antibiotic resistance has become a complex public health problem throughout the world, which causes the therapeutic effect to decrease to no effect and impacts various sectors, especially the economic and health sectors [1]. When an infection becomes resistant to first-line antibiotics, treatment must be switched to second-and third-line drugs, which are much more expensive and sometimes much more toxic, requiring longer hospital stays. Patients are at risk for worse clinical development, death, and high therapy costs than patients infected with non-resistant bacteria. Antibiotic resistance caused the annual cost of healthcare in the United States to soar from US$21 million to US$34 million, with an additional US$8 million in hospitalization costs and a decline in Gross Domestic Product (GDP) of 1.6% in 2008 [2]. The overuse and misuse of antibiotics further exacerbate antibiotic resistance without professional supervision. For example, antibiotics are often used by patients with viral infections such as flu and fever when antibiotics should be given to treat bacterial infections [3]. Based on the AMRIN study (Anti Microbial Resistance study in Indonesia) in 2001-2005, two types of resistant bacteria are commonly found, *Escherichia coli* and *Staphylococcus aureus* [4]. In 2012, the percentage of these bacteria increased, Extended-spectrum Beta Lactamase (ESBL) of *Escherichia coli* to 52% and Methicilin Resistant *Staphylococcus aureus* (MRSA) to 24% [3]. *Escherichia coli* (*E. coli*) is an opportunistic bacterium short rod-shaped (cocobasil), Gram-negative, facultatively anaerobic, and does not produce spores, often found as normal flora in the human gut. These bacteria have a size of 0.4-0.7 m x 1.4 m, most of these bacteria are positive motion, and some strains have capsules. *E coli* has several pathogenic factors: surface antigens as a means of attachment to host cells or tissues, enterotoxins, and hemolysins. This bacterium is one of the main causes of acute diarrhoea and Vibrio cholera, Shigella dysentriae, and Campylobacter Jejunum. These bacteria also often cause urinary tract infections, the most common cause of bacteremic infections in all ages, meningitis infections in the fetus, and intra-abdominal infections such as peritonitis and Skin and Soft Tissue Infection (SSTI) [5, 6]. *E coli* is resistant to the fluoroquinolone groups (e.g., ampicillin or amoxicillin). They are often used as oral antibiotics and third-generation cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone), often used in intravenous treatment. *E coli* strains resistant to third-generation cephalosporins produce Extended-Spectrum Beta-Lactamase enzymes (ESBLs). Because of this, this strain of bacteria is called ESBL *E coli*, which destroys many beta-lactam-producing antibiotic drugs [3]. *Staphylococcus aureus* (*S. aureus*) is a spherical bacterium clustered like grapes in an irregular arrangement, Gram-positive, facultatively anaerobic, living as normal flora on the skin and mucous membranes in humans. These bacteria have a diameter of 8-1 microns, do not produce spores and do not have locomotion. Colonies of this bacterium have a characteristic colour, namely golden yellow and are surrounded by a zone of hemolysis. *S aureus* pathogens are invasive. *S aureus* produces three

metabolites: non-toxin, exotoxin, and enterotoxin, where the pathogenicity of this bacterium is the combined effect of the various metabolites it produces. *S aureus* bacteria can cause various kinds of infections in the human body, namely, infections of the musculoskeletal system, pneumonia, and endocarditis and are widely known to cause SSTI, bacteremia. Several strains of *S. aureus* produce toxins and cause Staphylococcal Skin Scalded Syndrome, Staphylococcal food poisoning [7]. By 1940, *S. aureus* had developed resistance to drugs that produce beta-lactam enzymes such as penicillin, ampicillin and amoxicillin. It gradually resists stable beta-lactamase drugs (methicillin and cloxacillin) and beta-lactamase inhibitor drugs (clavulanic acid and sulbactam). The strain of *S. aureus* bacteria that has developed resistance to the drug is called Methicillin-Resistant Staphylococcus aureus (MRSA) and is the cause of Hospital Associated (HA) infections whose cases are increasing steadily in the world [8]. Therefore, it is necessary to find new therapies that can treat infections. Therapy using medicinal plants is beneficial in treating many diseases, and the natural rate of medicine is 100% safer to use than drugs made from synthetic substances and more accessible to the public. Plants are rich sources of bioactive secondary metabolites with various diversity, such as tannins, alkaloids, and flavonoids, which are reported to have antibacterial properties *in vitro* [8, 9]. *Garcia mangostana* Linn, widely known as mangosteen, is a widely found plant in Southeast Asia, especially in Thailand, Malaysia, and Indonesia. The mangosteen fruit is dubbed the "queen of fruits" because of its fragrant aroma, sweet taste and slightly sour but rich in health benefits. In Asia, especially in Indonesia, mangosteen peel has long been used to treat diarrhoea, skin infections and wound care. The rind of the mangosteen fruit contains large amounts of xanthone, resin, tannin, and flavonoid compounds. Xanthone compounds in the mangosteen rind are mangostin, garcinone E, and 9-hydroxycalaba-xanthone, which have many biological activities, especially antibacterial activity [10, 11]. Based on the description above, the authors are interested in examining the antibacterial activity of mangosteen peel extract in inhibiting the growth of *E. coli* and *S. aureus* bacteria. The second strain of bacteria used is the ATCC strain used by researchers around the world and has been shown to prevent poison because it can mimic metagenomic compounds in bacteria and offers a universal control for microbial analysis and research development [12]. Therefore, the research aimed to determine antibacterial potential of mangosteen rind extract to inhibit the growth of *E. coli* and *S. Aureus*.

Literature Review

Mangosteen is one of the local fruits favoured by both the Indonesian people and the international community. Mangosteen grows in a warm tropical climate, with high humidity and sufficient sunlight (the length of the day is usually more than 10 hours), rainfall is relatively high and evenly distributed throughout the year with a temperature of 25˚C-35˚C, and has a short dry season. Mangosteen grows well at an altitude of fewer than 800 m above sea level, with deep solum soil, pH ranging from 5.5 to 7.0 with, a groundwater depth of about 2 meters, relatively permeable with high humidity and organic matter content good drainage [13, 14]. Recently, mangosteen has been widely grown in Western Australia, South America, and South Africa. However, countries that grow many mangosteens are still found in the Southeast Asian region, especially Thailand, Malaysia, the Philippines and Indonesia. In Indonesia, mangosteen can be found on lands with a relatively high water level, such as swamps, riverbanks, irrigation canals, and near ponds or lakes that usually have pretty fertile growth. Mangosteen has many names internationally, namely mangosteen, queen of fruit (UK), Manggistan (Netherlands), Mangastane (Germany), Mangostao (Portuguese), Mangustan (Hindi), Mangostan (France), Mangusta (Malaysia), Manggustan (Philippines), Cay Mang Cut (Vietnam) and Mengop mengut (Myanmar). In Indonesia itself, the mangosteen fruit also has many local names. On the island of Sumatra, this fruit is called mangoita (Nangroe Aceh Darussalam), mangosta (West Sumatra), and manggus (Lampung). On the island of Java, this fruit is called manggu (West Java). In the eastern part of Indonesia, the mangosteen fruit is known by the name manggusto (North Sulawesi) and the name manggustan (Maluku) [15]. Linnaeus Garcin first discovered this plant species in 1753. He named this species *Garcinia mangostana* L and grouped it in the Clusiaceae tribe. Mangosteen is a plant that grows the slowest but has the most extended lifespan among other tropical fruit plants. This plant is a large and upright tree with a height that ranges from 6 to 25 meters and has a diameter (of 60 cm). The bark of the mangosteen tree is blackish-brown, and the surface tends to be rough and easily peeled off. The inner bark is yellowish, gummy and contains bitter latex [16, 17]. The morphology of the mangosteen leaf has an elongated or oblong shape, the edges of the leaves are flat, and the flesh of the leaves is thick. The leaves are alternate and in opposite directions. Mangosteen leaves are quite long, ranging from 9 to 25 cm and leaf widths from 4 to 5 cm. Young mangosteen leaves are reddish, then change with age to light green and finally dark green when the leaves are mature. Mangosteen leaves can last for several years [18]. Mangosteen is a dioecious plant, which is a plant whose male and female flowers are not in the same tree. Mangosteen flowers appear at the ends of the shoots, usually single but sometimes more than one, and there are even up to more than ten flowers/fruit in each shoot tip [19]. The crown of the mangosteen flower is large, thick, fleshy, oval in shape, yellowish-green with a pink border. Mangosteen flowers begin to open in the afternoon and by dusk are fully open. The flower's base has attached the ovary, which is almost round in shape with 4-8 chambers. These stamens are free, short, sometimes branched with a length of about 0.5 cm and have sterile pollen. The appearance of flowers in one plant is usually not simultaneous but gradual so that in 1 tree, you can find flowers that have just appeared (flower nipples), flowers that are in bloom, flowers that have formed fruit (fruit nipples) until the fruit grows to maturity. Thus, the fruit harvest period in 1 tree can take 1-2 months [16]. The mangosteen fruit is produced by parthenogenesis, and the seeds produced are apomixis. The mangosteen fruit is round or

slightly flat with a 4-7 cm diameter and weighs between 75-150 grams. At the end of the mangosteen fruit, there are usually 4-8 stigma spaces (pale) that remain attached until the fruit is ripe. However, in the Ratu Tembilahan mangosteen variety, the fruit can have more than eight spaces of stigma (cupat). Even in other mangosteen varieties, there are those with more than ten spaces of stigma. The number of stigmas indicates the number of segments of flesh contained in the mangosteen fruit [16]. The fruit's skin is smooth, fibrous, hard, 4-8 mm thick, at first green. Then, when the fruit is fully ripe, it turns red-purple on the outside and purple on the inside. The skin of this fruit contains a bitter yellow sap. The yellow sap will drip out if the young fruit is injured. In the ripe fruit, this rigid structure of the fruit skin is good protection for the soft flesh of the fruit during packing and transportation. Inside the mangosteen fruit, there is 4-8 mangosteen flesh which is white, juicy and soft. The flesh segments of this fruit are not the same size. Some of them contain seeds that develop or do not develop. The flesh segment that contains the seeds is larger than the segment that does not contain the seeds. Each fruit usually consists of 0-2 seeds, and it is rare to find a mangosteen fruit containing three perfect seeds [20]. The mangosteen plant contains abundant chemical compounds. According to the original Indonesian herbal crematorium, the roots, skin, stem and skin of the mangosteen plant contain saponins. Mangosteen root and bark contain flavonoids and polyphenols. 29 In the mangosteen rind, there are tannin compounds with 7-13% levels, triterpenoids, polyphenols, flavonoids, and alkaloids. Mangosteen rind also contains other substances such as calcium, phosphorus, iron, thiamin, riboflavin, niacin, and ascorbic acid. Secondary metabolic compounds result from a metabolic process and play a role in plant growth and fruit development. This compound acts as a form of plant adaptation to defend itself wherever it is located. This compound is also a biomolecule as a lead compound in discovering and developing new drugs from plants. Secondary metabolites commonly found in plants are alkaloids, flavonoids, saponins, terpenoids, tannins, and phenolics [19, 21, 22]. Several studies suggest that mangosteen rind is the primary source of phytonutrient compounds, such as anthocyanins and proanthocyanins, which belong to the group of flavonoid compounds, phenolic acids, and xanthones, which are also secondary metabolites of mangosteen rind. Mangosteen rind contains triterpenoid compounds, benzophenone, biphenyl, pyrrole, and benzofuran [23]. Although often a wasted part, mangosteen peel has many benefits in treating various diseases. The compounds contained in the mangosteen rind function as antioxidants, antitumor, anti-inflammatory and antiallergic, antiplasmodial, antiviral, antifungal, and especially antibacterial [24]. Enterobacteriaceae is a group of gram-negative rod bacteria, and their natural habitat is the intestinal tract of humans and animals. Enterobacteriaceae are widely cultured in the laboratory. Staphylococci and streptococci are the most common bacteria that cause disease. Escherichia belongs to the Enterobacteriaceae group. Escherichia coli is part of the normal flora present in the intestine and causes disease indirectly. However, salmonella and shigella are generally pathogenic bacteria to humans [25]. *E. coli* forms circular, smooth colonies with visible angles. These bacteria typically give positive results on indole, lysine decarboxylase, mannitol and lactose fermentation and produce gas from glucose. Some strains of Escherichia coli produce hemolysis on blood agar. The urine test can be identified quickly by the appearance of hemolysis on blood agar, characteristic colony morphology with various "glossy" colours on *Endo* methylene blue (EMB) agar, and a positive indole spot test. *E. coli* is a bacterium commonly found in the intestines of humans and warm-blooded animals. Most strains of *E. coli* are harmless. However, some strains, such as shiga toxin producing *E. coli* (STEC), can cause severe foodborne illness. The bacteria transmit the toxin to humans mainly by consuming contaminated food such as raw or undercooked meat products, raw milk, and contaminated raw vegetables. STEC can be turned off by thoroughly cooking food until all parts reach a temperature of 70˚C or higher. *E. coli* can cause disease because of its ability to multiply and spread widely in body tissues and the presence of several substances produced by these bacteria, including non-toxins and toxins. *E. coli* is a bacterium that often causes urinary tract infections, especially in young women. Clinical manifestations are increased urinary frequency, dysuria, hematuria and pyuria, and dull pain related to upper urinary tract infection. Urinary tract infections can cause bacteremia, with sepsis as the clinical symptom. Most urinary tract infections involving the bladder or kidneys in healthy hosts are caused by small amounts of O antigen acting specifically with virulence factors facilitating colonization and subsequent clinical infection, and this organism is a uropathogenic *E. coli* [26]. This organism produces hemolysin, is cytotoxic and facilitates tissue invasion. The bacterial strain that causes pyelonephritis expresses the K antigen and creates a specific type of pilus, Fimbrae P, which binds to the blood group antigen P. *E. coli* has become a significant pathogen. These organisms cause widespread disease due to their ability to possess plasmid-mediated resistance factors that encode resistance to lactam antibiotics, fluoroquinolones, and aminoglycosides. *E. coli* is a common cause of diarrheal disease worldwide. *E. coli* is grouped based on the characteristics of its virulence substances, and each group causes disease based on different mechanisms. Currently, there are five classifications, namely enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), and Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli (*EAEC). When the host's immune system is inadequate, *E. coli* can reach the bloodstream and cause sepsis. Newborns have a high chance of developing sepsis because they lack IgM antibodies. Sepsis may result from urinary tract infection and is associated with *E. coli* invasion. *E. coli* and group B streptococcus are the leading causes of meningitis in infants. About 80% of the *E. coli* bacteria that cause meningitis have antigens. Staphylococcus comes from the word staphyle, which means the group of grapes and coccus, which means round. These germs are often found as normal flora on humans' skin and mucous membranes, and there are three species of Staphylococcus, namely: *S. aureus*, *S. epidermidis*, and *S. saprophyticus*. Among these three species, the pathogen is *S. aureus* [27]. *S. aureus* is a spherical, Gram-positive bacterium, usually arranged in an irregular

series like grapes, and the diameter of the germ is between 0.8 – and 1.0 microns [28]. Some of them are classified as normal flora on humans' skin and mucous membranes. This bacterial infection can cause various skin infections, namely pus, abscess, and pyogenic infections. *S. aureus* is non-sporing and immobile. Colonies on solid media will be round, smooth, prominent, shiny, and form a golden-yellow pigment. Culture characteristics Staphylococcus aureus grows well on various bacteriological media under aerobic or microaerophilic conditions. These bacteria proliferate at a temperature of 37˚C, but the best pigment formation is at room temperature (20˚-35˚C). *S. aureus* can cause food poisoning. Poisoning occurs because food managed by humans is not clean or hygienic. These bacteria can grow either in foods that have been cooked or salted. The growth of *S. aureus* in foodstuffs produces enterotoxin toxins which, if ingested, can cause sudden attacks, namely stomach cramps accompanied by vomiting. *S. aureus* has two antigens: polysaccharides and protein A. Polysaccharides can inhibit phagocytosis, while protein A can interfere with the host immune system [29]. These two antigens also form the cell wall of bacteria. *S. aureus* can cause disease because of its ability to multiply and spread widely in body tissues and the presence of several substances produced by these bacteria. They are a) non-toxins (surface antigens, coagulase, hyaluronidase, fibrinolysin, proteases, penicillinase, catalase); b) toxins (exotoxins, enterotoxins, Exfoliative/ Epidermolytic Toxins; and c) Toxic Shock Syndrome Toxins (Toxic Shock Syndrome Toxin-1/ TSST1). The development of *S. aureus* infection is related to the organism's virulence and the resistance of the host itself. The skin acts as a barrier against external protection, one of which is infection. The presence of trauma or damage to skin tissue causes the entry of S. *aureus*, where bacteria can attach to mucosal cells mediated by teichoic acid present in cell walls. The ability of these bacteria to cause disease can be directly related to their ability to inhibit chemotaxis. Protein A in bacteria ii reacts specifically to IgG1, IgG2, and IgG4. This protein is located on the outermost bacterial membrane and can absorb serum immunoglobulins, preventing antibacterial antibodies from acting as opsonins and inhibiting phagocytosis. Leukocidine causes leukocyte degranulation, and hemolysis causes lysis of erythrocytes which also contributes to the virulence of these bacteria. The prevalence of other bacterial species also controls bacterial proliferation in the gastrointestinal tract. If the balance is disturbed during antibiotic therapy, bacteria become resistant so that they can proliferate and invade the intestinal wall. Signs and symptoms vary according to the site of infection, although they most often cause skin infections. Severity is usually the result of local suppression, systemic spread with metastatic infection, or systemic effects of production. Features of local infection *S. aureus* is an infection of the hair follicles or an abscess, usually a severe, localized, painful, inflammatory infection with central suppuration that resolves rapidly when the pus is drained. Some staphylococci are resistant to antibiotics, including MRSA (Methicilin Resistant *Staphylococcus aureus*) and VRSA (Vancomycin-Resistant *Staphylococcus aureus*) [30]. Most strains of *S. aureus* have been resistant to penicillin because the production of beta-lactamase enzymes can damage the beta-lactam structure of penicillins. To replace penicillin, *S. aureus* is treated with nafcillin or oxacillin. MRSA is a type of S. *aureus* already resistant to methicillin antibiotics and other drugs of the same class, such as penicillin, amoxicillin and oxacillin, used to treat it. MRSA was first discovered in patients treated in hospitals and other health facilities (HA-MRSA or healthcare-associated MRSA), especially in the elderly, seriously ill people, and those with open wounds (e.g., abrasions). Due to prolonged lying down), or patients who use a catheter. After that, MRSA was found in community-associated MRSA (CA-MRSA) associated with antibiotic use, sharing contaminated equipment, active skin disease or wounds, poor hygiene and living in a bad environment. Several *S. aureus* isolates, apart from being resistant to penicillin, were also resistant to methicillin (MRSA), so the antibiotic vancomycin had to be used. However, it has now been reported that *S. aureus* resistance to vancomycin MRSA has also been reported to increase in frequency in Latin America, England, Canada, Australia, Asia, and all parts of Europe. Extraction can be carried out from fresh or dried materials. Simplicia is a natural substance used as a medicine that has not undergone any processing and, unless otherwise stated, is in the form of dried material [31]. Vegetable simplicia are simplicia in the form of whole plants, plant parts or plant exudates, cell contents that spontaneously come out of the plant or cell contents that are removed from the cells in a certain way, or other vegetable substances which are separated in a certain way from the plant and have not been pure chemical. Simplicia will later be processed into extract. Simplicia is powdered to the desired degree of fineness. The degree of fineness of the powder affects the quality of the extract. Generally, hard materials such as seeds, wood, bark, and roots, are powdered before being extracted to expand the contact between the filter and the surface of the simplicia to facilitate filtering the active compounds [32]. However, if the powder is too fine, it will complicate the extract filtering process. Extraction is a dry, viscous or liquid preparation suitably prepared by a simplicia filter outside the influence of direct sunlight. Natural material extraction aims to extract the chemical components found in natural materials [33]. Extracts are dry, viscous or liquid preparations made by extracting simplicia according to a suitable method outside direct sunlight. The extract should be easily ground into a powder. This extraction is based on the principle of substance mass transfer into the solvent, where the transfer begins to occur in the interfacial layer and then diffuses into the solvent. Several methods are used in carrying out the extraction, namely extraction using solvents and steam distillation. Antibacterial testing method is the diffusion method, well method, and dilution method [34, 35].

Research Methods

The type of this research is experimental research with Post Test Control Group Design. The negative control used was sterile Mc Farland solution. The positive control used was ceftazidime antibiotic disc as a control against the growth of *E. coli* bacteria and cotrimokzasol antibiotic disc as a control against the growth of *S*

aureus bacteria. Mangosteen fruit is obtained from a fruit shop in Cawang. The method antibacterial test used is the agar diffusion test method (Kirby and Bauer methods), and the research was conducted at the Indonesian Christian University Microbiology Laboratory from September 2018 to November 2018. The bacterial samples used were *E. coli* ATCC 25922 and *S. aureus* ATCC 25933, obtained from the University of Indonesia. The purchased mangosteen fruit is 7 kg. The mangosteen fruit is washed first with distilled water, and then the skin is separated from the flesh. After that, the mangosteen rind was chopped into small pieces and dried in an oven at 40˚C for 45 hours. The simplicia was sieved and weighed to determine the shrinkage, and 200 grams of simplicia was taken from the mangosteen rind powder. Then, the mangosteen rind simplicia was extracted through a maceration process using 800 ml of ethanol p.a as a solvent and evaporated using a vacuum pan evaporator. The samples used in this study were mangosteen pericarp extract in various concentrations of 10%, 30%, 50%, 70%, 100% and antibiotics ceftazidime and cotrimokzasol as positive controls, and sterile distilled water as negative controls. In this study, mangosteen rind extract was diluted to obtain various concentrations in a test tube. After the desired concentration is formed, filter paper is given into it, which will be placed on the agar medium to see the zone of blandness of the growth of *E. coli* and *S. aureus* bacteria. This research will be repeated four times. All tools used in the research are washed, dried and wrapped in paper or aluminium foil first. Then, the tools were sterilized in an autoclave for 15 minutes. The pressure should be set to 1 atm and the temperature to 121˚C. The manufacture of mangosteen rind extract is the preparation of extract materials and the manufacture of extraction. Preparation of bacterial suspension is done by using the pure strain of *E. coli* and *S. aureus*. The suspension was made into tubes I (*E. coli*) and tube II (*S. aureus*) containing broth solution until turbidity was obtained, which was adjusted to the Mc Farland turbidity standard of 0.5 to obtain bacteria as much as 10^9 CFU/ml. After that, it was diluted three times to obtain a colony suspension of 10^6 CFU/ml. This bacterial suspension was used as an inoculum. Making media, namely making MHA (Mueller Hilton Agar) media, making EMB (Endo Methylene Blue) media, and making MSA (Mannitol Salt Agar**)** media. The data was obtained descriptively by recording the inhibitory zone results of Gram-negative bacteria Escherichia coli and Gram-positive bacteria *S. aureus* after being treated with mangosteen rind extract at concentrations of 10%, 30%, 50%, 70%, 100%, negative control (equates sterile), as well as positive controls (ceftazidime and cotrimoxazole). The data is presented descriptively in tables and graphs that are processed using the Microsoft Excel 2016 program.

Result and Discussion

From 200 grams of mangosteen rind powder, a thick extract weighing 10.31 grams was obtained. The result of of extraction of mangosteen peel can see in Figure 1.

Fig 1: Results of Extraction of Mangosteen Peel

The extraction method chosen is the maceration method because nutritious substances will be attracted from their natural ingredients by using this maceration method. The advantage of masrasi is that it is easy to do, and the equipment is cheap and simple.

The sensitivity test used is the Kirby Bauer disk diffusion method because although this method is an old and simple sensitivity test method, this sensitivity test is still relevant and includes the standard antibacterial test in CLSI [35].

1. Inhibition zone of mangosteen rind extract against *E. coli*

The inhibitory power of mangosteen rind extract (*Garcinia mangostana* Linn) on the growth of *E. coli* with concentrations of 10%, 30%, 50%, 70%, 100%, sterile distilled water as a negative control and ceftazidime antibiotics as a positive control can be seen in Figure 3.

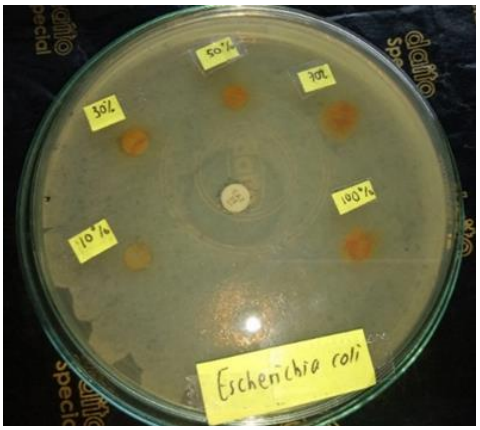

Fig 2: The results inhibition of the mangosteen rind extract against the growth *E. Coli*

Based on research that has been carried out through 4 treatment repetitions in a row, the results of measuring the inhibition zone diameter are shown in the Table 1.

Table 1: Results of *Inhibition* Testing of Mangosteen Peel Extract on the Growth of *E. coli* bacteria ATCC 25922

s	Sterile Aquades Control (-)	Ceftazidime Control (+)	Diameter of Inhibition Zone Mangosteen Peel Extract (mm)				
			10%	30%	50%	70%	100%
1	0	21	0	0	0	0	0
2	0	21	0	0	0	0	6,5
3	0	21	0	0	0	0	0
4	0	21	0	0	0	0	0
Average	0	21	0	0	0	0	1,625

The Table 1. shows no inhibition zone in the negative control treatment group with sterile distilled water, while in the positive control group with the antibiotic ceftazidime 30 mg, an inhibition zone of 21 mm was found. In the first repetition, no inhibition zone was found. In the second repetition, an inhibition zone was found at a 100% concentration of 6.5 mm. At other concentrations, there was no formation of an inhibition zone. In the third repetition, no inhibition zones were found at all concentrations. In the fourth repetition, no inhibition zones were found at all concentrations.

2. Inhibition zone of mangosteen rind extract against *S. aureus*

The inhibitory power of mangosteen rind extract (*Garcinia mangostana* Linn) on the growth of *S. aureus* with concentrations of 10%, 30%, 50%, 70%, 100%, sterile distilled water as a negative control and cotrimoxazole antibiotic as a positive control can be seen in the picture 4.

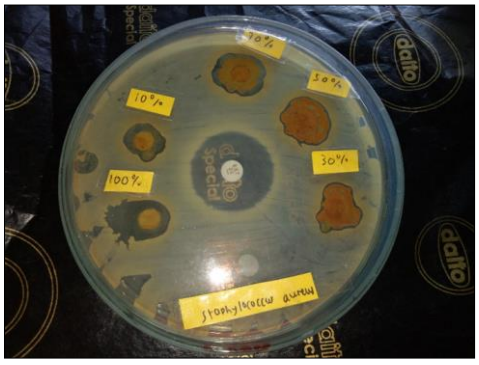

Fig 3: The results inhibition of the mangosteen rind extract against the growth *S. aureus*

Based on research that has been carried out through 4 treatment repetitions in a row, the results of measuring the inhibition zone diameter are shown in the Table 2.

Table 2: Results of Inhibition Testing of Mangosteen Peel Extract on the Growth of *S. aureus* ATCC 25923

Duplication	Control (-) Sterile Aquades	Control (+) Ceftazidime	Diameter of Inhibition Zone of *S. Aureus* Mangosteen Peel Extract				
			10%	30%	50%	10%	100%
1	0	19.5	11	13	15	16	20
2	0	19.5	11	11.5	13.4	16	20
3	0	25	9	10	11.4	16	20
4	0	25	9	9.4	9.8	16	18
Average	0	22.5	10.23	10.98	13.45	16	19.5

The Table 2 shows no inhibition zone in the sterile aqua dest negative control treatment group, while in the positive control group with the antibiotic cotrimoxazole 30 mg, an inhibition zone of 22.5 mm was found. In the treatment group, mangosteen rind extract with 10%, 30%, 50%, 70%, and 100% each had an average inhibition zone diameter of 10.23 mm, 10.95 mm, 13.45 mm, 16 mm, and 19.5 mm. In the Kirby Bauer disc diffusion sensitivity test method, the absorption of filter paper is very influential [36].

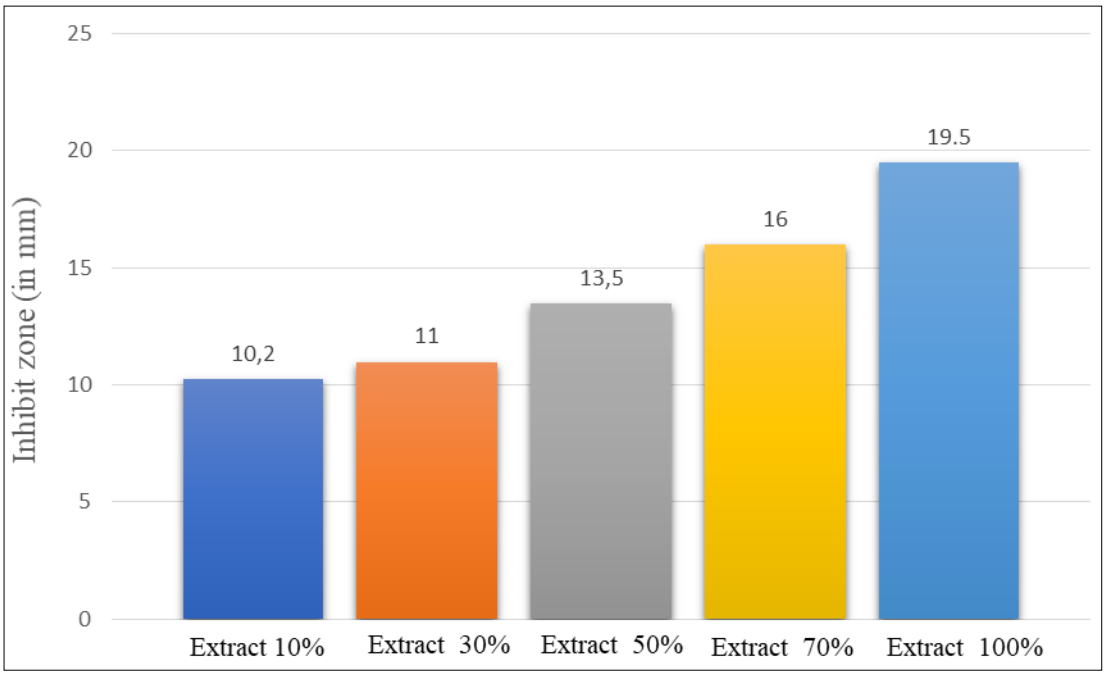

Fig 4: Graph of average inhibition zone diameter of *S. aureus* bacteria ATCC 25923

In the sensitivity test using the Kirby Bauer disc diffusion method, mangosteen rind extract was carried out by duplicating four times. The average inhibition zone diameter was 10.2 mm, 11 mm, 13.5 mm, 16 mm, and 19.5 mm, at the concentration of the mangosteen rind extract. 10%, 30%, 50%, 70%, 100%. It indicates an increase in the diameter of the inhibition zone formed against S aureus following the increase in the concentration of the mangosteen rind extract. This study looked at whether mangosteen rind extract inhibited the growth of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 *in vitro* by observing whether or not the diameter of the inhibition zone was formed. In this study, each treatment group was tested four times in repetition, according to Federer's formula, to produce consistent data, and the results obtained were not due to the opportunity factor but due to the influence of the treatment. The zone size can be affected by the density/viscosity of the culture medium, the rate of diffusion of the antibiotic medium, the antibiotic concentration in the filter paper disk, the sensitivity of the organism to the antibiotic and the medium used. In this study, the diameter of the inhibition zone in the negative control using sterile distilled water was not formed. It indicates that the antibacterial activity is not influenced by the solvent used, so the antibacterial activity analyzed is the potential of the mangosteen rind extract. Testing the antibacterial activity of mangosteen rind extract (*Garcinia mangostana* L.) against *E. coli* ATCC was carried out four times according to Federer's formula calculation. According to the statement of Geetha *et al*., the increase in the concentration of the extract increases the concentration of bioactive substances so that the inhibitory activity will also be higher [9]. The phenomenon is that the increasing concentration, the larger the diameter of the inhibition zone formed is similar to the susceptible dose-dependent category in measuring the effectiveness of an antimicrobial that was just added by CLSI (Clinical and Laboratory Standards Institute) in 2014. In this category, the sensitivity of a bacterial isolate depends on the drug dose. Suppose the bacterial sensitivity test results are in the susceptible dose-dependent or dose sensitive (SDD) category. In that case, it is very important to use a dosing regimen (increasing the dose, increasing the frequency of drug use, or both) that can increase drug exposure to a bacterium compared to the dose of the drug which is usually used to reach the sensitive category.

The concept of SDD is often included as a definition of antibacterial in the category of intermediates. However, clinicians and microbiologists did not understand this when intermediate results were reported. The SDD category may be used when doses above the dose normally used to achieve the sensitive category have been approved and used in the treatment and where there are sufficient data to confirm their use and have been discussed. The definition is fixed and does not change when the intermediate category is used. Clinicians widely use the SDD category to provide more definite sensitive criteria to provide maximum and more specific therapy. The intermediate criteria are often misinterpreted as resistance criteria because they have unclear boundaries with resistance criteria. A wise course of antibiotic administration by increasing the dose and administration of antimicrobials during therapy can also increase public awareness of the wise use of antibiotics. Only cefepime antimicrobials have been identified in this SDD category [38]. According to Greenwood's criteria, the inhibition zone size of 16-20 mm indicates strong inhibition zone activity. It means that at a concentration of 100%, mangosteen rind is a strong antibacterial against Staphylococcus aureus ATCC. It can be concluded that mangosteen rind extract (*Garcinia mangostana* L.) plays a role in inhibiting the growth of Staphylococcus aureus ATCC. Preliminary research results also reveal the same thing. According to research conducted by Yin Sze Lim *et al*., 70% methanol extract of mangosteen rind can inhibit the growth of *S. aureus* ATCC 11632 bacteria with an inhibition zone diameter of 6 mm at an extract concentration of 80%. It can inhibit the growth of *B. cereus* ATCC 10876 bacteria with a diameter of the inhibition zone was 2.06 mm at 80% extract concentration, while in the colonies of *E. coli* ATCC 10536, there was no inhibition zone formed which indicated that the 70% methanol extract of mangosteen rind could not inhibit the growth of *E. coli* bacteria ATCC 10536 [23]. According to Poelongan and Pratiwi 2008, 70% ethanol extract of mangosteen rind inhibited the growth of Gram-positive bacteria (*S. aureus* ATCC 25922 and *S. epidermidis*) by forming inhibitory zones with an average diameter of 11 mm and 12.3 mm. In Gram-negative bacteria (*S. enteritidis* B2284 and *E. coli*), there was no formation of the diameter of the inhibition zone. According to research conducted by Sujono and Anik Nuyati in 2017, methanol extract of mangosteen rind can inhibit the growth of *S. aureus* bacteria with an inhibition zone diameter of 10 mm at an extract concentration of 64% and no inhibition zone formation in *E. coli* bacteria [39]. The difference in the research results on the inhibitory power between *E. coli* ATCC and *S. aureus* ATCC was due to differences in the structure of the bacterial cell wall itself. The cell wall structure of Gram-positive bacteria only consists of a thick layer of peptidoglycan, little lipid, and contains polysaccharides (teichoic acid) which are easily soluble in water. In Gram-negative bacteria, the cell wall structure contains more lipids, less peptidoglycan and an envelope consisting of a plasma membrane, periplasm, peptidoglycan and an outer membrane containing three important components, namely oligosaccharides, proteins and lipids. The more complex structure of the cell wall in Gram-negative bacteria causes these bacteria to be more difficult to inhibit than Gram-positive bacteria, which have a simpler bacterial cell wall structure. Enveloping the outer membrane of Gram-negative bacteria and *E. coli* has the function of repelling both hydrophobic and hydrophilic molecules well. Large molecules cannot enter these bacteria [40]. The formation of the inhibition zone is influenced by the presence of compounds that have an antibacterial mechanism in the mangosteen rind. These compounds include Xanthones, Phenolic Acid, and Flavonoids. Xanthan compounds are the main bioactive compounds from mangosteen peel which have antibacterial activity. Antibacterial activity is found in xanthone, -mangostin and -mangostin derivative compounds because they have free C-6 hydroxyl groups and have good antibacterial activity. The possible target of the antibacterial mechanism of this compound is the bacterial cytoplasmic membrane, and the intracellular components of the bacteria become leaky. Xanthones also induce the release of lipoteichoic acid, which is an important component of Gram-positive bacteria that binds to the outer peptidoglycan as a protective bacteria, so that these compounds can bind to the bacterial cell wall and cause leakage of the intracellular components of bacteria [41]. The phenolic acid compounds contained in the mangosteen rind also have antimicrobial activity by destroying the cytoplasm of bacterial cells in the presence of hydroxyl groups attached to the cytoplasm of these cells. The accumulation of hydrophobic phenolic groups in the lipid bilayer of the bacterial wall can disrupt protein-fat interactions and increase membrane permeability. Furthermore, it causes chaos in the membrane structure, increases intracellular constituents' leakage, and ultimately destroys the integrity of the bacterial cell membrane [42]. Flavonoid compounds act as antibacterial by denaturing and damaging the cytoplasmic membrane, which can cause cell leakage. This damage allows nucleotides and amino acids to leak out and prevents the entry of active ingredients into the cell, leading to bacterial cell death [43]. Extraction is the process of pulling chemicals out of natural materials. In the extraction process, the level of the polarity of the solvent used greatly determines the amount of active substance because in the extraction process, the principle of "like dissolves like" applies where the substance is only properly dissolved and extracted if the solvent used has the same level of polarity. According to Nuttawan and Eshtiahi 2015, absolute ethanol solvent extracted more xanthone compounds than 50% ethanol solvent and 72.5% ethanol solvent. Xanthan is a polar compound with a weak polarity, while absolute ethanol is a solvent with a low polarity value so that more xanthone compounds come out [44]. More phenolic acid and flavonoid compounds were released when using organic solvents with lower polarity levels than water. The results showed that methanol solvent extracted the most phenolic acid and flavonoid content. However, it was found that absolute ethanol solvent could extract sufficient amounts of phenolic acid and flavonoid content. Researchers prefer absolute ethanol solvents over absolute methanol solvents because ethanol solvents are allowed to extract food in several countries such as the European Union, the United States of America, Japan, and Indonesia. Meanwhile,

the solvent for methanol is limited to the United States of America. Absolute ethanol solvent can also extract the antibacterial compound content of mangosteen rind in sufficient quantities [45].

Conclusion

The results showed that the mangosteen rind extract could not inhibit the growth of *E. coli* ATCC 25922, but there was can inhibit the growth of *S. aureus* ATCC 25933. Furthermore, further research is needed to investigate the Selective decontamination of the digestive phenomenon in mangosteen rind extract and the toxicity effect of mangosteen rind extract when the concentration is further increased.

References

1. Frieri M, Kumar K, Boutin A. Antibiotic resistance. Journal of infection and public health,20171:10(4):369-78.
2. Bisht R, Katiyar A, Singh R, Mittal P. Antibiotic resistance-A global issue of concern. Asian journal of pharmaceutical and clinical research,2009:2(2):34-9.
3. Hadi U, Kuntaman K, Qiptiyah M, Paraton H. Problem of antibiotic use and antimicrobial resistance in Indonesia: are we really making progress?. Indonesian Journal of Tropical and Infectious Disease,2013:1:4(4):5-8.
4. Lestari ES, Severin JA, Filius PM, Kuntaman K, Duerink DO, Hadi U. Antimicrobial resistance among commensal isolates of Escherichia coli and Staphylococcus aureus in the Indonesian population inside and outside hospitals. European Journal of Clinical Microbiology & Infectious Diseases,2008:27(1):45-51.
5. Kupp MA, Chatton MJ. Current medical diagnosis & treatment. InCurrent medical diagnosis & treatment, 2012, 1062-1062.
6. Longo D, Kasper D, Jameson J. All. E, editors. Harrison's Principles of Internal Medicine-18th editi.
7. Bhunia AK. Staphylococcus aureus. InFoodborne Microbial Pathogens, 2018, 181-192. Springer, New York, NY.
8. Lee AS, De Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A. Methicillin-resistant Staphylococcus aureus. Nature reviews Disease primers,2018:31:4(1):1-23.
9. Geetha RV, Roy A, Lakshmi T. Evaluation of antibacterial activity of fruit rind extract of Garcinia mangostana Linn on enteric pathogens-an in vitro study. Asian J Pharm Clin Res,2011:4(2):115-8.
10. Pothitirat W, Pithayanukul P, Chomnawang MT, Supabphol R, Gritsanapan W. Biological and chemical stability of mangosteen fruit rind extract. Functional Plant Science and Biotechnology,2009:3(1):16-8.
11. Soetikno JS, Handayani R, Rahayu RD. Assay for antimicrobial activity of mangosteen rind extracts. Journal of Innovations in Pharmaceuticals and Biological Sciences,2016:3(1):54-60.
12. Lopera J, Hunter M, Chuang RY, King S, Amselle M, Chase B. Development and Evaluation of Whole Cell-and Genomic DNA-based Microbiome Reference Standards.
13. Pao S, Long III W, Kim C, Rafie AR. Salmonella population rebound and its prevention on spray washed and non-washed jalapeno peppers and Roma tomatoes in humid storage. Foodborne Pathogens and Disease,2012:1:9(4):361-6.
14. Putraa SJ, Arhamb Z. Plant Suitability Evaluation of Mangosteen Plant with Geoprocessing Model Approach Using Upland Soil Test Parameters. InInternational Conference on Science and Technology (ICOSAT 2017)-Promoting Sustainable Agriculture, Food Security, Energy, and Environment Through Science and Technology for Development Atlantis Press, 2017, 99-103.
15. Hariana A. 262 medicinal plants and their properties. Group Self-Help Disseminator, 2013.
16. Orwa C. Agroforestree Database: a tree reference and selection guide, 2009. version 4.0. http://www. worldagroforestry. org/sites/treedbs/treedatabases. asp..
17. Cunha BL, França JP, Moraes AA, Chaves AL, Gaiba S, Fontana R. Evaluation of antimicrobial and antitumoral activity of Garcinia mangostana L.(mangosteen) grown in Southeast Brazil. Acta Cirúrgica Brasileira,2014:29:21-8.
18. Akao Y, Nakagawa Y, Iinuma M, Nozawa Y. Anti-cancer effects of xanthones from pericarps of mangosteen. International journal of molecular sciences,2008:14:9(3):355-70.
19. Ropiah S. Morphological and Physiological Development of Mangosteen (Garcinia mangostana L.) during Growth and Maturation.
20. Ansori AN, Fadholly A, Hayaza S, Susilo RJ, Inayatillah B, Winarni D. A review on medicinal properties of mangosteen (Garcinia mangostana L.). Research Journal of Pharmacy and Technolog,2020:1:13(2):974-82.
21. Gokhale M, Wadhwani M. Antimicrobial activity of secondary metabolites from plants-a review. International Journal of pharmacognosy,2015:22:23.
22. Anulika NP, Ignatius EO, Raymond ES, Osasere OI, Abiola AH. The chemistry of natural product: Plant secondary metabolites. Int. J. Technol. Enhanc. Emerg. Eng. Res,2016:4(8):1-9.
23. Sze Lim Y, Sze Hui Lee S, Chin Tan B. Antioxidant capacity and antibacterial activity of different parts of mangosteen (Garcinia mangostana Linn.) extracts. Fruits,2013:1:68(6).
24. Nauman MC, Tocmo R, Vemu B, Veenstra JP, Johnson JJ. Inhibition of CDK2/CyclinE1 by xanthones from the mangosteen (Garcinia mangostana): a structure-activity relationship study. Natural product research,2021:2:35(23):5429-33.

25. Abebe E, Gugsa G, Ahmed M. Review on major food-borne zoonotic bacterial pathogens. Journal of tropical medicine, 2020.
26. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nature reviews microbiology,2015:13(5):269-84.
27. Verstappen KM, Willems E, Fluit AC, Duim B, Martens M, Wagenaar JA. Staphylococcus aureus nasal colonization differs among pig lineages and is associated with the presence of other staphylococcal species. Frontiers in Veterinary Science,2017:23:4:97.
28. Valentini F, Calcaterra A, Ruggiero V, Pichichero E, Martino A, Iosi F. Functionalized graphene derivatives: antibacterial properties and cytotoxicity. Journal of Nanomaterials, 2019.
29. Kuipers A, Stapels DA, Weerwind LT, Ko YP, Ruyken M, Lee JC. The Staphylococcus aureus polysaccharide capsule and Efb-dependent fibrinogen shield act in concert to protect against phagocytosis. Microbiology,2016:162(7):1185.
30. Hasan R, Acharjee M, Noor R. Prevalence of vancomycin resistant Staphylococcus aureus (VRSA) in methicillin resistant S. aureus (MRSA) strains isolated from burn wound infections. Tzu Chi Medical Journal,2016:28(2):49-53.
31. Rosmi RF. The Effect of Drying Method on Turmeric Rhizome Simplicia's Quality. Indonesian Journal of Multidisciplinary Science,2021:1(3):274-82.
32. Toruan AL, Ayu GA. Integration of Herbal or Traditional Medicine Through Evidence Based Practice. Pokjanas T, 2016, 69.
33. Uwineza PA, Waśkiewicz A. Recent advances in supercritical fluid extraction of natural bioactive compounds from natural plant materials. Molecules,2020:(17):3847.
34. Sandle T. Pharmaceutical microbiology: essentials for quality assurance and quality control. Woodhead Publishing, 2015.
35. Damian L, Patachia S. Method for testing the antimicrobial character of the materials and their fitting to the scope. Bulletin of the Transilvania University of Brasov. Engineering Sciences. Series I,2014:7(2):37.
36. Vineetha N, Vignesh RA, Sridhar D. Preparation, standardization of antibiotic discs and study of resistance pattern for first-line antibiotics in isolates from clinical samples. International Journal of Applied Research,2015:1(11):624-31.
37. Biedenbach D, Lob S, Badal R, Sahm D. Variability of Susceptibility and Multidrug Resistance among K. pneumoniae from IAI in Asia/Pacific Countries–SMART 2012-2013. In International Journal Of Antimicrobial Agents PO BOX 211, 1000 AE Amsterdam, Netherlands: Elsevier Science BV,2015:45:S81-S82.
38. Sujono S. Antibacterial test of methanol extract of mangosteen rind (Garcinia mangostana L.) against Staphyllococcus aureus and Escherechia coli. Journal of Laboratory Technology,2017:25:6(1):24-9.
39. Yang R, Li P, Li N, Zhang Q, Bai X, Wang L. Xanthones from the pericarp of Garcinia mangostana. Molecules,2017:22(5):683.
40. Miladiyah I, Rachmawaty FJ. Potency of xanthone derivatives as antibacterial agent against Methicillin-Resistant Staphylococcus Aureus (MRSA). JKKI: Jurnal Kedokteran dan Kesehatan Indonesia,2017:15:8(2):124-35.
41. Merkl R, HRádkoVá I, FIlIp V, ŠMIdRkal J. Antimicrobial and antioxidant properties of phenolic acids alkyl esters. Czech Journal of Food Sciences,2010:28(4):275-9.
42. Toudert N, Djilani SE, Djilani A. Antimicrobial activity of flavonoids of Ampelodesma mauritanica. American-Eurasian J Sustainable Agric,2009:3:227-8.
43. Yoswathana N, Eshtiaghi MN. Optimization of subcritical ethanol extraction for xanthone from mangosteen pericarp. International Journal of Chemical Engineering and Applications,2015:6(2):115.
44. Haminiuk CW, Plata-Oviedo MS, de Mattos G, Carpes ST, Branco IG. Extraction and quantification of phenolic acids and flavonols from Eugenia pyriformis using different solvents. Journal of Food Science and Technology,2014:51(10):2862-6.
45. Chaovanalikit A, Mingmuang A, Kitbunluewit T, Choldumrongkool N, Sondee J, Chupratum S. Anthocyanin and total phenolics content of mangosteen and effect of processing on the quality of mangosteen products. International Food Research Journal,2012:19(3):1047.