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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SUGARCANE MOLASSES ETHANOLIC EXTRACT AND ITS BACTERIOSTATIC MECHANISMS AGAINST SELECTED FOODBORNE PATHOGENS

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By

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Sugarcane molasses is thick, dark, and sweet syrup derived from the sugar refining process and teems with numerous essential elements. Sugarcane molasses have various valuable phenolic constituents such as flavonoids and melanoidins, generating antioxidant and antibacterial properties. The significantly high number of bioactive compounds from plant by-products lead to evaluate health conferred materials from sugarcane molasses. Since the application of synthetic preservatives has increased food safety crisis, the pressure on food manufacturers and adverse health impacts such as allergies, asthma, gastrointestinal problems and even cancers, sugarcane molasses can be introduced as a natural preservative in the food industry. Therefore, the objectives of this study were 1) to characterise the composition of sugarcane molasses, 2) to determine the antioxidant and antibacterial activities of sugarcane molasses ethanolic extract against selected foodborne pathogens, and 3) to investigate the bacteriostatic mechanisms of sugarcane molasses ethanolic extract towards *Staphylococcus aureus* and *Escherichia coli*. The composition of sugarcane molasses was determined by proximate analysis, sugar and amino acid profiling. Antioxidant activities were estimated by a total phenolic compound assay and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. Meanwhile, antibacterial activities were carried out using disc diffusion, minimum inhibition concentrations (MICs) and minimum bactericidal concentrations (MBCs) assays. Bradford assay, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analysis were carried out to analyse the bacteriostatic mechanisms and morphological changes of the bacterial cell. The proximate composition of sugarcane molasses consisted of 75.10±0.7% carbohydrate, 17.55±0.2% moisture, 5.35±0.1% ash, 2.00±0.4% crude protein and zero fat. It was also rich in sugar content included sucrose, fructose and glucose. Sugarcane molasses ethanolic extract showed high total phenolic compounds; phenylvaleric acid, quinic acid, tannic acid, apigenin and gallic acid with high antioxidant activities and 50% inhibitory concentration (IC_{50} value) was about 0.79 mg QE/g. The inhibition zone against four foodborne pathogens, *Staph. aureus*, *Listeria monocytogenes*, *E. coli* and *Salmonella* Typhimurium, ranged from 8.82±0.3 mm to 25.05±1.6 mm. Meanwhile, the MICs of sugarcane molasses ethanolic extract ranged

from 3.125% to 6.25% v/v and MBCs were 6.25% to >12.5% v/v. These revealed the highest antibacterial activities were found in Gram-positive *Staph. aureus* and Gram-negative *E. coli*. The protein leakages of *Staph. aureus* and *E. coli* that treated with sugarcane molasses ethanolic extract were ranged from 1.91 µg to 2.53 µg and 1.09 µg to 2.29 µg, respectively. Results of SEM and TEM analysis suggested the cytoplasmic membranes of *Staph. aureus* and *E. coli* were damaged and ruptured. The sugarcane molasses ethanolic extract was found able to change the morphology of bacterial cells. In conclusion, sugarcane molasses is high in carbohydrate, mainly sucrose. They are also rich in phenolic compounds, which can be a potential source for antioxidant and antibacterial compounds.

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AKTIVITI ANTIBAKTERIA DAN ANTIOXIDA EKSTRAK ETANOL MOLASES TEBU DAN MEKANISME BAKTERIOSTATIKNYA TERHADAP PATOGEN MAKANAN TERPILIH

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Molases tebu adalah sirap pekat, gelap dan manis yang diperoleh daripada proses penapisan gula dan mempunyai banyak elemen-elemen penting. Molases tebu mempunyai pelbagai sebatian fenolik yang bernilai seperti flavonoid dan melanoidin, yang menghasilkan sifat antioksida dan antibakteria. Peningkatan yang ketara dalam pemulihan komponen bioaktif daripada sisa buangan tumbuhan membawa kepada penilaian bahan yang sihat daripada molases tebu. Oleh kerana penggunaan pengawet sintetik telah meningkatkan krisis keselamatan makanan, tekanan pada pengeluar makanan dan kesan yang buruk kepada kesihatan seperti alergik, asma, masalah gastrousus dan juga kanser, molasses tebu boleh diperkenalkan sebagai pengawet semulajadi di dalam industri makanan. Oleh itu, objektif-objektik kajian ini adalah 1) mencirikan komposisi molases tebu, 2) menentukan aktiviti antioksida dan antibakteria ekstrak etanol molases tebu terhadap patogen makanan yang terpilih, dan 3) mengkaji mekanisme bacteriostatik ekstrak etanol molases tebu ke atas *Staphylococcus aureus* dan *Escherichia coli*. Komposisi molases tebu telah ditentukan oleh analisis proksimat, profil gula dan asid amino. Aktiviti antioksida ditentukan dengan ujian jumlah sebatian fenolik dan ujian 1,1-diphenyl-2-picrylhydrazyl (DPPH). Sementara itu, aktiviti antibakteria telah dijalankan melalui asai penyebaran cakra, ujian kepekatan bakteriostatik minimum (MICs) dan kepekatan bakterisidal minimum (MBCs). Asai Bradford, analisis mikroskop elektron imbasan (SEM) dan mikroskop elektron penghantaran (TEM) dijalankan untuk mengkaji mekanisme bakterostatik dan perubahan morfologi pada sel bakteria. Keputusan komposisi proksimat molases tebu terdiri daripada 75.10±0.7% karbohidrat, 17.55±0.2% kelembapan, 5.35±0.1% abu, 2.00±0.4% protein dan kosong kandungan lemak. Ia juga kaya dengan kandungan gula termasuk sukrosa, fruktosa dan glukosa. Ekstrak etanol molases tebu menunjukkan sebatian fenolik yang tinggi; asid phenylvaleric, asid quinic, asid tanin, apigenin dan asid gallic bersama aktiviti antioksida yang tinggi dan 50% kepekatan perencatan oksida (nilai IC_{50}) adalah berjumlah 0.79 mg QE/g. Zon perencatan terhadap empat patogen makanan, *Staph. aureus, Listeria monocytogenes, E. coli* dan *Salmonella* Typhimurium berada di antara 8.82 ± 0.3 mm hingga 25.05 ± 1.6 mm. Sementara itu, MICs ekstrak etanol molases tebu adalah 3.125%

hingga 6.25% v / v dan MBCs adalah 6.25% hingga > 12.5% v / v. Ini menunjukkan aktivit antibakteria yang tinggi dijumpai pada Gram-positif *Staph. aureus* dan Gram-negatif *E. coli*. Kebocoran protein *Staph. aureus* dan *E. coli* yang dirawat dengan ekstrak etanol molases tebu berada dalam lingkungan 1.91 µg hingga 2.53 µg dan 1.09 µg hingga 2.29 µg, masing-masingnya. Keputusan analisis SEM dan TEM menunjukkan membran sitoplasma *Staph. aureus* dan *E. coli* rosak dan pecah. Ekstrak etanol molases tebu didapati dapat mengubah morfologi sel bakteria. Kesimpulannya, molases tebu mempunyai kandungan karbohidrat yang tinggi terutamanya sukrosa. Ia juga kaya dengan sebatian fenolik yang berpotensi menjadi sumber untuk kompaun antioksida dan antibakteria.

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Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
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%	Percentage
/	Per
±	Plus-minus sign
nm	Nanometre
µm	Micrometre
mm	Millimetre
G	Gravity
µg	Microgram
mg	Milligram
et al	et alia or and others
CFU	Colony-forming unit
min	Minute
H	Hour
mL	Millilitre
µL	Microlitre
mmHG	Milimetre high
rpm	Revolution per minute
ATCC	American Type Culture Collection
v/v	Volume over volume
w/v	Weight over volume
^{o}C	Degree Celsius
pH	Potential of Hydrogen
DIZ	Diameter of inhibition zone

CHAPTER 1

INTRODUCTION

1.1 Background

Sugarcane molasses is viscous and dark coloured concentrated liquid syrup derived from the sugar refining process (Arimi et al., 2014). It is the final liquid residue obtained in the preparation of commercial sugar by the repeated process of evaporation, crystallisation, and the centrifugation of juices from sugarcane (Arimi et al., 2014; Olbrich, 2006; Takara et al., 2007). Sugarcane molasses is also considered to be rich in the valuable compound. It is dominated by sucrose, fructose, glucose, trace elements, and minerals (Soukoulis & Tzia, 2018). Besides, sugarcane molasses has been reported to be an excellent source of organic compounds, i.e., phenolic compounds (Iqbal et al., 2017).

Approximately 10% of the phenolic compound is extracted after the crystallisation process with total content of 101.3 mg gallic acid equivalents (GAE)/g (Thao & Tuan, 2017). To the relevant, most of the phenolic compound in sugarcane molasses belong to flavonoids and lignan. Both flavonoids and lignan can exhibit biological activities by promoting chemopreventive properties (Pietta, 2000). Takara et al. (2007) found that rutin and chlorhexidine are compounds belonging to flavonoids and possess antimicrobial activities. Another by-product of sugarcane also showed high antibacterial activities against four foodborne pathogens; *Staphylococcus aureus, Listeria monocytogenes, Escherichia coli* and *Salmonella* Typhimurium (Zhao et al., 2015).

For centuries, human beings have been encountered with the crisis of food quality deterioration and foodborne illness (Mith et al., 2014). Aziman et al. (2014) have mentioned, more than one-third of the population in the developing country associated with foodborne illness annually caused by foodborne pathogens. The most frequent disease associated with food spoilage and foodborne is diarrhea. It has reported, approximately 550 million people in the world were attempted the disease (WHO, 2019). The infection of foodborne pathogen can be occurred due to the consumption of food that has been contaminated with harmful substances secreted from bacteria and their toxin (Akbar & Anal, 2011).

The crisis has brought food industry in seeking various methods in order to preserve food from being spoiled by microorganisms. Chemical synthetic preservatives have been applied in order to prevent or delay the spoilage of food (Jie et al., 2012). The preservatives were shown as the most effective method to prolong the shelf-life of food products while minimizing the cost of inventing natural preservatives (Sharma, 2015). However, the excessive use of chemical synthetic preservatives can build toxic reactions and detrimental impacts to human health (Rosculete et al., 2019). Certain food preservatives may cause allergies, asthma, dermatologic, gastrointestinal problem and even cancers (Khoshnoud et al., 2018; Sharma, 2015).

Therefore, the use of natural antibacterials are proposed since they offer several beneficial effects include; shelf life extension of food product, protection under temperature mistreatment, minimising the risk of foodborne pathogen transmission, ameliorate the economic losses due to food spoilage, minimising chemical preservative usage, organoleptic properties of foods, and satisfy the industrial and consumers demands (Campos et al., 2011). In this study, sugarcane molasses is used to derive numerous phenolic constituents that possess antibacterial and antioxidant properties against foodborne pathogens. The sugarcane molasses used was the unsulphured molasses which extracted from the matured sugarcane without any sulphur treatment. The molasses also came with high level of sugar which can act as natural preservative for food product. The molasses also known as backstrap molasses as it is type C molasses that has been extracted after third cycle of centrifugation, evaporation and crystallization process. Since the ripened blackstrap molasses was believed to has rich bioactive compound, the present study carried out to analyse the composition, antioxidant, antibacterial properties and the bacteriostatic mechanism of sugarcane molasses.

1.2 Problem Statement

The demands of a modern and globalized world have required the food industry to produce foods with instant preparation that rich in flavour, ready-to-eat, low-costing and long shelf life (Linke et al., 2018). Preservative agents have become the most essential additive to ensure the manufactured foods are remaining safe and unspoiled. According to Global Food Preservatives Market Size Report (2019), the application of antibacterial preservatives dominated half of the market as compound required for food with market share 56.7%. Unfortunately, synthetic type preservatives shown as the largest additive used compared to natural preservative with value 81.3% in the market share.

The extensively used chemical synthetic preservatives in the food product have become a significant concern to the consumer. These preservatives have led to the deterioration of food quality and safety to human health. Excessive intake of synthetic chemical preservatives can cause allergies, asthma, gastrointestinal problem and even cancers (Khoshnoud et al., 2018; Sharma, 2015). Therefore, new alternative preservative is required to replace the abuse usage of the chemical synthetic preservative. The market scenario nowadays also shows the rise in demand of natural food products. This free synthetic preservation can be derived from natural materials such as sugarcane molasses and contribute as new alternative preservation that can satisfy the consumer preferences to claim the ‘green food’. However, there are still limited studies were reported on the composition of sugarcane molasses. Even though many researchers have carried out studies to evaluate different epidemiological of sugarcane molasses, very limited research reported on its antioxidant properties, antibacterial properties and the activities of the antibacterial mechanism.

1.3 Objectives

The objectives of this study are:

1. To characterise the composition of sugarcane molasses.
2. To determine the antioxidant and antibacterial activities of sugarcane molasses ethanolic extract against selected foodborne pathogens.
3. To investigate the bacteriostatic mechanism of sugarcane molasses ethanolic extract against *Staph. aureus* and *E. coli*.

1.4 Hyphotheses

Sugarcane molasses which known as the plant that generated carbon fixation process may dominate with sugar compound and amino acid. Besides, the ethanolic extract of sugarcane molasses could exhibit numerous essential phenolic compound which possess various properties particularly antioxidant and antibacterial properties. The presence of antibacterial properties in sugarcane molasses ethanolic extract would be effectively used against selected foodborne pathogens including *Staph. aureus, L. monocytogenes, E. coli* and *Sal.* Typhimurium. The changes in morphology of bacterial structure can also be influenced by the treatment of sugarcane molasses ethanolic extract.

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