CURRENT BIOCHEMISTRY
ISSN: 2355-7877
e-ISSN: 2355-7931
Journal homepage: http://journal.ipb.ac.id/index.php/cbj
Journal E-mail: current.biochemistry@gmail.com

Antibiotic-Resistant-Bacterial Infection of Diabetes Mellitus-Induced Ulcers: A Narrative Review on a Healing Ointment

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Received: *29 September 2020;* Accepted: *16 December 2020*

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ABSTRACT

Gangrene is the main reason why diabetes mellitus patient's legs are amputated. Gangrene is a result of infection by bacteria such as Staphylococcus aureus. Natural compounds which are contained in coffee grounds extract, can actually act as antibacterial substances by inhibiting the growth of Methicillin-Resistant Staphylococcus aureus (MRSA) that is resistant to several antibiotics. Coffee grounds are discarded waste caused by people underusing this material, and there is a high production level of coffee grounds in Indonesia. The purpose of this study was to examine the content of antimicrobial compounds from coffee grounds that can heal wounds caused through hyperglicemia due to diabetes mellitus and to create a formulation of a coffee ground from based ointment for hyperglicemic-mediated infection. Methods used in this research are narrative review of the literature, normality tests, and T-tests. The conclusion of this narrative review is that natural compounds such a trigonelline, caffeine, and chlorogenic acid found in coffee grounds are capable of MRSA growth inhibition at a concentration of 44%. A coffee grounds based ointment with 1% (w/w) of ointment preparation is expected to reduce coffee grounds waste by developing a new product as for hyperglicemic-mediated wounding.

Keywords: *Antibacterial, Coffee Grounds, Diabetes Mellitus, Gangrene, MRSA*

1. INTRODUCTION

Coffee is currently one of the most popular commodities. This is supported by the fact that coffee is one of the plantation commodities that has long been cultivated by Indonesians growers (Rahardjo 2012). Indonesia itself is ranked third largest in coffee production in the world (Sativa *et al*. 2014). According to the Badan Pusat Statistika (2017), coffee production in 2015 was about 602.37 thousand tons, then there was an increase in 2016 by 4.92 percent to 632 thousand tons and in 2017 this increased by 0.74 percent to 636.7 thousand tons. Along with increasing coffee production, coffee consumption among the community also increased. Coffee products that have been processed into beverages will produce coffee grounds. Although the amount of coffee that is produced is abundant, the resulting coffee is not presently being utilized further.

Coffee grounds are known to still have active ingredients that can accelerate wound healing. Even in the past Indonesians have used coffee grounds as an alternative for wound healing (Kumar *et al*. 2007). Research that has been done by Artho *et al.* (2015) supports the previous statement by pointing out that rabbits whose wounds are given coffee grounds are healing faster so that their level of inflammation can be lowered.

The Indonesian Ministry of Health in 2012 stated that diabetes mellitus is in the top ten non-contagious diseases with a large number of patients (Rosa *et al*. 2019). Patients with diabetes mellitus left without good self-management, will develop into a more serious disease. In addition it can also cause complications such as the onset of gangrene. People with diabetes mellitus have a 29 times higher risk of gangrene. This is because diabetes mellitus are susceptible to infections closely related to the proliferation of bacteria in the environment with high glucose levels (Wahyuni and Arisfa 2015). Diabetic ulcers are complications of diabetes consisting of deep tissue lesions associated with neurological and circulatory disorders especially in the lower limbs (Hajimohammadi *et al.* 2019). Chronic hyperglycemia as found in the case of diabetes mellitus often leads to secondary complications such as in blood vessels, kidneys, nerves, visual impairment and infections. Damage to blood vessels can cause blood flow to decrease, resulting in nerve damage to the legs. This can increase the likelihood of an ulcer in the foot (diabetic foot) (Scobie 2007). Bacterial infections that slow healing lead to deformity and death. Bacteria that cause infection in diabetic ulcers are *Pseudomonas aureginosa, Streptococcus pyogenes, Staphylococcus aureus, Proteus mirabilis, Escherichia coli, and Klebsiella pneumoniae* (Sutjahjo 2013).

Coffee has been shown to contain antimicrobial compounds, such as caffeine, trigonelline, glyoxal, methylglyoxsal, and chlorogenic acid. The bioactive substances can be obtained by extraction technique (Juliantari *et al*. 2018). According to Bonyanian and Rose'Meyer (2015), caffeine in coffee grounds can cure diabetic wounds. Antimicrobial compounds owned by coffee can inhibit the growth of *Escherichia coli, Salmonella* sp., and *Staphylococcus aureus.* Experiments have been conducted both *in vivo* and *in vitro* to test the activity of antimicrobial compounds contained in coffee (Matheson *et al*. 2011). The results of the experiment showed that coffee, or coffee grounds that still has the content of antimicrobial compounds, can provide antimicrobial or antibacterial benefits to wounds when applied topically. The use of these substances derived from coffee and coffe grounds can reduce the absorption of iron. It is important because iron is needed for the growth of *Staphylococcus aureus* (Matheson *et al*. 2011).

Wounds caused by diabetes mellitus if not given proper treatment can lead to ongoing infections leading to amputation of limbs. There are several treatments for healing diabetes mellitus injury, which can be done by diabetic wound healing specialists, and drugs that can be smeared topically on the wound. However, such treatments are costly because drugs on the market are still relatively expensive. The use of antibiotics is not always effective in addressing this problem, as *Methicillin-Resistant Staphylococcus aureus* (MRSA) is still resistant to a number of antibiotics. In addition, coffee grounds have no economic value so are not utilized and just thrown away. Therefore, the use of coffee waste can be an alternative in treating wounds caused in diabetes mellitus.

2. METHODOLOGY

Tools and Material

This research used a narrative study method conducted on 44 papers. Tools used in this study are an ACER LAPTOP AS4739 and SPSS 16.0 software. The theoretical sources used in the study are:

1) the *National Library of Medicine database* site (http://www.pubmed.ncbi.nlm.gov)
2) *Researchgate* (http://www.researchgate.net)
3) *web of science* (https://www.webofknowledge.com)
4) Indonesian Scientific Repository (http://www.neliti.com/id/).

Research Procedure

The research procedure is described on Figure 1.

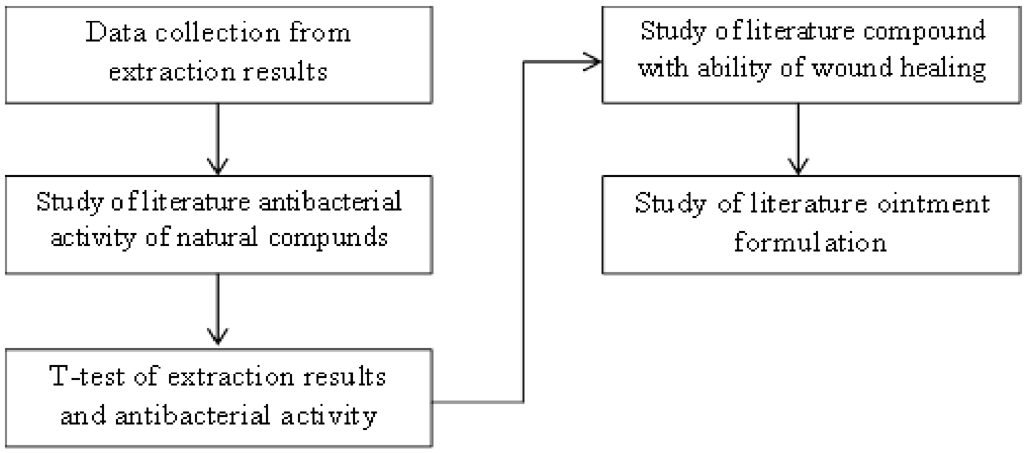

Figure 1. Research flow chart

Normality Test Of Inhibition Capability Data (Apriyono 2013)

Before the data from the concentration of robusta coffee compounds against bacterial resistance can be tested in T-test, the variable that is in this study must be measured first to know the validity of the data with data normality test. Normality testing is performed to see the normality of the data used, whether the data is distributed normally or not. The normality of the data is very important because normal distributed data considered to represent the population. The test used is the Kolmogorov-Smirnov (Liliefor) test which is calculated with the SPSS 16.0 program.

Paired T-Test of Robusta Coffee Compound Content Against Two Types of Bacteria (modified from Hati and Novita 2018)

Once it was known that the data distribution is normal, the researchers used *paired T-test* analysis using the SPSS 16.0

software. This analysis was used to determine significant differences between the resistance zone percentage of robusta coffee levels against wild type *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus*. The testing criteria is to use a significant value /P-Value. If the value is significant /P-Value > 0.05 then Ho is accepted, whereas if the value is significant /P-Value < 0.05 then Ho is rejected and an $H_{alternative}$ is accepted.

The coffee grounds based ointment used a modification of the method from Maya *et al.*(2017). The coffee grounds extract with the best concentration that can inhibit MRSA combined with white vaseline at concentration 1% (w/w) was identified. The homogenization should be done by heating coffee ground extract with ethanol 96 (v/v), then ed to the white vaseline.

3. RESULT AND DISCUSSION

Chlorogenic acid is an antibacterial substance that works by permeability of plasma membranes enhancement so that the defense function of bacterial cells will decrease and bacterial cells may disrupt. Trigonelline also has antibacterial activity similar to chlorogenic acid, precisely by disrupting the stability of bacterial cytoplasmic membranes. Membrane instability will inhibit the exchange of bacterial nutrients so that metabolism and growth of bacteria becomes inhibited (Lee and Lee 2018). Caffeine is an alkaloid compound high in nitrogen in its base group so that it can react with cell wall-building amino acids and bacterial DNA. This damage causes genetic changes and lysis in bacteria (Tanauma 2016).

Table 1 Coffee extraction yield value results of various solvents

Types of coffee	Types of treatment	Solvent types	Yield values	Sources
Robusta coffee	Maceration	Ethanol 70%	6.76±0.01%	Juliantari *et al.* (2018)
Robusta coffee	Maceration	Ethanol 80%	6.81±0.01%	Juliantari *et al.* (2018)
Robusta coffee	Maceration	Ethanol 90%	7.87±0.05%	Juliantari *et al.* (2018)
Arabica coffee	Soxhlet extraction	n-hexane	3.43%	Lamona and Nurman (2018)
Arabica coffee	Soxhlet extraction	Ethanol 70%	13.95%	Sri and Rubiyanti (2012)
Arabica coffee	Soxhlet extraction	Ethanol 96%	49.81%	Dewi *et al.* (2017)
Robusta coffee	Maceration	Ethanol 96%	10.80%	Wigati *et al.* (2018)
Robusta coffee	Maceration	Ethanol 96%	14.80%	Utami *et al.* (2018)
Robusta coffee	Sonication extraction	Ethanol 96%	20.50%	Utami *et al.* (2018)

The research of Juliantari *et al.* (2018) shows that from the results of diversity analysis, treatment of ethanol solvent concentration, maceration temperature and interaction between treatment has a very real effect (P<0.01) on robusta ground coffee rendment (*Coffea canephora* L.). The results also showed that the highest yield were obtained by the treatment of 90% ethanol solvent concentration and 60°C maceration temperature in robusta coffee grounds extract, which is 7.87±0.05%. The highest caffeine is obtained in this treatment with a rate of 0.70±0.01%. The highest total phenol was obtained at 11052.83±1124.30% mg GAE/100g, while the lowest yield was produced at 70% ethanol solvent concentration treatment with a maceration temperature of 75°C of 5.27±0.02%. The lowest levels of caffeine and phenols were obtained at 70% ethanol treatment with a maceration temperature of 45°C, caffeine levels obtained amounted to 0.51±0.01%, while phenol concentration obtained only 6449.62±54.28 mg GAE/100g on this treatment.

In contrast to robusta coffee, arabica coffee oil rendment (*Coffea arabica* L.) from soxhlet extraction results with variations in extraction time, based on the data obtained, it appears that the optimal yield is produced at a time of 180 minutes which is 3.43% with the weight of the type of arabica coffee bean oil produced which is 0.9184 g/cm (Lamona and Nurman 2018). In general, increased extraction time increases the number of yield. this is due to increased contact between samples and solvents (Aziz *et al.* 2009). Based on data obtained from GC-MS (*Gas Chromatography-Mass Spectrometry*), arabica coffee has several active compounds, six of which have an area above 4% namely 2-*propanone*,2.3-*dimethyl butane* 2-*chlorobutane, methyl cyclopentane, pentadecylid acid and* 1.2-*benzenedicarboxylic acid* (Lamona and Nurman 2018). Roasted arabica coffee beans have a caffeine content of 1.1-1.3 g/100 g, trigonellin levels of 1.2-0.2 g/100 g, and chlorogenic acid levels of 1.9-2.5 g/100 g (Farah 2012).

According to Sabarni and Nurhayati's research (2018), the temperature treatment and coffee roasting time will have a major influence on the types of compounds resulting from these processes. The roasting process is one of the important stages for producing thermally degraded compounds due to decaffeination. According to Ningsih (2014) caffeine in coffee can be obtained through extraction methods using organic solvents and extraction conditions are solvents, temperatures, time, pH, and solvent compositions ratio with ingredients so as to affect the efficiency of caffeine extraction. Trigonelline obtained based on the research of Ky *et al*. (2001) with dekok extraction method and using distilled water as solvent is 1.77% for arabica coffee type, and 1.24% for robusta coffee type.

Mangiwa *et al.* (2015) show that the method and the temperature of the roasting step affect the levels of CGA or chlorogenic acid in coffee beans. CGA levels obtained from coffee beans range from 6, 93 – 9.33 % with the lowest chlorogenic acid levels obtained at a roasting temperature of 75°C and the highest obtained at a roasting temperature of 150°C. The roasting process that is especially performed at temperatures above 180-200°C can result in major changes in the chemical compositions and bioligical activities of coffee as a result of Maillard and Strecker reactions (Belay and Gholap 2009). The selection of extraction methods will also affect the quality of the extract. The acidity level produced by the coffee extract becomes slightly higher with the soxhlet extraction method compared to the maceration method. Meanwhile, the chemical contents of coffee such as alkaloids, terpenoids, steroids, saponins, polyphenols and tannins in coffee beans extracted with soxhlet extraction method is higher than the maceration method. Therefore, it can be concluded that the extraction method affects the physical and chemical properties of coffee. The soxhlet extraction method is proven to be optimal in extracting coffee beans compared with the maceration method (Mangiwa *et al.* 2015).

Table 2 Micronutrients level of Arabica and Robusta coffee extract

Types of coffee	Micronutrients	Levels	Extraction Methods	Solvents	Sources
Robusta coffee	Caffeine	0.66±0.01% 0.68±0.01% 0.70±0.01%	Maceration	Ethanol 70% Ethanol 80% Ethanol 90%	Juliantari *et al.* (2018)
		84%	Supercritical Protein Extraction	CO_2 and water	Tello *et al.* (2011)
	Chlorogenic Acid	1.346%	NADES-UAE	Water	Syakfanaya *et al.* (2019)
		99.21%	Maceration	Ethanol 95%	Kaisangsri *et al.* (2019)
	Trigonelline	1.24%	Dekok extraction	Distilled water	Ky *et al.* (2001)
Arabica coffee	Caffeine	1.77%	Soxhlet extraction	Ethanol 96%	Dewi *et al.* (2017)
		57.83%	Liquid-liquid extraction	Chloroform	Sabarni and Nurhayati (2018)
		2.63%			Suwiyarsa *et al.* (2018)
		9.33%	Soxhlet extraction	Methanol	Mangiwa *et al.* (2015)
	Chlorogenic acid	5.07 ±1.0% 4.67 ±1.6% 5.28 ±1.2%	Maceration	Water Methanol 70% Isopropyl alcohol 60%	Sua´ rez-Quiroz *et al.*(2014)
	Chlorogenic acid	53.635%		Ethanol 95%	Kaisangsri *et al.* (2019)
	Trigonelline	0.96%	Dekok extraction	Distilled water	Yisak *et al.*(2018)
	Trigonelline	1.77%			Ky *et al.*(2001)

Before doing the Paired T-test, robusta coffee inhibition capability data on *Staphylococcus aureus* and MRSA bacteria were tested for normality first with Kolmogrov-Smirnov test to see whether or not the data spread was normal. After being tested, the data of the resistance of these two types of bacteria shows p-value > α, hence the H0 is accepted which means that both data sets spread normally. After that, a paired T-test can be conducted to see significant differences in robusta coffee inhibition capability to *Staphylococcus aureus* and *Methicillin-Resistant Staphyococcus aureus* bacteria. Result shows that this data has a p-value of 22.5% (p-value > α) so H0 is accepted. This means there is no significant difference between *Staphylococcus aureus* and *Methicillin-Resistant Staphylococcus aureus* test results at a real rate of 5%.

Coffee regulates the tissue condition by making wounds become acidic (Arimbi and Yuwono 2016). Areas of ulcers that are sufficiently acidic can damage abnormal collagen from the base of the ulcer and will reduce protease activity (MMPs) by inhibiting the expenditure of TNFα and increasing angiogenesis which is the activity of macrophags (Gethin 2007). Angiogenesis is considered to help hypoxia problems that are widely found in chronic wounds. Oxygen is required for fibroblast replication, migration, performing functions, as well as collagen maturation. Therefore, wound healing will be inhibited in hypoxia (Schreml *et al*., 2014). According to Marcone (2004), coffee grounds contain calcium. Calcium is known as one of the blood clotting factors (Yusmiati and Wulandari 2017).

According to the research of Maya *et al.* (2017) which has been modified, and based on the results of the literature study on the effectiveness of MRSA inhibition capability by coffee, the concentration of coffee grounds extract to be used in ointment formulations is 44%. This can inhibit MRSA growth with the inhibition zone of 14.5 mm (Pinandito 2018), in ointment preparation 1% (w/w). 0.1 grams of coffee with 10 grams of white vaseline mixed by heating with aqueous ethanol 96% (Maya *et al.* 2017).

Based on the data collected, extraction of coffee grounds with soxhlet extraction method is the best method in extracting coffee grounds with 96% (v/v) ethanol solvent. Coffee grounds contain natural compounds such as trigonelline, caffeine, and chlorogenic acid that can heal wounds and act as antibacterial substances by inhibiting MRSA growth. Formulation of ointment preparation 1% (w/w), with 0.1 grams of coffee grounds extract with the concentration of 44% mixed with vaseline 10 grams, heated together with 96% ethanol.

ACKNOWLEGDEMENT

This research was supported by Indonesian Ministry of Research, Technology and Higher Education who provided grant through Program Kreativitas Mahasiswa (PKM).

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