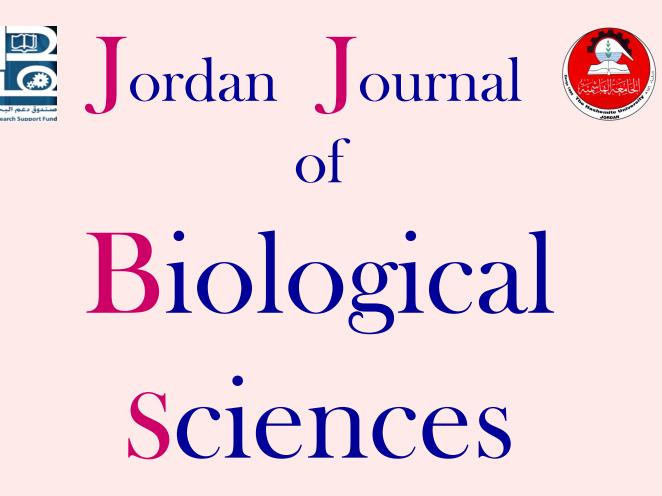


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CONTENTS

Original Articles

413 - 418	Evaluation of Pre-treatment Methods and Anaerobic Co-digestions of Recalcitrant Melanised Chicken Feather Wastes with other Wastes for Improved Methane and Electrical Energy Production <i>Ibrahim Yusuf, Kubra I. Arzai, and Abdulwahid S. Dayyab</i>
419 - 429	Characterization of Egyptian durum Wheat Genotypes using Biochemical and Molecular Markers Samira A. Osman and Walaa A. Ramadan
431 - 435	Heat Exposure Affects the mRNA Levels of Antioxidant Enzymes in Embryonic and Adult Broiler Chickens <i>Amneh H. Tarkhan, Khaled M. M. Saleh and Mohammad B. Al-Zghoul</i>
437 - 439	Diet Composition and Prey Selection in the Long-eared Owl, <i>Asio otus</i> in Jordan: the Importance of Urban Avifauna <i>Mohammad A. Abu Baker, Ratib M. Al-Ouran and Zuhair S. Amr</i>
441 - 451	Genetic Characterization of Algerian Minor Date Palms (<i>Phoenix dactylifera</i> L.) Cultivated in the Oases of Biskra using Nuclear Microsatellite Markers <i>Ahmed Simozrag and Ziane Laiadi</i>
453 - 461	Selenium-Supplemented Diet Influences Histological Features of Liver and Kidney in Tilapia (Oreochromis niloticus) Sonia Iqbal, Usman Atique, Muhammad Sharif Mughal, Muhammad Younus, Muhammad Kamran Rafique, Muhammad Sultan Haider, Hafiza Sundas Iqbal, Shahid Sherzada and Tanveer Ali Khan
463 - 468	Inclusion of <i>Myrmecodia pendens</i> bulb Extract in the Diet Stimulates Immune Response in <i>Clarias gariepinus</i> against <i>Aeromonas hydrophila Rudy A. Nugroho , Yanti P. Sari and Esti H. Hardi</i>
469 – 474	In vitro Antibacterial Activity of Cell Free Fermentation Supernatant of Passiflora edulis forma flavicarpa Sims. Fruit Fermented by de Man, Rogosa and Sharp Media Safarini Marwah, Iif H. Rosyidah,Ni M.Mertaniasih,Muhammad N.S.B.Hamzah, Kholis A. Novianti, Riesta Primaharinastiti, Dian Rahmawaty and Isnaeni Isnaeni
475 – 482	Prevalence of Capsular Polysaccharide Genes and Antibiotic Resistance Pattern of <i>Klebsiella</i> pneumoniae in Palestine Ghaleb M. Adwan, Dina M. Owda and Awni A. Abu-Hijleh
483 - 492	Prediction of Protein Secondary Structure from Amino Acid Sequences by Integrating Fuzzy, Random Forest and Feature Vector Methodologies Sivagnanam R. Mani Sekhar, Siddesh G. Matt and Sunilkumar S Manvi
493 – 498	Biogenic Silver Nanoparticle Synthesis, Characterization and its Antibacterial activity against Leather Deteriorates Savita Kate, Madhuri Sahasrabudhe and Archana Pethe
499 - 508	Pollen Morphological Variations among some Cultivated <i>Citrus</i> species and its Related Genera in Egypt <i>Wafaa K. Taia, Manaser M. Ibrahim and Mahmoud Abdel-Sattar</i>
509 - 518	Direct Somatic Embryogenesis and Regeneration of an Indonesian orchid <i>Phalaenopsis</i> <i>amabilis</i> (L.) Blume under a Variety of Plant Growth Regulators, Light Regime, and Organic Substances
519 - 523	Windi Mose, Budi Setiadi Daryono, Ari Indrianto, Aziz Purwantoro and Endang Semiarti Estradiol Affects Ultimobranchial Gland of a Freshwater Catfish, <i>Heteropneustes fossilis</i> kept in Different Calcium Environments Susmita Srivastav, Diwakar Mishra, Sunil K. Srivastav3, Nobuo Suzuki and Ajai K. Srivastav
525 - 533	Antioxidants Released from <i>Cichorium pumilum</i> Jacq. Amendment Mitigate Salinity Stress in Maize Nadia M El-Shafey and Hamada R AbdElgawad

535 - 541	Molecular Identification and Characterization of Parrotfish species from the Farasan Islands, Red Sea-Saudi Arabia
	Mohamed M. Hassan , Ayman Sabry and Mohamed Ismail
543 - 550	Fungal Endophytes from <i>Tabernaemontana heyneana</i> Wall. (Apocynaceae), their Molecular Characterization, L-asparaginase and Antioxidant Activities
	Naguvanahally S. Bhavana, Harischandra S. Prakash and Monnanda S. Nalini
551 – 557	Responses of <i>Lantana Camara</i> Linn. Callus Cultures to Heavy Metals Added to the Culture Media
	Reham W. Tahtamouni , Rida A. A .Shibli, Laila S.Younes , Saida Abu-Mallouh and Tamara S. Al- Qudah
559 - 565	Synthesis and Characterization of Zinc Nanoparticles by Natural Organic Compounds Extracted from Licorice Root and their Influence on Germination of <i>Sorghum bicolor</i> Seeds
	Mahmood A. S. Al-Shaheen, Mustafa N. Owaid and Rasim F. Muslim
567 - 574	Assessment of Antimicrobial and Anticancer Activity of Radish Sprouts Extracts Mahmoud Khalid, Reem Ayayda, Nameer Gheith, Zaidoun Salah, Saleh Abu-Lafi, Amal Jaber, Fuad Al- Rimawi and Ghassab Al-Mazaideh

In vitro Antibacterial Activity of Cell Free Fermentation Supernatant of *Passiflora edulis forma flavicarpa* Sims. Fruit Fermented by de Man, Rogosa and Sharp Media

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Abstract

Antibacterial activities of cell free fermentation supernatant (CFFS) of passion fruit (*Passiflora edulis forma flavicarpa* Sims.) fermented in de Man-Rogosaand Sharpe (MRS) broth media against *Staphylococcus spp., Methicillin-Resistant Staphylococcus aureus* (MRSA), and Extended Strain Methicillin-Resistant (ESBL) *Escherichia coli* have been investigated. The fermentation broth was derived from 24 and 48 hours cultures collection after rotary shaking incubation at 37°C. A bioassay was performed using well diffusion agar method on nutrient agar media, incubated at 37°C for 24 hours. Minimum inhibitory concentration and potency of the CFFS were determined using kanamycin, streptomycin, vancomycin, erythromycin, and amoxicillin as standards. It was found that the fermentation broth containing 32 x 10⁴ CFU/g exhibited inhibitory activity against *S. Aureus* ATCC 25923 and *S. epidermidis* FN-6 after 24 hours similar to 48 hours fermentation. The anti-bacterial activities of 24 hours fermentation supernatant against all the test bacteria were almost similar. The characteristic of the CFFS indicated the acid property with pH of 3 ± 0.1 . Lactic acid bacteria were detected by biochemical identification based on catalase, Gram staining, motility, H₂S, indol, Simon citrate, and Voges Proscower tests. Thin layer chromatography-contact bioautography was developed by KH₂PO₄ solution as eluent and *E.coli* as a test bacterium showed two spots by which two clear inhibition zones were obtained. The prospective of CFFS passion fruit as a potential antibacterial substance source is recommended for future development.

Keywords: Antibacterial activity, passion fruit, fermentation supernatant, Staphylococcus spp, ESBL, MRSA.

1. Introduction

Nowadays, the use of natural ingredients as raw materials in drug development is beginning to be in demand among the pharmaceutical industry communities. According to World Health Organization (WHO) data, about 80% of the world population are using products based on medicinal herbs. Plants, especially those with ethnopharmacological uses, have been the primary sources of medicine for early drug discovery (Sofija, 2017). During the last 10 years, the discovery of new antibiotic drugs is not considered comparable with the prevalence of antibacterial resistance, so research for the discovery of antibiotic raw materials began to be directed at natural sources (Asirvathamdoss, *et al.*, 2008).

Passion fruit (*Passiflora edulis*), a member of the Passifloraceae family, is also well-known as markisa fruit. It has more than 500 species (Paull and Duarte, 2012; Reis *et al.*, 2018). The plant originated from Brazil and has

scattered to other countries in Asia, Australia, Africa, India, South America, and the Caribbean. It has other variants that can be identified by the color of their fruits such as yellow which is P. edulis forma flavicarpa, purple which is P. edulis forma edulis, and orange which is P. edulis var. caerulea (Reis et al., 2018). The plant parts (flower, leaves, stem, fruits, and roots) have several medicinal effects and have been used traditionally. The flower is the part that is used mostly for its calming effect, anticonvulsant and antihypertensive properties, which are useful for patients with anxiety and insomnia. However, it is not as potent as Passiflorain carnata flower (Ramaiya et al., 2014). Meanwhile, the fruits are almost round or oval in shape; about 4cm to 8cm in diameter, and mainly used as food since they have a juicy orange pulp. The pulp is sweet-sour in flavicarpa variant and sweeter in both edulis and caerulea variant. The skin is tough, smooth, and waxy but wrinkles when it is ripe. The seed is numerous, hard, small, and pitted inside the fruit. It has many common names according to the country they are grown in such as

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"markisa" in Indonesia and Malaysia, "limangkan" in Laos, "maracuya peroba" in Portugal, "maracuja-do-campo" in Brazil (Paull and Duarte, 2012; Aziz, 2016).

The fruits contain good amount of nutrients which are good for dietary consumption and have numerous phytochemicals such as glycosides including flavonoids (Ingale and Hivrale, 2010), e.g. luteolin-6-C-chinovoside, luteolin-6-C-fucoside, cyanogenic glycosides, e.g. passibiflorin, epipassibiflorin, passicapsin, passicoriacin, epipassicoriacin, cyanogenic-b-rutinoside, epitetraphyllin B, amygdalin, prunasin, triterpenoid glycosides, e.g.passiflorine), and salicylate glycosides. Other chemicals such as b-carboline alkaloids harman, harmine, harmaline and harmalol, phenols, carotenes, and g-lactones are also found in the fruit (Bernes et al., 2007). Passion fruit is a fruit that has a high nutritional value where there are many multimineral contents and various vitamins, as well as high carbohydrates and water (Zibadi and Watson, 2004). These compounds may become the prospect of antimicrobial, antioxidant, anticancer, and anticarcinogenic (Ramaiya et al., 2014; Oliveira et al., 2016). Reis et al (2018) mention that the compound is also associated with antiplatelet, antiviral, antiallergic, and anti-inflammatory activities. Furthermore, the plant parts of the passion fruit including the peel extract and the pulp are positively tested for antibacterial and antifungal activity on certain microbial tested (4). It has been reported that antibacterial and antifungal compound has been isolated by Birner and Nicolls as cited by Ramaiya et al (2014).

In recent years, there have been many studies on the antimicrobial activity of the *Passiflora edulis* plant by which is strong to moderate inhibition both in Grampositive and Gram-negative has been exhibited. Extracts from the leaves and flowers of the plant *Passiflora edulis* are able to inhibit the growth of pathogenic bacteria such as *V. Cholerae, Pseudomonas aeroginosa, Escherichia coli, Bacilus subtilis* (Ingale and Hivrale, 2010), *Salmonella typhi, Staphylococcus aureus, Streptococcus pyrogens,* and *Bacebuspenilis* (Asirvathamdoss, *et al.,* 2008).

There have been numerous studies of passion fruit for its phytochemical properties; however, there are no reports concerning the antibacterial activities of the pulp of fruit fermented by de Man Rogose and Sharp (MRS) media against pathogenic bacteria. Zahroh (2014) has reported lactic acid bacteria (LAB) isolated from the passion fruit *Passiflora edulis* var. Sims. Some LAB have been known as sources of bioactive substances included antimicrobial. This study has performed antibacterial activities of cellfree fermentation supernatant (CFFS) of *Passiflora edulis forma* flavicarpa fruit against ESBL *Escherichia coli*, MRSA, and some species of *Staphylococcus* and non-ESBL *Escherichia coli*. Screening active substances by Thin Layer Chromatography-contact bioautography have also been reported.

2. Materials and Method

2.1. Plant source and determination

The yellow passion fruits (Figure 1) were collected freshly from a local farm in Sidoarjo, harvested in April 2019. The passion fruit plant was identified and determined based on the taxonomy character of leaf, flower, fruit, steam plant, and recommended by Herbarium Malangensis, Department of Biology, Faculty of Math and Science, Universitas Negeri Malang as *Passiflora edulis forma flavicarpa*, Sims.

2.2. Sample preparation, fermentation, and characterization

The passion fruits were washed and dried before they were divided into two parts and the 5 gram of fruit pulps were weighed and put into 50 mL of MRS broth media to be fermented with rotary shaker at 150 rpm and at 37°C for 48 hours. The fermentation broth was taken after 24 hours of fermentation, centrifuged, and the supernatant was collected for characterization and bioassay.

2.3. Determination of Total Plate Count (TPC)

The supernatant was then made into a serial dilution of 1:10 until 10⁷ using sterile normal saline solution. Each of the serial dilutions was inoculated on the MRS (Oxoid, UK) agar and incubated at 37°C for 24 hours. Cell growth was observed and the plating colonies were counted using bacteria colony counter.

2.4. Inoculum preparation.

The selected bacteria strain was transferred aseptically to sterile saline water, vortex, and then the turbidity was measured using spectrophotometer against the sterile saline water to obtain 25% Transmittance (about 10^9 CFU/ml of bacteria) turbidity or optical density at 580nm (Isnaeni *et al.*, 2019).

2.5. Bacterial inhibitory activity.

Screening inhibitory activity of the passion fruit was performed after 24 and 48 hours fermentation. The bioassay was done by well agar diffusion method using NA media and Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 8739, ESBL and MRSA bacteria obtained from RSUD Dr. Soetomo as test bacteria. The test media were prepared by pour plate method. 10-12 mL of melted NA (45-50°C) were poured into the empty sterile petri disk, used as a base layer. The seed layer was prepared by adding 3-5 µL of 25 % transmittance of the test bacteria inoculum into 8 mL of the melted NA, mixed well with vortex, poured on the surface of the base layer, and allowed it to solidify. The well was made by bored with 7 mm in diameter. Each well reservoir was filled with 50 µL of the solution test. Incubation was performed at 37°C for 24 hours. Growth inhibitory zone diameter was measured by digital caliper (Isnaeni, 2019).

2.6. Thin Layer Chromatography-contact bioautography

The developing chamber used was CAMAG Chamber (10x10) with lid and was prepared aseptically. Hence, the size of the TLC plate used was 6cm x 10cm and it was dried at 100°C for 20 minutes before developing. The solvent used was 7.5% of KH_2PO_4 solution. The samples used were 20μ L for each spot and "overspot" loading was used to concentrate the samples on one spot over again after the previous spot has dried. The samples were CFFS of passion fruit of 24-hour fermentation, streptomycin (100ul/ml), and kanamycin (100uL/ml) used as standard.

The Contact Bioautography was performed after the developed TLC plate had completely dried from the residual solvent and contacted, silica gel side down, onto the *Escherichia coli* seed layer as the test bacterium in the petri dish. The agar plate and the contacted plate were stored in the refrigerator for 1 hour to allow the diffusion

of the active substances in the chromatogram into the seed layer. Furthermore, the plate was removed from the agar plate, then incubated for 24 hours at 37° C. The growth of the *E. coli* and the inhibition zone were observed.

2.7. Minimum Inhibitory Concentration.

Determination of the minimum inhibitory concentration (MIC) of the passion fruit CFFS was done using the agar dilution method, where a number desired volume of the supernatant (1ml, 2ml, and 3ml) were mixed with the NA media. Then it was inoculated with six test bacteria by streaking using Öse needle about 1cm on the surface of the NA medium. Multiple replicates were performed on the same agar plate. The agar plates were then incubated at 37'C for 20-24hours. An agar plate without the passion fruit CFFS was performed as the negative control. Serial dilution of CFFS had been applied form 100%, 75%, 50%, 25%, 12.5%, and 6.25% of concentration on the paper disk with volume capacity of 10µL, placed on the surface of NA agar inoculated by E.coli as a test bacterium. Zone of growth inhibitory activity was observed after 20-24 hours of incubation.

3. Results

3.1. Characteristic of Free Cell Fermentation Supernatant

Performance of the fermentation broth of passion fruit in MRS media (Table 1) was brownies in color, pH value 6 ± 0.1 before and decreased to 3 ± 0.1 after 24 and 48 hours fermentation. Total plate count in MRS and NA media was $133\ 10^3$ and $32\ x\ 10^4$ CFU / mL respectively. Several colonies suspected Lactobacilli and Streptococci were found, based on the identification of the isolates with biochemical reactions (Gram staining, catalase, and motility test, conformed to vitek-2).

Table 1.Characteristic of CFFS passion fruit fermented in MRS media

Parameters	Characteristics					
Organoleptic	Liquid, browns colour					
Odor	Specific smell of passion fruit					
pН	6 ± 0.1 (0 hours), 3±0,1 (24 hours)					
Total plate count in MRS and NAMedia	133 x 10^3 and $32x10^4$ CFU/ml					
Inhibitory activity at 0 hour	- (S. aureus and E.coli)					
incubation						
Inhibitory activity at 24 hours	+ (S. aureus and E.coli)					
incubation						
Inhibitory activity at 48 hours	+ (S. aureus and E.coli)					
incubation						
Lactic acid bacteria screening	+ based on Gram staining,					
	morphology, catalase and motility test,					
	conformed to					
	VITEK-2					

3.2. Antibacterial activity

The CFFS of passion fruit screening growth inhibitory activity against *S. aureus* ATCC 25923 showed that the potency of the CFFS after 24 hours and 48 hours fermentation was almost similar (Table 2 and Figure 1), as well as the activity against *S. epidermidis* FN-6 (Figure 2), ESBL, and non-ESBL *E. coli* after 24 hours of fermentation (Figure 3). Subsequent tests were carried out for fermented broth after incubation for 24 hours on several *Staphylococcus spp.* and *E. coli* (Table 2). The agar well diffusion test performed to 5 different strains of *E. coli* showed that the CFFS of passion fruit presented inhibitory activity against five different strains of *E. coli* with the zone of inhibition diameter from 13.80 mm to 22.05 mm (Figure 4).

Table 2. Inhibitory activities of CFFSpassion fruit after 24 hours fermetation againts various test bacteria

Destaria	Zone of growth Inhibition (mm)								
Bacteria	CFFS	Van 8ppm	Cefat 8 ppm	Strep 8 ppm	Am 16 ppm	Ery 8 ppm			
Exteded Spectrum Beta Lactamase	$17,\!78\pm0,\!60$								
Escherichia coli ATCC 8739	$17{,}47\pm0.57$								
ESBL E.coli6110	$15{,}10\pm1{,}17$								
ESBL E.coli6024	$16,33 \pm 0,31$								
ESBL E.coli5949	$20{,}65\pm0{,}69$								
Staphylococcus aureus ATCC 25923	$17,\!76\pm1,\!12$	16.63 <u>+</u> 0.05	20.00 <u>+</u> 0.00	22.98 <u>+</u> 3.15	19.88 <u>+</u> 0.65	21.35 <u>+</u> 0.00			
MRSA	15.13 <u>+</u> 1.03								
Staphylococcus epidermidis FNG-1	$15{,}68 \pm 0{,}86$								
Staphylococcus epidermidis FNG- 2	$16{,}83 \pm 0{,}47$								
Staphylococcus epidermidis FNG- 3	$15{,}80 \pm 0{,}68$								
Staphylococcus epidermidis FNG-4	$16{,}48 \pm 0{,}41$								
Staphylococcus epidermidis FNG-5	$16{,}99\pm0{,}39$								
Staphylococcus epidermidis FNG-6	$15{,}43 \pm 0{,}44$								
Staphylococcus epidermidis FNG-7	$16{,}16\pm0{,}59$								
Staphylococcus epidermidis FNG-8	$17,03 \pm 0,24$								
Staphylococcus epidermidis FNG-9	$17,06 \pm 0,24$								
Staphylococcus epidermidis FNG-10	$17{,}80 \pm 1{,}12$								

Diameter of agar hole =7 mm.Van= Vancomycin, Cefad= Cefadorxil, Strep= Streptomycin, Am = Amoxycilin, Ery = Erythromycin

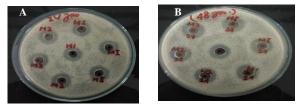


Figure.1. Growth inhibitory activity of passion fruit CFFS in MRS media against *S. aureus* ATCC 25923 after incubation at 37°C for 24 hours (A) and 48 hours (B).

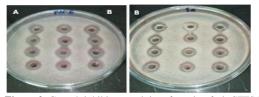


Figure.2. Growth inhibitory activity of passion fruit CFFS in MRS media against *S. epidermidis FN-6* (A) and *S. aureus* ATCC 25923 (B) after incubation at 37°C for 24 hours.

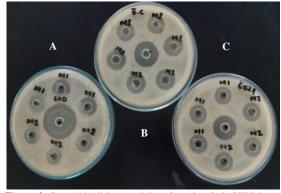


Figure.3. Growth inhibitory activity of passion fruit CFFS in MRS media against ESBL 6110 (A), non-ESBL *E.coli* (B), and ESBL 6024 (C) after incubation at 37°C for 24 hours.

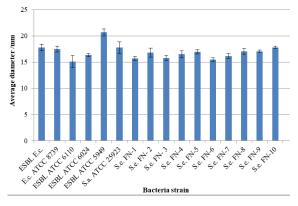


Figure 4.Average diameter (mm) of zone inhibition of CFFS againts test bacteria

3.3. Minimum inhibitory concentration

The MIC of CFFS passion fruit was performed after antibacterial sensitivity was done. Figure 5 depicted the sensitivity of CFFS against six test bacteria. The agar dilution test of the CFFS had shown that the negative control agar plate (without CFFS addition) was covered with full growth of all test bacteria, while the agar plate with 1mL of the CFFS had shown inhibition to most of the inoculated area with few insignificant bacterial growths. Furthermore, the agar plate with 2mL and 3 ml of the CFFS had completely inhibited all microbial growth (Table 3). Table 3 showed the susceptibility test of the agar dilution test against the test bacteria. In addition, determination of MIC using serial dilution of 10 µL CFFS 100%, 75%, 50%, 25%, 12.5%, and 6.25 % applied on the sterile paper disk indicated that 75% of exhibited zone of growth inhibitory activity were less than 13 mm, meanwhile the 50% CFFS solution did not exhibit inhibitory activity.

3.4. Statistical analysis

Statistical analysis using Kruskal Wallis test was performed to evaluate the significant difference of inhibitory activity of the CFFS against bacterial tests. From the analysis results, it was obtained Asymp sig value that was 0,000. CFFS provided the greatest inhibitory activity on ESBL *E. coli* 5949 and the smallest one in *Staphylococcus epidermidis* FNG-6.

	Volume of CFFS added to 10 ml nutrient agar media											
Bacteria	Positive Control (Without CFFS)		1mL		2mL			3 mL				
	R1	R2	R3	R1	R2	R3	R1	R2	R3	R1	R2	R3
ESBL	+	+	+	+	-	-	-	-	-	-	-	-
E ESBL6110	+	+	+	-	-	-	-	-	-	-	-	-
E ESBL6024	+	+	+	-	-	-	-	-	-	-	-	-
E ESBL5949	+	+	+	-	-	-	-	-	-	-	-	-
E ESBL8739	+	+	+	-	-	+	-	-	-	-	-	-
SA ATCC 25923	+	+	+	-	-	+	-	-	-	-	-	-

Table 3. Sensivitity test of six test microbial against passion fruit CFFS after 24 hours using streak method

 $\mathbf{R} = \mathbf{Replicate}$

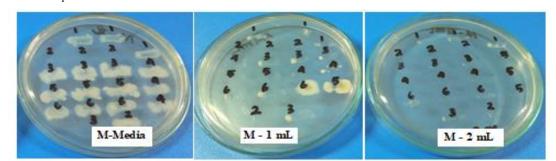


Figure 5. Sensitivity of six test bacteria against CFFS on Nutrient Agar mediaESBL *E. coli*, 2. NA-broth, 3. ESBL 6024, 4. ESBL 5949, non-ESBL *E. coli* ATCC 8739, *S. aureus* ATCC 25923, M-Media: media without CFFS, M-1 mL: NA Media + 1 mL of CFFS, M-2 mL: NA Media + 2 mL of CFFS.

4. Discussion

Passion fruit, as well as other fruits, is a suitable habitat for the growth of lactic acid bacteria (Askari et al., 2012) and even probiotics, due to their adequate nutritional content. White and Sharareh (2018) have reported the results of their research on the fermentation process of Lactobacillus rhamnosus GR-1 using apple, orange, and grape juice. The main fermentation products that can be directly detected and dominant are lactic acids and other organic acids, thereby reducing the pH value in the fermentation process. In this study, pH value of the CFFS decreased at 24 hours of evaluation from 6 to 3. It was reported that passion fruit by-product stimulates the growth and folate production by starters and probiotic cultures in fermented soymilk (Mac et al., 2017). The fermentation process using inulin apparently accelerates growth by up to 10 times compared to being stored in a refrigerator. The potential of the CFFS passion fruit as lactic acid bacteria by which many useful substances produced by fermentation processes might prospectively develop.

In this study, it has also been proven that before the fermentation process, the viability of the cell colonies in MRS media was less than 30 and increased to 32×10^4 CFU / mL after 24 hours of incubation. The MRS is a selective medium for the growth of lactic acid bacteria (Askari et al., 2012). Before the fermentation process, CFFS passion fruit also did not show inhibitory activity against all test bacteria (Table 1). Antimicrobial activity of dried fruit has been widely reported (Aziz et al., 2006). This phenomenon proves the importance of the presence of active compounds in fruit (Ingale and Hivrale, 2010; Reis et al., 2018) as antimicrobial. Asirvathamdos et al. (2008) have reported in-vitro antimicrobial activity of passion fruit extract, but the effect of the fermentation process in selective media MRS for probiotic growth and antibacterial activities has not yet been reported. Fermentation with MRS media leads to increase the population of lactic acid bacteria that are able to produce lactic acid and other organic acids, as well as other active ingredients that can act as anti-bacterial. Therefore, the CFFS passion fruit was not only effective as a source of active compounds, but also a source of lactic acid bacteria that have various activities. The accumulation of inhibitory activity against pathogenic microbes is very interesting to be further studied in order to detect the dominant component contributing as an antibacterial.

Pathogenic bacteria used as test bacteria ESBL, MRSA, and non-ESBL E.coli and Staphylococcus spp. are usually resistant bacteria group against methicillin and beta lactam antibiotics, in which in this research was represented by vancomycin, meropenem, cefadroxil, amoxicillin, kanamycin, and erythromycin. The streptomycin, antibiotics standard solutions used at the CFFS passion fruit showed intermediate until sensitive against some test bacteria at $100\%/50\mu$ L with MIC of 10% (Table 2, Figures 5 and 6). Antibacterial activity data from CFFS compared with various bacteria test were analyzed using the IBM SPSS ver 24 program. From the results of normality testing requiring one group of samples that had a sig value <0.05, we could obtain abnormal data distribution results.

Statistical analysis was using the non parametric Kruskall Wallis method with a confidence degree of 0.95 ($\alpha = 0.05$). From the analysis results, it was obtained Asymp sig value of 0,000, so that the p value was<0.05. It can be said that there were significant differences between the antibacterial activity of CFFS with the type of bacteria test. CFFS provides the greatest inhibitory activity on ESBL E. coli 5949 and the smallest one on Staphylococcus epidermidis FNG-6. However, this phenomenon cannot prove the inhibitory potential of bacteria based on their Gram bacteria. The CFFS has a broad spectrum activities, but its inhibitory potential will depend on these individual bacterial strains. Therefore, further research is needed for this matter. In the future, it is interesting to examine the passion fruit CFFS activities on Mycobacterium tuberculosis (MTB) and Multi Drugs Resistant (MDR)-TB and other MDRs. On the other hand, the CFFS has potential opportunity to improve as an antimicrobial instant preparation.

The results of active substances identification by TLCcontact bioautography showed that CFFS contained two active compounds and the TLC chromatograms could be detected by UV lamps (Figure 7), but the Rf value of active compound on the TLC-bioautograms was still needed to be further investigated, whether it was derived from the same compounds as detected with UV lights. Based on the TLC chromatogram developed by a single eluent of KH₂PO₄ solution with a pH of 6.5, which was able to eluate the active compound with Rf 0.4-0.5 (tailing), the active compound was predicted to be polar. The TLC-bioautography method was very suitable for screening active compounds for both identification and separation through eluent system optimization. A very simple method can separate the active compound components from natural ingredients, two or more compounds from the same class, for example, the antibiotic streptomycin aminoglycosides and kanamycin (Febri et al, 2019) that have been validated (Isnaeni et al., 2019).

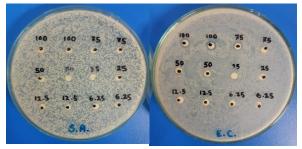


Figure 6. Determination of MIC of 10 µL CFFS on the paper disk against *S. aureus* (A) and *E. coli* (B) on NA mediaat 100%, 75%, 50%, 25%, 12.5%, and 6.25% concentration.

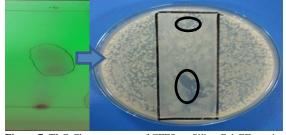


Figure 7. TLC-Chromatogram of CFFS on Silica Gel GF₂₅₄ using 7.5% KH₂PO₄ solution as eluent under UV lamp detection (A) and TLC-contact bioautogram using *E.coli* as a test bacterium (B).

5. Conclusion

Cell-free fermentation supernatant of passion fruit (*Passiflora edulis forma*.flavicarpa Sims.) is recommended to be developed as a source of active substances for antibacterial against pathogenic bacteria event for multi drugs resistant. The active substance might be a polar compound. Furthermore, isolation, separation, and purification to obtain the active isolates or novel substances are very interesting to be studied in the future.

References

Asirvathamdoss, Doss PA, Rangasamydhanabalan. 2008. In-vitro antimicrobial activity of extracts of passiflora edulis (Passifloraceae) and sphaeranthus indicus (Asteraceae). *Ethnobot leaflets.*, **12**: 728-733.

Askari GA, Azzeddine K, Khadija K, Reda C, and Zakaria M. 2012. Screening of lactic acid bacteria isolated from dried fruits and study of their antibacterial activity. *Middle East JSci Res.*, **11(2)**: 209-215.

Aziz N'A. 2016. A review of the antimicrobial properties of three selected underutilized fruits of Malaysia. *IJPCR.*, **8** : 1278–1283.

Bernes J, Anderson LA, Phillipson JD. 2007. Passion flower. In: Herbal Medicines, third ed. Pharmaceutical Press, United Kingdom

Febri A, Iftitahatur R, Asri D, and Isnaeni. 2020. Method validation of contact and immersion TLC-bioautography for determination of streptomycin sulfate shrimp. *Turk J Pharm Sci.*, (ahead of print)

Ingale AGand Hivrale AU. 2010. Pharmacological studies of Passiflora sp. and their bioactive compounds. *Afr J Plant Sci.*, **4(10)**: 417-426.

Isnaeni, Andri A, and Muhammad.Y. 2017. Validation of thinlayer-chromatography-bioatutographic method for determination of streptomycin. *JFIKI*, **4**(1): 32-38.

Mac A, Bedani R, LeBlanc JG, and Saad SMI. 2017. Passion fruit by-product and fructooligosaccharides stimulate the growth and folate production by starter and probiotic cultures in fermented soymilk. *Int J Food Microbiol.*,**261**: 35-41.

Oliveira CF, Gurak PD, Cladera-Olivera F, and Marczak LDF. 2016. Evaluation of physicochemical, technological and morphological characteristics of powdered yellow passion fruit peel. *Int Food Res J.*, **23**(**4**):1653–1662.

Paull RE and Duarte O. (Eds.) 2012. **Tropical fruits, Volume 2.** Available at: https://www.cabi.org/isc/datasheet/38799 (accessed on 5 November, 2019).

Ramaiya SD, Bujang JS, and Zakaria MH. 2014. Assessment of total phenolic, antioxidant, and antibacterial activities of passiflora species. *Sci World J.*, pp.1-10.

Reis RLC, Facco EMP, Salvador M, Flôres SH, and de Oliveira Rios A. 2018. Antioxidant potential and physicochemical characterization of yellow, purple and orange passion fruit. *J Food Sci Technol.*, **55**(**7**):2679–2691

Sofija MD. (Eds.) 2017. From medicinal plant raw material to herbal remedies. Available at: https://www.intechopen.com/books/aromatic-and-

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White J and Sharareh H. 2018. Development of probiotic fruit juice using *Lactobacillus rhamnosus* GR-1 fortified with short chain ang long chain fiber. *Fermentation.*, **4(27)**: 1-12.

Zibadi S, and Watson, RR. 2004. Passion fruit (*Passiflora edulis*) composition, efficacy, and safety. *JEBIM*,**1**(3): 183-187

Zahro, F. 2014. Isolasi dan identifikasi bakteri asam laktat asal fermentasi Markisa Ungu (Pasiflora edulis var. Sims) sebagai penghasil eksopolisakarida. Undergraduate thesis, Universitas Islam Negeri Maulana Malik Ibrahim, Malang. Jordan Journal of Biological Sciences

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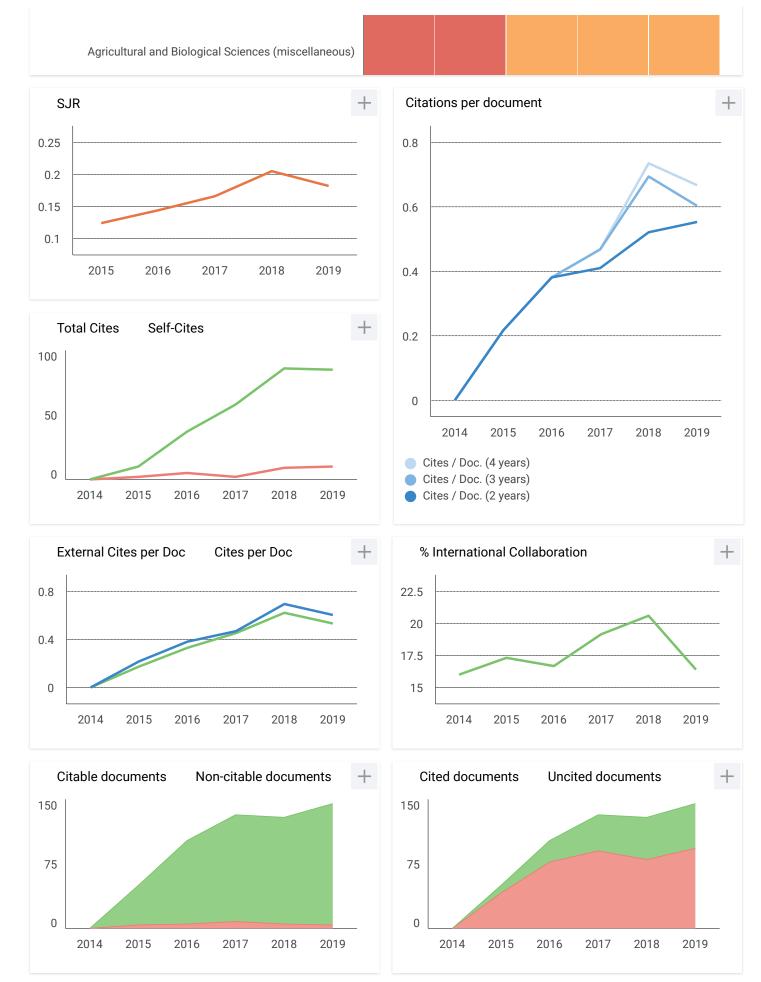
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