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## MICROBIAL CONTAMINATION AND BIOACTIVE COMPOUNDS OF JAMU BERAS KENCUR

#### Wani Devita Gunardi<sup>1\*</sup>, Virginia Marsella Teiseran<sup>1</sup>, Kris Herawan Timotius<sup>1,2</sup>

<sup>1</sup>Department of Microbiology, <sup>2</sup>Center for Jamu and Herbal Medicine, Faculty of Medicine and Health Sciences, Krida Wacana Christian University (UKRIDA), Jakarta, Indonesia \*Email: <u>wani.gunardi@ukrida.ac.id</u>

#### ABSTRACT

Background: Jamu Beras Kencur (JBK) is recognized as an herbal medicine, containing two main components: the rhizome of Kaempferia galanga and rice. While previous research has identified bioactive compounds in galangal rhizomes, such as Ethyl p-Methoxycinnamate (EPMC), Ethyl-cinnamate, and Kaempferol, there are few reports on polar or aqueous compounds in JBK. LC-MS/MS and GC-MS enable comprehensive analysis of bioactive compounds, with LC-MS/MS detecting non-volatile, polar, and thermally sensitive compounds like flavonoids and glycosides, while GC-MS analyzes volatile and semi-volatile compounds, such as terpenoids, providing precise separation and identification. Therefore, this study were to know the amount and the growth of contaminant bacteria, yeast and mold; to determine the main bioactive compounds in JBK; and to determine the bioactive compound in aqueous and ethanolic extracts of rhizome that analysed with LC-MS/MS and GC-MS. Methode: JBK samples were sourced from local producers in West Jakarta, freshly prepared, and immediately analyzed for microbial contamination and bioactive compounds. Result: The analysis revealed microbial contamination in JBK, including Escherichia coli, Staphylococcus aureus, Coliform, yeast, and mold. Additionally, three novel flavonoid glycosides were identified: Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6"-(2-methylbutyryl)]-glucoside, and Acacetin-7-galactoside. Conclusion: Therefore, from the pharmacological perspective, JBK has the potentials as a healthy herbal drink. However, further preclinical and clinical studies are essential to validate its safety and efficacy for clinical use, which could pave the way for its integration into mainstream healthcare as a natural therapeutic option.

Keywords: Flavonoid, GC-MS, Jamu beras kencur, Kaempferia galangal, LC-MS/MS

#### ABSTRAK

Latar Belakang: Jamu Beras Kencur (JBK) adalah obat herbal yang terbuat dari rimpang *Kaempferia galanga* dan beras. Meskipun studi sebelumnya telah mengidentifikasi senyawa bioaktif dalam galanga, seperti Ethyl p-Methoxycinnamate (EPMC), Ethylcinnamate, dan Kaempferol, informasi mengenai senyawa polar atau akua dalam JBK masih terbatas. LC-MS/MS dan GC-MS memungkinkan analisis komprehensif senyawa bioaktif; LC-MS/MS mendeteksi senyawa non-volatil, polar, dan sensitif terhadap panas seperti flavonoid dan glikosida, sementara GC-MS menganalisis senyawa volatil dan semi-volatil, seperti terpenoid. Penelitian ini bertujuan untuk menilai tingkat dan pertumbuhan bakteri, ragi, dan jamur kontaminan dalam JBK, mengidentifikasi senyawa bioaktif utamanya, serta menganalisis ekstrak akua dan etanol dari rimpang menggunakan LC-MS/MS dan GC-MS. **Metode**: Sampel JBK diperoleh dari produsen lokal di Jakarta Barat, disiapkan segar, dan segera dianalisis untuk kontaminasi mikroba dan senyawa bioaktif. **Hasil**: Analisis mengungkapkan kontaminasi mikroba dalam JBK, termasuk *Escherichia coli, Staphylococcus aureus*, koliform, ragi, dan jamur. Selain itu, tiga glikosida flavonoid baru diidentifikasi: Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O-[6"-(2-methylbutyryl)]-glucoside, dan Acacetin-7-galactoside. **Kesimpulan**: Dari perspektif farmakologis, JBK memiliki potensi sebagai minuman herbal yang sehat, tetapi studi praklinis dan klinis lebih lanjut diperlukan untuk memvalidasi keamanan dan efektivitasnya untuk penggunaan klinis.

Kata kunci: Flavonoid, GC-MS, Jamu beras kencur, Kaempferia galangal, LC-MS/MS

## INTRODUCTION

Jamu is a traditional herbal formula medicine that has been practised for many centuries in the Indonesian community to maintain good health and to treat diseases(Elfahmi et al., 2014). As one of the famous jamu, jamu beras kencur (JBK, galanga-rice jamu) is principally made from two main components, a rhizome of Kaempferia galanga and rice. Additional components such as brown sugar, cinnamon and ginger are often added. JBK is good for hypertension, pectoral and abdominal pains, headache, toothache, rheumatism, dyspepsia, coughs, and inflammatory tumour(Wang FangLin et al., 2013). JBK is well known for its antioxidant, antimicrobial, analgesic, anti-inflammatory, sedative, vasorelaxant, nematocidal, mosquito repellent, larvicidal, antiprotozoal and wound healing activities(Nag & Mandal, 2015). It is also good to help with restlessness, stress, anxiety, and depression(Wang FangLin et al., 2013). K. galanga is also known for its induction to the activity of Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) as an index of thermogenesis(Nishidono et al., 2017).

Previous has research reported the bioactive compounds from the rhizome of galanga, such as Ethyl p-Methoxycinnamate (EPMC), Ethyl-cinnamate, Kaempferol(Nag & Mandal, 2015). Most of the reported compounds can be extracted with organic solvents, either non or semi-polar solvents. However there are few reports on the polar or aqueous compounds found in JBK. The use of LC-MS/MS and GC-MS for analyzing bioactive compounds is based on their complementary strengths. LC-MS/MS is particularly effective for detecting non-volatile, polar, and thermally sensitive compounds, such as flavonoids and glycosides, with high sensitivity and precision(Pitt, 2009). On the other hand, GC-MS is ideal for analyzing volatile and semi-volatile compounds, such as terpenoids, providing excellent separation and identification, supported by extensive spectral libraries(Gomathi et al., 2015). These techniques together enable a comprehensive analysis of a wide range of bioactive compounds in complex mixtures.

The objectives of this study were to assess the amount and growth of contaminant bacteria, yeast, and mold; to identify the main bioactive compounds in jamu beras kencur; and to determine the bioactive compounds in aqueous and ethanolic extracts of the rhizome, analyzed using LC-MS/MS and GC-MS.

## MATERIAL AND METHOD

## Jamu Beras Kencur (JBK)

JBK was obtained directly from the local producers/sellers in West Jakarta (Figure 1). The samples were freshly prepared and directly counted for the contaminated microbe and for the GC-MS and LC-MS/MS analyses (Figure 2). After two days of storage in room temperature, the contaminated microbe in JBK was recounted. For the aqueous and ethanolic extracts, the rhizomes were obtained from the same producer/sellers.



Figure 1. Traditional carrier of jamu gendong (left) and a glass of "ready to drink" jamu beras kencur (right)

#### Microbial counting (CFU) with 3M Petrifilm

The JBK was first filtered using sterile cooking shovels strainers to eliminate the fibers and residues of the herbal drink. Then the JBK was diluted in Butterfield's Phosphat dilluent at various dilution factors. Each diluted sample was taken 1 mL and inoculated to Petrifilms-3M for total bacteria, Coliform, Staphylococcus, yeast and mold according to the instruction given by Petrifilm-3M. The incubation was at 37° ± 1°C. The incubation period for Staphylococcus aureus was 24 ± 2 hours, for Coliform was  $48 \pm 3$  hours, for Escherichia coli was  $24 \pm 2$  hours, and for veast and mold were 72-120 hours. After incubation, the CFU was counted. The before and after incubation, the CFU was counted. All measurements are triplicates.

#### LC-MS/MS analysis

Mariner Biospectrometry was used for the LC-MS/MS analysis. It was equipped with a binary pump (Hitachi L 6200). The equipment was interfaced with a Q-tof mass spectrometer fitted with an ESI (Electrospray Ionisation) source with positive and negative ion mode. Full-scan mode from m/z 100 to 1200 was performed with a source temperature of 140 °C. Shimp-pack C8, 150 × 6 mm i.d., was used as a column. The solvent was methanol with 0.3% formic acid and delivered at a total flow rate of 1 ml/min. A 5  $\mu$ I sample was injected and eluted isocratically.

#### GC-MS analysis

GC-MS was carried out on a GCMS-QP20105 SHIMADZU for the ethanolic extract of rhizome of *K. galanga*. The electron ionisation energy was 70 Ev Ion source temperature was 250°C and the interface temperature 305°C. An Abdel 5MS column (30 m x 0.25 mm i.d.and 0.25  $\mu$ m film thickness) was used. The oven temperature was programmed from 60°C –300°C at 10°C C/min. Data acquisition was performed with software for the mass ranges 28 – 600 amu with a scan speed of 1250. Carrier gas helium was used at the rate of 0.51 mL/min. Injection temp 300°C. Injection mode was splitless.



Figure 2. Research flowchart

Microbial Contamination and Bioactive Compounds... Gunardi et al.

#### **RESULT AND DISCUSSION**

## Microbial contamination of jamu beras kencur

After two days incubation of JBK at room temperature, the CFU number of coliforms, *Escherichia coli*, Pseudomonas,

Staphylococcus aureus, yeasts and molds increased significantly more than 100% (Table 1) (Figure 3). Meanwhile, the microbial count varies among the three samples, possibly due to different home industries which influenced the hygiene.



Figure 3. Representative colonies of 3M Petrifilm. a) *Staphylococcus aureus with the red colonies till the violet-black colour; b)* Coliform colonies: pale red with gas bubbles; c) *Escherichia coli*: with blue colonies and gas bubbles; and d) Yeast and mold: small blue colonies for the yeast, large blue colonies for the mold

Table 1. The amount	(CFU/ml) of	f contaminated	microbe in	jamu beras	kencur
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Contaminated Microbe	Sample	Jamu beras kencur		Increase (%)	
Stanbulananua auraun		Fresh	Two days		
Staphylococcus aureus	А	28.10 <sup>1</sup>	56.10 <sup>3</sup>	>100	
	В	15.10 <sup>4</sup>	28.10 <sup>4</sup>	86	
Coliform	С	74.10 <sup>3</sup>	90.10 <sup>3</sup>	21	
	А	15.10 <sup>3</sup>	30.10 <sup>3</sup>	100	
	В	9.10 <sup>3</sup>	88.10 <sup>3</sup>	>100	
	С	25.10 <sup>2</sup>	>100.10 <sup>5</sup>	>100	

Contaminated Microbe	Sample	Jamu be	eras kencur	Increase (%)
Escherichia coli	А	56.10 <sup>2</sup>	60.10 <sup>2</sup>	7.1
	В	10.10 <sup>3</sup>	13.10 <sup>3</sup>	30
	С	10.10 <sup>1</sup>	>100.10 <sup>5</sup>	>100
Yeast	А	10	10.10 <sup>5</sup>	>100
	В	3.10 <sup>1</sup>	3.10 <sup>4</sup>	>100
	С	10	70.10 <sup>4</sup>	>100
Mold	А	3.10 <sup>1</sup>	39.10 <sup>2</sup>	>100
	В	56.10 <sup>1</sup>	38.10 <sup>3</sup>	>100
	С	10	56.10 <sup>2</sup>	>100

# Glucosides content of jamu beras kencur (LC-MS/MS and GC-MS analysis)

Liquid chromatography (LC) is widely used to separate compounds in a sample prior to analysis, often in combination with mass spectrometry. The separation is based on interactions between the compounds and the mobile and stationary phases, with each compound's affinity for the mobile phase determining its separation. After elution from the column, the compounds are desolvated, ionized, and analyzed by a mass spectrometer(Ardrey, 2003; Pitt, 2009; Thermo Scientific, 2024). Similarly, in Gas Chromatography (GC), the sample is vaporized and separated into components by a capillary column, propelled by an inert gas such as helium, hydrogen, or nitrogen. The compounds elute at different times based on their boiling points and polarity, allowing GC to resolve complex mixtures with hundreds of compounds.

LC-MS/MS is particularly effective for detecting non-volatile, polar, and thermally sensitive compounds, such as flavonoids and glycosides, with high sensitivity and precision(Pitt, 2009). On the other hand, GC-MS is ideal for analyzing volatile and semi-volatile compounds, such as terpenoids, providing excellent separation and identification, supported by extensive spectral libraries(Gomathi et al., 2015). The use of these two methods aims to enhance analysis because some bioactive compounds cannot be detected by LC-MS but are found in GC-MS, and vice versa(Table 1).

	Jamu	Rhizomes	Ethano	lic
Name of compounds	beras	201100115	Linano	
	kencur	aqueous	LS- MS/ MS	GC-MS
2-O-α-D-Glycosides of galactose-1-deoxynojirimycin	LA	nd	nd	nd
Maokonine	LA	nd	LA	nd
7-O-α-L-Rhamnosyl-3-O-β-D-	LA	nd	nd	nd
glucopyranosyl kaempferol				

Table 2. Phytochemical constituents of jamu beras kencur and rhizome (LC-MS/MS and GC-MS)

7-O-α-L-Rhamnosyl-3-O-β-D-	LA	nd	nd	nd
glucopyranosyl kaempferol				
Hirsuteine	LA	nd	nd	nd
1-[(2E,4E)-2,4-Decadie-noyl]pyrrolidine	LA	Nd	nd	nd
3'-O-Methylviolanone	LA	nd	nd	nd
Patuletin-7-O-[6"-(2-methylbutyryl)]-	POS	nd	nd	nd
Glucoside				
Chrysoeriol-4'-O-β-D-glucopyranoside	POS	nd	nd	nd
Acacetin-7-galactoside	POS	nd	nd	nd
1,2,6-Tri-O-galloyl-β-D-	LA	nd	nd	nd
Glucopyranoside				
Isorhamnetin-3-O-β-gentiobioside	LA	POS	nd	nd
Quercetin-3',4',7-trimethyl ether	nd	POS	POS	nd

			Rhizomes		
Name of compounds		Jamu beras kencur		Ethanolic	
			aqueous	LS- MS/ MS	GC-MS
3-(4'-Hydroxy-benzyl)-5,7-dihydroxy-	6-methyl-8-		LA	nd	nd
methoxy-chroman4-one					
Picrasidine K		Nd	LA	nd	nd
Feralolide		nd	nd	POS	nd
Skullcapflavone II		nd	nd	POS	nd
Yakuchinone B		nd	nd	POS	nd
Neonootkatol		nd	nd	LA	nd
5-Hydroxyauranetin		nd	nd	LA	nd
Feroxin A		nd	nd	LA	nd
Quercetin-3-O-(2G-α-L-rhamnosyl)-		nd	nd	LA	nd
Rutinoside					
6-Isoinosine		nd	nd	LA	nd
Ethyl-p-methoxycinnamate		nd	nd	nd	91%

Note: LA: least abundance; POS: abundance; nd: not detected

LC-MS/MS analysis (negative mode) revealed the abundance presence of three glucosides in jamu beras kencur. They are Chrysoeriol-4'-O- $\beta$ -D-glucopyranoside, Patuletin-7-O-[6"-(2-methylbutyryl)]-glucoside, and Acacetin-7-galactoside (Figure 4). All

belong to the flavonoid compounds with the same flavone ring structure but different substitutions (Figure 5 and Table 2). The positive mode of LC-MS/MS analysis revealed only less abundance compounds (Figure 6).

2500 2000 Intensity [Counts] 1000 Chrysoeriol-4'-O-B-D-glu... 7.77 Patuletin-7-O-[6'... \$ 500 1 37 1,2,6-Tri-O-galloyl-B-D-g. 0 2 3 4 6 7 5 8 Retention time [min]

Figure 4. Chromatogram of LC-MS/MS (negative mode) of jamu beras kencur



Figure 5. a.) Flavon aglycones and their glucosides in jamu beras kencur and b.) Flavone aglycones and their glucosides in jamu beras kencur





Figure 6. LC-MS/MS Chromatogram (positive mode) of the rhizome aqueous extract

LC-MS/MS (positive mode) revealed that the positive compounds in the aqueous extract of rhizome contains Isorhamnetin-3-O- $\beta$ -gentiobioside and Quercetin-3',4',7-trimethylether (Figure 6). The ethanolic extract contains Quercetin-3',4',7-trimethyl ether, Feralolide, Skullcapflavone II, and Yakuchinone B (Figure 7) with have closed retention times, 16.73, 16.64, 16.67, and 16.96

respectively. The negative mode of LC-MS/MS revealed less abundance compounds in ethanolic extract. GC MS analysis revealed that the ethanolic extract of rhizome contains Ethyl p-Methoxycinnamate as the sole dominant component (Table 2, Figure 8). These constituents were not found in the aqueous and ethanolic extracts of the rhizome.



Figure 7. LC-MS/MS Chromatogram (positive mode) rhizome etanolic extract

JBK is easily contaminated by many microbe that may become a source of foodborne disease. The contamination is happened during the preparation and keeping of the product, particularly when it is kept at room temperature. The presence of E. coli and other coliform bacteria (Klebsiella and Enterobacter) are reported already(Jilan Maulida, n.d.; Walther et al., 2016). But, so far, no reports on the presence of Salmonella spp and Shigella spp(Walther et al., 2016). The contamination source of JBK may come from the water used during the preparative process of JBK, the ineffectiveness of the heating process in killing the contaminant microbe, the cleanness of the container and equipment particularly for grinding the material/rhizome, and the material or rhizome itself. The presence of Coliform, Escherichia coli, Staphylococcus aureus, yeast, and mold are indicative enough for the problem of contamination and the growth of the microbe in JBK. The JBK is not able to inhibit or limit the growth of contaminant microbe. Therefore, the amount of contaminant increased during

the keeping of the JBK. From this information, it is strong enough to say that the maker and the seller do not have enough good knowledge and skill on sanitation. This report is the first report on the growth of contaminant microbe in JBK.

It is also the first report on the pres-Chrysoeriol-4'-O-B-D-glucopyraence of noside, Patuletin-7-O-[6"-(2-methylbutyryl)]glucoside, and Acacetin-7-galactoside in JBK (Table 3). These flavonoid glycosides were not detected in the aqueous and ethanolic extract of the rhizome. Ethyl p-methoxy cinnamate is only detected by GC MS in ethanolic extract of the rhizome. This means that the preparative process of JBK is no guaranty that the essential bioactive compounds from the rhizome are available in JBK. But, a correlation between a microbial community in JBK and the quality of BJK is also subject to further research. The prospects and trends in evaluating natural glycosides in JBK can make it a new effective therapeutics(Bartnik & Facey, 2017).



Figure 8. GC-MS Chromatogram of the ethanolic extract of Kaempferia galangal

Many previous reports used extract obtained from the rhizome of K. galanga by the help of organic solvents, such as Ethyl p-Methoxycinnamate. This compound is detected clearly in ethanol extract but not in aqueous extract. Ethyl p-methoxycinnamate has antibacterial activity. The number of bacteria in jamu beras kencur, a water extract of rhizome mixed with other ingredients, is increased significantly. This antibacterial capacity is not expressed in jamu beras kencur because ethyl p-methoxycinnmate is not soluble in water.

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Compound	Reported Bioactivity
Acacetin-7-ga- lactoside	anti-inflammation, immunomodulation, neuroinflammation, (Ha et al., 2012; Zhao et al., 2014) reduce the inflammatory response of macrophages(Liou et al., 2017), anticancer, alleviate TPE (telomere position effect), deprotect telomeres against DNA damage response.(Boussouar et al., 2013; Punia et al., 2017) Anti-atrial fibrillation(Wu et al., 2011), inhibit lipid accumulation inadipocytes, reduce body weight and visceral adipose tissue weight, inhibit adipogenesis in adipocytes and obese mice (Liou et al., 2017)
Chrysoeriol-4'- Ο-β-D- glucopyra- noside	antioxidant and anti-inflammatory activities, (Cheng et al., 2013; Choi et al., 2005; Csupor et al., 2013; Nguyen et al., 2015) anti-Parkinson's disease, (Limboonreung et al., 2020) anticancer, anti-metastatic, cell cycle arrest, (Tanagornmeatar et al., 2014; Yang et al., 2010) antihyperglycaemic effect, anti-diabetic, (Rauter et al., 2010; Tofighi et al., 2014) inhibit palmitic acid uptake (Han et al., 2003) anti-pho- toaging agents of skin. (Kim et al., 2004)
methylbutyryl)]- glucoside	Antioxidant,(Corrêa et al., 2018; Daroui-Mokaddem et al., 2017) anti-inflammation, inhibit the production of pro-inflammatory factors such as ROS, IL-8,and TNF-alpha in stimulated neutrophils,(Corrêa et al., 2018; Pawłowska et al., 2018) potentialim- munosuppressive and anti-arthritic lead candidate.(Jabeen et al., 2016)

Aglycon of Acacetin, Chrysoeriol, Patuletin glucosides in JBK exerts a wide range of activities (Table 3). They are antioxidative, anti-inflammatory, and immunomodulators, and anticancer agents(Tanagornmeatar et al., 2014). Therefore, JBK is a promising herbal medicine that needs to be improved in its specificity and efficacy. Acacetin and Chrysoeriol may act as a potential therapeutic agent for brain diseases involving neuroinflammation such as Alzheimer's disease, Parkinson's disease,(Limboonreung et al., 2020) and ischemia.(Ha et al., 2012)

Table 4. Bioactive compound of aqueous and ethanolic of rhizome of Kaempferia galangal

Compound (extract)	Reported bioactivity
Isorhamnetin-3-O-β- gentiobioside (Aqueous)	No information available
Quercetin-3',4',7-trime- thylether (Aqueous and Ethanolic)	Increase or decrease tracheal relaxant activity, depending onits methyla- tion, vasorelaxant activity position(Guerrero et al., 2002; WC. Ko et al., 1999) $\alpha$ -Glucosidase inhibitors(Ezzat & Salama, 2014)
Feralolide (Ethanolic)	Antiviral(Abd-Alla et al., 2012), Antioxidant, (Lucini et al., 2015) anti-inflam- mation(Rauwald et al., 2021)
Skullcapflavone II (syn: neobaicalein) (Ethanolic)	Anticancer, inhibit prostate cancer cell proliferation(Bonham et al., 2005; Jang et al., 2012) Anti-inflamation, upper airway inflammation, anti-aller- gic, anti-asthma, inhibit cyclooxygenase/ lipoxygenase,(Bui et al., 2017; Chandrasekaran et al., 2011; Jang et al., 2012; H. Lee et al., 2021; You et al., 1999) inhibits osteoclastogenesis,(J. Lee et al., 2019) antioxi- dant,(Parsafar et al., 2020) Efflux pump Inhibitors and anti TB(Solnier et al., 2020)

Compound (extract)	Reported bioactivity
Yakuchinone B (Etha- nolic)	Antioxidant, (Bayati & Yazdanparast, 2011; Surh, 1999) anti-inflamma- tion, (Chun et al., 1999, 2002; Surh, 1999; Ullah et al., 2020) Anticancer, skin carcinogenesis, (Chun et al., 1999, 2002; Roy et al., 2002) inhibitor against Islet Amyloid Polypeptide Aggregation (IAPP) that asso- ciatedwith Type 2 diabetes mellitus (T2DM) (Hsu et al., 2021) inhibitors of acyl-CoA: cholesterol O-acyltransferase (Ohishi et al., 2001) tyrosinase inhibitory activity, (Shirota et al., 1994) suppress expression of leukocyte adhesion molecules on human vascular endothelialcells (Yama- zaki et al., 2000)
Ethyl p- Methoxycinnamate (Eth- anolic)	Antibacteria, antifungal, anti TB,(Arambewela et al., 1999; Gupta et al., 1976; Lakshmanan et al., 2011; Song et al., 2021; Swain et al., 2021) anticancer,(Amuamuta et al., 2017; Muhamad et al., 2020; Tritripmongkol et al., 2020; Xue & Chen, 2002) Antiangiogenic Sedative activity,(He et al., 2012; Umar et al., 2014) vasorelaxation,(Huang et al., 2008; Sri- vastava et al., 2021) anti-inflammatory, acute and chronic inflamma- tion(Jagadish et al., 2016; Umar et al., 2012) Hypopigmentary effects, a skin whitening agent(HJ. Ko et al., 2014) neu- roprotective effect in reversing an acute memory deficit,exert cognitive en- hancing property independent of direct AChE and glutamate receptor in- hibition.(Rijal et al., 2019)

Among bioactive compounds found in aqueous and ethanol compounds, ethyl pmethoxycinnamate has been reported in several reports.(Hasegawa et al., 2016; Kumar, 2020; Tritripmongkol et al., 2020; Yu et al., 2000). The other bioactive compounds are the first reported in this report (Table 4). They have wide biologically activities. Unfortunately, their presence is not detected in JBK. A combination between rice ('beras") and the rhizome of *Kaempferia galanga* (kencur) made jamu beras kencur unique in its composition. Not all bioactive compounds are extractable`e in JBK.

## CONCLUSION

Jamu beras kencur is contaminated with a lot of Gram negative and Gram positive bacteria, such as Coliform, *Escherichia coli*, Pseudomonas, Staphylococcus. Yeast and mold contaminated also jamu beras kencur. All of them are able to grow in jamu beras kencur. Jamu beras kencur is a habitat and good nutrients for the growth or fermentation of the microbe. It is recommended that jamu beras kencur should be made more hygiene to minimalize the contamination and store or sold in cooled condition. But on the other side, there is a possibility that special fermentation is happening during the making process of jamu beras kencur. This study reported for the first time, the results of LC-MS/MS analysis revealed the presence of three flavonoid glycosides, Chrysoeriol-4'-O-β-D-glucopyranoside, Patuletin-7-O- [6"-(2-methylbutyryl)]-glucoside and Acacetin-7-galactoside in jamu beras kencur. The LC-MS/MS analysis confirmed that components of jamu beras kencur are not similar with the components of aqueous and ethanolic extracts of the rhizome of *Kaemferia galanga*. GC-MS revealed that Ethyl-p-methoxycinnamate is found in rhizome ethanolic extracts.

From the pharmacological point of view, jamu beras kencur has the potentials as a healthy herbal beverage. But further preclinical and clinical studies are required to justify its clinical use.

## **CONFLICT OF INTEREST**

Authors declare no conflict of interest

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