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Original Article

Anti-fatigue activity of syrup containing ethanolic extract of kencur (*Kaempferia galanga* L.) rhizome in Wistar rats

[Actividad antifatiga del jarabe que contiene extracto etanólico de rizoma de kencur (*Kaempferia galanga* L.) en ratas Wistar]

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Abstract

Context: The Javanese culture includes the consumption of traditional herbal medicine known as jamu. One commonly consumed type of jamu to enhance physical fitness and alleviate fatigue is Beras Kencur, which contains the kencur *(Kaempferia galanga)* rhizome. Although it has been used for generations, scientific testing on the effects of *K. galanga* as an anti-fatigue agent remains rare.

Aims: To assess the anti-fatigue effects of *K. galanga* rhizome extract (KGE) through an endurance swimming test conducted on male Wistar rats.

Methods: This study involved 30 Wistar rats randomly allocated into 5 groups: a negative control group administered with syrup vehicle, a positive control group receiving 9 mg/kg body weight (BW) of caffeine syrup, and three treatment groups receiving KGE syrup at different doses - dose 1 (37 mg/kg BW), dose 2 (73.5 mg/kg BW), and dose 3 (147 mg/kg BW). The anti-fatigue effect was evaluated based on the duration of struggling in the water in the endurance swimming test.

Results: The results demonstrated that the treatment groups exhibited a significant anti-fatigue effect compared to the negative control group. The administration of a syrup formulation containing KGE at doses 1 (37 mg/kg BW), 2 (73.5 mg/kg BW), and 3 (147 mg/kg BW) revealed an anti-fatigue effect on male Wistar rats.

Conclusions: The administration of *K. galanga* demonstrated dose-dependent anti-fatigue activity in rats. The most effective dose was found to be 147 mg/kg body weight in rats.

Keywords: fatigue; jamu; kencur, *Kaempferia galanga*; swimming test.

Resumen

Contexto: La cultura javanesa incluye el consumo de la medicina herbaria tradicional conocida como jamu. Un tipo de jamu que se consume habitualmente para mejorar la condición física y aliviar la fatiga es el Beras Kencur, que contiene el rizoma de kencur (*Kaempferia galanga*). Aunque se ha utilizado durante generaciones, las pruebas científicas sobre los efectos de *K. galanga* como agente antifatiga siguen siendo escasas.

Objetivos: Evaluar los efectos antifatiga del extracto de rizoma de *K. galanga* (KGE) mediante una prueba de resistencia de nado realizada en ratas Wistar macho.

Métodos: En este estudio participaron 30 ratas Wistar asignadas aleatoriamente en 5 grupos: un grupo de control negativo al que se le administró jarabe de vehículo, un grupo de control positivo que recibió 9 mg/kg de peso corporal (PC) de jarabe de cafeína y tres grupos de tratamiento que recibieron jarabe de KGE a diferentes niveles. dosis: dosis 1 (37 mg/kg de peso corporal), dosis 2 (73,5 mg/kg de peso corporal) y dosis 3 (147 mg/kg de peso corporal). El efecto antifatiga se evaluó basándose en la duración del esfuerzo en el agua en la prueba de natación de resistencia.

Resultados: Los resultados demostraron que los grupos de tratamiento exhibieron un efecto antifatiga significativo en comparación con el grupo de control negativo. La administración de una formulación de jarabe que contiene KGE en las dosis 1 (37 mg/kg de peso corporal), 2 (73,5 mg/kg de peso corporal) y 3 (147 mg/kg de peso corporal) reveló un efecto antifatiga en ratas Wistar macho.

Conclusiones La administración de *K. galanga* demostró actividad antifatiga dosis-dependiente en ratas. La dosis más eficaz fue 147 mg/kg de peso corporal en ratas.

Palabras Clave: fatiga; jamu; *Kaempferia galanga*; kencur; prueba de natación.

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INTRODUCTION

Fatigue is a common experience in our everyday existence, reflecting a state of diminished energy, reduced vitality, and decreased motivation. This condition can significantly impact our daily activities. Conversely, the World Health Organization's constitution defines well-being as "a state characterized by overall physical, mental, and social health, rather than simply the absence of disease or infirmity" (Kaneko et al., 2022). Fatigue resulting from exercise is primarily linked to an excess of physical activity, leading to feelings of fatigue and/or discomfort. Fatigue is a continuous activity that impacts work performance, health, and safety. Consequently, rest or sleep is required for recovery. The potential effects of fatigue include reduced alertness, impaired judgment or thinking, drowsiness while driving, falling asleep at the wheel, decreased memory, and mood changes (Mafitri and Parmadi, 2018). To address fatigue resulting from the accumulation of lactic acid, stamina-enhancing supplements or tonics with effects that boost or strengthen organ systems and stimulate muscle tone repair may be utilized. Consequently, the exploration of novel anti-fatigue interventions designed to ameliorate the manifestations of fatigue is a dynamic area of scholarly interest (Chen et al., 2021; Lee et al., 2011).

One of the compounds frequently employed to enhance stamina is caffeine. The primary mechanism of caffeine involves acting as a competitive adenosine receptor within the central nervous system, particularly at the presynaptic terminal, where it regulates the release of neurotransmitters, such as acetylcholine, glutamate, and dopamine, and inhibits the occurrence of lipolysis. The consumption of 2-10 mg/kg of caffeine has been shown to increase alertness and prevent the onset of fatigue (Habibi and Artanty, 2019). However, long-term caffeine consumption can result in increased stomach acid, elevated total cholesterol levels, and cardiovascular issues such as hypertension, tachycardia, and arrhythmia (Nieber, 2017). Research on new compounds with safe and minimal side effects that have anti-fatigue effects is necessary to avoid these side effects. One approach is to utilize traditional medicines made from natural ingredients that have been proven effective and safe through generations, thus minimizing the occurrence of harmful side effects (Mafitri and Parmadi, 2018).

Indigenous Indonesian communities possess various local wisdom in utilizing plants as medicines to treat diseases, including sexual disorders. *Kaempferia galanga* L. (family *Zingiberaceae*), locally known as keciok, kencur, or kencor, is commonly used by the Singkil, Alas, Cirebon, Batak Simalungun, and Madura ethnic groups in Indonesia in aphrodisiac formulations, as it is believed to enhance sexual stamina (Fauzi et al., 2019; Silalahi et al., 2015). The stamina-enhancing effect of *K. galanga* is suspected to be due to its ability to reduce body fatigue and provide energy through muscle metabolism. Although *K. galanga* is widely used in traditional preparations, the number of traditional sellers has significantly declined. Therefore, there is a need for formulations that are easier to consume, easy to swallow and offer immediate benefits. For this reason, a syrup formulation is considered an alternative solution to address the existing issues in the community.

In developing *K. galanga* syrup formulations, it is essential to consider the chemical compounds present, particularly the dominant constituents. The primary and predominant component in *K. galanga* rhizome extract is ethyl p-methoxycinnamate (EPMC) (constituting up to 80.05% of the extract) (Umar et al., 2012). EPMC belongs to the group of ester compounds containing a benzene ring and methoxy groups, which are nonpolar, along with a carbonyl group binding ethyl that is slightly polar. Therefore, in its extraction, solvents of varying polarities, such as ethanol, ethyl acetate, methanol, water, and hexane, can be used (Setyawan et al., 2012). EPMC is suspected to have activity as an anti-fatigue by increasing the release of lactate dehydrogenase (LDH) in experimental rats (Sirsisangtragul et al., 2011). Thus, this study used ethanol extract of *K. galanga* (KGE) with standardized EPMC content administered orally to rats. The anti-fatigue effect was assessed based on an endurance swimming test.

MATERIAL AND METHODS

Chemicals

Caffeine was purchased from Sigma-Aldrich (Indonesia), high fructose syrup from Toffin (Indonesia), propylene glycol and sucralose from Brataco (Indonesia), while sodium benzoate and citric acid were acquired from Merck (Indonesia).

Plant material

Kaempferia galanga L rhizome was obtained from Batu (Malang, Indonesia). -7.8674939238633055, 112.51928912439544) and identified by UPT Laboratorium Herbal Materia Medica Batu with voucher code number 074/413.A/102.7/2020. Further extraction was conducted with the assistance of PT Agaricus Sido Makmur Sentosa. The extraction was performed using 70% ethanol as the solvent with a sample-to-

solvent ratio of 1:10. For the first 6 hours, the mixture was stirred, and then it was left to stand for a total duration of 24 hours. The extract was then evaporated using a rotary vacuum evaporator until a constant extract weight was achieved.

Determination of marker compound content

The p-methoxycinnamate (EPMC) content was measured using the thin-layer chromatography (TLC) densitometry method with a silica gel GF_{254} as a stationary phase, hexane: ethyl acetate (9:1), and two drops of formic acid (for 10 mL) mixture as a mobile phase. *K. galanga* extract (KGE), at a concentration of 0.2% in ethanol, was applied as 1.5 µL, and EPMC (400-1200 ng/spot) was spotted on the plate as the sample and reference, respectively. After elution with the mobile phase, the plate was observed at 254 nm.

Optimization and preparation of KGE syrup formulation

KGE extract at various doses (Table 1) was dissolved in propylene glycol and stirred for 5 minutes until fully dissolved (Solution 1). Solution 1 was then mixed with high fructose syrup and stirred for 90 minutes, followed by the addition of sodium benzoate solution. Citric acid was added last, and the solution was stirred until homogenous. The mixture was adjusted to a volume of 100 mL with distilled water.

Animal and experimental design

Male Wistar rats aged 2-3 months, weighing 200-250 grams, were obtained from the Veterinary Pharma Center (Pusvetma) institution under healthy conditions. The testing protocol received ethical approval from the Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga, with certificate number 2.KEH.038.04.2022. Rats were acclimated for one week and provided with a standard diet. On the 8th day, rats underwent a 12-hour fasting period before the experiment to eliminate potential interference from other food-related factors.

Anti-fatigue activity test

Rats were weighed and divided into five groups, each comprising six individuals. The groups included negative control (vehicle), positive control (caffeine), and treatment groups (KGE dose 1, dose 2, dose 3) as per Table 2. The sample was administered orally (p.o.) using an oral gavage feeding tube. Thirty minutes after administration, rats underwent an endurance swimming test adapted from the Forced Swimming test (Carter and Shieh, 2015; Porsolt et al., 1978; Yankelevitch-Yahav et al., 2015) in a glass container 20 × 45 cm filled with water to a height of 18 cm. The endurance swimming test was intended as a physical activity inducing fatigue in rats. The rat's swimming endurance was measured by the time spent floating on the water's surface, struggling, and making necessary movements until exhaustion, allowing its head to be submerged underwater for more than seven seconds. Struggling time represented the duration the rat needed to swim with maximum effort, characterized by keeping its head and both front limbs above the water surface (not sinking). Testing was halted when the rat exhibited fatigue, indicated by the lack of leg movement for swimming and allowing its head to stay submerged underwater for more than seven seconds. Struggling time was used as a parameter to assess anti-fatigue activity in rats.

Statistical analysis

The struggling duration data obtained were initially subjected to a homogeneity test. Upon achieving a homogenous data distribution, the analysis was carried out using the one-way ANOVA method, followed by post hoc Tukey and LSD tests to analyse the significant differences in struggling times between groups ($p<0.05$) by mean of Graphpad 9.0, GraphPad Software, Inc., USA. Data are represented as mean ± SEM.

Table 1. Syrup formulation.

Component	Formula 1 (mg/100 mL)	Formula 2 (mg/100 mL)	Formula 3 (mg/100 mL)
Kaempferia galanga extract	370	735	1470
Propylene glycol	15	15	15
Fructose	65	65	65
Sodium benzoate	100	100	100
Sucralose	50	50	50
Citric acid	250	250	250

Table 2. Animal grouping and treatment.

Group	Sample
Negative control	Syrup base (high fructose syrup, sorbitol 70%, propylene glycol, sodium benzoate, citric acid, sucralose, distilled water)
Positive control	Caffeine 9 mg/kg BW rats in syrup base
Dose 1	KGE 37 mg/kg BW
Dose 2	KGE 73.5 mg/kg BW
Dose 3	KGE 147 mg/kg BW

Table 3. Quality evaluation of the syrup formulations.

Parameter	Syrup formula		
	Dose 1	Dose 2	Dose 3
Color	Light yellow	Yellow	Light brown
Smell	A weak aromatic flavor	A weak aromatic flavor	Distinct aromatic scent
Taste	Sweet, slightly spicy, with a hint of tanginess on the tongue	Sweet, slightly spicy, with a lingering taste on the tongue	Slightly bitter, spicy, strong lingering taste on the tongue
Clarity level	Clear	Clear but with floating particles.	Clear but with floating particles.
pH	3.5	3.4	3.7
Specific gravity	1.3	1.3	1.4
Displaced volume test	100%	100%	100%

RESULTS AND DISCUSSION

Before KGE was used in syrup formulation and administered to experimental animals, the determination of the EPMC content was conducted. From the standard EPMC testing, a linear equation y= 7.8498x + 798.11 with an R-value of 0.988 was obtained. This equation was then used to calculate the EPMC content in the KGE. Based on the calculation, the average EPMC content in the extract was found to be 43.49% w/w. This content is relatively lower than the content measured in other research, which reported that the ethanolic (70%) extract of KGE contains 87.40% EPMC (Hikmawanti et al., 2021). The difference may be attributed to variations in the growth environment, plant age, and methods for determining the content. Despite the significant differences in content, quantifying EPMC levels can serve as a standardization parameter and a justification for the dosage in the utilized extract.

The KGE was then formulated into three syrup formulations with varying amounts of extract. The best formula was determined based on the evaluation results. In the organoleptic test, the three formulations were directly observed visually. A good syrup is characterized by taste, aroma, and colour distinctive to the used extract and good clarity. The results of the organoleptic test for the three formulations are shown in Table 3.

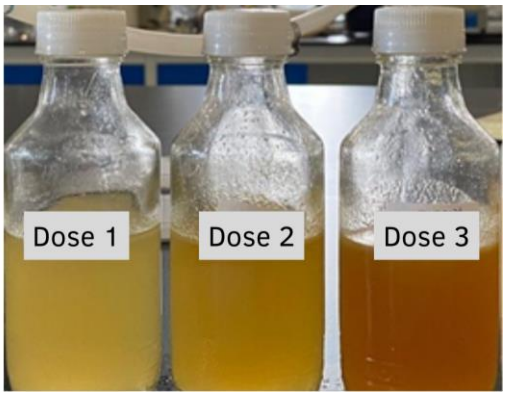

Figure 1. Physical appearance of syrup preparation.

The results of organoleptic evaluations on the three formulations of syrup containing KGE indicate varying colors that intensify with increased extract dosages. The resulting color becomes more concentrated with higher extract dosages (Fig. 1). Regarding odor, all three formulations exhibit a distinctive aromatic scent, with the dose 3 formula having a more pungent aromatic odor than the dose 1 and dose 2 formulations. Observations of syrup clarity indicate that the dose 1 formula meets the criteria for good syrup. In contrast, the formulations

for doses 2 and 3 do not fulfill the requirements for good clarity as syrups due to the presence of floating particles. This suggests that the dose 2 and dose 3 formulas cannot be classified as syrup formulations but are more suitable as suspension formulations since they contain non-soluble solid particles dispersed in the liquid phase. The presence of non-soluble solid particles might be attributed to the higher amounts of extract added to the dose 2 and dose 3 formulas, given at 2 and 4 times more, respectively, compared to the dose 1 formula. The additional propylene glycol, functioning as a cosolvent, was provided at the same concentration of 15% in all three formulations. Therefore, optimization of the KGE in formulation is necessary to ensure complete solubility of the extract. For using propylene glycol as a cosolvent in oral solutions, concentrations ranging from 10% to 25% are recommended (Rowe et al., 2009). pH measurements of the three formulations yielded values exceeding 3, satisfying the criteria for good syrup (pH 3-6). Density measurements indicated that all three syrup formulations met the requirements for good syrup density (≥1.3 g/mL). Transfer volume tests also showed that the volume was 100% for all three formulations, ensuring that the syrup provides the specified volume when transferred from its original volume, thus ensuring dose accuracy.

This study used the endurance swimming test method to conduct an anti-fatigue assessment on white rats. The endurance swimming test employed in this study adopts the Porsolt test, which involves placing rats in a confined and limited space, such as a cylinder with sufficient water (half the size of the cylinder), until the rats are unable to touch the bottom of the cylinder with its hind legs (Porsolt et al., 1978). In the initial phase, rats' activity is notably high in an attempt to escape, ultimately leading to the cessation of activity, commonly referred to as immobility (a non-moving posture). Furthermore, in the final stage, the rats only perform movements necessary to keep their heads above the water surface (Carter and Shieh, 2015).

The endurance swimming test was conducted as a physical activity to induce fatigue. In the endurance swimming test, the ability of rats to struggle was assessed. This method can test the tonic/anti-fatigue effects of formulations that strengthen the body and enhance physical performance during work-related activities. The principle of testing the anti-fatigue effect using the endurance swimming test method is to evaluate the effect of formulation on the test animals based on the observed increase in activity, manifesting as an extension of the time the test animals spend swimming in a container of water (Gillis, 1965). The test animals are considered fatigued when they do not move their legs to swim, their tails remain still, and they allow their heads to stay below the water surface for more than seven seconds. The fatigue time is recorded as the interval from the time the test animals are introduced into the water tank until fatigue occurs (Gillis, 1965). The advantages of the endurance swimming test method include its relatively short duration, the spontaneous observation of anti-fatigue effects through increased work capacity, and the simplicity of the equipment used. However, a limitation of the endurance swimming test method is that it can only assess the increase in physical activity.

The negative control group comprised a syrup vehicle containing high fructose syrup, propylene glycol, sodium benzoate, citric acid, sucralose, and distilled water. Meanwhile, the positive control group consisted of a caffeine syrup formulation with a 9 mg/kg BW of mice. A KGE syrup was administered in the treatment groups, with 37 mg/kg BW, 73.5 mg/kg BW, and 147 mg/kg BW. The administration was performed orally, with a volume of 2 mL per dose, using an oral gavage feeding tube.

The observation data of swimming endurance duration, employed as a test for anti-fatigue activity, were initially subjected to normality and homogeneity tests using the SPSS application. Based on the normality and homogeneity test results, $p>0.05$ were obtained, indicating that the data for the duration of swimming endurance in rats were normally distributed and homogeneous. The data from the swimming test were subsequently analysed using One Way ANOVA, and $p<0.05$ was obtained, allowing the conclusion that the average swimming endurance times among the five test groups of rats were significantly different. To identify pairs of test groups with significant differences, a post hoc test using the Tukey and LSD methods was conducted. The results of the post hoc LSD and Tukey tests analysing the swimming endurance times among the mouse groups revealed that all compared groups exhibited significantly different outcomes ($p<0.05$).

In the endurance swimming test graph (Fig. 2), a clear observation emerges, indicating a significant increase in the duration of struggling time among rats between the control and treatment groups. The order of magnitude, from smallest to largest, is negative control<positive control<treatment 1<treatment dose 2<treatment dose 3. Between-group comparisons within the treatment groups reveal statistically significant differences with a $p<0.05$, where the mean struggling duration increases proportionally with the dosage; this suggests a dose-dependent anti-fatigue

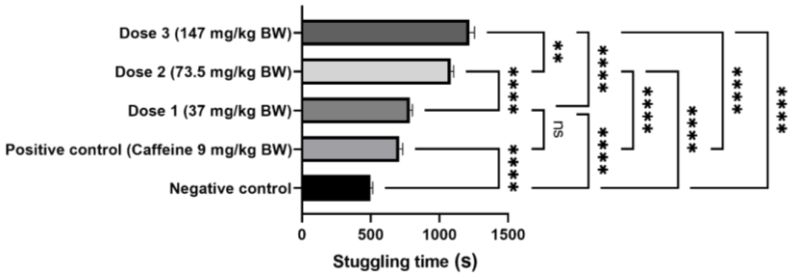

Figure 2. Endurance swimming test result.

Data represent the mean ± SEM (n = 6). **P<0.05; ****p>0.01 one-way ANOVA method, followed by post hoc Tukey and LSD tests.

effect within the treatment groups. Specifically, the administration of dose 1 (37 mg/kg BW) shows a not significantly different struggling time from the positive control at a 9 mg/kg BW dose. This result is likely related to the presence of different active compounds between the positive control group (caffeine, acting as a central nervous system stimulant) and the treatment group, which contains multi-component compounds with various mechanisms of action.

In the 100 mg of KGE, EPMC was present at 43.5%, which works by enhancing the release of LDH (Sirsisangtragul et al., 2011). LDH is essential for maintaining glycolysis and ATP production in conditions of minimal oxygen by catalysing the conversion of lactate to pyruvate, reducing NAD^+ to NADH (Harahap and Marpaung, 2022). LDH in muscles catalyses the reduction of pyruvate to lactate during increased glycolytic activity of muscle contraction. Consequently, EPMC in KGE can contribute to minimizing lactic acid accumulation in the body during intense physical activity. Other compounds in the KGE, such as flavonoids and alkaloids, are believed to have tonic/anti-fatigue activities, prolonging swimming time in mice, increasing hepatic glycogen content in muscles, and reducing lactic acid content in muscles. Therefore, flavonoids have the potential to act as anti-fatigue agents (Li and Zhang, 2013; Zhou and Jiang, 2019). Additionally, the essential oil in ginger exhibits vasodilatory activity, enhancing blood flow to effectively clear increased lactic acid concentrations (unpublish data). The essential oil acts as a tonic/anti-fatigue agent by penetrating mucosal membranes, activating neurotransmitter secretion, resulting in vasodilation of the parasympathetic nerves and vascular smooth muscle relaxation. Based on this mechanism, KGE essential oil widens blood vessels (vasodilation). It increases blood flow, effectively clearing increased lactic acid concentrations, which can reduce fatigue in the body engaged in physical activities (Lin et al., 2018).

Several studies also highlighted the anti-inflammatory and antioxidant properties of *K. galanga*, which could indirectly enhance stamina and reduce fatigue. Studies have shown that *K. galanga* extracts possess anti-inflammatory effects by inhibiting inflammatory mediators such as TNF-α, IL-1β, and nitric oxide (NO) (Andriyono, 2019). Additionally, the extract has been reported to have antioxidant properties due to the presence of phenolic and flavonoid compounds like luteolin and apigenin (Kurniawan et al., 2022). These properties could potentially help in reducing muscle fatigue by combating oxidative stress and inflammation. Moreover, *K. galanga* has been found to exhibit neuroprotective effects, as seen in studies related to traumatic brain injury, where it reduced caspase-3 expression and suppressed brain edema development (Niantiarno et al., 2023; Suryo et al., 2024). These neuroprotective effects could indirectly contribute to improving overall physical performance and stamina. Although direct studies on *K. galanga*'s impact on muscle LDH activity in rats are lacking, its anti-inflammatory, antioxidant, and neuroprotective properties suggest that it may have the potential to enhance stamina and reduce fatigue. Further research specifically focusing on its effects on muscle function and LDH activity would be beneficial to fully understand its impact in this area.

While the anti-fatigue effects of *K. galanga* are rarely documented, its family member in the *Zingiberaceae* family, *K. parviflora*, has been reported in several studies. These studies highlight the potential benefits of *K. parviflora* in enhancing physical fitness and acting as an anti-fatigue agent. Research findings indicate that *K. parviflora* extract can improve physical performance, oxidative status, and cardiovascular health across various populations, suggesting its promise as a natural supplement for those seeking to enhance overall fitness levels (Muchimapura et al., 2012; Promthep et al., 2015; Sripanidkulchai et al., 2020; Wattanathorn et al., 2023). However, the specific compounds responsible for these effects or the major

constituents in *K. parviflora* have not yet been identified. It is hypothesized that the major compounds in *K. parviflora* are similar to those found in *K. galanga*. The *Zingiberaceae* family includes numerous plants with medicinal properties, such *as K. parviflora* and *K. galanga*, both traditionally recognized for their health benefits.

CONCLUSION

The administration of *Kaempferia galanga* L. extract demonstrated a significant dose-dependent anti-fatigue activity by increasing the duration of struggling time among rats in the endurance swimming test. Remarkably, the most effective dose was found to be 147 mg/kg body weight in rats.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Tupenalay DJ	Sukardiman	Suciati	Oktarina RD	Handayani R
Concepts or ideas		x			
Design		x	x		
Definition of intellectual content	x	x	x	x	x
Literature search	x				
Experimental studies	x	x	x	x	
Data acquisition	x				x
Data analysis	x				x
Statistical analysis	x				x
Manuscript preparation					x
Manuscript editing					x
Manuscript review	x	x	x	x	x

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