

Pediculicidal Activity of Cem-ceman White Cempaka Flower (*Michelia alba* DC.) against *Pediculus humanus capitis*

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ABSTRACT

The prevalence of pediculosis reported in Asia has ranged from 0.7% to 59%. High resistance and side effects is a challenge in using the pediculicidal agents. The widely use and empirical as well as scientific studies of traditional plants for anti-lice were already done, one of them is cempaka. The aim of this study was to determine the pediculicidal activity of cem-ceman white cempaka flower (*Michelia alba* DC.) on *Pediculus humanus capitis* in vitro. The white cempaka flowers made into cem-ceman preparations by mixing and soaking in coconut oil until 3 days. Pediculicidal activity was tested by filter paper diffusion test method with 3 replications. Data were analyzed using Kruskal-Wallis followed by post hoc Mann-Whitney Method test with software SPSS v.16. The phytochemical screening showed that sample contains alkaloid, tannin, saponin, and triterpenoid. Pediculicidal activity test showed that cem-ceman white cempaka flower has pediculicidal effect in 10% concentration (w/v) with 66.67% mortality and 15% concentration (w/v) with 77.33% mortality. In conclusion cem-ceman white cempaka flowers has pediculicidal activity at concentration 10% and 15% but lower than permethrin 1% ($p < 0.05$).

KEYWORDS: pediculosis, cem-ceman, white cempaka flower, pediculicidal

INTRODUCTION

Pediculosis is a disease caused by *Pediculus humanus capitis*. Pediculosis is endemic and commonly infected in primary school age children, especially in girls (Leung, Fong, & Pinto-Rojas, 2005). The prevalence of pediculosis in the world ranges from 1-61% (Falagas, Matthaiou, Rafailidis, & Panos, 2008). The prevalence in Indonesia is not known with certainty, but 21 from 40 childrens were screened in one elementary school in Gerung, West Nusa Tenggara diagnosed pediculosis (Sari, Tresnani, & Pratama, 2018).

Pediculosis can be transmitted through direct or indirect transmission (Natadisastra &

Agoes, 2009). Pediculosis can cause severe itching, allergies, scalp irritation, lack of sleep, and can cause bacterial infections such as *Bartonella quintana*, *Acinetobacter baumannii* and *Borrelia recurrentis*. These symptoms are caused by the saliva those the lice secreted when they suck blood (Bonilla, Kabeya, Tlenn, Kramer, & Kosoy, 2009; Bouvresse, et al., 2011; Dent, 2014).

Pediculosis can be treatment by non-pharmacological and pharmacological therapy. Non-pharmacological therapy were done by routine combing, haircut, and maintain personal hygiene. The Pharmacological therapy were done by using the pediculicidal agents (Verma & Namdeo,

2015). The pediculicidal agents currently used are organochlorine, permethrin, organophosphate, and carbamate groups. Permethrin is still recommended as the first line of pediculosis today because it has efficacy more than 95%, fast onset, and can be obtained without a prescription (Zaoutis & Chiang, 2007). The limitation of these agents can cause resistance due to mutations of the T9291 and L932F genes, as well as other groups such as organophosphates and carbamates. The long-term use of organochlorines has a risk of neurotoxicity which can lead to death (Yoon, et al., 2003).

The widely use and empirical as well as scientific studies of traditional plants for anti-lice, one of which is white cempaka. White cempaka flower (*Michelia alba* DC.) is empirically used in hair care (Hasanuddin, 2015; Zumaidar, 2009). Secondary metabolites such as alkaloids, flavonoids and essential oils in these flowers have potential as pediculicidal agents. The study of cempaka leaves in *cem-ceman* preparation has been tested *in vitro* and *in vivo* (Sari, Tresnani, & Pratama, 2018). The limits research on Cempaka flower is encouraging for further research.

MATERIAL AND METHODS

Chemicals and Instrument

The chemicals and materials used are amyl alcohol, ammonium hydroxide, hydrochloric acid 37% v/v, sulfuric acid 97% v/v, iron (III)

chloride, cempaka flower, filter paper, chloroform, 70% methanol, coconut oil, Dragendorff's reagent, Meyer's reagent, 1% permethrin (Peditox®), and magnesium powder. The instruments used are petri dishes, beakers, HVS paper, plastic clips, digital microscope, test tubes, tweezers, head lice combs, and plastic containers.

Extraction

The preparation of *cem-ceman* white cempaka flower by soaking the flower slices into coconut oil solvent for 3 days (Wirakusumah, 2007; Octovina, 2006). The mixed results was filtered and stored in a closed container and then the phytochemical screening of the extract was identified by color reaction test (Nugrahani, Andayani, & Hakim, 2016). The extract was made into various concentration (2.5%; 5%; 10%; 15%; and 20%).

Identification of Lice

The Lices were obtained from hair combing from the probands. The collected lice were stored in plastic clips along with the hair strands of the proband, then identified in the Research Laboratory of the Pharmacy Study Program, Faculty of Medicine, Universitas Mataram. The lice identified using a digital microscope and referring to the morphology of *Pediculus humanus capitis* in literature The Biology and Taxonomy of Head Lice (Bonilla, Durden, Eremeeva, & Dasch, 2013).

Table 1. Phytochemicals screening of cem-ceman white cempaka flower

Phytochemical	Result
Alkaloid	+
Flavonoid	-
Tannin	+
Saponin	+
Steroid	-
Triterpenoids	+

Pediculicidal Activity Assay

Pediculicidal activity assay of the *cem-ceman* white cempaka flower used the filter paper diffusion test method with 3 replications. Coconut oil was used as negative control, and permethrin 1% (Peditox®) as positive control. After randomization, Ten lice were placed on filter paper in petri dish of each group experiment. Next, 0.5 ml of each test solution (2.5%; 5%; 10%; 15%; and 20%), permethrin 1% as positive control, and coconut oil as negative control) spread over filter paper. The mortality of lice checked after 6 hours under digital microscope with 50x magnification. The mortality criterion were no movement of external and internal organs of lice such as antennae, mouth, legs, thorax, abdomen, or digestive system with or without stimulation. The total of lice deaths after 6 hours from each group was converted as the percentage of mortality.

Data Analysis

The average of percentage lice mortality were analyzed normality and homogeneity test with *Shapiro-wilk* and *Levene* method test. The activities pediculicidal were analyzed

using *Kruskal-Wallis* followed by *post hoc Mann-Whitney* Method test with software SPSS version 16.

RESULTS AND DISCUSSION

Phytochemicals Screening

The organoleptics of *cem-ceman* white cempaka flower (Figure 1) shown that light yellow color, fragrant like cempaka flower, and sweet taste. The results of phytochemicals screening test for *cem-ceman* white cempaka flower shown in Table 1. In general, the phytochemical content of white cempaka flower were correspond to the results of Deepti's Screening (Deepti, 2013) except for alkaloid and flavonoid compounds.

Identification of Lice

The identification shows that the animal used in this research is *Pediculus humanus capitis*. The identification results (Figure 2) show that the experimental animals are dark color, flat dorsoventral shape, has 5-segmented antennae, indentation in the stomach (spiracles), and has 6 legs with claws (tarsal claw). Based on the morphology indicates those animals were *Pediculus humanus capitis* (Goddard, 2007).

Male and female lice can be identified from size, structure, and life cycle. Based on Figure 3-a, the experimental animal has a body length of 2.8 mm with bifurcate genitals, while Figure 3-b shows a body length of 2.3 mm with aedeagic genitals. Based on the literature, it is shown that the

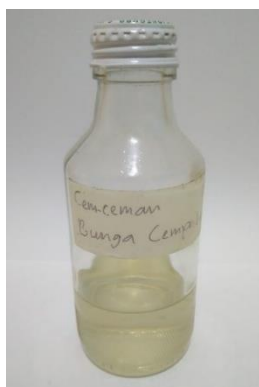


Figure 1. Cem-ceman white cempaka flower

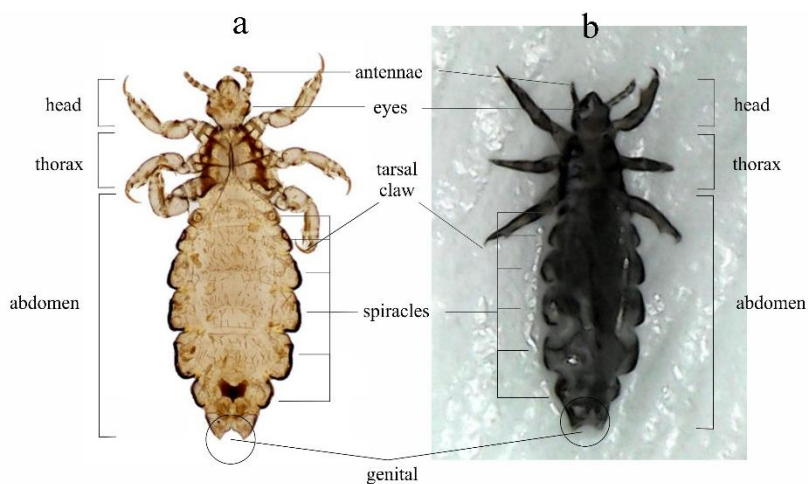


Figure 2. Identification experimental animal: (a) literature (Bonilla, Durden, Ereemeeva, & Dasch, 2013) ; (b) The results of observation



Figure 3. The result of identification types experimental animal: (a) female, (b) male

test animals used were female and male lice (Madke & Khopkar, 2012).

Pediculicidal Activity Assay

The results of the pediculicidal activity of *cem-ceman* white cempaka flower were shown in Table 2. The results of the *Kruskal-Wallis* test showed that there was a significant

difference in the percentage of mortality after 6 hours of giving the extract ($p < 0.05$). This method used to determine if there are statistically significant differences between all groups to show the research done is correct. The observations were made after 6 hours because in the previous study, the Lethal Time (LT) of *cem-ceman* cempaka leaves

Table 2. Percentage of mortality after 6 hour *in vitro*

Group	Mortality after 6 hour (%)
Extract 2.5%	43.33 ± 4.71
Extract 5%	63.33 ± 9.43
Extract 10%	66.67 ± 9.43
Extract 15%	73.33 ± 4.71
Extract 20%	56.67 ± 16.99
Positive control	93.33 ± 8.16
Negative control	40.00 ± 4.71

against *Pediculus humanus capitis* is 350 minutes or approximately 6 hour (Sari, Pratama, & Tresnani, 2021). The *Mann-Whitney* test used for determine significant differences between 2 groups to identification the location of the differences and the effects each experiment group. Based on the results of the *Mann-Whitney* test (Table 3), it shows that 10% and 15% white cempaka flower have a pediculicidal effect which is indicated by a significant difference with negative control ($p < 0.05$). The results of the pediculicidal activity test showed that the 15% *cem-ceman* white cempaka flower had the highest activity even though it was lower than the positive control ($p < 0.05$).

Generally, the mortality were increased from 2.5% until 15% concentration of *cem-ceman*, but the mortality decreased at concentration of 20%. This can be caused by several factors. Firstly, realibility or instability during the testing process, indicated by a fairly large standard deviation, especially at 20% concentration. Besides these factors, it may also be caused by the preparations consisting of a mixture of many compounds which can have a synergistic and antagonistic effect. The antagonistic effect allows for decreased activity (Galardo, Picollo, Gonzalez-Audino, & Mougabure-Cueto, 2012; Heukelbach, Canyon, & Speare, 2007).

Theoretically, alkaloids, tannins and triterpenoids are compounds that have a pediculicidal effect (Postma, 2009; War, et al., 2012; Campli, et al., 2012) and the compound content in the white cempaka flower (such as alkaloids, tannins, and triterpenoids) also gave a pediculicidal effect. The content of these metabolites is thought to have a suffocation mechanism so that it will

Table 3. The results of *Mann-whitney* test

Group	<i>p</i> value	Group	<i>p</i> value
Negative Control - 2.5%	0.046*	Extract 2.5% – Extract 5%	0,068
Negative Control - Extract 5%	0.261	Extract 2.5% – Extract 10%	0,043*
Negative Control - Extract 10%	0.046*	Extract 2.5% – Extract 15%	0,043*
Negative Control - Extract 15%	0.046*	Extract 2.5% – Extract 20%	0,346
Negative Control - Extract 20%	0.261	Extract 5% – Extract 10%	0,822
Negative Control - Positive Control	0.046*	Extract 5% – Extract 15%	0,197
Positive Control – Extract 2.5%	0.043*	Extract 5% – Extract 20%	0,653
Positive Control – Extract 5%	0.043*	Extract 10% – Extract 15%	0,361
Positive Control – Extract 10%	0.043*	Extract 10% – Extract 20%	0,369
Positive Control – Extract 15%	0.043*	Extract 15% – Extract 20%	0,369
Positive Control – Extract 20%	0.046*		

*Significant difference: $p < 0.05$ between treatment groups

interfere with the respiratory system by attacking the cuticles to the trachea of lice. In other study, also reported that the extract of *Michelia champaca* L. has acaricidal (Chungsamarnyart, Jiwajinda, & Jansawan, 1992) and insecticidal effect (Jacobsson, Kumar, & Saminathan, 1995). The research about Cempaka (*Michelia alba* DC.) as pediculosis are very limited, so there needs to be further development.

CONCLUSION

Based on the results obtained, it can be concluded that *cem-ceman* white cempaka flower has pediculicidal effect. The pediculicidal effects were presented at concentration 10% with 66.67% mortality effect and at concentration 15% with 73.33% mortality effect but its lower than permethrin 1% ($p < 0.05$).

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