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Research Article

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Tonic Effects Test of Pegagan (*Centella Asiatica L*) Syrups on White Male Mice

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ABSTRACT

Objectives: *Centella asiatica L or known as pegagan leaf, empirically can be used as a tonic for organs such as liver, kidneys and brain. It also can increase memory. In our society, Centella asiatica L hasn't been optimally processed as a tonic. Commonly, it is processed by boiling or grating the leaf. Raw consumption of this leaf inflicts bad taste. The aim of this research is to find out tonic effect and determine the effective dose of herbal syrup Centella asiática L . The effect of tonic can be determined using natatory exhaustion method, by observing stimulant effect of a certain drug to an animal.*

Methods: *This research used male mice divided into 5 groups of treatment. The 1st group: negative control without herbal syrup Centella asiatica L substance, positive control 50 mg/kg body weight, first dose group 120 mg/kg mice's body weight, second dose group 150 mg/kg mice's body weight, third dose group 180 mg/kg mice's body weight. The effect of tonic was observed trough the increasing duration of the mice's ability of self-defense when it was swum.*

Results: *This research resulted that the use of herbal syrup Centella asiatica L gave tonic effect on dose 120 mg/kg mice's body weight, 150 mg/kg mice's body weight, and 180 mg/kg mice's body weight. The most effective dose is the use of herbal syrup Centella asiatica L on dose 180 mg/kg mice's body weight.*

Keywords: Syrup; Tonic; *Centella asiatica L*

INTRODUCTION

Fatigue can cause weaken of activity, decrease of focus, anxiety and confused which can lead to disease and infection, so that the ability of the body stamina to fight the disease will diminish [1]. We need tonic to stimulate body activity so the feeling of tired, exhausted, and weary can be relieved. Besides the tonic can strengthen the body, recover stamina, and improve the vitality of the body [2]. *Centella asiatica L* is one of the plants that are nutritious for improving stamina. This plant is empirically used as a tonic [3]. This plant contains asiaticoside with trisaccharides units attached to Asiatic acid. Asiaticoside and madecasol a tirtepen saponin in which the saponigenin is useful for medication, thankuniside, isothankuniside, brahmoside, brahmic acid, madasitic acid, hydrocotyline, mesoinositol, centellosa, caretenoid, mineral salt (kalium salt, natrium, magnesium, calcium, and iron) are used as tonic [4]. The previous research about *Centella asiatica L* resulted tonic effect on dose 50 mg/kg body weight, 100 mg/kg body weight, 150 mg/kg body weight showed by the increase of stamina counted from the deviation of swimming duration before treatment [5]. In society, *Centella asiatica L* hasn't been optimally processed as a tonic. Commonly, it is processed by boiling or grating the leaves. Raw consumption of this leaves inflicts bad taste. Based on the previous analysis researchers need to do an advance research about the effect of tonic of *Centella asiatica* leaves syrup to male mice, so the use can be scientifically accountable, simpler, efficient, and acceptable.

MATERIALS AND METHODS

Material

Centella asiatica herbs taken from the Research and Development Center of Herbal and Traditional Medicine, Tawang Mangu, Central Java, white male mice, natrium benzoate, cafein, oleum menthae, citric acid, aquades, reagent *Liebermann-Bouchard*, Reagen Dragendrof, and Mg powder in amyl alcohol. Permision and approval for animal studies were obtained from Faculty of Medicine, Sebelas Maret University and Surakarta.

Methods

Centella asiatica powder 200 g, soak in 2 liters ethanol 96% for 3 days, shaken occasionally, than strained using clean cloth. Repeat the process 3 times using the same solvent. Filtrate is combined and concentrated by evaporator until resulting thick extract.

Phytochemical Screening

Conducted testing of alkaloid, flavonoid, triterpenoid and saponin towards concentrate extract of *Centella asiatica* herb.

Making *Centella Asiatica* Herbs Syrup Formula

Weigh 42 g concentrate extract plus sucrose and add natrium benzoate 0.6 g, then, add aquadest until boiling add 60 mL take and remove the foam by filtering it then pour into the bottle, add oleum menthae and citric acid sufficiently until pH 5 then shake it until homogeneous.

Tabel 1: Centella asiatica herbs syrup formula

Composition	Formula I	Formula II	Formula III	Formula IV	Formula V
Concentrate extract	-	-	0.12 g	0.15 g	0.18
Sucrose	42 g	-	42 g	42 g	42 g
Na benzoat 0,1%	0.6 g	-	0.6 g	0.6 g	0.6 g
Cafein	-	20 mg/10 ml	-	-	-
Oleum menthae	Qs	-	Qs	qs	qs
Citric Acid	Qs	-	Qs	qs	Qs
Aquadest	ad. 60 ml	-	ad. 60 ml	ad. 60 ml	ad. 60 ml

Physical Characteristic Test of Syrup

Viscosity test:

Measurement of consistency was done using *Viscometer Oswald*. Easy or not to be poured test. Poured 100 ml syrup at ± 45^o slant and recorded the time until the syrup is decanted thoroughly (Table 1).

pH test:

pH test was conducted by measuring pH indicator; take a little syrup then put the pH paper into the syrup. Compare the color change of pH paper to pH indicator.

Density:

Clean, dry and calibrated pycnometer was used by setting pycnometer density.

Organoleptic:

Organoleptic test was conducted by observing the change of flavor, taste, and color of syrup in a certain period.

Taste test response:

Taste test response was conducted using random sampling of heterogeneous population of 20 respondents. Taste test response was divided into good, pretty good and not good. The data was presented in form of table according to the percentage of respondents with the taste response given. In this taste test response, the respondents were asked to drink the syrup.

Activity Test of Pegagan (*Centella Asiatica L)* Syrups on White Male Mice

Test animal used in this research are 25 male mice Balb/C, weight 23-35 g, 8 weeks old. The mice were weighted and divided into 5 groups. After that, each mouse was given treatment by orally feeding tube the test material. The divisions of groups are; positive control caffeine 50 mg/kg body weight, negative control was only given CMC Na 0.5%, treatment 1 was extract syrup preparation dose 120 mg/kg body weight, treatment 2 was extract syrup preparation dose 150 mg/kg body weight, treatment 3 was extract syrup preparation dose 180 mg/kg body weight. The method use is natatory exhaustion. This test was conducted to the mice using swim container with C and water level was 18 cm, temperature 20 ± 0.5, given artificial waves generated from an air pump, additional equipment must be placed out the container so that it didn't disturb the swimming activity [6]. Swimming duration before treatment was the swimming duration of the testing animal before the animal was given dose treatment. It was counted starting from when the animal put into the aquarium until when it showed fatigue sign; the testing animal let it head under water for 7 seconds. Then, it would be taken from the water and the duration was recorded. The animal was given 30 minutes rest time, and then given orally treatment. After 30 minutes rest, the animal would be put into the water again and the fatigue time was recorded. The fatigue parameter was the animal didn't move their feet to swim, the mice' body was upright to the water and let it head under water for 7 seconds. The addition of durability or tonic effect was the deviation between swimming duration after treatment and swimming duration before treatment. The data of effect tonic was the addition of durability obtained from the deviation of swimming duration after and before the treatment.

RESULTS AND DISCUSSION

Identification of Chemicals Compound

Chemicals compound identification in *Centella Asiatica* herbs syrup in powder was aimed to check the active compound contain in sample that responsible as an tonic effects.

Table 2: Identification of chemicals compound triterpenonoid, saponin, alkaloid

Preparation	Senyawa		
	Triterpenonoid	Saponin	Alkaloid
Powder	-	-	-
Pegagan leaf	Formed red color	Still foamed	There are deposits of white and brown precipitate
Powder	-	-	-
Pegagan leaf	Formed red color	Still foamed	There are deposits of white and brown precipitate

The yield obtained from maseration is ethanol concentrate extract 8.52%. The result of qualitative identification can be concluded that pegagan leaf (*Centella asiatica L)* contains alkaloid, saponin and triterpenoid. According to Table 2 Phytochemical extract test result indicated alkaloid, triterpenoid dan saponin compound. Alkaloid hydrocotylin ($C_{22}H_{35}NO_8$) was isolated from dried pegagan leaf. Saponin was found in all parts of the plants; centellasaponin B, C, dan D [7]. Triterpenoid compound of pegagan leaf are asiatikosida, centellosida, madekasosida dan asam asiatik [8]. Flavonoid of pegagan leaf is minor compound such as 3-glikosilkuersetin, 3- glukosilkaempferol dan 7-glikosilkaempferol [9].

The Result of Physical Characteristic of Syrup Preparation

Viscosity test:

Vicosity test syrup preparation conducted using Viskometer Oswald. Viscosity is statement custody of a fluid to flow, the higher the viscosity the greater custody [10]. Viscosity test results on each syrup can be seen in the Table 3 below:

Table 3: Vicosity centella asiatica herbs syrup

Replication	Formula I	Formula II	Formula III	Formula IV	Formula V
I	47,086	46,657	48,123	45,787	48,653
II	46,601	45,551	47,581	45,091	47,753
III	45,785	45,409	46,779	45,481	48,427
Mean	46,491	45,939	47,494	45,453	48,277
SD	0,0657	0,0124	0,0676	0,0349	0,0756

Based on the data above, the mean result of viscosity test of each syrup is constant. So it can be concluded that the syrups have good stability.

pH test:

pH test was conducted to make the pH of the syrup was in the range of pH 3-7, the syrups made have been checked on PH 7 (normal) but to make the syrups weren't easily grown by bacteria and fungi then syrups were made at pH 5 by adding citric acid sufficiently [11] (Tables 4 and 5).

Table 4: pH test

	Formula I	Formula II	Formula III	Formula IV	Formula V
pH grade	6.9	7.2	6.8	6.7	6.3

Density:

Table 5: Density

Sample	Empty pycno (g)	Pycno + Content (g)	Content (g)	density (g/ml)
Formula III	20.09	61.94	43.85	1.236
Formula IV	17.68	67.39	45.71	1.346
Formula V	32.00	72.15	47.85	1.325

Based on the data, the density of syrup formula I was 1,236 g/ml, Syrup formula II 1,346 g/ml and syrup formula III was 1,325 g/ml. The result indicated that syrups preparation has been fulfill the requirement of density > 1 g/ml (Table 6).

Organoleptic:

Table 6: Organoleptic test result

Parameter	Formula 1	Formula 2	Formula 3	Formula 4	Formula 5
Color	Yellowish white	colorless	Dark chocolate	Dark chocolate	Dark chocolate
Taste	Sweet, slightly sour taste	Asour and little bitter	sweet, sour, bitter	sweet, sour, bitter	sweet, sour, more bitter
Flavor			Mintz	Mintz	Mintz

Describe flavor, color and taste. Preparations produced should have an interesting color, a pleasant flavor and delicious taste.

Activity Test of Pegagan (*centella asiatica l)* Syrups on White Male Mice

Statistical test results using the method oneway ANOVA showed no significant difference in swimming endurance of five groups before treatment and after treatment ($p<0.05$). Mann Whitney test results indicated a significant difference between the negative control group with the positive control group, a group a dose of 120 mg/kg BW dose group of 150 mg/kg BW, and a dose of 180 mg/kg BW ($p>0.05$). The method used in this research is the Natatory Exhaustion method, a pharmacological screening method to determine the effects of drugs that work in coordination of movement, especially a decrease in neural control center [6] (Figure 1).

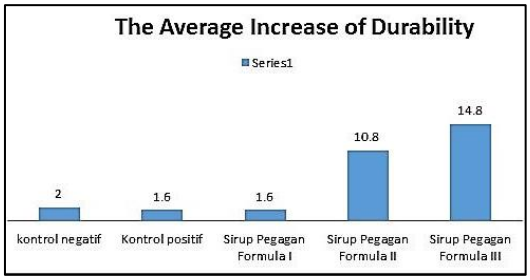

Figure 1: Histogram relationship between the average increase of endurance (minutes) to the treatment groups at the tonic test

The histogram indicated that the herb pegagan leaf (*Centella asiatica L)* syrup at a dose of 180 mg/kg has a tonic effect greater than the pegagan leaf (*Centella asiatica L)* dose of 120 mg/kg body weight in mice and pegagan leaf (*Centella asiatica L)* syrup dose of 150 mg/kg body weight in mice. pegagan leaf (*Centella asiatica L)* has several compounds which have different mechanism. Triterpenoids works by revitalizing the blood vessels so that blood circulation becomes smooth, has a calming effect and lead to better stamina [12]. Pegagan leaf (*Centella asiatica L)* also contains several vitamins that may serve to increase stamina and vitality as well as antioxidants [13]. Saponins allegedly gave a tonic effect on this study because Pegagan leaf (*Centella asiatica L)* contains lead compounds saponin with triterpene acid in the form of esters from sugar. Triterpen acids are Asiatic acid, madekasic acid and asiatikosida; are the most important compounds for the treatment and vascularity. Asiatikosida is efficacious as anksiolitic, anti-inflammatory, antioxidant, and antiulcer [14]. Three groups of trisaccharides attached to the aglycone asiatic acid containing OH groups. The antioxidant activity through the capture of free radicals associated with the dissociation energy of the OH group. The ability to ward off free radicals is associated with the activity of solubility. Through the liposomes model consisting of lipophilic and hydrophilic parts, which are polar sugar groups, will be in the water phase. Because reactive oxygen radicals are also produced in the aqueous phase, it will be captured by antioxidant molecules which are polar and are in the water phase. So that oxidation of the fatty parts will be reduced [15]

CONCLUSION

The research findings indicated that Centella Asiatic leaf herbal syrup on dose 120 mg/kg mice's body weight, dose 150 mg/kg mice's body weight, and dose 180 mg/kg mice's body weight gave tonic effect. The effective dose of Centella Asiatic leaf herbal syrup was on dose 180 mg/kg mice's body weight which was proofed rising body endurance.

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