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Research Paper

Comparisonof Analgeticactivities Ethanol Extractandrographis Paniculata Nees (Sambiloto) Andmoringa Oleifera Lam (Kelor) On Mice

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***ABSTRACT**: Sambiloto and kelorhad been used empirically by some society to treat and lighten disease simptomsand decreasepain (analgetic), however still not evidence scientifically.It will be correlated with the composition of somesecondary metabolitecompound andother chemicals in sambilotoand kelorsuch as alkaloid, flavonid dan terpenoid. This studywas to examine phytochemicallyandanalgesic activitiesof thecombinationof sambiloto and kelorethanol extract insome comparison ofconsentrationgiven orallyto male mice inductioned by aseticacidintraperitoneally. The objective of this study wasto get analgeticsubstrat as alternativeof naturematerialwhich easy to obtain, rasionally dan economics. Sambilotoand kelor extract prepared bymaserationused ethanol 70%. Phytochemicalscreening examined to thesambiloto and kelor ethanol extract (EES andEDK). The combinationof EES dan EDK formulatedin some variation of consentration (0:10); (2,5:7,5); (5,0:5,0); (7,5:2,5); (10:0), examined analgesic activitiesto the male miceinductioned by aseticacid intraperitoneally, observedtime of pain responresistancyafterformulated extract was given, anddiclofenacsodiumascontrolled, each 5 minutesfor 30 minutes. Obtained data counted as analgetic potencyand analysed statistically.From phytochemical examination obtained that sambiloto dan kelorconsist of secondarymetabolite such as alkaloid, flavonoid, glycoside, tannin dan terpenoid. Inanalgetic activitiesobtained thatthecombination of EES dan EDK withcomparison 0:10 dan 2,5:7,5 hadanalgetic activitiesresembledas diclofenac sodium.*

***KEYWORDS**: Sambiloto, kelor, ethanol extract, analgetic activities.*

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I. INTRODUCTION

Sambiloto(*Andrographis paniculata*Nees) wasone of plantused as tradisional medicine came fromIndia andcan growup at lowland or highland.Sambilotohave properties in treating some diseases and common used by people. Sambilotoleaves used as medicines since 1919. Active compound in sambilotosuch asandrographolidmakesambilotobecome one of medicines component.Part ofsambilotousedasmedicinefor snake or insect bite, fever, dysentery, rheumatic, tubercullosis, digest infection, andalso for inflamation, infection, breathless andtoimprove heart function.It also caused by the composition in sambiloto such as andrographolid, saponin, flavonoid, alkaloid andtanin. These compounds were very useful for human body [1].

Kelor (*Moringa oleifera* Lam) have been known for many years as a multifunction plant, full of nutrition and have medication effect. Kelorwas knownconsist of more than 90 items of nutrition such as essential vitamin, mineral, amino acid, antiaging, dan antiinflamation. Kelorconsists of 539 compounds which was known in African and Indian traditional medicineand have been used in traditional medicine to protect more than 300 diseases.Some parts of kelorwere worked as heartstimulant andblood circulation, havefunction asantitumour, antipyretic, antiepileption, antiinflamation, antiulcer, diuretic, antihipertension, decreasecholesterol, antioksidan, antidiabetik, antimicroba dan antifungi[2].

Painwas common in human being and one of the very often reason to visite the doctorbecause pain can raise inconvenience and disturb social functionand quality of life. Inflamationwas the manifestation of tissue damage signed bypresense of pain [3]. *The U.S. Centre for Health Statistics*study for 8 yearssaid that 32% of Americanpeople suffer chronic pain. Study of WHO which involved more than 25.000 patientsfrom 14 countries said 22% patientssuffer from pain at least for 6 months.Pain will be accompanied stress respons such as decrease of ansiety, heart rate, blood pressure and breath rate. Continued pain or not adequate handling can

rise long stress response which decrease body resistance by decrease immune function, hasten tissue demage, metabolic rate, blood coagulation and urine retentionand at the end decrease health quality[4].

Based on above conditions, we had done the screening to ethanol extract of AndrographispaniculataNees (sambiloto) andMoringa oleifera Lam (kelor), and formulated combination of ethanol extract ofAndrographispaniculataNees (sambiloto) and Moringa oleifera Lam (kelor). Then the effectivity of the extract was examinated as analgetic to male white mice usingchemist methodebygivingaseticacid injection intraperitoneally anduse diklofenac sodium as controlled.

II. METHODE

The designe of this study was experimental study. Variation ofconsentration sambiloto andkelorethanol extract formulatedas independent variable, andsome parameter examination testsasdependent variable. This study consists of some steps such as sample preparation, animal preparation, extraction, phytochemical screening and analgetic activities test.

Sambilotodan kelorfresh leaves collected andcleaned from garbage by washing with clean water, drained, and dried in cabinet. The samplewasdried if it was broken when crushed. Then the sample was delicatedby using blender, so we got simplisia, and stored in containare.

About 3 kilogramsofsambiloto/kelorleaves was wet sortationedtodisappear contaminantbywashingusedrunning water 3 times andthendriedunder sunshine in a few minutes and passed the wind. Sambiloto/kelorcleaned and dried leaves delicated by using blender. Thenabout 200 grams sambiloto/kelorsimplicia put in closed container and add ethanol 70%about1500 mlto submersed,thenstir for a few minutes, andstored for 5 days. Stored in protected from sunshineplace. During that the container stir for a few times everyday. After 5 days filterred with flannel cloth and put in extraction bottle. Residu was maseratedby 500 ml ethanol 70% andstoredfor 2 days. Duringthat the container stir for a few times everyday.Filterred andthe result combined to extraction bottle. Then the maserat distilled in low pressure at not more than 60^0 C using rotary evapouratoruntil thick extract obtained. Dried by *freeze dryer*forabout 24 hoursuntil sambiloto/kelor extract obtained, calledekstraketanol sambiloto (EES) andekstrakdaunkelor (EDK)[5].

Phytochemical screening had been done to find out the category of secondary metabolite chemistry compound in ethanol extract of sambiloto andkelorsuch as alkaloid, flavonoid, tannin, saponin, steroid/triterpenoid, glycoside, andessential oil so we could know the potency ofethanol extractof sambiloto andkeloras analgetic.

About 0,5 gfresh extract(sambiloto/kelor)put in reaction tubes, added 1 ml chlorideacid 2N dan 9 ml aquadest, heated on waterbad for 2 minutes.When it was cold then filtered it.Filtratwas used as:

a. 1ml filtrat added 2 drops Mayerreagen, formedwhite or yellow sediment
b. 1 ml filtrat added 2 drops Bouchardatreagen, formed chocolate or black sediment
c. 1 ml filtrat added 2 drops Dragendorffreagen, formed red or chocolate sediment

If there was only feculentthen continued with as followed:

8 ml filtrat added 5 drops concentrate ammonia then stir with 10ml mixeter-chloroform (3:1) dan letit seperated, took ether-cloroform layer, add a few ofanhydrat sulphate sodium, filterred andevaporatedinarlojiglass on waterbad.The residudissolvedwith a few ofchloride acid 2N. Alkaloid positifif there was sediment or muddy at least in 2 reactions of 3 above trials.

About 10 gramsfresh extract(sambiloto/kelor)put inerlenmeyerflask, added 10 ml methanol, refluxabout 10 minutes,filtrated when hot. Filtratediluted with 10 ml aquadest, added 5 ml petroleum eter, stirred gently andabandoned.Took methanol layer, evaporatedat 40^0C, diluted residu in 5 ml acetic ethyl, then filtered. Filtrat usedfor flavonoid testas followed:

a. About 1 ml filtratevaporatedto dried, residudilutedin 2 ml ethanol 96% then add 0,5 g zinkpowder and 2 ml chloride acid 2N, abided 1 minute. Added 10 dropsconcentrate chlorideacid. If in 2-5 minutes there formed intensifred showed flavonoid (glycoside-3-flavonol)
b. 1 ml filtratevaporated to dried, residudilutedin 1 ml ethanol 96% thenadd 0,1 g magnesium powder and 10 drops concentrate chlorideacid. If there formed orange-redtopurple-redshowed flavonoid.

About 3 gramsfresh simplisia(sambiloto/kelor)maseratedwith 30 ml mixture of 70parts ethanol 96% dan 30parts aquadest. Add concentrate sulphat acid and refluxabout 10 minutes, filtered when cold. Then tookabout 20 ml filtrateadded 10 ml aquadest and 10 ml Pb(II) acetat 0,4 M, stirred, abided about 5 minutethenfilterred. Filtratemaseratedwith 20 ml mixture ofchloroform and isopropanol (3:2), remaserated 3 times. Examination had done as followed:

1. Examination of sugar compound

a. Took about 1 ml upper layer (water extract) evaporatedonwaterbad. Added 2 ml aquadestand 5 dropsMolishreagen to the residu of evaporation, andadded carefullyconcentrate sulphate acid, formedpurple ring at the fluid border, this reaction showed the presence of sugar binding.

b. Took 1 ml upper layer (water extract) evaporated on waterbad. Added Fehling A and Fehling B (1:1) to the residu of evaporation, then heated it. The presence of brick red sediment showed reduction sugar.

2. Examination of non sugar compound

Took aboud 1 ml lower layer (organic solvent extract), evaporated on waterbad at not over then 60^0C.The residusoluted in 2 ml methanol, added 20 drops glacial aceticacid and 1 dropconcentrate sulphate acid (Lieberman-Bouchardreagen), if there were blue colour, green, purple red, or purplr, it would be positivefornonsugar[6].

About 0,5 gramfresh extract(sambiloto/kelor)put in reaction tube, added 10 ml hot water, colded andgently stirredfor 10 second. If there was stable foam at 1-10 cm heigh at least 10 minute andwhen added chloride acid 2N the scum was not disappeared, showedthe presence of saponin.

About 1gramfresh extract(sambiloto/kelor)added with 20 ml eterthen filterred. Took about 5 mletersolution, evaporated on waterbad, then to the residu added 20 drops glacial acetic acid and 1 dropconcentrate sulphateacid (Lieberman-Bouchardreagen). If there was blue or green showed the presence of steroid, andifthere wasred or purple color showed triterpenoide.

About1 gramfresh extract(sambiloto/kelor) boiled about 3 minutesin 100 ml aquadest, coldedandfiltrated. Added 1-2 dropFe (III) klorida 1% to the filtrate,if there was black blue or black green color showed the presence of tannin.

The steps of analgetic activities combinationof fresh ethanol extractsambiloto (EES) andkelor (EDK) which formulatedin some consentrationon male micewere as followed:

All of the mice adaptedthen measured the body weighandgave the sign. The mice dividedin 7 groups and every group consisted of 5 mice. The extracts and controlled one were given orally. The groups were classificated as followed:

1. Group I : Given F-1 (EES 0 :EDK 10),
2. Group II : Given F-II (EES 2,5 :EDK 7,5),
3. Group III : Given F-III (EES 5,0 :EDK5,0),
4. Group IV : Given F-IV (EES 7,5 :EDK 2,5),
5. Group V : Given F-V (EES 10 : EDK 0)
6. Group VI : Given CMC as negative control
7. Group VII : GivenDiclofenacsodiumas positive control :

:

All of the mice which given formulas based on body weigh orally then injected asetic acid solution intraperitoneally. Time of mice resistance to pain response of chemical stimulation was noted. Pain responsesigned bythe mice stretching.Observation time in this study was about30 menitat interval: 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, 30 minutes[7].

Data obtained as time in secondfor resistance to pain responsefrom every group of mice, analysedstatisticcallyto knowdata distribution and homogeneity. If the data wasin normal distribution andhomogenthencontinuedwith variansanalysis test (ANOVA) one waywith 95% significance using SPSS 22 version.

III. RESULT

Fromphytochemical screeningobtained thatethanol extractof sambiloto and kelor consisted ofsecondarymetabolitecompound such as alkaloid, flavonoid, glycoside, saponin, steroid, triterpenoid dan tannin as stated at table 1.

Table 1.Phytochemical Screening

NO	Examination	Result	
		Ethanol extract ofSambiloto	Ethanol extract ofKelor
1.	Alkaloid	(+)	(+)
2.	Flavonoid	(+)	(+)
3.	Glycoside	(+)	(+)
4.	Saponin	(+)	(+)
5.	Steroid	(+)	(+)
6.	Triterpenoid	(+)	(+)
7.	Tannin	(+)	(+)

Fromaverage of mice stretching every 5 minutesfor 30 minutesas statedat table2 andfigure 1 below.

Tabel 2. Average ofmice stretchingevery5 minutesfor 30 minutes

Group	Minute					
	5	10	15	20	25	30
I	3,8 ± 0,84	3,4 ± 0,55	3,0 ± 0,71	2,4 ± 0,55	2,2 ± 0,45	1,6 ± 0,55
II	3,4 ± 0,55	3,2 ± 0,45	2,4 ± 0,55	1,8 ± 0,45	1,2 ± 0,45	1,2 ± 0,45
III	6,4 ± 0,55	6,2 ± 0,45	5,4 ± 0,55	5,2 ± 0,45	4,2 ± 0,45	3,2 ± 0,45

IV	8,6 ± 0,55	8,2 ± 0,45	7,8 ± 0,45	7,2 ± 0,45	6,8 ± 0,45	6,2 ± 0,45
V	10,6 ± 0,55	9,8 ± 0,45	9,0 ± 0,71	8,6 ± 0,89	7,6 ± 0,89	6,8 ± 0,84
VI	14,6 ± 0,55	14,0 ± 0,71	13,4 ± 0,55	13,0 ± 0,71	12,4 ± 0,89	12,2 ± 0,89
VII	3,2 ± 0,45	3,2 ± 0,45	2,8 ± 0,45	2,2 ± 0,45	2,2 ± 0,45	1,6 ± 0,55

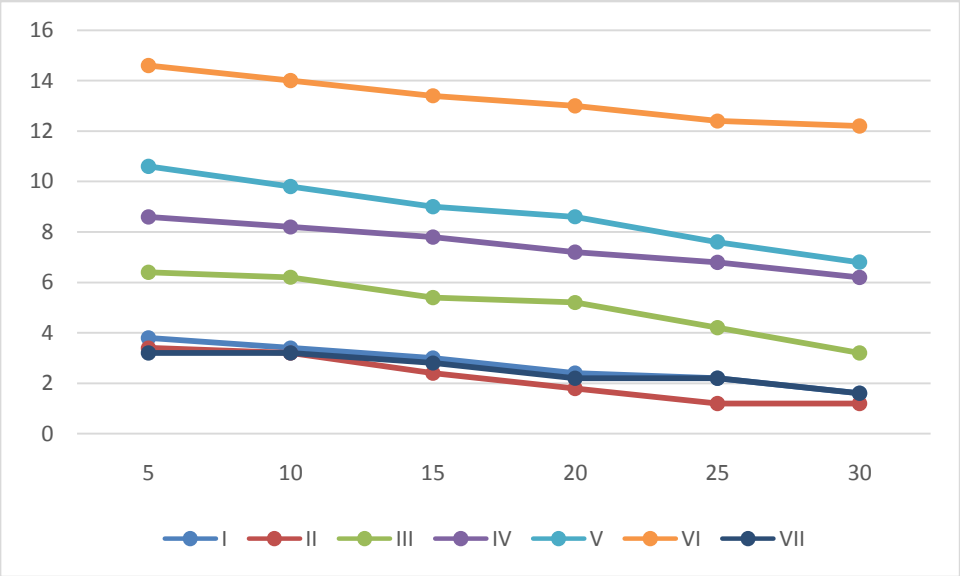

Figure 1. Average of mice stretching chart every minute for 30 minutes

Amount of mice stretching in every group for 30 minutes was stated on table3below.

Table3. Amount of mice stretching in every group for 30 minutes

Mice number	Group						
	I	II	III	IV	V	VI	VII
1	14	15	30	45	47	84	15
2	18	15	30	48	53	82	17
3	21	12	30	44	54	79	17
4	15	13	34	44	56	79	14
5	14	11	29	43	52	74	13
Avg	16,4	13,2	30,6	44,8	52,4	79,6	15,2

Protection persentageateach group for 30 minuteswas stated attable4below

Table4. Protection persentageat each group

Group	Stretching	% protection
I	16,4	79,40
II	13,2	83,42
III	30.6	61,56
IV	44,8	43,72
V	52,4	34,17
VI	79,6	0
VII	15,2	80,90

From table4 we knew that there was mice stretching after asetic acid injectionhad given intraperitoneally. There was bigger mice stretchinge few minutes after asetic acid injection had given intraperitoneally. After that mice stretching was decreased. Group I, II dan VII showed almost same mice stretching. Meanwhilein group III, IV dan V mice stretchingincreased in line with the increasing of sambiloto ethanol extract anddecreasingof kelor ethanol extract. At least amount of mice stretching(at most analgeticeffect) was obtainedinsambiloto extractcompared to kelor 2,5 : 7,5 (Group. II). From picture 1 weknewthat group I, group II andgroup VII showed almost same chartand can be stated that the three groups produced least mice stretching (most analgetic effect).

From Post Hoc test obtained that there was not significantdifference ($p > 0,05$) in analgetic testforgroup I, group II andgroup VII. Meanwhile there was significant different ($p < 0,05$) in analgetictest for group I withgroup III, group IV, group V andgroup VI.

From table4we can see that in group I, II and III there wasstretching protectionmore than 50%,meanedthat combination ethanol extract of sambiloto and kelor inspesific comparison hadperipheral analgetic effect. Most tretching protectiun (83,42%) obtainedatcombinationethanol extractof sambioloto and kelor 2,5 : 7,5 that more than stretching protectionof diclofenac sod (80,90%).

IV. CONCLUTION

1. Ethanol extractofAndrographispaniculataNees (sambiloto) andMoringa oleifera Lam (kelor)consisted of secondarymetabolite such as alkaloid, flavonoid, glycoside, saponin, steroid, triterpenoid dan tannin.
2. Ethanol extractin combination ofAndrographispaniculataNees (sambiloto) andMoringa oleifera Lam (kelor)had analgeticactivities on male mice which was given sense of pain by acetic acid injection intraperitoneally.
3. Ethnolextractin combination ofAndrographispaniculataNees (sambiloto) dan Moringa oleifera Lam (kelor)2,5 : 7,5hadbest analgetic effectand not signifikandifference withdiclofenacsodium.

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