



## COMPARATIVE EFFECTIVENESS OF DEFATTED HYPOTENSIVE CRUDE EXTRACT, ETHYL ACETATE AND BUTANOLIC FRACTIONS OF *CASSYTHA FILIFORMIS* L. ON DIFFERENT MODELS OF HYPERTENSIVE RATS

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Article Received on  
27 September 2014,

Revised on 21 Oct 2014,  
Accepted on 16 Nov 2014

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### ABSTRACT

The hypotensive effectiveness of defatted crude ethanolic extract, ethyl acetate and butanolic fractions of *Cassythia filiformis* L. have been compared on anesthetized prednisone-saline-induced (PN) and Prednisone-saline-LNAME -induced (PNL) hypertensive rats. Extract/fractions were given at the dose of 5 mg/kg each while a group treated by 100  $\mu$ mol/kg ofttempol was used as comparison. Extract/fractions/vehicle were commence in 3 consecutive doses intravenously every one hour interval. The systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) of the animals were measured (Biopac<sup>®</sup> MP 150

Data Acquisition System). Data were presented as the percentage changes of those parameters and analyzed by three way ANOVA followed by Duncan's Multiple Range Test. Results showed that the ethanolic defatted extract and tempol decreased animal SBP, DBP, MAP and HR significantly ( $p < 0.05$ ) while ethyl acetate and butanolic fractions did not ( $p > 0.1$ ). The average percentage decrease of animal SBP and MAP on PNL rats were higher ( $p < 0.05$ ) as compared to those on PN rats, while DBP and HR of those groups of animal were not significantly different ( $p > 0.1$ ). Repeated dose of all samples tent to decrease animal SBP and HR ( $p < 0.1$ ) but not their DBP and MAP ( $p > 0.1$ ). These results indicated that the hypotensive effectiveness of the defatted ethanolic extract of *Cassythia filiformis* is better as compared to its ethyl acetate and butanolic fractions and these effects are greater on oxidative stress related hypertensive rats.

**KEYWORDS:** hypotensive activity, prednisone-saline induced, prednisone-saline-L-NAME induced, *Cassytha filiformis*.

## INTRODUCTION

Hypertension is the most prevalent cardiovascular disorder with increasing prevalence year by year in the global world, including in Indonesia. It remains the major public health challenge that put a potential risk for organ damage, including the heart, brain, and kidney. More than 90% of the case are essential hypertension which is progressing without any identifiable medical causes. One of the most common proposed etiology is an imbalance between reactive oxygen species and nitric oxide (Dornas & Silva, 2011).

Stress oxidation can trigger many degenerative diseases. The reactive oxygen species (ROS) and nitric oxide (NO) unbalance will cause pathologic destruction of blood vessels, heart, liver and pancreas. Hypertension may be the impact of cardiovascular deterioration due to this condition which in a long period will result in the organ damage due to microcirculatory alterations (Cohuet *et. al.*, 2006).

*Cassytha filiformis* has been reported to have antioxidant activity (Mythili *et al.*, 2011 Dhanalakshmi *et al.*, 2012) and vasorelaxant (Tsai, *et. al.*, 2008), increase the bleeding time on mice (Armenia, 2007), decrease the body weight on high fat diet mice (Armenia, 2010) and reduce blood glucose if it is used for a longer period. The extract showed a low toxicity (Babayi *et al.*, 2007). According to our previous work, the ethanolic extract of this plant reduced blood pressure of hypertensive rats induced by prednisone-NaCl (Yori *et. al.*, 2013). In this research, we compare the effectiveness of defatted crude extract, butanol and ethyl acetate fractions of *C. filiformis* in lowering blood pressure on hypertensive rats induced by Prednison-NaCl and Prednison-NaCl-LNAME. This research result will support the government in developing a high quality of traditional medicine.

## MATERIAL AND METHODS

The n-hexane defatted ethanolic extract, ethyl acetate and butanol fractions of the dried *Cassytha filiformis* plant were used in this study.

### *Animal Preparation*

A number of 30 of 3-4 months old male Sprague-Dawley rats with the body weigh of 210 - 250g were divided into two groups. In Group 1 (PN), hypertension was induced by giving

prednisone-saline (prednisone 2.5 mg/kg and 2% salt administered through oral gavage for 2 weeks ) and In group 2 (PNL), hypertension was induced by giving prednisone-saline-L-NAME (combination of prednisone 2.5 mg/kg and 2% salt for 2 weeks + 20 mg/kg of oral LNAME for 2 days). The rats were used for further experiment when their systolic blood pressure  $\geq 150$  mmHg at the end of hypertensive induction process.

### ***Antihypertensive Activity Evaluation***

Each models of hypertensive rats were divided into 5 subgroups. Subgroup 1 of each group were as Control; subgroup 2, 3 and 4 were treated with defatted EtOH extract, EtOAc and BuOH fractions of the plant, respectively at the dose of 5 mg/kg, While subgroup 5 was treated with tempol 100  $\mu$ mol/kg. Direct measurement of blood pressure and heart rate was conducted through carotid artery (Biopac<sup>®</sup> MP150 Data Acquisition System) on anaesthetized rats (pentobarbital sodium 60 mg/kg i.p). The doses were commenced in 3 consecutive intravenous administrations (0.1% of BW in mL) every one hour interval. The systolic and diastolic blood pressure (SBP & DBP), mean arterial pressure (MAP) and heart rate (HR) of the animals were measured (Biopac<sup>®</sup> MP 150 Data Acquisition System). The percentage changes of the parameters were calculated in each periods relative to baseline values.

### **Data Analysis**

Data were presented as the percentage changes  $\pm$  SEM and analyzed by the three-way ANOVA followed by Duncan's Multiple Range Test. The confidence interval was taken at  $p < 0.05$ .

## **RESULTS AND DISCUSSION**

### **Results**

The average MAP of PNL group of the rats was significantly higher ( $p < 0.05$ ) as compared to that of PN animals (Figure 1). SBP and HR of the animal were significantly ( $p < 0.05$ ) influenced by the models of hypertension, extract/fractions and repeated doses, MAP was significantly ( $p < 0.05$ ) influenced by the models of hypertension and extract/fractions while DBP was influenced by extract/fractions. There was no significant ( $p > 0.1$ ) interactive influence of the model of hypertension, extract/fraction and repeated doses to, extract/fractions and repeated doses influences to SBP, MAP, DBP and HR of the animals. SBP, DBP, and MAP of the animal treated with Ethanolic extract of *C. filiformis* were significantly lower as compared to control group ( $P < 0.05$ ) but not in the HR and ( $p > 0.1$ ).

These effects were not significantly different than that of Tempol group. On the other hand,, BuOH and EtOAc fractions of this plant did not change all parameters significantly as compared to control group ( $P>0.1$ ). All of the parameter responses were greater on PNL as compared to those on PN animals ( $p<0.05$ ) except for HR, no significant different was obtained between these two groups (Fig 2 - 5).

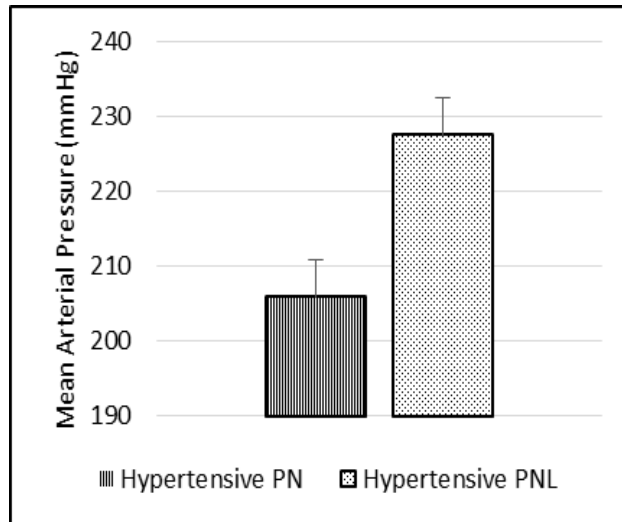


Fig 1: Comparative elevation of MAP between PN and PNL groups.

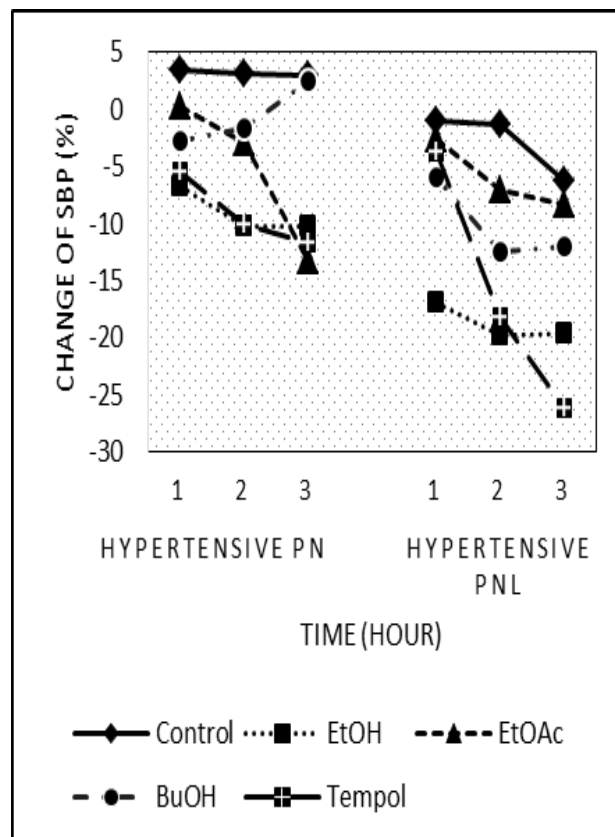
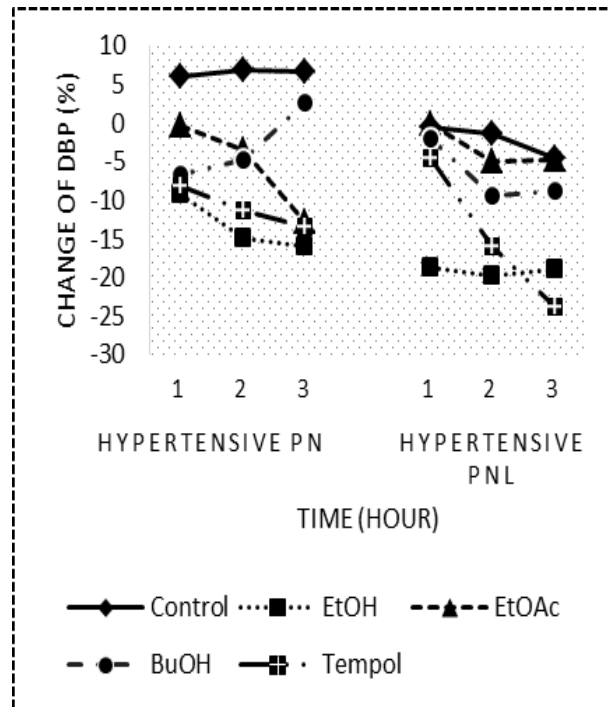
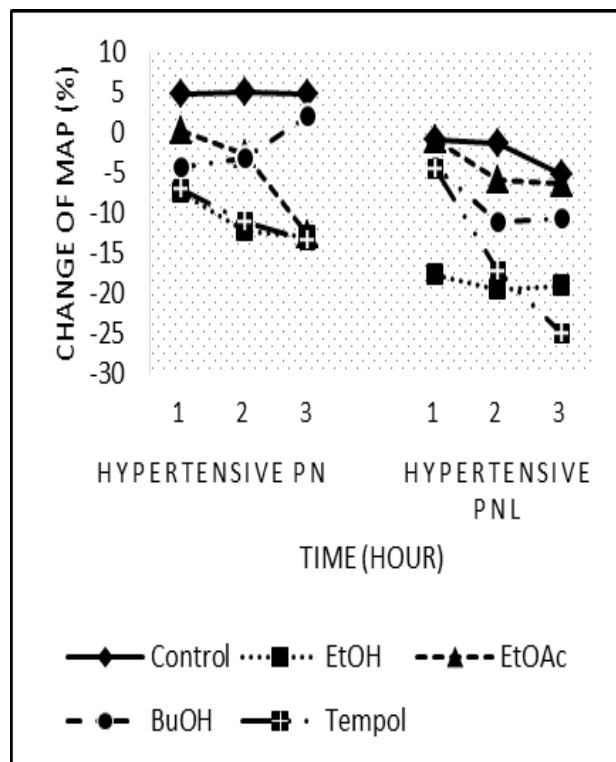


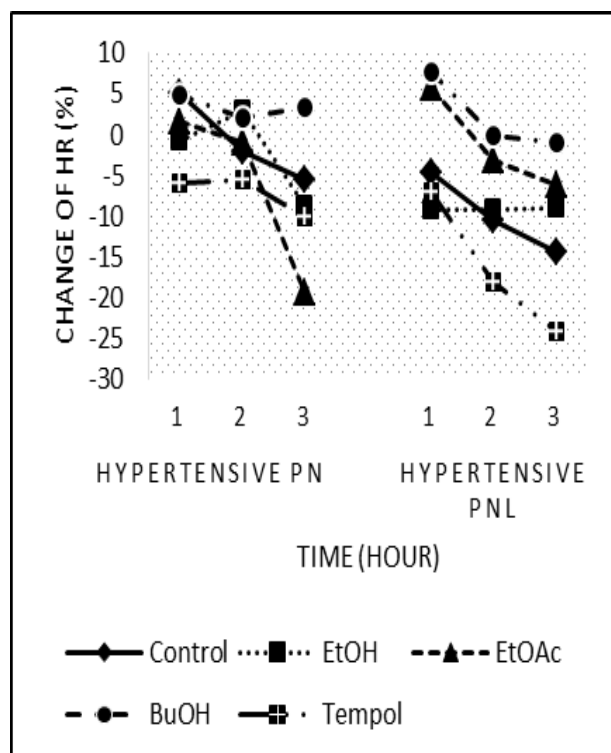
Fig 2. Change of SBP due to i.v. administration of *C. filiformis* extract/fractions in both hypertensive PN and PNL rats



**Fig 3: Change of DBP due to i.v. administration of *C. filiformis* extract/fractions in both hypertensive PN and PNL rats.**



**Fig 4: Change of MAP due to i.v. administration of *C. filiformis* extract/fractions in both hypertensive PN and PNL rats**



**Fig 5: Change of HR due to i.v. administration of *C. filiformis* extract/fractions in both hypertensive PN and PNL rats.**

## DISCUSSION

Our previous work has revealed a very potential blood pressure lowering activity of the crude extract of *Cassiytha filiformis*. In this study, further investigation was made to differentiate antihypertensive effectiveness of various fractions and the crude defatted extract itself on salt-prednisone and PN and PNL induced hypertensive rats. This will lead other researcher to or not to study the effect of single isolated compound(s), or prepare them as drug materials. It has previously been reported that potential pharmacological-active constituents of the plant are ranging from the very non-polar extract to the semi-polar fractions (Tsai *et al.*, 2008). That's why we fractionated the plant with those different solvents. We expect from those fractions, we would be able to obtain one or more fraction(s) which have potential effect as anti-hypertension. The use of various animal models of hypertension has been suggested to evaluate antihypertensive activity of plants since it may represents different pathogenesis and etiology of the disease (Badyal *et al.*, 2003; Manpreet *et al.*, 2011). A combination of oral prednisone and salt (PN) used to obtain hypertensive rats was firstly introduced in our previous work and has proven to effectively increase the blood pressure of the animals (Armenia *et al.*, 2007). Prednisone-salt combination was expected to cause endocrine-related

hypertension, just like one previously described by Badyal *et al.*, (2003) and Manpreet *et al.*, (2011).

Additional hypertensive type used in this study was the N<sup>G</sup>-nitro-L-arginine methyl ester (L-NAME) 20 mg/kg (PNL-type) for two days following the 14 days of oral administration of prednisone-salt. The chronic blockage of NO synthase (Ribeiro *et al.*, 1992) by L-NAME is expected to interfere its balance with reactive oxygen species. Actually, the addition of L-NAME caused some obvious extra elevation of blood pressure of the animal as compared to those induced by oral prednisone-salt only.

Extract and fractions were prepared to be administered intravenously through jugular vein. Hence, an n-hexane defatting process was taken to the crude ethanolic extract. All defatted EtOH extract and EtOAc and BuOH fractions of the plant were prepared as homogenous solution by adding Tween<sup>®</sup> 80.

Tempol (4-hydroxy TEMPO) has been a very popular referral for the evaluation of pharmacological active compounds of the plants related to oxidative stress. It exhibits both antioxidant and antihypertensive activity (Wilcox & Pearlman, 2008). In the present study, all extract/fractions showed potential blood pressure lowering effect in both models of hypertension compared to vehicle control group, especially the ethanolic extract while the BuOH and EtOAc fractions were less effective. The effectiveness of EtOH extract at 5 mg/kg is equal with Tempol of 1mg/kg. This hypotensive effect is in agreement with Tsai *et al.*, (2007) who reported that alkaloids and flavonoids content of this plant have vasorelaxant effect. The greater effectiveness of ethanolic extract compared to BuOH and EtOAc fractions indicated that there is an additive effect among components in the extract. This finding is in agreement with Chang *et al.*, (1998) who reported that *C. filiformis* contains 6 compounds in the methanolic extract that exhibit vasorelaxation. He further described that ocotein that available in this extract produced an alpha 1 adrenoceptor blocking activity. Similar result from ethanolic extract is also reported by Tsai *et al.*, (2007) due to its aporphin, morphinan, flavonoids and some other compounds. Accentuation of antihypertensive effectiveness of these extract/fraction in PNL animal can be understood since *C. filiformis* contains some good antioxidant activity compounds, especially in the ethanolic extract, as also reported by Mythili *et al.*, (2011).



## CONCLUSIONS

From this study it can be concluded that *C. filiformis* ethanolic extract exhibits a very potential and promising BP lowering effect as compared to its fractions. This activity was greater in the stress oxidative related hypertension. Therefore, it could be considered to be developed as a phytopharmaca.

## ACKNOWLEDGMENT

This study was supported by The Directorate General for Higher Education, Ministry of Education and Culture of Indonesia under the funding of “Fundamental Research Grant” (2013-2014).

## REFERENCES

1. Armenia. Efek ekstrak butanol tali putri sebagai antikoagulan pada mencit putih jantan. Padang: Universitas Andalas (Unpublished), 2007.
2. Babayi H M, Joseph J I, Udemé J A, Abalaka J I, Okogun O A, Salawu D D, Akumka Adamu S S, Zarma B B, Adzu S S, Abdulmumuni K, Ibrahim B B, Elisha S S, Zakariys U S Inyang. Effect of oral administration of aqueous whole extract of *Cassytha filiformis* on haematograms and plasma biochemical parameters in rats. *Journal of Medical Toxicology*, 2007; 3(4): 146-151.
3. Badyal D K, mH Lata and A P Dadhich, Animal Model of Hypertension and Effect of Drugs, *Indian J. Pharmacol*, 2003; 35: 349-362.
4. Chang F R, Chao Y C, Teng C M et al., Chemical constituents of *Cassytha filiformis* II, *J. Nat. Prod*, 1998; 61(7): 863-6.
5. Cohuet G, Struijker-Boudier H. Mechanisms of target organ damage caused by hypertension: therapeutic potential. *Pharmacol Ther*, 2006; 111: 81-98.
6. Dhanalakshmi P, Sagadevan E, Jaya Prakash Priya A, Chandra Sekhar Kommana, Jhansi A, Ramakrishna Rao M, Aroumougame S, Arumugam P, Umargani Jamal Mohamad and Mathivanan N. *International Journal of Biotechnology*, Phytochemical screening and in vitro antioxidant potential of *Cassytha filiformis*, 2012; 5(1): 001-007.
7. Dornas W C & M E. Silva. Animal Model for the Study of Hypertension, *J. Biosci*, 2011; 36: 731–737.
8. Manpreet, K., A. C. Rana, and K. Sunil. Induction of hypertension by various animal models. *Int J Pharm Bio Sci*, 2011; 1(3): 335-340.



9. Mythili, S., A. Sathiavelu, and T.B. Sridharan. Evaluation of antioxidant activity of *Cassythia filiformis*. *IJABPT*, 2011; 2(2): 380-385.
10. Ribeiro, M. O., E. Antunes, N.G. De, S.M. Lovisolo, and Zatz R. Chronic inhibition of nitric oxide synthesis. A new model of arterial hypertension. *Hypertension*, 1992; 20: 298-303.
11. Tsai, T. H., Wang G. J., and Lin L. C. Vasorelaxing alkaloids and flavonoids from *Cassythia filiformis*. *J Nat Prod*, 2008; 71: 289-291.
12. Wilcox, C. S. & A. Pearlman. Chemistry and antihypertensive effects of tempol and other nitroxides. *Pharmacol Rev*, 2008; 60: 418-469.
13. Yuliandra Y., Armenia and H. Arifin. Efek antihipertensi tumbuhan tali putri (*Cassythia filiformis* L.) pada tikus hipertensi yang diinduksi prednison dan garam, Prosiding Seminar Nasional “Perkembangan Terkini Sains Farmasi dan Klinik, 2013; 3: 264– 270.