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Research Article

Evaluation Antidiabetic Activity of Various Leaf Extracts of *Pluchea* indica Less

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ABSTRACT

Pluchea indica Less leaf extracts of various solvents (methanol, water, ethyl acetate) proven have antioxidant ability using DPPH radical scavenging effect, reducing power, total phenolic content and total flavonoid. However anti-diabetic ability and toxicity level of each extract have not even determined. This study was conducted to determine the effect of different polarity of solvent to phytochemical screening that cause reducing of blood glucose and influence toxicity level of laboratory animal.Our results showed that the water extract was the most potential as anti-diabetic agent. Its blood glucose reducing capability of Rattus Norvegicus Rats Winstar strain was 56.37 % that was the higher than glibenclamide (49.59%) and the other extracts (ethyl acetate extract = 19.11% and methanol extract = 24.27%). The water extract was also safe to the body healthy of Mus Musculus Mice (20-30 g), Balb/c strain. The male mice oral administration of water extract at 2.6 mg/20g b.w. increased motoric activity, however female mice at 1.3 and 2.6 mg/20 g b.w., respectively oral administration experienced motoric activity increasing and retablishment capacity decreasing. The potency of *Pluchea* leaf water extract as anti-diabetic agent was predicted that there was cardiac glycoside compounds contribution to reduce blood glucose.

Keywords: *Pluchea*indica *Less,* Anti-diabetic agent, Various Solven

INTRODUCTION

Type 2 diabetes mellitus (DM) is a disease of carbohydrate metabolism characterized by a number of metabolic abnormalities, including impaired pancreatic beta cell functions and insulin resistance in the skeletal muscles, adipose tissue, and liver[19,15,8]. This disease can be a major health problem and one of the leading causes of death. Recent record indicates that in 2010, an estimated 285 million people had diabetes of which type 2 were about 90% of the case[18,2]. WHO predicts that diabetes mellitus patients in Indonesia increase from 8.4 million at 2000 to around 21.3 million at 2030. According to International diabetes Federation (IDF) is predicted diabetes mellitus patients increasing to be 12 million at 2030. Hyperglycemia is a widely known cause of enhanced plasma free radical concentration and development of oxidative stress. Impaired generation of naturally occurring antioxidants in diabetes can also be expected to result in increased oxidative cell damage[19]. The dietary antioxidants can reduce toxicity of free radicals and protect the body from oxidative damage[15]. Herb plants are usually used as anti-hyperglycemicagent because they are composed phytochemical compounds acted as antioxidant. Bioactive compounds of plants can be effective anti-diabetic agents if they have capacity as anti-hyperglycemicand antioxidant agents[5,6]. Leaf of *Pluchea* indica Less has been used by people as traditional medicine because this herb plant has been proven containing many phytochemical compounds as lignin, terpene, phenylpropanoid, benzoid, alkanes[17], sterol, 2-(pro-1-unyl)-5-(5,6-dihydroxy hexa-1,3-diunyl)-thiophene, (-)-catechin[7], alkaloid[4], saponin, tannin, phenol, hydroquinone, flavonoid[23,24,25], cardiac glycoside[25], flavonol (quercetin, kaempherol, myricetin)[3]. Widyawati et al. (2014) informed that different polarity of solvent (methanol, ethanol, ethyl acetate, hexanes, and water) can extract different phytochemical compounds that have various antioxidant activities. Methanolic extract of *Pluchea* leaf is the highest potential as antioxidant agent (DPPH scavenging activity and iron ion reducing power, 794,9 mg/GAE/g samples dry base and 2,14 mg GAE/g samples dry base, respectively) because contains the biggest phenolic and flavonoid contents, 1185,2 mg GAE/g samples dry base and 911,9 mg CE/g samples dry base). Pramanik et al. (2006) also said that methanolic extract of *Pluchea* can reduce in blood glucose in normal (35,12% and 36,01%for 200 and 400 mg/kg, respectively) and in streptozotoxin induced diabetic rats (36,10% and 41,87% for 200 and 400 mg/kg, respectively). Srisook et al. (2012) has studied that hot water extract from *Pluchea*, herbal tea shows good antioxidant activity based on DPPH, superoxide and hydroxyl scavenging activities, ferric

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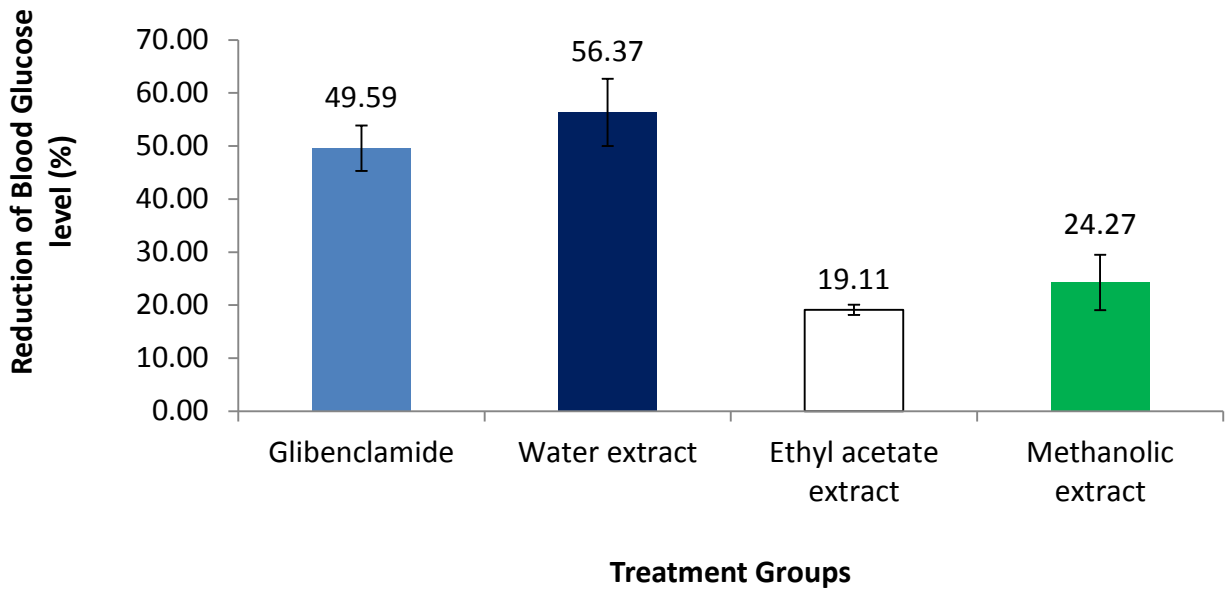

Figure 1. The effect of various extracts of *Pluchea*indica Less leaf of blood glucose level

ion reducing power, and ferrous ion chelating. Thus, the objective of this research was to determinethe effect of different polarity of solvent to phytochemical screening that cause reducing of blood glucose and influence toxicity level of laboratory animal.

MATERIALS AND METHODS

Plant material

Leaves of *Pluchea* indica Less at 1-6 segment levels were collected from areas at East Coast, Bendul Merisi, Keputih, and Wiyungin Surabaya and Kertosono, East Java, Indonesia.

Chemicals and reagents

Glibenclamide was purchased from medicine store in Surabaya, Indonesia, glucose assay kits (Sigma, USA). All the solvents and chemicals used were analytical grade.

Preparation of the plant extract

1-6 segment level of *Pluchea* leaves from peak was used as sample[24,9]. These leaves were dried at ambient temperature and grinded with 45 mesh size. Dried flour of *Pluchea* leaves was measured moisture content. And then this flour was extracted by different polarity solvent (water, methanol, and ethyl acetate) with soxhlet extractor at a boiling point for three hours. Extract was evaporated by rotary evaporator. The extract was stored at 4ºC in black glass bottle until analysis further.

Phytochemical screening

The freshly prepared crude extracts were qualitatively tested for presence of chemical constituents. These were identified by characteristic color changes using standard procedure. Phytochemical compounds in *Pluchea* leaves extract detected included alkaloid, flavonoid, phenolic, sterol, terpenoid, saponin, tannin, and cardiac glycoside[13].

Experimental animals

All the experiments were carried out using Mus Musculus Mice (20-30 g), Balb/c strain and Rattus Norvegicus Rats (125-150 g), Wistar strain of either sex that obtained from Rachmadi Priyadi's mice/rat husbandry, Surabaya, East Java, Indonesia. Theanimals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk asbedding. Animals were housed at a temperature of 24 ± 2ºC and relative humidity of 30–70%. A 12:12 light:day cycle was followed. All animals were allowed free access to water (*ad libitum*) and fed.The animals were used accordingly to guidelines of the Committee on Care and use of Experimental Animal Resources of LPPT-UGM, Jogjakarta, Indonesia.

Subchronic toxicity studies

Subchronic toxicity study was performed as per OECD-407 (1995). Albino mice was divided into 7 groups (n=8) of either sex selected by random sampling technique were used for the study. The animals were adapted in environmental and fed for one week before they were used. The animals were kept fasting for 8 hours providing only water, after that the suspended extracts (methanol, ethyl acetate, and water) with CMC-Na 0.5% were administered orally at the dose level 1.3 mg/20g b.w. by intragastric tube and observed for 28 days. The effect of clinicalof the experiment animal was observed for 4 hours at first day and the next day observation was done for 2 hours including deviation activity related to pharmacological screening assay and body healthy of the experiment animal. Morbidity and mortality of animalswere observed at least 2 times a day including motoric activity, haging activity, retablisment, flexion, cornea condition, pineal, haffner, posture, catalepsy, and straub. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not

Table 1: Phytochemical compounds in various *Pluchea* leaves extracts

Extract types	Color intensitas of phytochemical compounds							
	Terpenoid	Sterol	Flavonoid	Saponin	Tannin	Phenolic	Alkaloid	Cardiac glycoside
Aquadest	-	-	+++	++	+	+++	+++++++	+++++
Methanol	-	+++	+++++	++++	++++	+++++	++++++	+++
Ethyl acetate	-	+++	++	+	-	++	+++	+

Note: + detected based on color intensity, - not detected based on color intensity

Table 2. Effect of *Plucea*indicaLessleaf extract on mortality in experimental animals

Groups	Sample	Day									Mortality
		1	2	3	4	5	6	7	8	9-28	
Male mice											
I	Control	0	0	0	0	0	0	0	0	0	0
II	Water extract at 1.3 mg/20g b.w.	0	0	0	0	0	0	0	0	0	0
III	Water extract at 2.6 mg/20g b.w.	0	0	0	0	0	0	0	0	0	0
IV	Methanol extract at 1.3 mg/20g b.w.	0	0	2	0	1	0	1	0	0	4
V	Methanol extract at 2.6 mg/20g b.w.	0	0	1	2	1	2	0	2	-	8
VI	Ethyl acetate extract at 1.3 mg/20g b.w.	0	1	3	1	2	1	-	-	-	8
VII	Ethyl acetate at 2,6 mg/20g b.w.	0	5	3	-	-	-	-	-	-	8
Female mice											
VIII	Control	0	0	0	0	0	0	0	0	0	0
IX	Water extract at 1.3 mg/20g b.w.	0	0	0	0	0	0	0	0	0	0
X	Water extract at 2.6 mg/20g b.w.	0	0	0	0	0	0	0	0	0	0
XI	Methanol extract at 1.3 mg/20g b.w.	0	0	2	0	0	0	1	0	2	5
XII	Methanol extract at 2.6 mg/20g b.w.	0	3	5	-	-	-	-	-	-	8
XIII	Ethyl acetate extract at 1.3 mg/20g b.w.	0	2	5	1	-	-	-	-	-	8
XIV	Ethyl acetate at 2,6 mg/20g b.w.	0	4	3	1	-	-	-	-	-	8

observed, the procedure was repeated for further higher such as 2.6 mg/20g b.w.

Effect of *Pluchea* extracts on normal blood glucose level

Rats were divided into five groups (n = 5) andfasted for 10 h before the experiment. Rats were administered 50% (5 ml/kg b.w.) load followed by suspended extracts (with CMC-Na 0.5%) of *Pluchea* indica Less leaves and reference drug suspended glibenclamide (with CMC-Na 0.5%) orally by a cannula just after checking the fasting blood glucose. First group received the control vehicle 0.5% w/v CMC-Na. The methanol, ethyl acetate, and water extracts of *Pluchea* indica Less leaves (9 mg/200g animal body weight) were administered to second until fourth groups, respectively. The fifth group received the standard drug glibenclamide (0.126 mg/200 gb.w.) for assessingthe comparative pharmacological significance[21,11]. Blood was withdrawn from the tail vein by cutting the tip of the tail at 0, 15, 30, 60, 90 and 120 minutes of glucose administration and glucose levels were estimated immediately using compatible blood glucose test strips of glucometer (GlucoDr).

Table 3. Pharmacological screening assay of experimental animal after oral administration

Groups	Number	Motoric activity	Haging activity	Retablisment	Flexion	Cornea Condition	Pineal	Haffner	Posture	Catalepsy	Straub
Male mice											
Control	1	+	+	+	+	+	+	-	+	-	-
	2	+	+	+	+	+	+	-	+	-	-
	3	+	+	+	+	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	-	+	-	-
	6	+	+	+	+	+	+	-	+	-	-
	7	+	+	+	+	+	+	-	+	-	-
	8	+	+	+	+	+	+	-	+	-	-
	1	+	+	+	+	+	+	-	+	-	-

Table 3. Pharmacological screening assay of experimental animal after oral administration

Groups	Number	Motoric activity	Haging activity	Retablisment	Flexion	Cornea Condition	Pineal	Haffner	Posture	Catalepsy	Straub
Water extract at 1.3 mg/20g b.w.	2	+	+	+	+	+	+	-	+	-	-
	3	+	+	+	+	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	-	+	-	-
	6	+	+	+	+	+	+	-	+	-	-
	7	+	+	+	+	+	+	-	+	-	-
	8	+	+	+	+	+	+	-	+	-	+
Water extract at 2.6 mg/20g b.w.	1	++	+	+	+	+	+	-	+	-	-
	2	++	+	+	+	+	+	-	+	-	-
	3	+++	-	+	+	+	+	+	+	-	-
	4	+	+	+	+	+	+	-	+	-	-
	5	+++	+	-	+	+	+	-	+	-	-
	6	+	+	+	+	+	+	-	+	-	-
	7	+++	+	+	+	+	+	-	+	-	-
	8	+++	+	+	+	+	+	-	+	-	-
Methanol extract at 1.3 mg/20g b.w.	1	+	+	+	+	+	+	-	+	-	-
	2	+	+	+	+	+	+	-	+	-	-
	3	+	+	+	+	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	-	+	-	-
	6	+	+	+	+	+	+	-	+	-	-
	7	+	+	+	+	+	+	-	+	-	-
	8	+	+	+	+	+	+	-	+	-	-
Methanol extract at 2.6 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†
Ethyl acetate extract at 1.3 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†
Ethyl acetate at 2,6 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†
Female mice											
Control	1	+	+	+	+	+	+	-	+	-	-
	2	+	+	+	+	+	+	-	+	-	-
	3	+	+	+	+	+	+	-	+	-	-
	4	+	+	+	+	+	+	-	+	-	-
	5	+	+	+	+	+	+	-	+	-	-
	6	+	+	+	+	+	+	-	+	-	-
	7	+	+	+	+	+	+	-	+	-	-
	8	+	+	+	+	+	+	-	+	-	-
Water extract at 1.3 mg/20g b.w.	1	++	+	-	+	+	+	-	+	-	-
	2	++	+	-	+	+	+	-	+	-	-
	3	++	+	-	+	+	+	-	+	-	-
	4	++	+	-	+	+	+	-	+	-	-
	5	++	+	+	+	+	+	-	+	-	-
	6	++	+	-	+	+	+	-	+	-	-

Table 3. Pharmacological screening assay of experimental animal after oral administration

Groups	Number	Motoric activity	Hagging activity	Retablisment	Flexion	Cornea Condition	Pineal	Haffner	Posture	Catalepsy	Straub
	7	++	+	-	+	+	+	-	+	-	-
	8	+	+	-	+	+	+	-	+	-	-
Water	1	++	+	-	+	+	+	-	+	-	-
extract at	2	++	+	-	+	+	+	-	+	-	-
2.6 mg/20g	3	++	+	--	+	+	+	-	+	-	-
b.w.	4	++	+	-	+	+	+	-	+	-	-
	5	++	+	--	+	+	+	+	-	-	-
	6	++	+	-	+	+	+	+	-	-	-
	7	++	+	-	+	+	+	+	-	-	-
	8	++	+	-	+	+	+	+	-	-	-
Methanol	1	+	+	-	+	+	+	+	-	+	-
extract at	2	+	+	--	+	+	+	+	-	-	-
1.3 mg/20g b.w.				(drop after large time)							
	3	+	+	--- (drop directly)	+	+	+	+	-	-	-
	4	+	+	--- (drop directly)	+	+	+	+	-	-	-
	5	+	+	--- (drop directly)	+	+	+	+	-	-	-
	6	+	+	--- (drop directly)	+	+	+	+	-	-	-
	7	+	+	--- (drop directly)	+	+	+	+	-	-	-
	8	+	+	--- (drop directly)	+	+	+	+	-	-	-
Methanol extract at 2.6 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†
Ethyl acetate extract at 1.3 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†
Ethyl acetate at 2,6 mg/20g b.w.	1-8	†	†	†	†	†	†	†	†	†	†

Note :+ or - = deviation of experimental animal, + = normal condition at motoric and hanging activities, retablishment, posture, flexion, cornea condition, and pineal, - = normal condition at haffner, straub, catalepsy, and straub, † = mortality

RESULTS

Phytochemical compositions of various extracts of *Pluchea* leaves wereshowed at Table 1. Data informed that phytochemical compositions of three types of *Pluchea* leaf extract were different. The qualitative analysis based on color intensity showed that methanol solvent was the most effective to extract phytochemical compounds compared with the other solvents.Their compounds detected in methanol extract included sterol, flavonoid, saponin, tannin, phenolic, alkaloid and cardiac glycoside. Whereas water extract wasn't detected sterol and ethyl acetate extract wasn't detected tannin. Sub chronic study showed that methanol and ethyl acetate extracts of *Pluchea* leaves at 1.3 and 2.6 mg/20g b.w. caused mortality effect whereas the water extract are safe for long term administration (Table 2). Pharmacological screening effect (Table 3) showed that the oral administration of water extract at male mice was safe, but its consumption at 2.6 mg/20g b.w. could cause motoric activity increasing. Whereas female mice that was given water extract of *Pluchea* leaves at 1.3 and 2.6 mg/20 g b.w., respectively showed motoric activity increasing and retablishment capacity decreasing. Methanol extract given at 1.3 mg/20g b.w. was no clinical effect at male experimental animals but it gave effect at female experimental animals. Methanol extract at 2.6 mg/20g b.w. and ethyl acetate at 1.3 and 2.6 mg/20g b.w., respectively caused mortality. Blood glucose level at experimental animal after oral administration from various extract of *Pluchea* indica *Less* showed at Figure 1. Water extract of *Pluchea* leaves was highest potentially to reduce blood glucose level compared with the other extract.

DISCUSSION

Our study showed that *Pluchea* leaves extract with various polarity of solvent possessed antidiabetic activity based on blood glucose reducing capacity. Adesegun et al. (2013); Kwon et al. (2006) informed that postprandial hyperglycemia is one of the risk factors associated with type 2 diabetes mellitus. Mohammed et al. (2006) said that hyperglycemia is caused by free radical accumulation on plasma and development of oxidative stress. *Pluchea* indica Less leaves contained phytochemical compounds so that could act as natural sources of antioxidants useful to health. Widyawati et al. (2014) has showed that leaves extract of *Pluchea* with various polarity of solvent can scavenge DPPH free radical and reduce iron ion. The observed activity may be due to the presence ofchemical constituents such as phenolic compounds (tannins and flavonoids) in the extract. This argument is supported by[10,14]. Phenolics have been reported to inhibit α-amylase activities. They also haveanti-hyperglycemic activity and inhibit the development of diabetes[12,26]. The antidiabetic activity of water extract was the highest, notiwithstanding Widyawati et al. (2014) said that its DPPH scavenging activity is lower than methanol extract. It means that antidiabetic activity is not only depended by hydrogen donating capacity of phenolic compounds. It was predicted that there were involvement constituent chemical reducing glucose (cardiac glycoside test). The oral administration test also showed that water extract was safe that it was potentially as antidiabetic agent.

CONFLICTS OF INTEREST

All contributing authors declare no conflicts of interest.

CONCLUSION

This study showed that the *Pluchea*leaf water extract possessed anti-diabetic activity based on blood glucose reducing capability. Its ability was 56.37 % that was the higher than glibenclamide (49.59%) and the other extracts (ethyl acetate extract = 19.11% and methanol extract = 24.27%). The water extract was safe to the body healthy. The anti-diabetic activity of this extract was supported by cardiac glucose compounds content.

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