

Diuretic Activity of Kalumpang (*Sterculia foetida* L) Methanolic Leaf Extract in Male Albino Sprague Dawley Rats

Laarni Hannah Lacorte, Jia Lianne Ang, Donna Ferrer, Samantha Eryll Lorenzo, Marianne Khate Oandasan, Stephanie Ann Santos, Audrey Mae Tan Hoc and Eunice Camille Umayam

Department of Medical Technology, Far Eastern University Manila, Nicanor Reyes Street, Sampaloc, Manila 1008, PHILIPPINES.

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ABSTRACT

Introduction: Diuretics are used in patients with hypertension, edema and congestive heart failure. It functions by increasing water secretion into the urine causing a reduction in blood volume and pressure although most diuretics in the market have side effects in contrast to herbal medication with less toxicity. Leaves of *Sterculia foetida* (Kalumpang) has been used as diuretics in folkloric practices in the Philippines. **Aim:** This study was conducted to evaluate the diuretic activity of the methanolic extract of Kalumpang in male albino Sprague Dawley rats. **Methods:** Five groups were utilized in the study, negative control, positive control with drug furosemide and experimental group with extract of Kalumpang prepared in low to high concentrations 150, 250 and 350mg/kg. Urinary volume, pH, specific gravity and urinary electrolytes were measured in 5 hour. Data examined using one-way analysis of variance (ANOVA) and *t*-test, diuretic property of the extract was measured by modified Lipschitz method. **Results:** The result indicated that *S. foetida* in low concentration (150mg/kg) has a moderately positive diuretic index capacity of 1.17, and lipschitz value of 0.66 with an increase in potassium secretion and low sodium ions in the urine. Percentage increase in urine volume was measured hourly and was noted at 1st (12.75%) and 2nd hour (337.5%). Phytochemical analysis revealed presence of metabolites – alkaloids, flavonoids, sterols, tannins and triterpenes. **Conclusion:** The finding of this study provided scientific credential for the folkloric use of *Sterculia foetida* L (Kalumpang) plant extract using quantitative analysis in the diuretic property.

Key words: Diuretics, *Sterculia foetida* L, Folkloric, Diuretic activity.

Correspondence:

Laarni Hannah Lacorte,
Department of Medical
Technology, Far Eastern
University Manila, Nicanor
Reyes Street, Sampaloc
Manila 1008, PHILIPPINES.

E-mail: llacorte@feu.edu.ph

INTRODUCTION

According to World Health Organization in a study conducted in 2014, congestive heart failure is the leading diagnosis of hospitalized patient among adults aged 19 years and above in the Philippines and its 17 regions^[1]. CHF consists in a gradual reduction in heart function wherein there is an insufficient supply of oxygenated blood to the organs of the body. A serious medical condition known as “hypertension” or “high blood

pressure” occurs when the blood passes through the blood vessels of the heart with a greater force than the normal.^[2] Heart attack is the most common cause of death among Filipinos. A study conducted in 2008 conducted by Philippine Society of Hypertension showed that 21 percent of Filipino adults has hypertension^[3]

One of medications prescribed to help treat hypertension and congestive heart failure is by taking diuretics, also known as “water pills”. The use of diuretics is to increase the drainage of water and salt (sodium) into the urine, causing a reduction of blood pressure, a decrease in blood volume, and thus lowering resistance to the flow of blood^[4]. However, most diuretics in the market are allopathic which can have side effects. These side effects such as dizziness, thirstiness, rash or itching,

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ringing in the ears (loop diuretics), potassium, and/or magnesium levels in the blood (loop diuretics), high potassium levels in the blood (potassium-sparing diuretics), higher blood glucose or cholesterol level.^[5-6] In contrast, herbal medications are free from side effects and toxicity compared to allopathic medicines.

The plant Kalumpang (*Sterculia foetida* L.) belongs to the family of Malvaceae. It can be found in the stretch of Northern Luzon to Palawan and Mindanao. Researches states the other uses of plant Kalumpang (*Sterculia foetida* L.) which includes antimicrobial, antioxidant, anti-convulsant, antidiabetic, and so on. Literature reviews suggested that the Kalumpang (*Sterculia foetida* L.) leaf extract's diuretic property is only known to be folkloric and no significant studies has been made yet. Such evidence is needed to provide scientific credentials as to the folkloric use in diuretics of the plant Kalumpang (*Sterculia foetida* L.) which may aid in the development of medicinal science.

Thus, this study is conducted to evaluate and provide information regarding to the diuretic activity of the folkloric plant, Kalumpang (*Sterculia foetida* L.) in albino methanolic leaf extract in male albino Sprague Dawley rats.

METHODOLOGY

Plant collection, authentication and extraction

Kalumpang leaves were collected at Nasugbu, Batangas. It was then authenticated and certified at the Forest Products Research and Development Institute in Department of Science and Technology, Laguna. A sample of the Kalumpang leaf was brought to the institute for verification of its kind. It is believed that the plant is classified as *Sterculia foetida* L. (Kalumpang) plant for it has the characteristics present of the plant – smooth, leathery, with pointed tip.

From the collected kalumpang leaves, it was processed for drying in sunlight and pulverized leaves using Wiley mill. The 675.0 g obtained from the pulverized leaves was soaked in 4.0 L of methyl alcohol for 48 hours to produce a mixture. After 48 hours of soaking, the mixture was filtered and the filtrate obtained was concentrated using rotary evaporator at 60°C under vacuum for three hours. To obtain a semi-solid extract, the concentrated extract was further evaporated using water bath at 60°C. The crude extraction of dried kalumpang leaves, 675.0 g produced 3.5 L methanolic extract and the concentration of the filtrate yielded 31.0 g of a semi-solid extract with a percentage yield of 4.6%.^[7]

Experimental Animals: Male Albino Sprague Dawley rats

Thirty male albino Sprague Dawley rats were used as the test sample in the study randomly assigned into five groups containing six animals in each. Each male rats were classified to be at least 11 – 12 weeks old weighing about an average of 220 – 280 grams. Under the ethical considerations of the animals, they are subjective to treat with an extent comfort. The animals was kept under standard condition in an animal housing facility located in Department of Science and Technology (DOST) – Standard Test approved by the committee and also under the supervision of a veterinarian. The rats was housed in polypropylene cages with bedding and maintained at 27°C; the animals were given standard diet (commercial pellets) prior to the experimentation. To exclude external factors in the study, 12-16 hours prior to commencement of the experiment, the rats underwent fasting with no access to food or water and was then subjected to weighing to give the concentrations assigned to each.

Preparation of Control and Standard

The preparation of the control and experimental groups are discussed as follows. Rat samples were weighed first to determine the concentration that will be given to each sample. Three (3) increasing doses of the test sample – the control group, Kalumpang extract in 150mg/kg, 250mg/kg and 350mg/kg, the positive (Furosemide) and negative (Normal Saline Solution) controls and 2.5ml NSS/100g body weight were administered orally thru gavage and measured every hour for five (5) hours. The formula used for the basis of computation of concentration dosages given to each animal depending on the weight are as follows concentration per animal = computed weight times dosage over concentration.

The study utilized an experimental design to determine the diuretic activity of *Sterculia foetida* L. (Kalumpang) methanolic leaf extract in male albino Sprague Dawley

Table 1: Sample population and the treatment assigned per group in the study

Group	Dosage	Sample Population
Negative (NSS)	Normal Saline solution	6
Positive (Furosemide)	20 mg/kg	6
Kalumpang extract	150 mg/kg	6
Kalumpang extract	250 mg/kg	6
Kalumpang extract	350 mg/kg	6
TOTAL		30 albino rats

rats. Controls were prepared using the standard saline solution. Urine output of the samples were analyzed; parameters such as volume, pH, specific gravity and urinary electrolytes were measured to evaluate the significant effect of the *Stercula foetida* L. (Kalumpang) extract in low, moderate to high concentrations 150mg/kg, 250mg/kg and 350mg/kg respectively. Thirty (30) male albino Sprague Dawley Rats were randomly assigned into five groups containing 6 animals in each. Negative control with normal saline solution. Positive control provided with furosemide 40 mg/kg per body weight while Group III, IV and V were the experimental group which received the *Stercula foetida* L. (Kalumpang) leaf extract in 150mg/kg, 250mg/kg and 350 mg/kg per body weight accordingly.

Collection of Urine & Measurement of Outputs

Each group containing six rats in each was put in a metabolic cage, a urine collecting device to gather the urinary output excreted by the rats in a 5 hour experimentation study. The metabolic cage is made up of fiber glass with a food and feeds supply connected to the cage, beneath it is a strainer-like metal to separate the urine from the feces excreted during the experimentation. The urine will freely flow into the metal tube into the beaker. The urine collected will then be subjected to measurement of volume using graduated cylinder in an hourly manner and measurement of urinary pH and specific gravity with the use of reagent strips. Data were examined using one way analysis of variance (ANOVA) followed by T test, diuretic property of the extract was measured by modified Lipschitz method.

Analytical Procedure

In this study, the rat's urinary electrolytes such as Na⁺, K⁺, and Cl⁻ were also measured. To test the electrolytes of the urine, the machine used in the study was EasyLite Analyzer Machine that uses principle of ISE (Ion Selective Electrode) technology. 100 ul urine samples were added to 900 ul urine diluents to become diluent sample with the ratio of (1:10). The sample container is positioned so that the probe hole is well below the surface of the sample. Hold the sample container in place until the probe automatically rises and wait until analyzing the specimen is finished and record the results for Na⁺, K⁺, and Cl⁻. Ratios of electrolytes were calculated to evaluate the natriuretic, saluretic inhibitory and carbonic anhydrase activity of the extract used.

Phytochemical Analysis

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the

unmatched availability of chemical diversity [8]. Phytochemical screening test was done on the Kalumpang extract using standard procedures to identify the presence of metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols, tannins, and triterpenes to identify which metabolite contributes to the diuresis component of the extract.

Acute Toxicity Test (LC₅₀)

Brine Shrimp Lethality Assay was used to determine the toxicity of the various concentrations and pure compounds present in the extract used in the study. Brine shrimp eggs, *Artemia salina* were hatched in artificial sea water with a light source for 48 hours. An average of 50 brine shrimp nauplii were selected and transferred in vials which contains the solution of the crude plant extract. Determination of lethal concentration LC₅₀ was calculated from the mean survival nauplii present in each vials. The importance of Acute Toxicity in the study is to determine the concentration of a drug that can caused adverse effect in a particular animal species. It is attained by a single or multiple exposures of an animal in a substance in short period of time, usually less than or within twenty – four hours. The LC₅₀ (Lethal Concentration 50) is defined as the concentrations of a substance that kills 50% of the population and usually measured using the unit of mg/ml.

RESULTS

Urinary volume, pH and specific gravity

Table 2: Urine volume, pH and sg of the samples in 5 hour collection period

Group	Total urine Volume (mL)	Mean urine volume (mL)	pH	sg
Negative (NSS)	38.3	7.66	7	1.05
Positive (Furosemide)	68.6	13.72	6.5	1.10
Kalumpang Extract (150mg/kg)	45.1	9.02	6.5	1.10
Kalumpang Extract (250mg/kg)	33.1	6.62	6.5	1.10
Kalumpang Extract (350mg/kg)	34.3	6.68	6.5	1.10

Table 2.2: Percentage increase of urine volume by hour

Group	1 st hour	2 nd hour	3 rd hour	4 th hour	5 th hour
Positive (Furosemide)	37.25	396.88	33.87	100	76
Kalumpang extract 150mg/kg	12.75	337.5	0	0	0
Kalumpang extract 250mg/kg	0	9.37	0	0	12
Kalumpang extract 350mg/kg	0	21.88	35.48	6.67	0

Table 2.3: Ttest and sig p value of extract in Negative and Positive

Kalumpang Extract Conc	Mean Difference (rel. to the Negative Control)	t Value	Sig. (p-value)
Kalumpang 150mg/kg	-1.36	-0.258	0.803
Kalumpang 250mg/kg	1.04	0.221	0.830
Kalumpang 350mg/kg	0.8	0.198	0.849
Concentration	Mean Difference (rel. to the Positive Control)	t Value	Sig. (p-value)
Kalumpang 150mg/kg	4.7	0.812	0.440
Kalumpang 250mg/kg	7.1	1.35	0.215
Kalumpang 350mg/kg	6.86	1.46	0.195

Urinary Electrolytes

Table 3: Urinary electrolytes, Saliuretic index, Natriuretic Index and Carbonic Anhydrase ratio

Group	Urinary Electrolyte Concentration (mmol/L)			Saluretic index			Natriuretic index	Carbonic anhydrase
	Na ⁺	K ⁺	Cl ⁻	Na ⁺	K ⁺	Cl ⁻		
Negative (NSS)	160.40	88.20	213.90				1.82	0.86
Positive (Furosemide)	196.60	211.30	267.90	1.23	2.40	1.25	0.93	0.66
Kalumpang 150 mg/kg	132.40	163.90	197.00	0.83	1.86	0.92	0.81	0.66
Kalumpang 250 mg/kg	157.70	207.30	230.30	0.98	2.35	1.08	0.76	0.63
Kalumpang 350 mg/kg	97.50	113.10	204.50	0.61	1.28	0.96	0.86	0.97

Diuretic Action and Activity

Table 4: Measuring diuretic action and activity.

Group	Diuretic Action	Diuretic Activity (Lipschitz Value)
Negative (NSS)	1	
Positive (Furosemide)	1.79	1
Kalumpang 150mg/kg	1.17	0.66
Kalumpang 250mg/kg	0.86	0.48
Kalumpang 350mg/kg	0.89	0.5

Phytochemical Analysis

Table 5: Phytochemical Analysis

Phyto-constituents	<i>Stercula foetida</i> L. leaf extract
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	-
Sterols	++
Tannins	++
Triterpenes	+

(-) absence of constituents, (+) traces, (++) moderate and (+++) abundant
Source: Pharmacognosy, 15th edition, 2002, Trease & Evans. Data are approved by MA. Rachel V. Parcon, RCh (Head, Organic Chemistry Section at DOST).

Acute Toxicity Test

Table 6: Acute Toxicity Test

Group Number	Dosage (mg)	Number of brine shrimp	Mortality	Mortality %
I	150 mg	50	33/50	66%
II	250 mg	50	38/50	76%
III	350 mg	50	41/50	82%
IV	500 mg	50	47/50	94%
V	1000 mg	50	50/50	100%

DISCUSSION

Urinary volume, pH and specific gravity

Table 2.1 shows the total and mean urine volumes computed for the five-hour duration for the different controls and treatments. Between the three (3) concentrations of Kalumpang, it is noted that the concentrations 250mg/kg and 350 mg/kg have total volumes below the total volume of the negative control. Only the 150 mg/kg concentration has total urine volume greater than that of the negative control. Meanwhile, urinary pH and sg of the positive group (furosemide 40mg/kg) and of the Kalumpang extract in different concentrations has the same measured acidic pH of 6.5 and significant gravity of 1.10. While the negative group with the normal saline solution has a neutral pH of 7 and sg of 1.10.

Table 2.2 depicts the percentage increase in urinary output per hour in a span of 5 hour experimentation proper was also measured in the study. The positive group exhibited the highest increase in one hour of urine excretion with 37.25% increase followed by the Kalumpang extract in 150mg/kg with 12.75% while the other concentrations of Kalumpang did not exhibit a percentage increase at the first hour. During the second hour of excretion both the positive group (396.88%) and Kalumpang extract with 150mg/kg concentration (337.5) showed a peak in percentage increase with the two being closely related. On the fourth and fifth hour, the positive control group showed a decline increase in urine with 33.87%, 100% and lastly 76%. Both the extract of Kalumpang in 150mg/kg and 250mg/kg showed no increase in the last three hours of urine collection period.

The regression slopes which depict the decline in the urine volume during the 5-hour period are shown. It is noted that the positive control (-5.11) and the 150 mg/kg concentration (-5.26) showed close regression slope values which means that the rates of decrease in the urine volume during the 5-hour period are the same for both. This means that, in general, the positive control and the 150 mg/kg concentration of Kalumpang exhibited the same trend in terms of the urine volume. On the other hand, the negative control (-3.3) and the 250 mg/kg (-3.44) and 350 mg/kg (-2.59) concentrations of Kalumpang exhibited almost the same regression slopes.

Table 2.3 shows the result of the One-Way Analysis of Variance (ANOVA) to test the significant difference between the three concentrations of Kalumpang. The null hypothesis that the three concentrations have no significant difference in terms of the urine volume for

the 5-hour period is tested at 0.05 level of significance. The ANOVA showed a p-value of 0.860 which means that there is no conclusive evidence to reject the null hypothesis. This result may suggest that there is no significant difference between the three concentrations in terms of the urine volume for the 5-hour period.

Statistically, the data suggests that there is no conclusive evidence to suggest that the three (3) concentrations have similar or different performance between each other and with the positive and negative controls. However, Tables 1 and 2 numerically suggest that the 150 mg/kg concentration of Kalumpang is the only concentration with higher urine volume compared to the control. Moreover, regression slopes on urine volumes over the 5-hour period suggest that the positive control and the 150 mg/kg concentration of Kalumpang exhibited similar trend and possibly the same diuretic activity.

Urinary Electrolytes

In table 3, urinary electrolytes were also measured to evaluate the concentrations of ions secreted by the rats' sample during the 5 hour collection period. The positive group (furosemide) as predicted showed an increase in all parameters of urinary electrolyte concentrations, sodium, potassium and chloride ions in comparison to those of the negative group which received NSS treatment.

Saliuretic Activity

Saluretic index of the table above shows highest ratio in Na⁺ (Sodium) of the positive control which is the Furosemide, with 1.23. Followed by 250 mg/kg, 150 mg/kg, and 350 mg/kg of kalumpang with values of 0.98, 0.83, and 0.61 respectively. It is observed that the K⁺ (Potassium) and Cl⁻ (Chloride) content of the solutions has increased values compared with the Na⁺. Furosemide has still the highest ratio of 2.40 (Potassium) and 1.25 (Chloride), still followed by 250 mg/kg of Kalumpang with 2.35 (Potassium) and 1.08 (Chloride). But the lowest ratio of Cl⁻ is different from the lowest ratio of K⁺ and Na⁺. The lowest ratio of Cl⁻ can be seen in Kalumpang that has 150 mg/kg solution with the ratio of 0.92 as to compare with K⁺ and Na⁺ that can be seen in 350 mg/kg of Kalumpang solution with the ratio of 1.28 and 0.61 respectively.

Natriuretic Activity

Natriuretic activity index is used to measure the excretion of sodium in urine. The value of Na⁺/K⁺ ratio that is greater than 2.0 indicates a favorable natriuretic effect and ratios greater than 10.0 is indicative of potassium – sparing effect. The negative (NSS) with the ratio of 1.82, the nearest result of all the variables to the favorable natriuretic effect ratio, is not considered to be significant

in the excretion of sodium in the urine. The table above shows that the use of Kalumpang extract with different dosages results have no known potential to increase the excretion of sodium in urine.

Carbonic Anhydrase

The carbonic anhydrase inhibition is excluded with ratios between 1.0 and 0.8. Lower than these ratios is an indicative of strong inhibition of carbonic anhydrase. Inhibition of carbonic anhydrase in the body causes diuresis or increase production of urine. The kalumpang extract with the dosage of 250 mg/kg has the ratio of 0.63 is strong indicative of carbonic anhydrase inhibition. Compared with the other Kalumpang dosages and the standard furosemide their results clearly shows that 250 mg/kg of Kalumpang extract has greater effects in carbonic anhydrase inhibition.

Diuretic Action and Activity

Table 4 shows the diuretic action and activity were measured to compare the urinary output of the five groups. Kalumpang extract in 150mg/kg concentration showed 1.17 diuretic action compared to the negative and 0.66 in contrast with the positive. While other values of Kalumpang in 250mg/kg and 350mg/kg were lower than those of in 150mg/kg concentration. The value obtained in diuretic action of the Kalumpang in 150mg/kg is much closer to the negative group which indicates that active component/s of Kalumpang extract could produce diuresis but not comparable to the standard drug used in the study.

Diuretic index of >1.50 indicates good diuretic activity, if between 1.00 to 1.50 moderately diuretic activity, 0.72 to 1.00 indicates little diuretic activity and if it is less than 0.72 indicates no diuretic activity^[9].

Phytochemical Analysis

Table 5 shows the phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities (Sofowra, 1993). Analysis of the plant *Sterculia foetida* L. (Kalumpang) methanolic extracts revealed the presence of phytochemicals such as alkaloids, tannins, flavonoids, glycosides, sterols, and triterpenes. Tannins and sterols are observed to be present in moderated proportion. While traces of alkaloids, flavonoids, glycosides and triterpenes are present and saponin being absent.

Acute Toxicity Test

Acute Toxicity Test of Kalumpang (*Sterculia foetida* L) methanolic extract was conducted in *Artemia salina* shrimp. From table 6 shown above, Group I which

received the lowest dosage of 150 mg of methanolic extract resulted to 66% mortality, an average of 33 nauplii brine shrimp were counted dead over the median brine shrimp population in each tube which is 50. Group II showed 76% mortality, Group III with 82% and Group IV with 94%. Group V which received 1000 mg of the extract resulted in a 100% mortality rate of the population.

CONCLUSION

This study revealed that methanolic extract of Kalumpang (*Sterculia foetida* L) showed moderately positive in diuretic activity in terms of both increase in urine volume and also in increase in concentration of potassium ions in the urine. Greater diuretic activity or index of Kalumpang extract was observed in 150mg/kg per body weight with diuretic index of 1.17, while 250mg/kg concentration showed 0.86 and lastly the highest concentration of the extract exhibited 0.89 diuretic activity.

It can also be concluded that the Kalumpang methanolic extract exhibits the mechanism of a loop diuretic. It can be noticed that the extract of different concentrations displays almost the same result to furosemide with excretion of high potassium ions

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the study of this paper.

AUTHORS' CONTRIBUTIONS

The authors of this study contributed in the following parts of the study. Ang, Jia Lianne and Ferrer, Donna and performed the statistical analysis and interpretation of the data. Lorenzo, Samantha Eryll designed the research framework and flowchart needed for the methodology of the study. Oandasan, Marianne Khate and Santos, Stephanie managed the literature studies needed to build a solid background information regarding the sample, experimental procedure and evaluation of results. Umayam, Eunice helped in the course of experimental process of the study done in clinical laboratories. Tan Hoc, Audrey Mae was responsible for the first draft of the manuscript,

editing and revisions needed. All authors read and approved the final manuscript. Ms. Laarni Hannah C. Lacorte, supervised the whole research experiment.

ETHICAL APPROVAL

Under the ethical considerations of the test animals, they are subjective to treat with an extent comfort. The animals was kept under standard condition in an animal housing facility located in Department of Science and Technology (DOST) – Standard Test approved by the committee and also under the supervision of a veterinarian. The rats was housed in polypropylene cages with bedding and maintained at 27°C; the animals were given standard diet (commercial pellets) prior to the experimentation.

Ethical committee clearance was obtained from IACUC (Institutional Animal Care and Use Committee) in Department of Science and Technology - Industrial Technology Development Institute. The study was also done in accordance to the Republic Act 8485 of the Animal Welfare Act of 1998 as amended by RA 10631.

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