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Diuretic activity of *Pandanus tectorius* (Pandanaceae)

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ABSTRACT

*Previous study on the crude leaf extract of Pandanus tectorius showed a diuretic activity using Sprague-Dawley rats. To corroborate this claim, the crude extract was fractionated and the isolation of squalene (**1**) was done on the chloroform fraction. A positive diuretic effect on three increasing doses of the chloroform extract in comparison to the negative control was observed. The highest diuretic effect was observed at a dose of 500 mg/kg BW and a diminished effect occurred at the highest dose of 1000 mg/kg BW which may indicate a low-ceiling loop diuretic mechanism. A positive diuretic activity on **1** was observed in test animals administered with 15 and 60 mg/kg BW doses. Although both **1** and chloroform extract exhibited positive diuretic effects, the results were not comparable to the positive control, Furosemide, which is a known potent diuretic agent.*

Keywords: Diuretic, Pandanus, Squalene, Pandanus tectorius, Pandanaceae

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INTRODUCTION

Diuretics are agents that increase the rate of urine formation and alter pH and ionic composition of urine by inhibiting ion transports that decrease the reabsorption of sodium ions at different sites of the nephron [1]. These agents have been widely used as first-line antihypertensive therapy and have shown to prevent cardiovascular morbidity and mortality [2]. Aside from their use in the management of cardiovascular diseases, diuretics have other clinical applications such as in diabetes insipidus, glaucoma, edema, hypokalemic alkalosis, nephritic syndrome, mountain sickness, urolithiasis and renal failure [3]. Diuretics are often classified based on their principal sites of action on the renal tubule and the resulting increase in sodium excretion and urine volume into thiazide, loop, potassium sparing diuretics and carbonic anhydrase inhibitors.

In an ethnopharmacological survey by Quisumbing (1978), more than 200 plant species in the Philippines are used as diuretics, including the *Pandanus tectorius* Parkins. species belonging to the Pandanaceae family. Ethnomedical information on *P. tectorius* includes its used to treat dysuria, leprosy, headaches and rheumatism [4]. Filipinos used the water from a cut near the base of the trunk to stimulate urination [5]. To validate the diuretic potential of *P. tectorius*, the crude and semi-crude extracts were subjected to diuretic test using male Sprague-Dawley rats. The diuretic assay of squalene which was isolated from the semi-crude chloroform fraction of *P. tectorius* will also be reported.

MATERIALS AND METHODS

General Consideration

NMR spectra were recorded on a JEOL JNM A-500 spectrometer using $CDCl_3$ as solvent and TMS as reference. VLC was performed using silica gel Merck 7739 while silica gel Merck 7734 was used for gravity column chromatography. Single-distilled or analytical grade solvents were used for the extraction and purification procedures.

Plant Material

Fresh leaves of *Pandanus tectorius* Parkinson ex. Z. were collected at the University of Santo Tomas, Manila, and identified at the Philippine National Museum with Botany Division Reference No. 2000-180.

Extraction and Isolation

Air-dried, ground leaves of *P. tectorius* were exhaustively extracted with distilled methanol (21.4 L) and filtered. The filtrate was concentrated under reduced pressure to obtain the crude methanolic extract (Pt-M, 110.5 g). A portion of the crude extract (40 g) was dissolved in 20% methanol in water and filtered. The filtrated was partitioned exhaustively with hexane. The combined hexane fractions was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to afford the hexane extract (Pt-H, 6.26 g). The aqueous layer was partitioned exhaustively in the same manner with $CHCl_3$, EtOAc and n-BuOH. Each fraction was concentrated under reduced pressure to afford the $CHCl_3$ extract (Pt-C, 2.22 g), EtOAc extract (Pt-E, 0.98 g) and n-BuOH extract (Pt-B, 10.68 g).

A portion of Pt-C (1.50 g) was subjected to vacuum liquid chromatography using neat hexane, hexane:$CHCl_3$ (75:25, 50:50, 25:75), neat $CHCl_3$, $CHCl_3$:acetone (75:25, 50:50, 25:75), neat acetone, acetone:MeOH (50:50) and neat MeOH as mobile phases. The process yielded 6 pooled fractions (Pt-CA – Pt-CF) after concentration under reduced pressure and TLC. Pt-CA was subjected to gravity column chromatography using 2% increments of $CHCl_3$ in hexane until hexane:$CHCl_3$ (8:2) and increasing to 5% increments of $CHCl_3$ in hexane until hexane:CHCl3 (1:1). Nine pooled fractions (Pt-CA1-Pt-CA9) were obtained based on TLC and were concentrated under reduced pressure. Pt-CA4 appeared as single on TLC with various solvent systems and was subjected to spectroscopic measurements.

Diuretic Assay

The crude and semi-crude extracts and squalene were sent at the Industrial Technology Development Institute-DOST, Philippines for the diuretic assay. Briefly, three increasing doses of the test material (250, 500, 1000 mg/kg for the crude and semi-crude extracts; 15, 30, 60 mg/kg for squalene) , the positive control (Furosemide) and the negative control (10% Tween 80 in normal saline solution, NSS) each with 2.5 mL NSS/100 g body weight were given orally to the fasted male Sprague-Dawley rats (310-400 g) in groups of two, respectively. Urine was collected and measured every hour for five hours. Percentage change (increase in urine volume) was computed based on the negative control (NSS).

% Increase (urine volume) = ((mL treated – mL negative control)/mL negative control) x 100

RESULTS AND DISCUSSION

The fractionation of the alcoholic leaf extract of Pandanus tectorius afforded the hexane (Pt-H), chloroform (Pt-C), ethyl acetate (Pt-E) and n-butanol (Pt-B) extracts. Subsequent purification was done on Pt-C based on the preliminary diuretic assay results conducted at the ITDI-DOST. Moreover, most secondary metabolites with diuretic property are commonly found in the semi-polar fraction.

Chromatographic purification of the Pt-C fraction afforded a colorless oily isolate (Pt-CA4, **1**, 18.1 mg) which showed a single-spot on TLC. The ^{1}H-NMR of **1** integrated for 25 protons which were assigned to 12 methyl protons (δ 1.60, 9H, s and δ 1.68, 3H, s), 10 methylene protons (δ 2.01 – 2.07, 10 H, m) and 3 olefinic methine protons (δ5.12, 3H, m). The ^{13}C-NMR spectra showed 15 carbons while the MS spectrum gave the molecular ion peak at m/z 410, thus, revealing the symmetrical nature of **1**. Comparison of the NMR data of 1 with those of other *Pandanus* compounds identified **1** as squalene. This is not the first report of squalene being isolated for Pandanus species. It was also isolated from *Pandanus dubius* [6], *Pandanus tectorius* var. *laevis* [7] and *Pandanus amaryllifolius* [8].

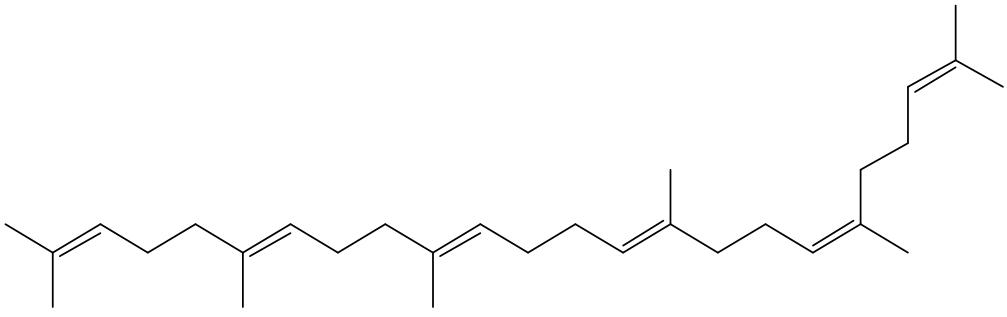

Figure 1. Structure of compound 1 (squalene).

Previous preliminary study on the diuretic activity of *P. tectorius* showed significant increase in urine volumes of experimental animals with inhibition of Na^+ reabsorption and K^+ excretion. It also inhibited the H^+ secretion as indicated by an increase in urine pH [9].

In this research, the chloroform extract (Pt-C) and standard squalene (98% pure, Sigma Aldrich) were subjected to diuretic assay at the Standards and Testing Division, Industrial Technology Development Institute-Department of Science and Technology. Their diuretic activities were based mainly on their effects on urine output of experimental animals. Pt-C produced positive diuretic effect as evidenced by the increase in total urine output of experimental animals after a five-hour urine collection in comparison to the negative control. A parameter known as percentage increase in urine volume was calculated for the treated groups of SD rats administered with 1st dose (250 mg/kg BW), 2nd dose (500 mg/kg BW) and 3rd dose (1000 mg/kg BW) (Table 1).

Table 1. Diuretic Assay Results of the Chloroform Fraction, Pt-C

Group No.	Drug	n	Dose (mg/kg BW)	Urine Volume Voided, mL	% Increase
I	10% Tween 80 in NSS	2	0	4.2	---
II	Furosemide	2	30	23.2	452.38
III	Pt-C	2	250	5.2	23.81
IV	Pt-C	2	500	11.4	171.43
V	Pt-C	2	1000	5.8	38.1

In-depth and more conclusive statistical analysis to determine whether the percent increase in urine output is significant or not was not included due to the following reasons: (1) the population of test animals used for the assay was not sufficient to qualify for statistical analysis; (2) only one trial was done for the three different doses of Pt-C; and (3) the diuretic assay was conducted only to find out whether the chloroform extract of P. tectorius exhibits diuretic effect by increasing the urine output. The results as presented in Table 1 indicate that Pt-C exert their action by causing dieresis. A dose-dependent increase in urine output was observed from the 1st dose (250 mg/kg BW) up to the 2nd dose (500 mg/kg BW) and a diminished diuretic effect occurred at higher dose (1000 mg/kg BW). Co-extracted substances in the Pt-C may interfere with absorption, distribution or binding to the receptor of the active component(s). The highest percentage increase in urine volume occurred at 500 mg/kg dose, which may indicate that Pt-C could have a mechanism similar to the low-ceiling loop diuretic agents, where increasing the dose beyond a certain amount has diminished or no corresponding effect [10]. The onset of diuretic action for all three doses was extremely rapid and it also had a fairly long duration of action which is suggestive of loop diuretics.

The diurectic activity of **1** (identified as squalene), isolated from the chloroform extract, was extrapolated from the results of the diuretic assay conducted on 98% pure squalene purchased from Sigma. The standard squalene was used in lieu of **1** because the amount of the isolate was insufficient for the assay. The same diuretic procedure adopted for Pt-C was applied to squalene except the use of a lower concentration (15, 30, and 60 mg/kg BW) (Table 2). The use of lower doses is expected and ideal with pure substances where higher concentration for the active component is present.

Table 2. Diuretic Assay Results of the Pure Squalene

Group No.	Drug	n	Dose (mg/kg BW)	Urine Volume Voided, mL	% Increase
I	10% Tween 80 in NSS	2	0	5.3	---
II	Furosemide	2	30	36.7	592.50
III	Squalene	2	15	9.4	77.36
IV	Squalene	2	30	5.1	-0.04
V	Squalene	2	60	8.9	67.00

A positive diuretic effect was observed in groups given with 15 and 60 mg/kg doses of squalene in comparison to the negative control. The results showed late onset of diuretic effect for the 1st and 3rd doses. These results, however, were not consistent with the diuretic action of the chloroform extract where **1** was isolated. The results obtained from the assay of the chloroform extract and 98% squalene suggested that the diuretic action of Pt-C may be due to the synergistic action of other constituents present. Although a positive diuretic effect was observed for Pt-C and squalene, this was not comparable with the effect of the positive control, Furosemide, which is a powerful loop diuretic agent.

The results of this study supported the ethnomedical use of *P. tectorius* as a diuretic agent. However, further experimentation such as determination of ionic composition (Na^+, K^+ and H^+) of urine excreted by the experimental animals is needed in order to understand the precise mechanism of action for the diuresis.

CONCLUSION

The chloroform fraction of *Pandanus tectorius* was subjected to diuretic assay using male Sprague Dawley rats. A positive diuretic effect was observed in three different doses as evidenced by the percentage increase in urine volume with reference to the negative control group. The onset of diuretic action is suggestive of loop diuretics. Compound **1**, isolated from the chloroform fraction and identified as squalene, showed minimal diuretic effect from the results obtained as extrapolated using a commercially available 98% squalene. This study is also the first report on the preliminary diuretic assay of squalene.

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