DIURETIC ACTIVITY OF MATOA LEAVES EXTRACTS AND FRACTIONS (POMETIA PINNATA J.R. FORSTER & J.G FORSTER) AND ITS INFLUENCE ON POTASSIUM AND SODIUM LEVELS

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ABSTRACT

Objective: The goal of this research was to determine diuretic activity of matoa leaves (Pometia pinnata) and its fractions and its influence on potassium and sodium levels.

Methods: Matoa leaves were extracted by reflux method followed by evaporation using rotary evaporator. The subjects were male Wistar rats that were divided into 11 groups: Furosemide (3.6 mg/kg bw); control group carboxymethylcellulose 0.5%; matoa leaves extracts with doses of 50 mg/kg bw 100 mg/kg bw, and 150 mg/kg bw; matoa leaves aqueous fraction with dose of 10.94 mg/kg bw, 21.88 mg/kg bw, and 32.82 mg/kg bw; matoa leaves ethyl acetate fraction (MEF) with dose of 4.35 mg/kg bw, 8.71 mg/kg bw, and 13.06 mg/kg bw. Rats were placed in metabolic cages during an observation study. Urine volume was measured for 5-24 hrs. Potassium and sodium levels in urine were determined using atomic absorption spectrophotometry.

Results: The effective dose of matoa leaves extract and fractions for diuretic activity was matoa leaves MEF 8.71 mg/kg bw which could increase the excretion of sodium and potassium in the urine of the male Wistar rats.

Conclusion: Matoa leaves extract and fractions could increase the excretion of sodium and potassium in the urine of the male Wistar rats.

Keywords: Matoa (Pometia pinnata), Diuretic, Urine volume, Potassium levels, Sodium levels.

INTRODUCTION

Diuretics are drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations [1]. Diuretics are drugs act on kidneys to increase urine output and sodium excretion [2]. Diuretics reduced the amount of fluid in the bloodstream, therefore some diuretics are used to treat high blood pressure. Urine is a mixture of water with polar compounds that must be removed from the body. If urinary excretion is not smooth from the bladder or kidneys can cause crystallization of substances that should be discarded [3,4].

Fluid retention is a key of acute heart failure, which was manifested as ankle swelling, ascites, and/or pulmonary edema. Therapeutic strategies to control fluid balance, and shift of fluid out of the interstitium, lead to significant symptomatic relief, and improved health-related with the quality of life [5]. Medicinal plants contain known and unknown important medicinal substances. The previous study [6] estimated that over 75% of the world’s population still depends on plant derived medicines for the treatment of common ailments. One of the reasons to use herbal medicines is probably the presence of synergistic and or side effects which can neutralize combination of phytochemical constituents [7]. Another reason for the shifting trends toward natural products is the harm effects of synthetic chemicals [8].

The previous study by Suedee et al. [9] succeed to isolate epicatechin, kaempferol-3-O-rhamnoside, quercetin-3-O-rhamnoside, glycolipid, 1-O-palmitoyl-3-O-[6-(galactopyranosyl)-(1→6)-B-galactopyranosyl]-sn-glycerol, steroid glycosides, stigmasterol-3-O-glucoside and triterpenoid saponin pentacyclic, 3-O-a-arabinofuranosyl-(1→3)-[a-rhhamnopranosyl-(1→2)]-e-arabinopyranosyl hexadecenin from matoa leaves extract that had activity as anti-HIV.

Based on the background mentioned above, related to the extent of diuretics in the treatment, as well as the lack of utilization of matoa as a diuretic, the study aims to test diuretic activity of matoa extract and fractions and its effect on potassium and sodium levels in urine. Determination of potassium (K) and sodium (Na) levels in urine can be done by atomic absorption spectrophotometry (AAS) method. The AAS has several abilities: It has a high sensitivity (lower detection limit of <1 µg/ml), wide boundary determination level (from µg/ml until %), the implementation is relatively simple, and a little interference. AAS based on the absorption of visible light or ultraviolet light by atoms of neutral [10].

MATERIALS AND METHODS

Materials

Leaves of matoa (Pometia pinnata), nitric acid, potassium, sodium standard, furosemide, carboxymethyl cellulose (Laboratory of Pharmacology Setia Budi University), and distilled water.

Preparation of sample

Samples (leaves) of P. pinnata were collected from Sukoharjo, Center of Java, Indonesia. Leaves sample was thoroughly washed with tap water, sorted while wet, cut, dried at 50°C for 5 days, and grinded into powder (40 Mesh).

Extraction

Sample was extracted by reflux using 96% ethanol. 500 g of crude drug was refluxed with 1.5 l of 96% ethanol for 3 hrs, done triplicate (named as maximum likelihood estimation [MLE]). Liquid extract was filtered and then evaporated using rotary evaporator at 40°C and speed of 40 rpm.

Fractination

Extract was fractionated by liquid-liquid extraction (LLE) with increasing polarity solvent. Ethanol extract 20 g was added 200 ml of hot...
water then LLE using n-hexane 200 ml. LLE was performed triplicate. Furthermore, the residue was LLE with ethyl acetate 200 ml carried out triplicate, and the residue was water fraction. Hence, there were three kinds of extracts: N-hexane fraction (named as multiple hearth furnace), matoa ethyl acetate fraction (MEF), and matoa water fraction (MWF). The obtained fractions were concentrated by rotary evaporator.

**Diuretic activity**

This study used 55 male rats weighing between 130 and 170 g. The rats were weighed and marked, respectively, were randomly divided into 11 groups, each group consisted of 5 rats. Previously rats were fasted for 12 hrs. Before treatment, the rat was given warm water 4 ml/200 g body weight (bw) (loading dose). Group I was matoa leaves extract with dose of 50 mg/kg bw (MLE 1). Group II was matoa leaves extract 100 mg/kg bw (MLE 2). Group III was matoa leaves extract 150 mg/kg bw (MLE III). Group IV was MEF 4.35 mg/kg bw (MEF 1). Group V was MEF 8.71 mg/kg bw (MEF 2). Group VI was MEF 13.06 mg/kg bw (MEF 3). Group VII was MWF 10 mg/kg bw (MWF 1), Group VIII was MWF 21.88 mg/kg bw (MWF 2). Group IX was MWF 32.82 mg/kg bw (MWF 3). Group X was the negative control group aqueous, and Group XI was furosemide 3.9 mg/kg bw. Immediately after administration sample or standard and vehicle, animals were placed in metabolic cages individually. During this period, no water and feed was available to animals. Urine was taken for 5 and 24 hrs [11,12]. Total concentration of Na and K were measured by AAS [13].

**Ethics committees**

The permission for conduction of these experiments was obtained from the relevant ethics committees, School of Pharmacy, Bandung Institute of Technology, number 02/KEPHP-ITB/12-201.

**Statistical analysis**

Data were expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance followed by post hoc Tukey. Significant differences were set at values <0.05.

**RESULTS**

**Urinary excretion**

Ethanol leaves extract and fractions of matoa which was given orally could increase urinary excretion (Table 1).

Study regarding relationship between observation time (hours) against the average volume of urine for 5 until 24 hrs, revealed that all of extracts sample showed diuretic effect. Matoa leaves extract with dose of 150 mg/kg bw (MLE 3) showed the highest urinary volume, which was comparable with furosemide as control.

In Table 2, it could be seen that excretion urine volume (EUV) of all of treated extract (except MEF 3, MWF 1, MWF 2, MWF 3) had no significant difference with furosemide.

Matoa leaves extract with dose of 8.71 mg/kg bw (MEF 2) gave the highest sodium levels which was not significantly different compared to furosemide.

**DISCUSSION**

The results of urine volume for five up to 2 hrs after treated with different dose extracts and fraction of matoa was given as in Table 1, which demonstrated that MLE 3 bw had the highest urinary excretion and was not different statistically with furosemide. The average volume of urine of rat on 24 hrs in control group was 3.94±0.74 ml and in furosemide group was 11.57±0.90 ml. The extracts expressed higher urinary volume than control. Statistically, urine volume in MLE 1, MLE 2, MLE 3, MEF 2, MWF 2, MWF 2 and MWF 3 showed significantly difference compared to control group (p<0.05), and not significantly different compared to furosemide. Based on the result, it can be concluded that ethanol extract of leaves and fraction of matoa had diuretic effect and their activity similar with furosemide. The previous study which was conducted by Sa’roni [12] demonstrated that ethanol leaves extract of Desmodium triquetrum with doses of 3.1 mg, 9.3 mg and 31 mg/100 g body weight in mice, showed significantly difference in urine volume compared to distilled water (p<0.05) but also had a significant difference with hydrochlorothiazide. It means that its diuretic effect was lower compared to potassium. Previous research [13] reported that ethanol extract of Rumex vesicatorius (500 and 1000 mg/ml) gave high urinary excretion more than furosemide standard.

In the Table 2, it can be seen that ethanol leaves extract of matoa with dose of 150 mg/kg bw had % EUV which was almost the same with furosemide. This study reported that ethanol matoa extract and fraction had the higher diuretic activity than control, but lower activity than furosemide. Statistically, diuretic activity of MLE 1, MLE 2, MLE 3, MEF 2 and MEF 3 significantly different with control (p<0.05), while MEF 1, MWF 1 and MWF 2 had no significant difference with control. Based on statistically analysis, it can be shown that all of the treatment extracts (except MWF 3) had no significant different in diuretic activity with furosemide standard. Hence, it can be concluded that extract and fraction of matoa had % EUV (except MWF 3). Study by Roa et al. [2] exhibited that diuretic activity of ethanol extract of R. vesicatorius with dose of 1000 mg/ml higher than furosemide standard.

**Fig. 1** exposed that potassium levels in MLE 1, MLE 2, MEF 2, MWF 2, MWF 3 and had no significant difference with furosemide.

**Table 1: Urinary excretion extracts and fractions of P. pinnata on 24 hrs**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urine volume (ml) on hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>MLE 1</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>MLE 2</td>
<td>0.71±0.12</td>
</tr>
<tr>
<td>MLE 3</td>
<td>0.65±0.06</td>
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<tr>
<td>MEF 1</td>
<td>0.76±0.09</td>
</tr>
<tr>
<td>MEF 2</td>
<td>0.79±0.11</td>
</tr>
<tr>
<td>MEF 3</td>
<td>0.92±0.11</td>
</tr>
<tr>
<td>MWF 1</td>
<td>0.55±0.06</td>
</tr>
<tr>
<td>MWF 2</td>
<td>0.49±0.09</td>
</tr>
<tr>
<td>MWF 3</td>
<td>0.59±0.12</td>
</tr>
<tr>
<td>Aquous</td>
<td>0.77±0.05</td>
</tr>
<tr>
<td>Furosemide</td>
<td>1.07±0.32</td>
</tr>
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</table>

P. pinnata: Pomelita pinnata
Table 2: Excretion urine volume of extract and fraction P. pinnata

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hours</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>24</th>
</tr>
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<tr>
<td>MLE1</td>
<td></td>
<td>9.00±2.15</td>
<td>78.12±12.05*</td>
<td>117.87±18.43*</td>
<td>132.69±19.77*</td>
<td>154.30±21.00*</td>
<td>368.33±61.83*</td>
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<tr>
<td>MLE2</td>
<td></td>
<td>15.71±5.12</td>
<td>74.14±14.60</td>
<td>130.24±25.10*</td>
<td>147.49±28.34*</td>
<td>149.55±29.10*</td>
<td>388.47±63.11*</td>
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<tr>
<td>MLE3</td>
<td></td>
<td>13.96±3.47</td>
<td>74.39±12.25</td>
<td>121.03±22.09*</td>
<td>139.09±25.43*</td>
<td>140.78±25.21*</td>
<td>413.43±70.84*</td>
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<tr>
<td>MEF1</td>
<td></td>
<td>16.34±9.98</td>
<td>74.30±5.05</td>
<td>109.98±6.93*</td>
<td>124.73±9.42*</td>
<td>136.53±11.72</td>
<td>276.73±24.44</td>
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<tr>
<td>MEF2</td>
<td></td>
<td>16.89±2.28</td>
<td>82.38±19.32*</td>
<td>108.10±20.47*</td>
<td>138.76±25.39*</td>
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<td>325.55±65.65*</td>
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<td></td>
<td>21.32±5.74</td>
<td>79.84±10.37*</td>
<td>129.57±24.01*</td>
<td>161.69±26.09</td>
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<td>284.71±45.41</td>
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<td>11.48±3.80</td>
<td>64.34±33.33</td>
<td>92.12±14.82</td>
<td>128.86±23.94*</td>
<td>136.24±29.92*</td>
<td>244.41±34.18</td>
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<tr>
<td>Aqueous</td>
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<td>16.31±2.67</td>
<td>51.04±9.48</td>
<td>67.04±13.08</td>
<td>81.62±17.28</td>
<td>87.09±20.65</td>
<td>170.20±35.21</td>
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<tr>
<td>Furosemide</td>
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<td>37.48±6.95</td>
<td>94.26±15.65</td>
<td>117.08±17.19</td>
<td>132.76±19.19</td>
<td>151.58±19.96</td>
<td>383.68±41.88</td>
</tr>
</tbody>
</table>

*Not significantly different compared to furosemide, P. pinnata: Pometia pinnata, MWF: Matoa water fraction, MLE: Maximum likelihood estimation

Fig. 1 demonstrated the results of sodium levels in urinary excretion. Control group gave the lowest sodium levels in urinary excretion, while furosemide had the highest sodium levels. Sodium levels of all of matoa extracts and fractions were higher than sodium levels in control group. MEF2 had sodium levels which was similar with sodium levels in furosemide group (p<0.05). Research by Sa'roni [12] reported that sodium levels of ethanol leaves extract of D. triquetrum groups with doses of 9.3 and 31 mg/100 g bw significantly different with sodium levels in aquadest group (p<0.05), but no significant difference with sodium levels which was given by hydrochlorothiazide.

The results of measurements of potassium levels can be seen in Fig. 2. The lowest potassium level was given by control group and the highest levels for furosemide group. Statistically potassium levels of all of matoa extract and fraction were significantly different with control group (p<0.05) but it had no significant different with furosemide group. MEF2 gave the highest sodium levels compared to the other extracts, and this result was lower than furosemide group. Previous research by Sa'roni [12] exhibited that potassium levels of ethanol leaves extract of D. triquetrum with dose of 31 mg/100 g bw was greater than potassium levels of distilled water group and had no significant different with potassium levels hydrochlorothiazide 0.16 mg/100 g bw group. Study by Roa et al. [2] expressed that the ethanol extract of R. vesicarius induced the urinary output which was accompanied by increasing in Na+, K+, Na/K ratio. In general, these observations suggested that matoa extract act as diuretic, which were usually given intravenously and inert by pharmacologically. The results of this study show that the amount of potassium excreted by all extracts and fractions matoa shows differences in the control group. The MEF2 could increase potassium excretion better than the others.

This study showed that MLE 3 had the highest diuretic activity, but lower effect in excretion of sodium and potassium ion. In general, the matoa leaves extract had diuretic activity but not saluretic while matoa ethyl fractions possessed diuretic and saluretic effect. Increasing in number of potassium in the blood as resulting in renin secretion is reduced and increasing in excretion of Na. If renin secretion is reduced, then it will not be changed angiotensinogen to angiotensin I, and thus the levels of angiotensin II would be decreased. As a result, vasoconstriction effect of angiotensin II and aldosterone secretion to reabsorb sodium and water will be reduced. This was followed by vasodilation of blood vessels of the kidneys that eventually increases blood flow to the kidneys and then urine excretion volume increased [14]. Increasing in potassium levels can cause saluretic because it leads increasing in excretion of sodium, so the low amount of sodium will reduce blood pressure.

Matoa plant contains alkaloid and flavonoid compounds. The previous study by Melendez-Camargo [15] reported that alkaloid would inhibit or reduce the reabsorption of water and electrolytes in the tubules which can cause saluretic effect [16,17].

CONCLUSION

Ethanolic extract and fraction of matoa had diuretic activity in male Wistar rats. Extract leaves of matoa and the fractions can affect the amount of sodium and potassium levels in urinary excretion. The effective dose of matoa leaves extract and fractions for diuretic activity was matoa leaves ethyl acetate fraction 8.71 mg/kg bw which could increase the excretion of sodium and potassium in the urine of the male Wistar rats.

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REFERENCES