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The attached manuscript has been submitted to the Tropical Journal of Natural Product Research [https://www.scopus.com/sourceid/21100933230](https://www.scopus.com/sourceid/21100933230) for consideration and possible publication. Your assistance is needed to help review the manuscript whether it is suitable or not for publication by TJNPR. We have contacted you based on your record as a competent expert in this area of study.

I would be grateful if you would kindly agree to act as a reviewer for this paper and send your valuable comments as soon as possible (27th April 2021)

**Title:** Potential Antidiabetic Activities Of Akar Kuning (*Fibraurea Tinctoria* Lour) Extract In Aloxan Induced Diabetic Rats

Thank you very much for your kind consideration.

Best regards

Abiodun

Professor Abiodun Falodun, PhD

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### A. MANUSCRIPT

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<td>Potential Antidiabetic Activities Of <em>Akar Kuning</em> (<em>Fibraurea Tinctoria</em> Lour) Extract In Aloxan Induced Diabetic Rats</td>
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<td>Erwin</td>
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### B. REVIEWER’S SPECIFIC COMMENTS PER SECTION OF MANUSCRIPT

<table>
<thead>
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| Abstract                 | - Fibraurea victoria Lour fixed to *Fibraurea tinctoria* Lour  
                          | - The word aloxan is fixed to alloxan |
| Introduction             | - Sentence: Diabetes mellitus is one of metabolic syndromes that have become highly prevalent globally, added "the" becomes: Diabetes mellitus is one of "the" metabolic syndromes that have become highly prevalent globally  
                          | - The words "and" are deleted one in the last sentence of the second paragraph of the introduction |
| Methodology              | - add a space at the beginning of the second sentence in the extraction  
                          | - the word "were" is changed to "have" in the second sentence and "were" becomes "was" in the last sentence in "diabetes induction" |
| Results                  | - |
| Discussion               | - Fibraurea victoria Lour fixed to Fibraurea tinctoria Lour  
                          | - Writing Ca2+ ions fixed to Ca2+ ions  
                          | - The word aloxan is fixed to alloxan  
                          | - 200 mg / KgBB is fixed to 200 mg / Kg BW |
| Conclusion               | - Add the word "the" at the beginning of the sentence in the acknowledgment |
| References               | - |
| Figures, Tables          | - |
C. REVIEWER’S GENERAL COMMENTS AND REMARKS

Comments may be continued onto another sheet if necessary.

This article worthy of publication after a few improvements.

D. REVIEWER’S RECOMMENDATION

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<td>Accept with major corrections (the article should be thoroughly changed)</td>
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E. REVIEWER’S INFORMATION

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<tr>
<th>Name</th>
<th>Erwin</th>
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<tr>
<td>Official title</td>
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<tr>
<td>Affiliation</td>
<td>Muawarman University</td>
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<tr>
<td>Specialization</td>
<td>Natural product chemistry</td>
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ABSTRACT----- In this study, we aimed to investigate the effects of oral administration of *akar kuning* (*Fibraurea tinctoria* Lour.) ethanol extract in alloxan-induced diabetic rats. The experimental animals were divided into 5 groups, where each one consisted of 3 male white rats. Group 1 and 5 (negative and control groups) consisted of diabetic rats that were given 0.3% NaCMC and 0.65 mg/KgBW glibenclamide respectively, while groups 2, 3, and 4 were given *Fibraurea tinctoria* Lour ethanol extract with doses of 50, 100, and 200 mg/kg respectively. The preliminary test was carried out by comparing the group of rats with and without alloxan (normal group). Before treatment, the rats were administered intraperitoneally with alloxan monohydrate of 175 mg/kgBW. The ethanol extract was given once a day for 15 days and blood glucose levels were observed. The ethanol extract administered also caused significant changes in glucose levels of the rats' blood (p <0.05). This shows that ethanol extract of *Fibraurea tinctoria* Lour has an antidiabetic effect at doses of 50, 100, and 200 mg/KgBW. In the negative control, there was an increase in blood glucose levels during the treatment, this was because this group was only given 0.3% NaCMC, an inert substance that has no effect on lowering blood glucose levels. In conclusion, the ethanol extract showed a significantly antidiabetic effect (p <0.05) reducing blood glucose levels of diabetic male white rats. Furthermore, a dose of 100 mg / KgBW is most effective in lowering blood glucose levels.

**Keywords:** Akar kuning; blood glucose; alloxan; dysentery; headaches; eye pain.
In recent times, the pattern of disease has started to shift from infectious diseases to metabolic syndromes. Diabetes mellitus (DM) is a diverse and complex metabolic disorder that occurs due to the disturbances in fat, proteins and carbohydrate metabolism in response to insulin deficiency or insensitivity [26]. Diabetes mellitus is one of the metabolic syndromes that have become highly prevalent globally. In 2003, the number of people worldwide suffering from this disease within the age range of 20 to 79 years was 149 million (5.1%) and was estimated to reach 333 million (6.3%) in 2025 (International Diabetes Federation, 2003). There are two types of diabetes mellitus, namely type 1 which depends on insulin and type 2 is not dependent on insulin. Type 2 diabetes can be treated by regulating blood sugar levels using hypoglycemic compounds, alpha-glucosidase enzyme inhibitors, and a low-sugar diet [17].

The development of herbal medicine in Indonesia has been fostered by the biodiversity of forest plants in Kalimantan and the traditional knowledge of various tribes in the community such as Fiberura tinctoria Lour. It is a medicinal plant that grows in the Samboja Special Purpose Forestry Area (KDKT), Kutai Kartanegara Regency, East Kalimantan. It also has been used as a traditional medicine to treat malaria, dysentery, and fever in the area [15]. Furthermore, akar kuning (Fiberura tinctoria Lour) is a woody liana whose roots and stems are used by the Dayak, Banjar, and Kutai tribes to treat jaundice (liver), malaria, and is used to boost stamina [10].

In addition, scientific studies revealed the potential of secondary metabolites in Fiberura tinctoria Lour root and stem extracts having anticancer, antioxidant, antimicrobial, hepatoprotector, and antidiabetic properties [18, 20, 21, 25]. This plant is becoming well known for its potential to treat liver damage, increase endurance, and act as an anti-diabetic because it
contains berberine compounds [1, 3, 12, 13]. Tryacontanyl caffeat isolated from *Fibraurea tinctoria* Lour trunk was shown to possess potential as an antioxidant [9]. The ability of this plant to treat these diseases may be caused by the phenolic compounds contained in it. Therefore, this research was conducted to explore the potential of *Fibraurea tinctoria* Lour ethanol extract as an antidiabetic by using alloxan-induced male white rats.

**Materials and Methods**

The equipment used in this study included glassware, analytical balance, test tube rack, spatula, grinder, maceration container, a set of distillation and rotary evaporators, volumetric pipette, test tube, measuring flask, measuring cup, bulb, cup, and a blood glucose measuring device (Gluko Dr Blood Glucose Test Meter, Gluko Dr Blood glucose Test Strips).

The materials used in this study were ethanol, Na CMC, alloxan monohydrate, glibenclamide. All chemical compounds were obtained from sigma Aldrich. Moreover, extracts of (*Fibraurea tinctoria* Lour), aquadest, and physiological NaCl were obtained from the Laboratory of Sekolah Tinggi Ilmu Kesehatan Samarinda, Indonesia.

**Extraction**

After the (*Fibraurea tictoria* Lour) were washed in water to eliminate dirt, dried at room temperature until the stable dry weight was achieved [22]. The maceration extraction method was carried out using 95% of ethanol solvent. As many as 200 grams of simplified (*Fibraurea tictoria* Lour) which has been sieved with 40 mesh sieves was macerated with 2 liters of 95% of ethanol solvent (1:10) slowly while keeping the stirring process until the solvent soaks all *Fibraurea tinctoria* Lour powder. Then, it was macerated for 2 hours and soaked up to 24 hours and filtered using filter paper. The
Re-maceration process was performed twice. The macerate that has been produced was then concentrated with a rotary evaporator at 500°C and then evaporated in the water until a thick extract was obtained. This extract was then tested for antidiabetic activity in vivo against male white rats [19].

**Experimental animals**

This study made use of 21 male white rats aged 2-3 months having body weights of 150-250 grams.

**Preparation of experimental animals**

The rats were acclimatized under laboratory conditions for one week with sufficient food and drink. During this period, body weight was measured. In addition, the rats used in this study were healthy, with no change in body weight exceeding 10% of that during acclimatization and they all exhibited normal behavior. The study was approved by the Faculty of Pharmacy Mulawarman University, Animal Experiments Local Ethics Committee /"ETHICAL EXEMPTION" No.50/KEPK-FFUNMUL/EC/EXE/01/2021.

**Diabetes induction**

Induction of diabetes was performed according to Misra and Aiman (2012), with modification. [24]. The rats were have fasted for 18 hours (drinking water was still given), and then injected intraperitoneal (i.p) with alloxan solution at a dose of 175 mg/kgBW. They were given food and drink containing 10% glucose for 2 days after induction. From the 3rd day, 10% glucose was replaced with plain drinking water and the rats were transferred to a cage, each one
containing a single rat. Furthermore, on the third day, the rats blood glucose levels were
determined using a device (Gluko Dr. Blood Glucose Test Meter, Gluko Dr. Blood glucose Test
Strips). The next category of rats used in this study were diabetes-induced, characterized by
blood glucose levels of ≥ 200 mg/dl. [19].

**Development of test preparations**

The test preparation was made by mixing the *akar kuning* ethanol extract with a suspension
which was created by heating 0.3% NaCMC over hot water 20 times. Subsequently, the
preparation was weighed, crushed, and added to the volume aquadest until the mark was hit.

**Preliminary Test**

The preliminary test was carried out by comparing the groups of rats with and without
alloxan (normal group). Furthermore, preprandial and postprandial blood glucose levels were
measured.

**Provision of test preparations**

Male white rats were divided randomly into 5 experimental groups with each one
containing 3 (three) rats and the body weight was measured for 2 weeks. Group 1 consisted of
normal rats that were only given drinking water and food during the treatment. In addition, this
group was also given 0.3% NaCMC. The rats in group 5 were given glibenclamide at a dose of
0.65 mg/kg BW. Meanwhile, groups 2, 3 and 4 consisted of diabetic rats which were given an
oral test extract, once a day for 15 days at a dose of 100, 200, and 300 mg/kg BW, respectively
[19].

**Determination of blood glucose levels**
On 5th, 10th, and 15th day after induction with alloxan tetrahydrate, blood was taken from the rats. Consequently, blood glucose levels were determined using the Gluko Dr Blood Glucose Test Meter, and the Gluko DrTM Blood glucose Test Strips. Furthermore, the percentage reduction in glucose levels was calculated with the following formula:

\[
\text{The percentage decrease in blood glucose levels} \% = \frac{K_o - K_d}{K_o} \times 100\% ................. (1)
\]

Description:

\(K_o\) = blood glucose levels on the first day when diabetes was identified

\(K_d\) = blood glucose levels on the 5th, 10th, and 15th day after diabetes was identified

**Data Analysis**

The research data was analyzed statistically using the Tukey test and one-way ANOVA for blood glucose parameters.

**Results and Discussion**

**Results of Determination**

The taxonomic identity of the plant was confirmed by Bina Swasta Sitepu, M.Sc. The voucher specimens were deposited in the herbarium Wanariset (WNA); herbarium numbers: 129/AK/2020 by using W252 specimens as comparative specimens. The results of the determination showed that the tuber used in the study was true of *Fibraurea tinctoria* Lour From the Iridaceae family Menispermaceae. *Fibraurea tinctoria* Lour was purchased from Samboja Special Purpose Forestry Area (KDKT), Kutai Kartanegara Regency, East Kalimantan, Indonesia.

**Preliminary Test**
44.25 grams of thick ethanol extract was obtained by the extraction and maceration of 800 grams of *Fibraurea tinctoria* Lour plant samples. This extract was then tested for its anti-diabetic activity in vivo against male white rats induced by alloxan tetrahydrate intraperitoneally at a dose of 175 mg/KgBW. Alloxan tetrahydrate is a diabetogenic substance that selectively acts on pancreatic β cells that produce insulin. Alloxan in the blood binds to GLUT-2 (glucose transporter) thereby allowing it to enter the β cytoplasm of the pancreas. Within these cells, alloxan causes excessive depolarization of the mitochondria as a result of the entry of Ca\(^{2+}\) ions and the excessive use of energy which leads to lack of energy in the cell. These two mechanisms cause damage to both the number and mass of pancreatic cells resulting in a decrease in insulin release, which leads to hyperglycemia [7].

**The level of preprandial blood glucose**

The rats preprandial blood glucose level after intraperitoneal administration of alloxan showed that the increase in these levels was significantly different than normal rats (p <0.05).

Based on table 1, it can be seen that an increase in blood glucose levels was higher in the group of rats with Alloxan compared to the group of normal rats. The results of statistical analysis using the independent sample-t test showed a significant difference (p <0.05). This indicates that alloxan significantly affects the preprandial blood glucose levels of diabetic rats compared to normal ones.

**Table 1.** The Level of Preprandial Blood Glucose After Intraperitoneal administration of Alloxan,

<table>
<thead>
<tr>
<th>Observation time (days)</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group</strong></td>
<td><strong>Alloxan Rats</strong></td>
<td>104.67±4.67</td>
<td>121.33±3.84</td>
<td>153.67±4.67</td>
</tr>
<tr>
<td><strong>Normal Rat</strong></td>
<td>86±7.02</td>
<td>105.33±9.61</td>
<td>121.67±7.26</td>
<td>154.33±25.91</td>
</tr>
</tbody>
</table>
Postprandial blood glucose levels

3 days after alloxan administration, results showed that the increase in postprandial blood glucose levels of the alloxan group of rats was significantly different from normal rats (p <0.05).

Based on table 2, it appears that an increase in blood glucose levels was higher in the fat-fructose group of rats than in the normal group. The results of statistical analysis with the independent sample-t test, showed a significant difference (p <0.05) on the 1st, 2nd and 3rd day. This indicates that alloxan administration significantly affects postprandial blood glucose levels of rats as compared to normal ones.

Table 2. The level of postprandial blood glucose after alloxan provision until day 3

<table>
<thead>
<tr>
<th>Observation time (days)</th>
<th>0</th>
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<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alloxan Rats</td>
<td>112.67±7.67</td>
<td>132±3.78</td>
<td>166,667±1.67</td>
<td>289±4,16</td>
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<tr>
<td>Normal Rats</td>
<td>95±12.53</td>
<td>118,67±9.26</td>
<td>145±10,41</td>
<td>174,33±35,89</td>
</tr>
</tbody>
</table>

Weight

Body weight data was used to determine the significant increase between the treated and normal rat groups. The results are presented in Table 3.

Table 3. The mean body weight (BW) of the rats after treatment of the days 0, 7 and 14

<table>
<thead>
<tr>
<th>Mean Weight on day-</th>
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<th>7</th>
<th>14</th>
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<tr>
<td>Group</td>
<td>0</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>1</td>
<td>191.86</td>
<td>214.47</td>
<td>237.54</td>
</tr>
<tr>
<td>2</td>
<td>170.31</td>
<td>200.54</td>
<td>224.03</td>
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<td>3</td>
<td>213.3</td>
<td>239.94</td>
<td>249.16</td>
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<td>4</td>
<td>204.68</td>
<td>230.24</td>
<td>248.18</td>
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<tr>
<td>5</td>
<td>203.75</td>
<td>243.98</td>
<td>286.24</td>
</tr>
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</table>
According to table 3, there was an increase in body weight across all groups. This parameter is one characteristic of insulin resistance that supports the assumption that the animals are already resistant.

Figure 1 shows the results of the anti-diabetic test of *Fibraurea tinctoria* Lour ethanol extract. Administering ethanol extract at the doses of 50, 100, and 200 mg / KgBW reduces blood glucose levels in diabetes induced rats induced. Furthermore, there was a significant difference in the negative control (p <0.05). The extract administered also caused significant changes in glucose levels of the rats' blood (p <0.05).

![Graph showing decrease in blood glucose levels of male white rats (mean)](image)

*Figure 1.* Graph showing decrease in blood glucose levels of male white rats (mean)

This shows that ethanol extract of *Fibraurea tinctoria* Lour has an antidiabetic effect at doses of 50, 100, and 200 mg/KgBW. In the negative control, there was an increase in blood glucose levels during the treatment, this was because this group was only given 0.3% NaCMC.
an inert substance that has no effect on lowering blood glucose levels. The antidiabetic effect of *Fibraurea tinctoria* Lour is assumed to be due to its berberine content. This substance helps to stimulate insulin secretion and regulate its sensitivity, therefore berberine has antidiabetic properties. Furthermore, it inhibits the synthesis of cholesterol and triglycerides in human liver cells, and it also activates the adenosine monophosphate-activated protein kinase (AMPK) pathway which is essential to body homeostasis. This substance is used to inhibit the oxidation of glucose within the mitochondria, thereby leading to an increase in AMPK in cells and the prevention of adipogenesis [7].

Berberine also has antidiabetic properties which work by inhibiting the α-glucosidase enzyme therefore reducing glucose levels in the blood. This mechanism is brought about by slowing the breakdown of carbohydrates into glucose, thereby allowing blood sugar levels to reach normal limits. Furthermore, the α-glucosidase inhibitor enzyme reduces postprandial blood sugar levels [11, 23]. Berberine belongs to a class of chemical compounds called alkaloids. These substances are polar chemical compounds; therefore, they will be bound in ethanol solvent that is polar. Based on research by Supomo, et al, *Fibraurea tinctoria* Lour contains secondary metabolites namely alkaloids, flavonoids and saponins [16].

Flavonoids are also chemical compounds with antidiabetic properties and are polar because they have a hydroxyl group (-OH) where hydrogen can be formed. Flavonoids and alkaloids are easily dissolved and bound in ethanol solvent because they are both polar [6]. Saponins are also substances found in *Fibraurea tinctoria* Lour and have antidiabetic properties because they can inhibit the work of the α-glucosidase enzyme in the intestine which functions to convert carbohydrates into glucose [4]. Saponins are also polar chemical compounds that can dissolve in ethanol solvent.
The blood glucose levels of the rats were determined using a Gluco Dr Blood Glucose Test Meter. Furthermore, the advantages of this method are that it is fast, precise, can be used in the 10-600 mg/dL range and only requires a few drops of blood. Diabetes is a disease characterized by an increase in glucose levels in the blood. An abnormal increase in blood glucose levels affects the work of the heart, liver and kidneys and other internal organs. The results of statistical analysis using the Tukey test revealed that the 50, 100, and 200 mg/KgBW group showed a significant difference (P <0.05) when compared to the negative control group (meaning that the extract has the potential to be an oral hypoglycemic drug). Comparison between the 100 mg and 200 mg groups showed (p> 0.05) there was no significant difference, therefore the 100 mg/kgBB dose was selected.

**Conclusion**

Based on the results, it can be concluded that the ethanol extract of *akar kuning* showed an antidiabetic effect at doses of 50, 100, and 200 mg / kgBW by significantly (p <0.05) reducing the blood glucose levels of diabetic male white rats (*Mus musculus* L). Furthermore, the 100 mg / KgBB dose was most effective in lowering blood glucose levels.

**Acknowledgement**

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1. **Conflict of interest**

The authors declare no conflict of interest.
2. Ethical approval

The study was approved by the Faculty of Pharmacy Mulawarman University, Animal Experiments Local Ethics Committee /"ETHICAL EXEMPTION" No.50/KEPK-FFUNMUL/EC/EXE/01/2021, on January 5th 2021.

3. Author contributions

All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript. Concept – S., E.S.S, H.S., K., H.A.W.; Design – S. K., E.S.S.; Supervision – S., E.S.S., H.S., K., H.A.W.; Resources – S., E.S.S., H.S., K., H.A.W.; Materials – S., E.S.S., H.S; Data Collection and/or Processing – S., E.S.S., H.S; Analysis and/or Interpretation – S., E.S.S., K.; Literature Search – S., E.S.S, H.S., K., H.A.W.; Writing – S., K., E.S.S.

References


[26] Belayneh YM, Birru EM. Anti-diabetic Activities of Hydromethanolic Leaf Extract of Calpurnia aurea (Ait.) Benth. Subspecies aurea (Fabaceae) in Mice. Hindawi Evidence-Based Complementary and Alternative Medicine, 2018, Article ID 3509073, 9 pages