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Jatropha curcas L. and *Mimosa pudica* L. as Potential Sources of Xanthine Oxidase and α-Glucosidase Inhibitors

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ABSTRACT

Medicinal plants have been of interest in discovering new xanthine oxidase inhibitors (XOIs) and α-glucosidase inhibitors (AGIs) in recent years. The aim of this study was to evaluate the XOI and AGI capacities of *Jatropha curcas* L. and *Mimosa pudica* L. from Itogon and Mankayan, Benguet. The methanol crude extracts (M), ethyl acetate fractions (E), and n-hexane fractions (H) of *J. curcas* (JM, JE, JH) and *M. pudica* (MM, ME, MH) were used. Thin-layer chromatography (TLC) direct bioautography detected the presence of XOIs in the fractions, and spectrophotometry evaluated the XOIs in the crude extracts and fractions. Samples were also subjected to an AGI assay. The fractions exhibited positive results, with yellow and white spots. MM (79.09 µg/ml and 74.35 µg/ml), ME (72.66 µg/ml and 69.90 µg/ml), and JE (82.03 µg/ml and 60.42 µg/ml) had the lowest XOI half-maximal inhibitory concentration (IC_{50}). The positive control, allopurinol, had an IC_{50} of 8.68 µg/ml. *M. pudica* demonstrated better AGI than *J. curcas*. MH showed higher inhibition at 97.36 ± 1.23% and 98.33 ± 0.78%, for Itogon and Mankayan, respectively, than acarbose, at 92.58 ± 0.63%. Both plants may be used as sources of lead compounds beneficial for patients with gout, while *M. pudica* is a potential source of new antidiabetic compounds upon clinical trials. Further studies should be conducted to purify and identify the bioactive compounds.

Keywords: xanthine oxidase inhibitor, α-glucosidase inhibitor, *J. curcas*, *M. pudica,* thin-layer chromatography direct bioautography

INTRODUCTION

Gout affects almost 1.6 million Filipinos, and the prevalence has been continuously growing over time as a result of an unhealthy diet and lifestyle (Crisostomo, 2015). Hyperuricemia, the primary etiologic factor of gout, results from an overproduction and underexcretion of uric acid (UA; Chen et al., 2016). It is also associated with other conditions like diabetic ketoacidosis, pernicious anemia, psoriasis, obesity, congestive heart failure, renal dysfunction, and Down's syndrome (Benn et al., 2018; DiPiro et al., 2008).

Xanthine oxidase (XO) is an enzyme responsible for the production of UA. Hence, XO inhibitors (XOIs) are employed

to block the biosynthesis of UA, thereby reducing UA levels in the body (Kostić et al., 2015). XOIs allopurinol and febuxostat have been proven effective in treating gout by reducing UA levels. However, these drugs have limitations due to various side effects (Guerrero & Guzman, 1998; Hendriani et al., 2016) and reduce the tolerance to long-term treatment. Febuxostat has limited accessibility because of its cost, especially in developing countries (Duong et al., 2017). The latest commercial XOI, topiroxostat, has high bioavailability and is safe for animals, but there is limited information on its adverse effects. Therefore, there is still a continuous and intensive effort to develop and discover XOI drugs that could be alternatives or be used in conjunction with the current XOIs to minimize the adverse effects. In addition, increased XO in gout was also seen as an additional source of reactive oxygen species leading to chronic oxidative injury as well as endothelial dysfunction in type 2 diabetes mellitus patients with poor glycemic control, contributing over time to the development of diabetic peripheral neuropathy (Mirić et al., 2016). Desco et al. (2002) concluded that XOIs completely inhibit superoxide formation and may have clinical significance in preventing late-onset vascular complications of diabetes. This has piqued interest in testing plant extracts and fractions for their antidiabetic property.

Diabetes is another condition that affects many Filipinos being the fourth cause of mortality in the years 2021 and 2022 in the Philippines (*2022 Causes of Deaths in the Philippines*, 2023) and described as a global burden with 10.5% recorded cases among the adult population (20–79 years; International Diabetes Federation, 2023). α-Glucosidase inhibitors (AGIs) play an important role as part of combination therapy of diabetes, especially in people who consume high-carbohydrate diets and have high postprandial glucose levels, as it works by preventing the conversion of complex, nonabsorbable carbohydrates into simple, absorbable ones in patients (Akmal, 2022). Two AGIs are on the market, namely, acarbose and miglitol.

The plants of interest, *J. curcas* and *M. pudica,* locally known as "tagumbao" and "makahiya," respectively, were used by some locals as traditional medicine for rheumatism in Sablan, Benguet (Balangcod & Balangcod, 2015). Both plants are widely distributed in Benguet and accessible to the locals. *M. pudica* extracts also possess antibacterial, antivenom, antifertility, anticonvulsant, antidepressant, aphrodisiac, and various other pharmacological activities (Ahmad et al., 2012). It also showed anti-inflammatory activity (Mistry et al., 2022; Nair & Nair, 2017; Sumiwi et al., 2014) and antidiabetic properties (Tunna et al., 2015). In the study for XOI of selected Philippine plants, *M. pudica* exhibited the lowest IC_{50} (Apaya & Chichioco-Hernandez, 2011). *J. curcas* has anti-inflammatory properties and other medicinal uses (Uriarte & Culaba, 2008). An in silico study on *J. curcas* by molecular docking showed that it could be a source of novel XOI leads (Mohapatra et al., 2015). It is also one of the plants identified to be used traditionally as an antidiabetic medicinal plant in Congo (Kasali et al., 2021). However, Ogunjinmi et al. (2023) tested ethanol leaf extracts of *J. curcas* for its α-glucosidase inhibitory capacity, and it exhibited the least inhibitory effect among four plants tested in the study. Methanol extracts by maceration and homogenized-assisted extraction of *J. curcas* were studied, and the stem extracts displayed better α-glucosidase inhibitory activity than the leaf extracts (Zengin et al., 2021). Hence, hexane and ethyl acetate were also used as solvents in this study.

In 2005, researchers developed a rapid thin-layer chromatography (TLC) bioautographic method for detecting XOIs (Ramallo et al., 2005). It is a planar chromatography integrated with biological detection used to detect antimicrobial

activity, antioxidant activity, and enzyme inhibition like XO inhibition (Zang et al., 2020). It can also detect the presence of estrogenic compounds (Dewanjee et al., 2015). However, there are no available studies that used the method to identify the presence of XOIs in *J. curcas* and *M. pudica*; therefore, it was used in this study.

Karimi et al. (2020) have identified that edaphic factors like soil type, pH, moisture, and composition influence plant compounds. Climatic variations due to differences in elevation like temperature, precipitation, solar irradiation, and relative air humidity affect the chemical profile of plants (De Sá Filho et al., 2022; Pietrzak & Nowak, 2021). High elevation is associated with low mean temperature, short growing season length, high precipitation, high winds, low soil quality, high ultraviolet (UV) intensity, and low partial pressure of carbon dioxide while lower elevation is characterized by high mean temperature, long growing season, low precipitation, low wind, high soil quality, low UV intensity, and high partial pressure of carbon dioxide (Richardson & Friedland, 2009). Soil type affects the plant growth as it influences the mineral contents, organic matter composition, and activities of soil enzymes of the soil (Quan & Liang, 2017). Therefore, a comparison of the XOI and AGI of the plants from the two sites was conducted.

The present study aimed to identify the presence of XOIs and AGIs in the traditional medicinal plants *J. curcas* and *M. pudica*, which are widely distributed in Benguet. It contributes to the existing knowledge on *J. curcas* and *M. pudica* as sources of XOIs but with the use of TLC direct bioautography, which has not been previously used in plant extracts. This provides supporting data on the currently known AGI potential of *M. pudica* and generated in vitro data on the AGI potential of *J. curcas* not found in previous studies. Therefore, the results of this study may aid future studies on new leads for antidiabetic and anti-gout drugs. Additionally, because the plants were obtained from two separate sites, the data were compared to evaluate whether the plants' geographic distribution affected their XOI and AGI potential.

MATERIALS AND METHOD

Sample Collection and Extraction

Healthy leaves of *J. curcas* and the whole plant of *M. pudica* were collected from Dalupirip, Itogon, Benguet (16°18'01.2"N 120°44'05.4"E) and Paco, Mankayan, Benguet (16°52'31.8"N 120°46'23.5"E). The samples were garbled and washed with distilled water prior to drying. Herbarium specimens of the plants shown in Figures 1–4 were identified and authenticated by Dr. Jones T. Napaldet, associate professor of the Department of Biology at Benguet State University, La Trinidad, Benguet.

Figure 1. Voucher specimen of the collected *M. pudica* from Mankayan, Benguet.

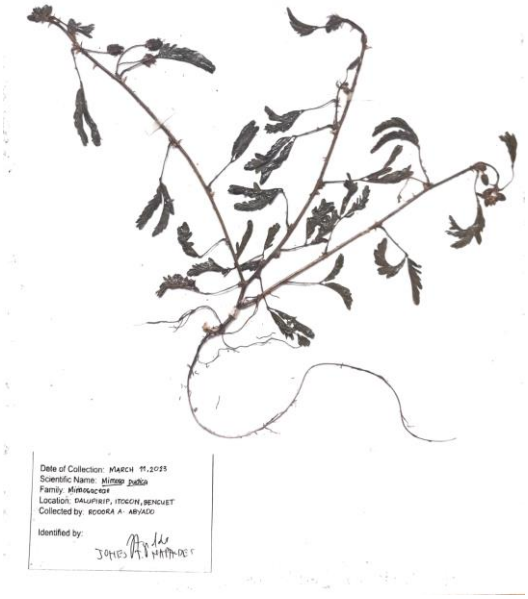


Figure 2. Voucher specimen of the collected *M. pudica* from Itogon, Benguet.

Figure 3. Voucher specimen of the collected *J. curcas* from Mankayan, Benguet.

Figure 4. Voucher specimen of the collected *J. curcas* from Itogon, Benguet.

The plants were dried in an oven at 60°C for not more than 8 hr. Then, powdered and macerated in methanol for 24 hr prior to extraction. Maceration and extraction were done three times. Lastly, the extracts from the three extractions were combined and concentrated under vacuum at a temperature of 60–65°C and 80 rpm in a Heidolph® rotary evaporator.

Liquid-Liquid Extraction of the Crude Extracts of J. curcas and M. pudica

The methanol crude extract was subjected to solvent partitioning using solvents with increasing polarity. The crude extract was dissolved with distilled water and poured into a separatory funnel. Then, n-hexane was added to remove nonpolar compounds. After n-hexane was removed, ethyl acetate was added to extract polar compounds. Hexane and ethyl acetate extraction were done three times. Then, the extracts from the three extractions were combined.

TLC Analysis of the Ethyl Acetate and Hexane Fractions of *J. curcas* and *M. pudica*

Materials and Equipment

Materials include TLC Silica Gel 60 F254 plates 20 cm × 20 cm, a twin-trough chamber (TTC) 10 cm × 10 cm (250-ml beaker or a TTC), capillary tubes, and 254-nm/366-nm CAMAG® UV lamp for visualization. The reference standard, allopurinol (Sigma A8003), was purchased from Sigma Aldrich, as well as xanthine (Sigma X7375), XO (Sigma X4376), and Nitroblue tetrazolium (NBT; Sigma N6B76). The TLC Silica Gel 60 F254 was from Merck. The chemicals methanol, ethyl acetate, n-hexane, chloroform ($CHCl_3$), sodium phosphate (Na_3PO_4) buffer, ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were also used in this research. All reagents used were analytical grade.

Sample Preparation

Ten milligrams of the extracts was dissolved well in 1 ml of MeOH using the Biobase® UC-100A ultrasonic water bath and Corning® LSE™ vortex mixer.

TLC General Procedure

The TLC Silica Gel 60 F254 plates were cut, and lines were drawn with a pencil, approximately 1 cm from the bottom and the other 1 cm from the top of the plates. The plates were then placed in an oven preheated at 105°C to eliminate moisture and cooled in a desiccator for 10–15 min. After cooling, the TLC plates were transferred to a flat and steady surface. Using a capillary tube, approximately 1–2 µl of the samples were gently and quickly spotted on the plates. After the spots were fixed on the plates, the plates were developed in the solvent system.

XOI TLC Direct Bioautography

The initial solvent systems used had different ratios of chloroform$_3$, methanol, hexane, and ethyl acetate. Appropriate adjustments were made to achieve good resolutions of the spots. The n-hexane-to-ethyl-acetate (6:4) ratio was selected to evaluate the ethyl acetate fractions of *J. curcas* (JE), the chloroform$_3$-to-methanol (6:4) ratio was selected for the ethyl acetate fractions of *M. pudica* (ME), and the n-hexane-to-chloroform (4:6) ratio was selected for the n-hexane fractions of *J. curcas* (JH) and *M. pudica* (MH).

A modified TLC direct bioautography to identify the presence of XOIs was used based on the protocol of Kong et al. (2018). The plates were stored for 24 hr to allow

complete drying after development. The plates were then dipped in the XO solution (0.1 U/ml) and incubated at 37°C for 6 min in a closed vessel. After incubation, the plates were dipped in the substrate solution, which is a mixture of xanthine (9 mM) and NBT (18 mM) in phosphate-buffered saline (PBS) (50 mM) to start the reaction, followed by incubation at 37°C for 20 min. Positive results were visually observed as yellow or white spots against a purple background.

Various factors were carefully monitored in performing the TLC direct bioautography including the ratio of the polar and nonpolar components of the solvent system, drying time of the plate after development, concentration of the substrate and indicator, pH of the buffer, incubation time, and temperature.

XO Inhibitory Assay

The 0.2 mg of methanol crude extracts, ethyl acetate fractions, and n-hexane fractions was dissolved in 1% DMSO to make 200 µg/ml and subsequently diluted with sodium phosphate buffer (0.15 M) to 100 µg/ml and 50 µg/ml. The allopurinol at 200 µg/ml, 100 µg/ml, and 50 µg/ml served as the positive control. The total assay solution was 1.6 ml, which consisted of a 0.5-ml sample at different concentrations, 50 µl of xanthine (1 mM), 50 µl of XO solution (2 U/ml), 0.5 ml of sodium phosphate buffer (0.15 M), and 0.5 ml of HCl (1 N) (Azmi et al., 2012). The solution without xanthine and HCl was first incubated at 37°C for 15 min, followed by the addition of xanthine to start the reaction. Then, it was incubated at 37°C for another 30 min. After incubation, the HCl was added to stop the reaction. Lastly, the absorbance was measured at 295 nm using an ultraviolet-visible (UV-Vis) spectrophotometer. The percent inhibition was calculated using the following formula:

$$\% \ inhibition = 1 - (\frac{\beta}{\alpha}) \times 100$$

where β is the absorbance with enzyme activity and α is the absorbance of the solution without enzyme activity (Azmi et al., 2012).

The mean values and standard deviation were calculated from triplicate measurements of the samples, and IC_{50} values were calculated from the concentration and percent inhibition through a linear regression analysis plot using MS Excel.

α-Glucosidase Inhibition Assay

All the extracts were analyzed at the Terrestrial Natural Products Laboratory of the Institute of Chemistry, University of the Philippines, Diliman. The assay used was based on the action of α-glucosidase on p-nitrophenyl α-D-glucosidase (p-NPG), which produces p-nitrophenol, a yellow product that can be spectrophotometrically detected. The rate at which the yellow color develops is correlated with enzyme activity; therefore, the presence of an inhibitor slows down this rate. The concentration of the samples used was 10 µg/ml, and the acarbose was at 1,000 µg/ml.

For each sample, a stock solution of 10,000 ppm and a working solution of 300 ppm in DMSO were prepared. Samples were homogenized using a vortex mixer, sonicated, and centrifuged. Each well of a 96-well quartz microplate consists of 190 µl of 50 mM PBS containing 100 mM sodium chloride (NaCl) at pH 6.8, 50 µl of 120-mU/ml enzyme solution, and 10 µl of 300-ppm sample. Each test sample solution has two trials and two replicates ($n = 2,\ t = 2$). The 200 µl of acarbose (well concentration of 1,000 ppm) was placed in the wells for the positive control, and 200 µl of 5% DMSO was placed in PBS (solvent well concentration of 3.33%) for the negative control. After incubation for 10 min at 37°C, 50 µl of 1.86 mM *p*-nitrophenyl-α-D-glucopyranoside was added to start the

reaction. The absorbance of the liberated *p*-nitrophenol was measured at 405 nm every 30 s for 30 min using a Multiskan Go® UV-Vis Spectrophotometer.

The inhibitory activities of the samples and the positive control (acarbose) were determined based on the average slope of each replicate using the following equation:

$$\%\ inhibitory\ activity = \frac{slope_{uninhibited} - slope_{inhibited}}{slope_{uninhibited}} \times 100$$

where $slope_{uninhibited}$ is the slope of the line from the absorbance versus time plot of the negative control group and the $slope_{inhibited}$ is the slope of the line from the absorbance versus time plot of the samples or positive control.

A sample is considered active if the percent inhibition is greater than or equal to 50% and if there is a significant mean difference with negative control at $p < 0.05$. The Kolmogorov–Smirnov test, Levene's test, Brown–Forsythe test, Welch test, and one-way analysis of variance using SPSS 25.0 were employed to statistically analyze the data.

Statistical Analysis

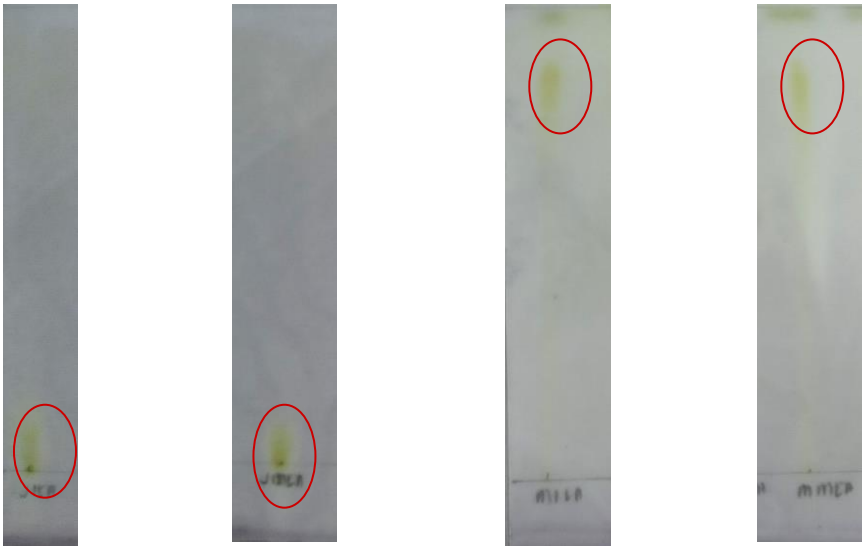
The XOI IC_{50} and AGI percent inhibition at 10 µg/ml of the samples from the two different sites was compared if there was a significant difference at $p < 0.05$. The unpaired *t*-test using QuickCalcs 2024 GraphPad® by Dotmatics Software was used to analyze the data.

RESULTS AND DISCUSSION

TLC Direct Bioautography of Ethyl Acetate and Hexane Fractions

The TLC direct bioautography was used to detect the presence of XOIs in ethyl acetate and hexane fractions of *J. curcas* and *M. pudica*, as shown in Figures 5–6. Yellow or white spots were observed and were indicative of the presence of XOI and superoxide scavenging activity in the samples, as described by Kong et al. (2018) in their development of an improved TLC direct bioautography assay.

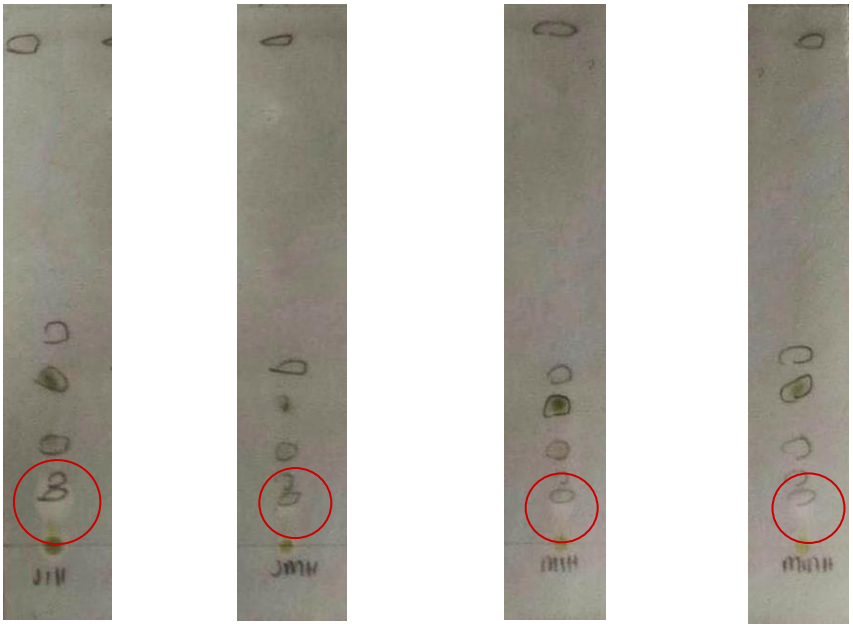
The ethyl acetate fractions showed yellow spots against a purplish background. The retention factor (Rf) of the yellow spots were seen at Rf = 0 for JE and at Rf = 0.86 for ME. For the hexane fractions, white spots at Rf = 0.07 and Rf = 0.057 of JH and MH, respectively, were observed. The spots with Rf = 0 indicate that the sample did not move at all while Rf > 0 indicates that the separation of the sample has occurred. Spots with lower Rf are more polar as they adhere more to the polar stationary phase and vice versa.



J. curcas Itogon | *J. curcas* Mankayan | *M. pudic*a Itogon | *M. pudica* Mankayan

Ethyl Acetate Fractions

Figure 5. TLC direct bioautography of *J. curcas* and *M. pudica* ethyl acetate fractions.



J. curcas Itogon | *J. curcas* Mankayan | *M. pudica* Itogon | *M. pudica* Mankayan

Hexane Fractions

Figure 6. TLC direct bioautography of *J. curcas* and *M. pudica* hexane fractions.

Previous works on direct bioautography used observations on different parameters, including color and Rf values. In a previous high-performance thin-layer chromatography (HPTLC) direct bioautography on the ethyl acetate extract of *Filipendula ulmaria*, active fractions were seen as yellow spots against a purple background. After further analysis, spots at Rf 0.6 were identified to contain mostly spiraeoside, those at Rf 0.9 contain quercetin, and those at Rf 0.95 have kaempferol. These turn out to be strong inhibitors (Gainche et al., 2021). Another TLC direct bioautography study has successfully identified well-known XOIs, astragaloside IV, kaempferol, and quercetin, from plant extracts and fractions. These identified compounds appeared as clear white zones against a purple background. Similarly, XOIs in *A. membranaceus* var. *Mongholicus* root extracts were also seen as white spots (Kong et al., 2018). Although white and yellow spots against a purplish background may be indicative of the presence of XOIs in the fractions, it may also indicate the presence of superoxide radical scavengers. Hence, a control assay may be used to differentiate pure XOIs from superoxide radical scavengers (Ramallo et al., 2005). Alternatively, in this current study, a spectrophotometric method was used to further test for the presence of XOIs in the samples in this study.

XO Inhibition Assay

The results of the spectrophotometric XOI test are shown in Table 1. It shows the inhibition of the methanol, ethyl acetate, and hexane extracts at different concentrations of the two plants, *J. curcas* and *M. pudica,* collected from two different sites, Itogon, Benguet, and Mankayan, Benguet.

The methanol crude extracts of *M. pudica* (MM) from Itogon and Mankayan had 64.23 ± 0.14% (IC_{50} = 79.09 µg/ml) and 60.78 ± 0.13% (IC_{50} = 74.35 µg/ml) inhibition at 100 µg/ml. This is comparable to previous studies of the methanol and ethanol leaf extracts of *M. pudica* with 62.36% (IC_{50} = 32.8 µg/ml) and 62.26 ± 6.26% (IC_{50} = 83.12 µg/ml) inhibition (Apaya & Chichioco-Hernandez, 2011; Hendriani et al., 2016). JE has lower IC_{50}, with 82.03 µg/ml and 60.42 µg/ml, than ME samples while for the hexane fractions, MH has lower IC_{50} than JH.

Table 1. Xanthine Oxidase Percent Inhibition and IC_{50} of the Methanol, Ethyl Acetate, and Hexane Extracts of *J. curcas* and *M. pudica*

Sample	Xanthine Oxidase Inhibition			IC_{50}
	200 µg/ml	100 µg/ml	50 µg/ml	
Allopurinol (positive control)	90.66 ± 0.44	81.58 ± 0.15	52.54 ± 0.63	8.68
Methanol				
JM Itogon	74.01 ± 0.11	58.37 ± 0.08	33.02 ± 0	96.64
JM Mankayan	84.12 ± 0.05	63.20 ± 0.12	30.19 ± 0.13	89.55
MM Itogon	80.85 ± 0.03	64.23 ± 0.14	36.29 ± 0.11	79.09
MM Mankayan	78.48 ± 0.21	60.78 ± 0.13	41.09 ± 0.28	74.35
Ethyl acetate				
JE Itogon	59.01 ± 0.10	57.24 ± 0.13	43.32 ± 0.20	82.03
JE Mankayan	95.35 ± 0.34	74.56 ± 0.50	39.22 ± 0.36	60.42

ME Itogon	72.28 ± 0.26	55.19 ± 0.15	31.76 ± 0.11	104.65
ME Mankayan	71.45 ± 0.20	56.10 ± 0.25	27.61 ± 0.19	109.23
Hexane				
JH Itogon	66.46 ± 0.07	44.16 ± 0.10	24.46 ± 0.44	134.98
JH Mankayan	76.65 ± 0.05	54.25 ± 0.11	23.25 ± 0.15	112.57
MH Itogon	73.80 ± 0.06	63.29 ± 0.10	40.23 ± 0.06	72.66
MH Mankayan	74.87 ± 0.05	52.92 ± 0.04	48.09 ± 0.09	69.9

Note. JM = methanol crude extracts of *J. curcas*; MM = methanol crude extracts of *M. pudica*; JE = ethyl acetate fractions of *J. curcas*; ME = ethyl acetate fractions of *M. pudica*; JH = n-hexane fractions of *J. curcas*; MH = n-hexane fractions of *M. pudica*.

Extracts with IC_{50} < 100 µg/ml may have the potential for further development, whereas extracts with IC_{50} > 100 µg/ml have a lesser potential for further development (Hendriani et al., 2016). This may warrant further investigation of the extract or fractions. Almost all extracts except that of ME and JH have IC_{50} < 100 µg/ml as seen in Figure 7, which means that the majority of the extracts have XOI potential. The standard allopurinol had an IC_{50} of 8.68 µg/ml.

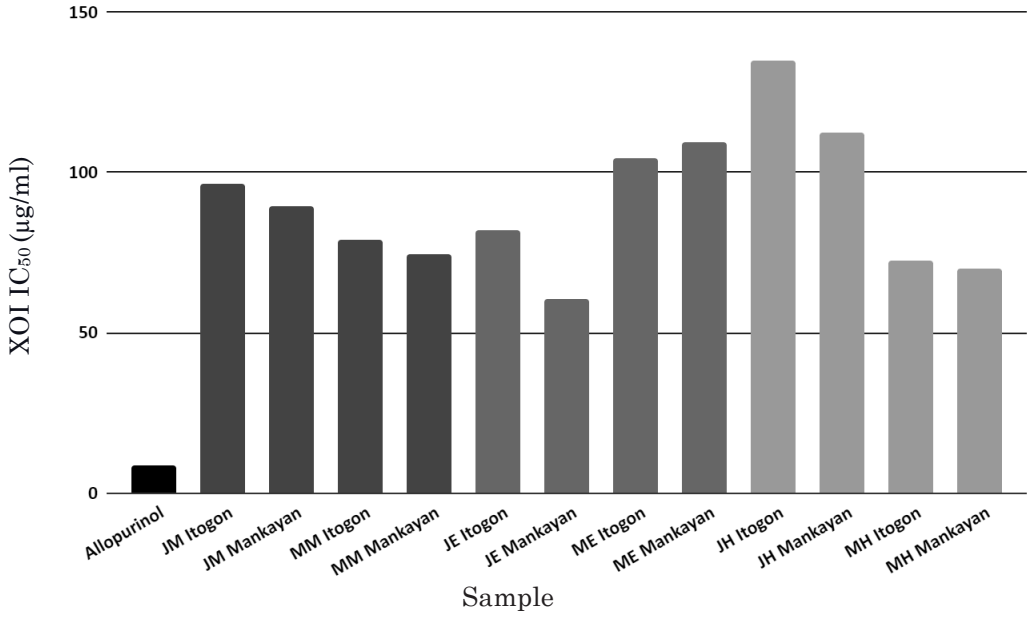


Figure 7. Xanthine oxidase inhibition IC_{50} of the samples and positive control, allopurinol. *Note.* JM = methanol crude extracts of *J. curcas*; MM = methanol crude extracts of *M. pudica*; JE = ethyl acetate fractions of *J. curcas*; ME = ethyl acetate fractions of *M. pudica*; JH = n-hexane fractions of *J. curcas*; MH = n-hexane fractions of *M. pudica.*

A comprehensive review of the *Mimosa* genus showed that the common metabolites present in the methanolic extract of *M. pudica* are tannins, flavonoids, and phenols (Rizwan et al., 2022). Studies on various extracts and fractions have shown that cardiac glycosides, flavonoids, phenolics, tannins, anthocyanins, and terpenoids can be isolated from plant extracts with XOI activity (Bakar et al., 2018). In contrast, the latex of *J. curcas* contains the alkaloids jatrophine, jatropham, and curcain, which have anticancer properties. It has also been applied externally to livestock to treat skin diseases and sores. *J. curcas* leaves contain metabolites such as apigenin, vitexin, and isovitexin, which are beneficial against malaria rheumatic and muscular pains. The seeds contain the alkaloid curcin, which makes them unsuitable for human consumption. In addition to the presence of alkaloids in various parts of the plant, the methanolic leaf extract was found to contain high amounts of flavonoids and cardiac glycosides (Oyama et al., 2016).

Certain polyphenols like flavonoids and tannins may range from weak to strong XOIs (Gainche et al., 2021). Terpenoids and cardiac glycosides did not have XOI activity (Apaya & Chichioco-Hernandez, 2011). Phenolic compounds, coumarins, flavonoids, and steroids have shown relatively high XOI activity, and some became leads for more detailed structure-activity studies (Pacher et al., 2006). Therefore, flavonoids, tannins, and phenols present in *M. pudica* and *J. curcas* might be responsible for the potential XOI properties exhibited in the TLC direct bioautography and the spectrophotometric XOI assay.

α-Glucosidase Inhibition Assay

Figure 8 presents our results on the AGI assay. *M. pudica* extracts and fractions exhibited high AGI activity comparable with the positive control, acarbose. In addition, it showed higher AGI potential than *J. curcas*. MH showed the highest inhibition at 97.36 ± 1.23% and 98.33 ± 0.78%, for Itogon and Mankayan, respectively, which is higher than the positive control, acarbose, at 92.58 ± 0.63%.

Previous studies demonstrated that the ethyl acetate extract is more active than hexane extracts of *M. pudica* (Afham et al., 2022; Sapkota et al., 2022). Methanol crude extracts of *M. pudica* with 95.65% inhibition at 1 mg/ml, which was described as a good inhibitory action, were also reported in a previous study (Tunna et al., 2015). The higher AGI activity of *M. pudica* than the positive control may be attributed to the plant parts used. Most of the previous experiments were conducted using *M. pudica* leaves; however, for this study, the whole plant, including the roots, was utilized. Hence, with the consideration that *M. pudica* is a creeping plant belonging to the legume family Fabaceae, this may imply a higher concentration of the probable metabolite responsible for its activity in roots. Legumes produce high diversity of secondary metabolites for their defense mechanism and to facilitate pollination (Wink, 2013). Limited studies on AGI activities of extracts of *J. curcas* can be found.

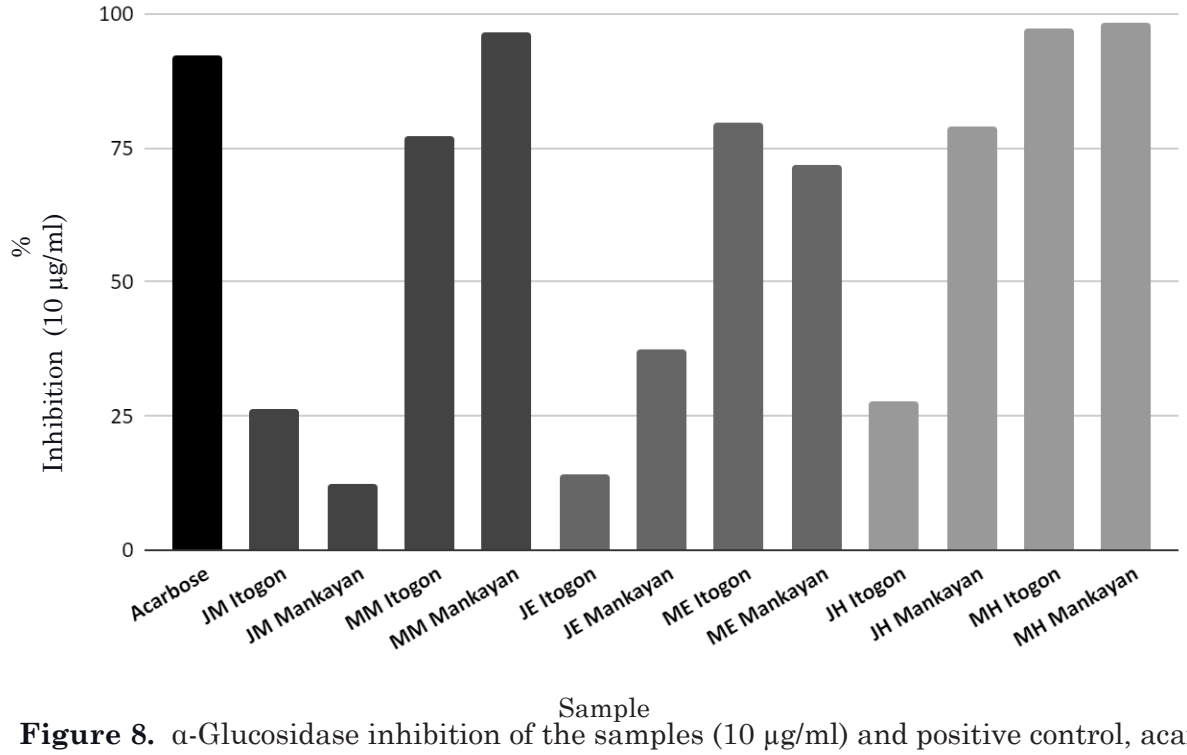


Figure 8. α-Glucosidase inhibition of the samples (10 µg/ml) and positive control, acarbose (1,000 µg/ml). *Note.* JM = methanol crude extracts of *J. curcas*; MM = methanol crude extracts of *M. pudica*; JE = ethyl acetate fractions of *J. curcas*; ME = ethyl acetate fractions of *M. pudica*; JH = n-hexane fractions of *J. curcas*; MH = n-hexane fractions of *M. pudica.*

Table 2 shows the activity of the methanol, ethyl acetate, and hexane extracts against α-glucosidase. Samples that have percent inhibition greater than or equal to 50% and are significantly different against negative control at $p < 0.05$ are considered active against α-glucosidase.

Table 2. α-Glucosidase Percent Inhibition of the Samples at 10 µg/ml and Positive Control (Acarbose) at 1,000 µg/ml

Sample	% Inhibition
Acarbose (positive control)	92.48 ± 0.63*
Methanol	
JM Itogon	26.38 ± 7.58
JM Mankayan	12.23 ± 2.38
MM Itogon	77.36 ± 8.85
MM Mankayan	96.67 ± 1.35*
Ethyl acetate	
JE Itogon	14.05 ± 2.22*
JE Mankayan	37.50 ± 2.52*
ME Itogon	79.67 ± 4.17*

ME Mankayan	71.93 ± 4.46*
Hexane	
JH Itogon	27.70 ± 3.06*
JH Mankayan	78.92 ± 10.09
MH Itogon	97.36 ± 1.23*
MH Mankayan	98.33 ± 0.78*

Note. JM = methanol crude extracts of *J. curcas*; MM = methanol crude extracts of *M. pudica*; JE = ethyl acetate fractions of *J. curcas*; ME = ethyl acetate fractions of *M. pudica*; JH = n-hexane fractions of *J. curcas*; MH = n-hexane fractions of *J. curcas*.
*Statistically significant against the negative control at 0.05 level.

The AGI activity of *M. pudica* may be from the compounds isolated and identified from previous studies. These compounds are stigmasterol, quercetin, and avicularin (Tasnuva et al., 2017), as well as protocatechuic acid and syringic acid isolated from the 80% ethanol extract of *M. pudica* (Lan et al., 2023). Moreover, the in silico toxicity study of the methanolic extract of *M. pudica* roots revealed no toxicity with the Ames test, no hepatotoxicity, and no skin sensitization (Shrestha et al., 2022).

Based on the results of the XOI and AGI assays, it can be concluded that *J. curcas* and *M. pudica* are good candidates as anti-gout drug leads because of their XOI action. At the same time, *M. pudica* may be a source of antidiabetic compounds because of its AGI action.

Comparison of the Plants From the Two Collection Sites

The current study also compared the activities of the two species from two collection sites in Benguet Province. In Table 3, we show that there was a significant difference in the mean XOI IC_{50} of *J. curcas* and *M. pudica* collected from the two different sites, Itogon and Mankayan.

Table 3. Comparison of the Xanthine Oxidase Inhibition IC_{50} of *J. curcas* and *M. pudica*

Sample		*t*	*d*	Mean Difference	Std Error Difference	*p*-Value at <0.05
J. curcas	M	42.66	4	7.16	0.17	<0.0001*
	E	22.57	4	21.70	0.96	<0.0001*
	H	44.02	4	22.35	0.51	<0.0001*
M. pudica	M	9.08	4	4.69	0.52	0.0008*
	E	11.72	4	−4.54	0.39	0.0003*
	H	10.60	4	2.94	2.17	0.0004*

Note. M = methanol crude extracts; E = ethyl acetate fractions; H = n-hexane fractions.
*Statistically significant.

Similarly, in Table 4, there was a significant difference in the mean percent xanthine inhibition at 10 µg/ml of the *J. curcas* and *M. pudica* (except ME) collected from the two different sites, Itogon and Mankayan.

Table 4. Comparison of the α-Glucosidase Inhibition at 10 µg/ml of *J. curcas* and *M. pudica*

Sample		*t*	*d*	Mean Difference	Std Error Difference	*p*-Value at <0.05
J. curcas	M	3.56	6	14.15	3.97	0.0119*
	E	13.97	6	−23.45	1.68	<0.0001*
	H	9.72	6	−51.22	5.27	<0.0001*
M. pudica	M	4.31	6	−19.31	4.48	0.0050*
	E	2.54	6	7.74	3.05	0.0444*
	H	1.33	6	−0.97	0.73	0.2312

Note. M = methanol crude extracts; E = ethyl acetate fractions; H = n-hexane fractions.
*Statistically significant.

The results of both experiments showed that the difference in XOI and AGI activities of the plants gathered from two different sites were statistically significant. The differences might be attributed to the difference in soil types: Itogon has sand loam and clay loam, while Mankayan has sand loam (DENR-PENRO-Benguet, 2019). Karimi et al. (2020) have identified that edaphic factors like soil type, pH, moisture, and composition influence plant compounds. Additionally, the collection site in Mankayan has a higher elevation at 3,169 feet than that of Itogon at 1,247-feet elevation, hence the difference in climatic conditions. Studies have shown that climatic variations like temperature, precipitation, solar irradiation, and relative air humidity affect the chemical profile of plants (De Sá Filho et al., 2022; Pietrzak & Nowak, 2021).

Pant et al.'s (2021) study on the effects of numerous environmental variables on the production and accumulation of secondary metabolites stated that plants create a certain quantity and quality of secondary metabolites in response to diverse environmental stresses. These variables cover soil water, soil salinity, illumination, temperature, carbon dioxide, and soil fertility. Thus, even plants of the same species grown in different environments have different concentrations of a certain secondary metabolite. The amount of rain and changes in temperature were observed to cause seasonal patterns in the levels of phenolics in the leaves and stems of *Tithonia diversifolia* (Sampaio et al., 2016). There was an increase in the flavonoid content of *Centella asiatica* (L.) after exposure to elevated carbon dioxide (Moghaddam et al., 2017). The phenol chlorogenic acid is higher in *Erigeron breviscapus* grown in areas exposed to sunlight than in those in shaded areas (Zhou et al., 2016). The total flavonoid

content of wild *Dendrobium officinale* Kimura & Migo was higher than that of plants cultivated bionically and in a greenhouse (Yuan et al., 2020). Total phenol, flavonoid, and saponin contents of *Mentha piperita* and *Catharanthus roseus* plants decreased in response to drought and heat stress; however, levels of other secondary metabolites, including tannins, terpenoids, and alkaloids, increased under stress in both plants (Alhaithloul et al., 2019).

Indeed, the geographical location of plants affects biological activities due to variations in phytochemical levels, which is why it is suggested that this must be considered when utilizing plants as raw materials for industrial and traditional applications (Khattak & Rahman, 2015).

CONCLUSION

The *M. pudica* and *J. curcas* plant extracts were shown to possess XOIs and AGIs. *M. pudica* hexane fraction demonstrated higher inhibition than the positive control, acarbose, and in general, *M. pudica* has a higher α-glucosidase inhibitory capacity than *J. curcas*. Our results suggest that *M. pudica* may be a potential source of antidiabetic compounds. In addition, both plants are good candidates for possible anti-gout compounds.

Further studies to identify and to isolate the XOI and AGI compounds present in the plants and to evaluate their safety relative to their anti-gout and antidiabetic uses are recommended. Most importantly, a detailed study of the influence of location on the chemical composition of plants must be conducted to determine the reason for this variation.

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