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Inhibition of xanthine oxidase activity from extracted *Agathis philippinensis* (Almaciga), *Tabernaemontana pandacaqui* (Banana bush), and *Cymbopogon citratus* (Lemon grass)

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Abstract

Xanthine oxidase is a terminal enzyme for the breakdown of both hypoxanthine and xanthine to uric acid which has an essential role in conditions like hyperuricemia and gout. The "Malaueg" Community of Rizal, Cagayan identified *Tabernaemontana pandacaqui* (Banana bush) as having wound healing properties and *Agathis philippinensis* (Almaciga) and *Cymbopogon citratus* (Lemon grass) capable of easing arthritis. This study determined the phytochemical profiles of the plants and xanthine oxidase inhibition (XOI) potentials using UV-Vis spectrophotometer. The aqueous extract of the plants was screened for the presence of phytochemicals. *A. philippinensis* showed slightly active signals for flavonoids and tannins. *T. pandacaqui* showed active signals for coumarins, flavonoids, and tannins and very active signals for saponins. *C. citratus* showed slight active signals for tannins, active signals for flavonoids and very active signals for coumarins. The aqueous extracts were used for xanthine oxidase inhibition. Aqueous extracts of *T. pandacaqui, C. citratus,* and *A. philippinensis* elicited an XOI percentages of 57.53 ±4.169, 54.96 ± 3.030, and 22.63 ±0.7898, *respectively*. The positive control, Allopurinol obtained a percentage inhibition of 88.82 ±1.507. *T. pandacaqui* and *C. citratus* are potential herbal plants for xanthine oxidase inhibition and for drug discovery.

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Introduction

Xanthine oxidase is a molybdenum-protein enzyme that catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid, which is a breakdown product of endogenously synthesized and ingested purines (Kostic *et al.*, 2015). In humans, however, uric acid does not undergo further metabolism and is excreted through the kidneys and intestine. High uric acid levels may be a response to increased xanthine oxidase activity and oxidative stress. This waste product accumulates in the synovial fluids of the joints, connective tissue or in the kidneys in the form of calculi, causing decreased flexibility of joints. This condition is called gout and causes rheumatic pain and arthritis (Cheng *et al.*, 2015 and Singh *et al.*, 2010). Over the century, several attempts have been made to provide evidence and elicit relevant information associated with hyperuricemia and gout and the risk factors that each can contribute to its pathogenesis. Pharmaceutical drugs like allopurinol which inhibits xanthine oxidase activity, can lower uric acid levels and had been reported to have adverse side effects such as producing reactive oxygen species (ROS) that may cause wide spectra of physiological and pathological diseases (pain and bleeding when urinating, sore throat and headache, nausea, loss of appetite and jaundice) (Cantu-Medellin *et al.*, 2013).

Medicinal plants that have a folkloric use are subjected into the study to really see if they exhibit therapeutic activity and can serve as an alternative to commercially available drugs. *Agathis philippinensis*, *Tabernaemontana pandacaqui,* and *Cymbopogon citratus* are commonly known as Almaciga, Banana bush, and Lemon grass, *respectively*. These plants are widely distributed in some parts of the Philippines like Northern Luzon, Babuyan Islands, Palawan and Mindanao and other provinces within the country. And were reported to have medicinal properties in which several groups of people within the country like Kankana-ey people of Benguet, Ifugao-migrants of Sierra Madre and Ayta people of Porac, Pampangga had used these for therapeutic purposes for dysentery, ulcer, arthritis, colds, asthma, wound healing, bladder problems and rheumatism (Stuart, 2016a,b,c).

The identification of bioactive compounds is an important preliminary step in determining the plant's potential as an herbal drug, such as the ability to inhibit xanthine oxidase with fewer side effects. Thus, the activity of these three plants to inhibit xanthine oxidase using aqueous extracts has yet to be studied.

Materials and methods

Collection of Sample

Mature leaves were collected in Rizal, Cagayan and was identified and authenticated by Bureau of Plant Industry of Department of Agriculture. In the collection area, the leaves were wiped clean and stored in vacuum sealed bags for transport to the laboratory.

Preparation of Sample

Plant leaves were brushed briskly to remove visible soil and dust particles with deionized water as quickly as possible. Leaves were dried and water was blotted off with filter paper, weighed and placed in an oven at 40°C for 12 to 72 h. Dried samples were ground finely in a heavy duty grinder (Polymix PX–MFC–90 D). Then it was kept in vacuum sealed bags that were properly labeled and stored prior to analysis. Containers were kept in a cool, dry place.

Aqueous Extraction of Sample

Thirty grams of dried material with constant weight was soaked in 90mL of double distilled water (w/v). After soaking, the material was placed on a hot plate at 50°C for 2 hrs with constant agitation at 250 rpm. After 2 hrs, the material was kept overnight in a refrigerator at 4°C. On use, the material was placed on a hot plate at 50°C for another 2 hrs with constant stirring. Then the extract was collected and subjected to qualitative testing subsequently. However, samples that were subjected to xanthine oxidase assay were concentrated using the sample concentrator (SP Genevac EZ-2).

Qualitative Analyses of Phytochemical

Phytochemical screening of plant extracts was carried out using the following standard methods (Edeoga *et al.*, 2005 and Harborne, 1984).

a. Coumarins. Plant samples consisting of 0.5mL of the extract were mixed with 5 drops of NaOH, 0.5mL

of ethanol was added to the mixture. Yellow color formation indicates the presence of Coumadin's.

b. Flavonoids. Plant samples consisting of 0.5mL of plant extract was boiled with 2.5mL of distilled water for 5 minutes and was filtered while hot. Few drops of 20% sodium hydroxide solution were added to the cooled filtrate. A change from yellow color to colorless solution upon addition of 10% hydrochloric acid indicates the presence of flavonoids.

c. Saponins. Plant samples consisting of 0.5mL of plant extract and 1mL of distilled water was separately boiled in a water bath for 10 minutes. While hot, the mixture was filtered and cooled at room temperature; 1.5mL of the filtrate was diluted with 5mL of distilled water and was shaken vigorously for 2 minutes. Frothing indicates the presence of saponins in the filtrate.

d. Tannins. Five drops for ferric chloride solution was added to 0.5mL of the extract. Formation of the greenish brown, brown or black solution indicates the presence of tannins.

Xanthine Oxidase Inhibitory Assay

The xanthine oxidase activity was measured using a 9100 UV-Vis Spectrophotometer (Palintest 9100) and the assay was adapted from Owen and John, 1999 with some modifications. 50mM of phosphate buffer solution was prepared by dissolving potassium phosphate, (Sigma Prod. No. P-5379) in deionized water. The pH was adjusted to 7.5 at 25°C using 1 M of KOH. 0.15mM of xanthine solution was initially prepared by dissolving xanthine (from Sigma Aldrich) in a minimal volume of NaOH and adjusting the pH to 7.5 using 0.1 M of KOH or 1% of HCl. The plant solution was prepared by adding 0.4mg/mL of 50mM potassium phosphate buffer with a pH of 7.5. The xanthine oxidase from bovine milk was prepared by diluting 24µL of 5U/0.2mL of xanthine oxidase solution to a final volume of 3mL of cold 50mM potassium phosphate buffer reagent. The positive control, allopurinol, was prepared by dissolving 5 mg in 5mL of 50mM phosphate buffer, pH 7.5.

The total volume of the test sample consists of 0.750mL of plant extract/ allopurinol/ deionized water, 1.150mL of 50mM of potassium phosphate buffer, and 0.990mL of 0.015mM xanthine solutions. It was pre-incubated for 10 minutes at 25°C. After incubation, it was monitored for 3 minutes with absorbance reading increments every 30 seconds at 295 nm. 0.105mL of xanthine oxidase solution was added into the test sample to initiate the reaction and was set again for absorbance reading under the same conditions.

Xanthine oxidase activity was expressed as the percentage inhibition of XO in the above assay system calculated as:

% Inhibition = $\{(A-B) - (C-D) / (A-B)\} \times 100$

Where A is the activity of the enzyme without the fraction, B is the control of A without the fraction and enzyme; C and D are the activities of the fraction with and without XO, respectively.

Statistical Analysis

Data obtained from three replicates were expressed as mean ± standard deviation and statistically analyzed to verify accuracy and sensitivity of the measurements.

Results and discussion

Three Philippine medicinal plants were screened for their qualitative phytochemical properties as described by Edeoga *et al*., 2005 and Harborne, 1984; the results are shown in Table 1. While xanthine oxidase inhibition activity was measured using a modified procedure of Owen and John, 1999. The plant extracts and Allopurinol were evaluated for their ability to inhibit xanthine oxidase and thus prevent uric acid production. It revealed that the absorbance at 295 nm, *A. philippinensis* as shown in Table 2, has a percentage inhibition against xanthine oxidase of 22.63±0.7898 and obtains a slight active signal in flavonoids and tannins. This plant has been reported to have folkloric medicinal use in conditions such as arthritis (Stuart, 2016a). While *C. citratus* aqueous extract has a percent enzyme inhibition of 54.96±3.030 and phytochemicals reveal that it has a very active signal for coumarins; an active signal for flavonoids; and a slightly active signal for tannins (as shown in Table 1).

In various folkloric uses of this plant, it is reported to be used as a remedy for back pain and rheumatism. Among the three plants tested, *T. pandacaqui* elicited the highest XOI percentage of 57.53±4.169, and the bioactive compounds present in this plant are coumarins, flavonoids, saponins, and tannins, which have an anti-inflammatory activity as reported by Taesotiku *et al.*, 2003. The positive control, Allopurinol, showed 88.82±1.507 percent XOI activity. The differences in the XOI of the plant samples suggest the presence of phytochemicals which tend to increase the inhibition rate.

Table 1. The Results of the Phytochemical Screening of Aqueous Plant Extracts.

Phytochemical	*A. philippinensis*	*T. pandacaqui*	*C. citratus*
Coumarins	-	++	+++
Flavonoids	+	++	++
Saponins	-	+++	-
Tannins	+	++	+

Legend: + indicates slightly active signal, ++ indicates active signals, and +++ indicates very active signal

Table 2. The Result of Xanthine Oxidase Inhibition Percentage of Plant Extracts and Allopurinol.

Test Samples	Mean of% XOI
Allopurinol	88.82 ±1.507
Agathis philippinensis	22.63 ±0 .7898
Tabernaemontana pandacaqui	57.53 ±4.169
Cymbopogon citratus	54.96 ± 3.030

Values are mean ± S.D. of three parallel measurements.

The presence of the phytochemicals is important in the inhibitory activity against xanthine oxidase. In previous studies conducted by Cos *et al.*, 1998 and Jiao *et al.*, 2006, flavonoids had competitive inhibition interaction against xanthine oxidase, which prevented the activation of xanthine and resulted in a reduction of O2- generation and also uric acid formation. According to D'Mello *et al.*, 2011, flavonoids, mainly flavones and flavonols, also showed COX-2 inhibition in silico procedure, thus they have anti-inflammatory potential. A class of phenol compounds, including tannins to have the potential to inhibit xanthine oxidase which have non-selective binding of the enzyme (Owen and John, 1999 Anam *et al.*, 2017).

In the study of Lin *et al.*, 2008, the structural analogues of coumarins exhibit xanthine oxidase inhibition in a competitive manner by effectively docking on the active site of the molybdopterin domain in the xanthine oxidase enzyme by forming several hydrogen bonds with residues of polypeptide. Calderon *et al.*, 2016 also reported saponin to exhibit xanthine oxidase inhibitory activity.

To the best of our knowledge, leaf extracts of *Agathis philippinensis, Cymbopogon citratus,* and *Tabernaemontana pandacaqui* have not yet been studied for their ability to inhibit xanthine oxidase. As a result of this research these three plants could be studied further to develop a xanthine oxidase inhibitor.

Conclusion

The results showed that all three plant samples exhibited xanthine oxidase inhibition though the positive control, Allopurinol, still elicited the highest enzyme inhibition activity. The bioactive compounds present in each plant shows a parallel result with regards to the xanthine oxidase inhibition percentage.

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