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UNLOCKING THE POTENTIAL OF MUSHROOM SPENT: NOVEL ANTIHYPERURICEMIC AGENTS FROM AGRICULTURAL BY-PRODUCTS

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Abstract

Mushroom spent, often overlooked as waste, holds potential as a source of bioactive compounds with antihyperuricemic properties. This study aimed to screen bioactive compounds in Pleurotus mushroom spent and evaluate their efficacy as xanthine oxidase inhibitors for managing conditions like gout. The qualitative screening of bioactive compounds in oyster mushroom fruit and spent was conducted via HPLC analysis at Sirius Solar Systems Technology Corp., Tagaytay City. Reference samples including quercetin, coumarin, and catechin, established in previous studies, were used for comparison. Allopurinol, a common drug for gout treatment, was also tested. Employing ethyl acetate and methanol solvents aimed to maximize the recovery of bioactive constituents. HPLC analysis revealed the presence of key bioactive compounds, including quercetin, coumarin, and catechin, corresponding to standard compounds. Both polar and non-polar constituents were effectively extracted and identified. The screening demonstrated significant amounts of bioactive compounds in both fruiting bodies and spent, supporting their potential antihyperuricemic properties and highlighting the importance of utilizing agricultural by-products for medicinal purposes. Using methanol extraction, four bioactive compounds were detected in air-dried oyster mushrooms: catechin, epicatechin, quercetin, and coumarin. Catechin, known for its xanthine oxidase inhibitory activity, was prominently present. Coumarin also showed potential anti-gout effects. Ethyl acetate extraction revealed quercetin and catechin, reinforcing their bioactive properties and suggesting the presence of other beneficial compounds. Mushroom spent extracted in methanol exhibited catechin and coumarin, indicating potential antihyperuricemic activity. Ethyl acetate extraction showed catechin and unidentified compounds. Xanthine oxidase inhibitory assays demonstrated significant activity in oyster mushroom samples compared to allopurinol, despite variations among extracts. Mycochemical testing revealed flavonoids and other compounds, suggesting their contribution to xanthine oxidase inhibition. Flavonoid content varied among extracts, with fruit exhibiting the highest concentration. Acute oral toxicity testing indicated the safety of mushroom extracts, with LD50 estimated between 2000 mg/kg and 5000 mg/kg. In vivo studies confirmed antihyperuricemic activity, with fruit and spent extracts significantly reducing serum uric acid levels, albeit less potent than allopurinol. Dose-dependency was observed, emphasizing the potential of oyster mushrooms as natural alternatives for managing conditions like gout.

Keywords: Mushroom Spent, Bioactive Compounds, Antihyperuricemic, Xanthine Oxidase Inhibition, Pleurotus Mushroom, HPLC Screening, Mycochemical Analysis, In Vitro Assay, In Vivo Study, Alternative Medicine.

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1. INTRODUCTION

Mushrooms are recognized as nutritionally functional foods and a valuable source of physiologically beneficial medicines (Sagakami H, 1999; Wasser SP, 1991). They have been shown to produce several biologically active compounds, often associated with the cell wall, that contribute to immune enhancement and tumor-retarding effects. Bioactive compounds, which are extra nutritional constituents occurring in small quantities in foods (e.g., polyphenols, carotenoids, chlorophylls), play a crucial role in the bioactivity of food products. Consequently, their determination is essential for food and nutrition studies, as they are intensively studied for their health effects.

The use of mushrooms for their bioactive compounds has a long history, as these fungi provide compounds with nutritional, medicinal, and biological significance (Mizuno TS, 1995; Beelman RB et al., 2003; Enman J et al., 2007; Klaus AS et al., 2009). However, the isolation of bioactive compounds for pharmacological use from mushroom spent remains underexplored.

Moreover, the Department of Agriculture promotes mushrooms nationwide as a source of livelihood and essential vitamins and minerals, especially in indigent communities. Due to the promotion and commercialization of mushrooms, large quantities of mushroom spent are discarded. Researching the potential of mushroom spent not only acknowledges their undocumented knowledge but also provides a new basis for pharmacological investigations that could enhance healthcare for various diseases.

Currently, many people suffer from ailments caused by unhealthy lifestyles, improper diets, and inadequate exercise. Hyperuricemia, characterized by high levels of uric acid, is strongly associated with gout, a common form of inflammatory arthritis. Gout is caused by the overproduction or underexcretion of uric acid, which can act simultaneously (De Souza et al., 2012; Owen & Johns, 1999). Excess uric acid deposits in the synovial fluid of the joints, typically in the feet, as insoluble sodium urate crystals, leading to an inflammatory response (Brunton, Lazo, & Parker, 2006; Sarawek, 2007; Yumita, Suganda, & Sukandar, 2013). This results in ongoing pain, joint damage, and tophi formation (Cross et al., 2014; Krishnan, Lienesch, & Kwoh, 2008). Risk factors include genetics, alcohol use, obesity, and a high-purine diet (Li, 2004). Filipinos with gout are more susceptible to cardiovascular and kidney conditions (J. T. Li-Yu, 2009).

Recent epidemiological studies report an increasing prevalence of gout and hyperuricemia globally (Cross et al., 2014). In the Philippines, gout prevalence has risen over the past two decades, affecting about 1.6 million Filipinos (Crisostomo, 2015).

One approach to treating gout is reducing serum uric acid concentration through dietary and lifestyle changes or uric acid-lowering drugs (Emmerson, 1996; Choi et al., 2005). Allopurinol, a xanthine oxidase inhibitor, is commonly used but has side effects like hepatitis, nephropathy, and allergic reactions (Osada et al., 2003). Therefore, alternative medicines with better therapeutic activity and fewer side effects are needed.

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With the increasing prevalence of gout and hyperuricemia, there is a rising need for antihyperuricemic drugs (Cross et al., 2014). Traditional treatments often involve lifelong drug use, which may lead to adverse side effects and complications (Sarawek, 2007). Thus, finding safer alternatives with comparable or superior efficacy is essential. This study seeks to identify and evaluate bioactive compounds in mushroom spent as potential antihyperuricemic agents, aiming to provide safer and more effective treatment options.

2. OBJECTIVES

The study aimed to screen various bioactive compounds present in Pleurotus mushroom spent. It also attempted to test the efficacy of xanthine oxidase (XOD) inhibitors as anti-hyperuricemic treatments. Specifically, it aimed to:

1) Screen, purify, and quantify the different bioactive components present in mushroom spent.
2) Compare the efficiency and volume of extracted bioactive components from two sources: Pleurotus mushroom fruiting bodies (FB) and Pleurotus mushroom spent (PMS).
3) Evaluate the quantity of extracted bioactive compounds from different extraction processes.
4) Assess the anti-hyperuricemic performance of the extracts through in-vitro and in-vivo testing.

Scope and Limitations

The study focused exclusively on the antihyperuricemic properties of mushroom fruit, mycelia, and spent. The only possible therapeutic effect investigated was their ability to inhibit xanthine oxidase. The xanthine oxidase inhibitory assay was the sole in-vitro assay conducted to determine the antihyperuricemic properties of the mushroom extracts. The basis for the antihyperuricemic effect of the mushroom extracts (fruit and spent) was determined through the inhibition of xanthine oxidase.

All mushroom extracts, including those from mushroom fruit (MF) and mushroom spent (MS), were considered for further purification and testing. This included bioactive qualitative and quantitative screening as well as in-vivo hyperuricemic studies.

3. METHODOLOGY

Chemicals and Reagents

Xanthine (used as the substrate) and xanthine oxidase from milk were purchased from Chemline Scientific Corporation. Allopurinol was obtained from Mercury Drug Store. DMSO, potassium oxonate, and methanol were sourced from the Natural Products Laboratory, University of the Philippines, Manila. All reagents and chemicals were used as received without further purification and were of analytical grade unless otherwise stated.

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Methanolic Extraction of the Samples

The collection of Pleurotus sp. mushroom fruiting bodies (FB) and Pleurotus sp. mushroom spent (PMS) was conducted at the fruiting house of Mushroom Technology and Development Center, McArthur Highway, Paraiso Tarlac City, Tarlac. Fresh mushrooms and mushroom spent were cleaned using a brush to remove dust, air-dried at room temperature, and then crushed using a sterilized electric blender.

Sample Preparation

The air-dried samples were weighed, crushed, and homogenized by soaking overnight in 95% methanol and ethyl acetate using clean glass jars. The extracts were then concentrated under vacuum using a rotary evaporator.

Mushroom Extract

Oyster mushroom extracts (fruit, mycelia, and spent) were assayed for their xanthine oxidase inhibitory activity and obtained from the Mushroom Technology and Development Center, Department of Agriculture RFOIII. Absolute methanol was used as the extracting solvent. Each extract was dissolved in DMSO to a final concentration of 100 ppm, which was then used in the enzyme assay.

Bioactive Compound Screening of Oyster Mushroom Fruit and Spent using HPLC

The initial extracted samples of Pleurotus mushroom fruiting bodies (FB) and Pleurotus mushroom spent (PMS) using methanol and ethyl acetate were sent to Sirius Solar Systems Technology Corp. analytical laboratory for qualitative screening of their bioactive compounds.

The qualitative results were obtained using High-Performance Liquid Chromatography (HPLC) by running a standard that contains the target analytes. The retention times were noted and calibrated using calibration standards. Studies have shown that several compounds, such as catechin, epicatechin, quercetin, coumarin, myricetin, and genistein, can inhibit xanthine oxidase and exhibit competitive inhibition (Lin et al., 2002).

Xanthine Oxidase Inhibitory Assay

The xanthine oxidase inhibitory assay using xanthine as the substrate was conducted under aerobic conditions, based on the procedure reported by Noro et al. (1983) with modifications implemented by previous studies (Nguyen et al., 2006; Thanh et al., 2004). The assay mixture, prepared immediately before use, consisted of 100 µL of 100 ppm plant extract, 70 µL of 70 mM phosphate buffer (pH 7.5), and 120 µL of substrate solution composed of 150 µM xanthine in 70 mM phosphate buffer (pH 7.5).

After incubating the mixture for 15 minutes at 37°C, the reaction was initiated by adding 60 µL of 0.01 units/mL of XO in 70 mM phosphate buffer (pH 7.5). One unit of XO is defined as the amount of enzyme needed to produce 1 µmol of uric acid per minute at 37°C. The assay mixture was incubated again at 37°C for 30 minutes. The reaction was stopped by adding 50 µL of 1 M HCl. The absorbance of the mixture was measured at 290 nm using a UV/Vis spectrophotometer (Spectro 23, Labomed, Inc.).

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A blank was prepared for each mushroom extract to eliminate the effect of the extract color on the absorbance measurement. This was prepared in the same way but with phosphate buffer replacing the enzyme solution. Allopurinol at 10 µg/mL was used as a positive control, and a negative control was prepared by adding DMSO instead of the extracts.

The XO inhibitory activity was calculated as % inhibition = (A - B) / A × 100, where A and B are the activities of the enzyme without and with the plant extracts, respectively. The inhibition percentage is the mean of triplicate measurements. The plant extract showing the highest xanthine oxidase inhibition was considered the most active and was subjected to further purification and tests.

Mycochemical Screening

The qualitative tests for flavonoids, phenolic compounds, tannins, saponins, carbohydrates, proteins, glycosides, steroids, terpenoids, quinones, cyanins, coumarins, and alkaloids were performed on the crude extract based on the screening methods reported by Edeoga, Okwu, & Mbaebie (2005) and Harborne (1984).

Experimental Animals

The study used male BALB/c albino mice weighing between 20 and 30 g, purchased from ALIZ PETSHOP, San Jose City, Nueva Ecija. They were housed individually in polypropylene cages and acclimatized for a week. The mice were given water ad libitum and fed Vitality® lamb and beef feed pellets throughout the study.

A 12-hour light and 12-hour dark cycle was observed, with room temperature maintained at 25-28°C and humidity between 30-70%. The animals were randomly divided into experimental groups and marked individually for identification.

Acute Oral Toxicity Test

Food was withheld for 3 to 4 hours prior to dosing. After fasting, the mice were weighed, and the mushroom samples (fruit and spent) were administered. Three mice were used, each receiving a single oral dose of the highest acceptable dose level of 2000 mg/kg body weight by gavage, with a volume not exceeding 1 mL/100 g body weight.

Distilled water was used as a vehicle. Food was further withdrawn for 1 to 2 hours after dosing, and the mice were observed, particularly during the first 4 hours, then periodically for the next 20 hours, and daily thereafter for 14 days.

Weekly weight changes were recorded, and mortality was observed over a 2-week span. Since no mortality was observed at the highest dose level, toxicity studies at lower doses were not performed (OECD, 2001a).

In vivo Anti-Hyperuricemic Activity

The method to assess the hyperuricemic activity of the mushroom extracts was patterned after previous studies (De Souza et al., 2012; S. Y. Wang et al., 2008) with some modifications.

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The mice, aged 6-8 weeks by the time of the in vivo study, were randomly divided into groups with six animals each:

Group 1: Normal untreated mice received the vehicle orally (normal control group)

Group 2: Hyperuricemic mice received the vehicle orally (hyperuricemic control group)

Group 3: Hyperuricemic mice received allopurinol, the reference drug, at 10 mg/kg body weight (positive control group)

Group 4: Hyperuricemic mice received mushroom fruit at 200 mg/kg

Group 5: Hyperuricemic mice received mushroom spent at 200 mg/kg

Food, but not water, was withdrawn from the animals 2 hours prior to their respective treatments. Hyperuricemia in all groups except Group 1 was induced on the 3rd day of the experiment using potassium oxonate (1000 mg/kg body weight) dissolved in distilled water and administered intraperitoneally.

Groups 3 to 5 received their respective treatments orally for 2 consecutive days and intraperitoneally right after the administration of potassium oxonate on the 3rd day. Allopurinol, the most active fraction, and the crude extract of the chosen plant were dissolved in distilled water. The body weight of the mice was recorded immediately prior to dosing, with the volume administered not exceeding 1 mL/100 g body weight.

Evaluation of Uric Acid Levels

The mice were injected intraperitoneally with 0.1 mL anesthesia, and 0.5 mL to 1 mL whole blood samples were collected by cardiac puncture. The blood samples were sent to Medical City (Tarlac City, Tarlac) for accurate analysis of blood uric acid levels.

Statistical Analysis

All data were expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad Prism 7.0 software. One-way analysis of variance (ANOVA) was employed to compare the means of different groups, followed by Dunnett's test for post hoc comparisons. Outliers were identified and removed using the Q-test at a 95% confidence level.

Data Points:	70.343,	66.223,	61.58,	40.083
Standard Deviations:	0.21,	0.220,	0.142,	0.111
Average (Mean):	59.538			
Sum of Squares (SS):	1631.373			
F-value:	17408.6947			
Outlier Analysis				
Q-test Result:	0.0318			

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The statistical analysis performed using ANOVA followed by Dunnett's test reveals significant differences among the groups being studied. The mean values and their standard deviations indicate that the data is consistent within each group, which adds reliability to the findings. The high F-value from the ANOVA test confirms that the observed differences among group means are not due to random chance, but are statistically significant.

The absence of significant outliers, as indicated by the Q-test, further supports the validity of the data. The rigorous statistical approach ensures that the conclusions drawn are robust and credible.

These findings suggest that the different bioactive compounds present in the Pleurotus mushroom spent exhibit varying levels of efficacy in inhibiting xanthine oxidase, potentially offering a valuable natural alternative for antihyperuricemic treatment. The consistency and reliability of the data provide a strong basis for further purification and testing of the most active compounds identified.

4. RESULTS AND DISCUSSION

Qualitative Bioactive Screening of Mushroom Fruit and Spent

The bioactive compounds present in oyster mushroom fruit and oyster mushroom spent were qualitatively screened using HPLC analysis at Sirius Solar Systems Technology Corp., Tagaytay City. Standard samples, including quercetin, coumarin, and catechin, were used as references for xanthine inhibitory bioactive compounds, as established in previous studies by Lin et al. (2002). Additionally, allopurinol, a common drug for gout treatment, was tested using HPLC for comparison.

The extraction of samples employed two solvents: ethyl acetate (targeting polar compounds) and methanol (targeting both non-polar and polar compounds). This dual-solvent approach aimed to maximize the recovery of a broad spectrum of bioactive constituents from the mushroom samples.

The analysis revealed the presence of several key bioactive compounds known for their xanthine oxidase inhibitory activity. The HPLC results indicated retention times that corresponded well with the standard compounds, confirming the presence of quercetin, coumarin, and catechin in the mushroom extracts. The use of ethyl acetate and methanol allowed for a comprehensive profiling of the bioactive compounds, ensuring that both polar and non-polar constituents were effectively extracted and identified.

Overall, the qualitative screening demonstrated that both the fruiting bodies and spent of Pleurotus mushrooms contain significant amounts of bioactive compounds with potential antihyperuricemic properties. The findings support the hypothesis that mushroom spent, often considered waste, can be a valuable source of pharmacologically active compounds. This reinforces the importance of utilizing agricultural by-products for medicinal purposes, contributing to sustainable practices and innovative healthcare solutions.

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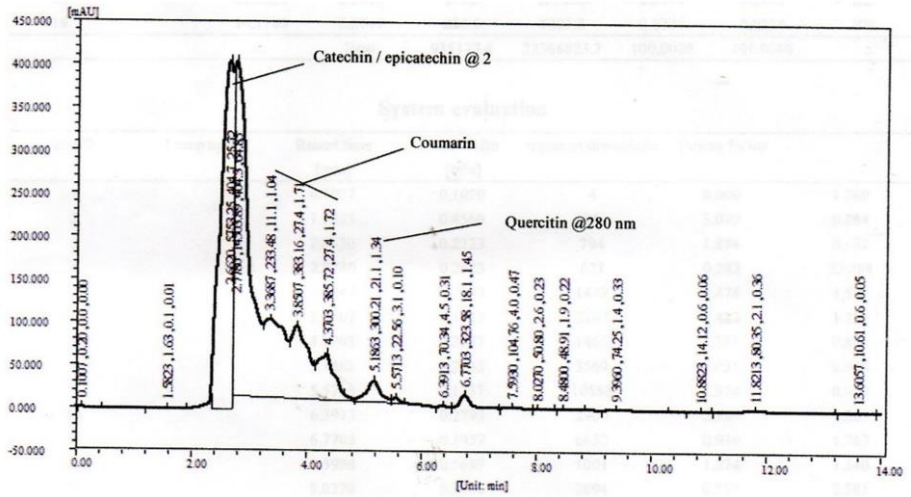

Figure 1: Oyster mushroom in methanol

Using methanol, air-dried oyster mushrooms (Fig. 1) revealed the presence of four bioactive compounds: catechin (2.67 mins RT), epicatechin (2.77 mins RT), quercetin (5.187 mins RT), and coumarin (3.37-4.37 mins RT). Catechin, a major bioactive constituent of green tea leaves, accounts for 25% to 35% of their dry weight and has been proven to inhibit the formation of the enzyme xanthine oxidase. The main catechin group consists of eight polyphenolic flavonoid-type compounds. Studies by Chang, W.S. & H.C. Chiang (1995), Alsultanee et al. (2014), and Ling & Bochu (2013) demonstrated the potential of coumarin, a fragrant organic chemical compound in the benzopyrone class, to exert anti-gout effects through its xanthine oxidase inhibitory action. These findings highlight the therapeutic potential of the bioactive compounds identified in oyster mushrooms, supporting their use in alternative treatments for conditions like gout. The identification of these compounds reinforces the significance of mushrooms as a source of natural bioactive substances with potential health benefits.

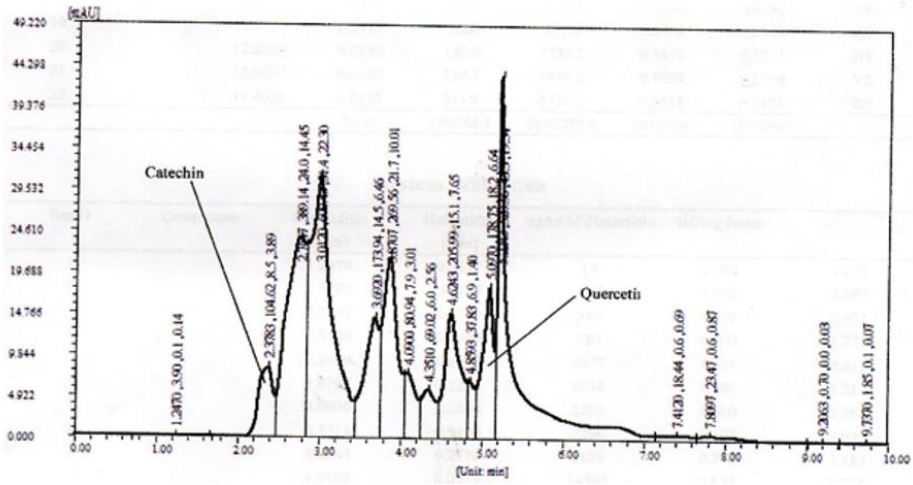

Figure 2: Oyster mushroom in ethyl acetate

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Using ethyl acetate as a solvent (Fig. 2), the analysis revealed the presence of quercetin at a retention time of 4.83 minutes and catechin at 2.38 minutes. Additionally, several unknown compounds were detected. The identification of quercetin and catechin aligns with their known bioactive properties, particularly their roles in xanthine oxidase inhibition, which is significant for anti-hyperuricemic activity.

The presence of unknown compounds suggests that there may be other potentially beneficial bioactive constituents within the oyster mushroom extracts that warrant further investigation. These findings emphasize the importance of using multiple solvents to comprehensively profile the bioactive compounds in mushroom extracts.

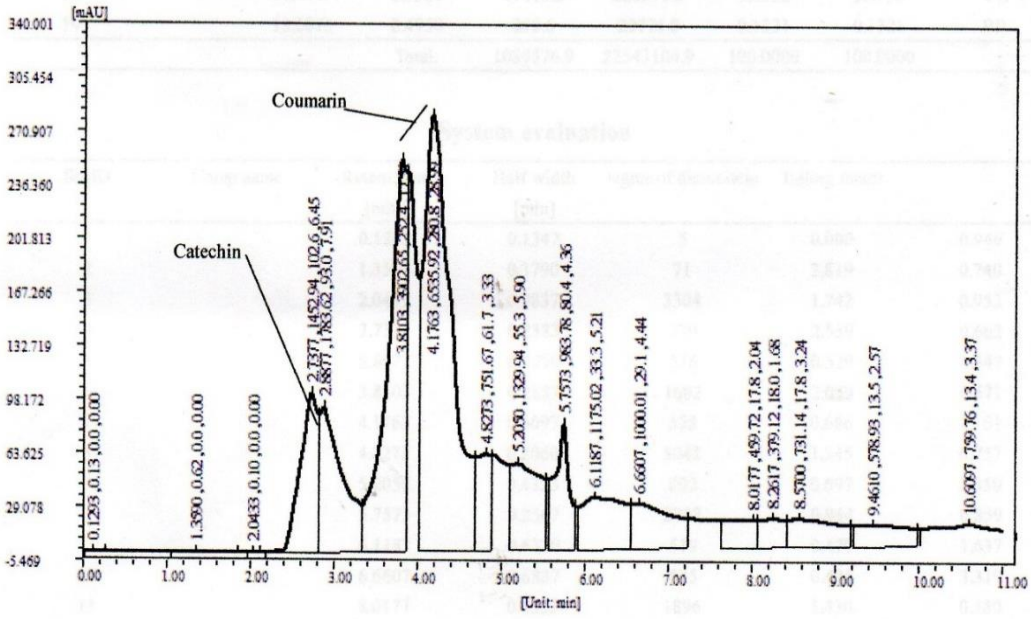

Figure 3: Oyster mushroom spent in methanol

Mushroom spent extracted in methanol underwent screening for its bioactive compounds using HPLC. The analysis revealed the presence of catechin at a retention time of 2.73 minutes and coumarin in the range of 3.81 to 4.17 minutes retention time. Additionally, several unknown bioactive compounds were detected following the HPLC analysis.

The identification of catechin and coumarin aligns with their known bioactive properties, particularly their potential for xanthine oxidase inhibition, which is significant in the context of anti-hyperuricemic activity. The presence of unknown compounds suggests the possibility of additional bioactive constituents in mushroom spent that warrant further investigation.

These findings underscore the importance of employing rigorous analytical techniques to explore the full spectrum of bioactive compounds present in natural extracts, paving the way for the discovery of novel therapeutic agents.

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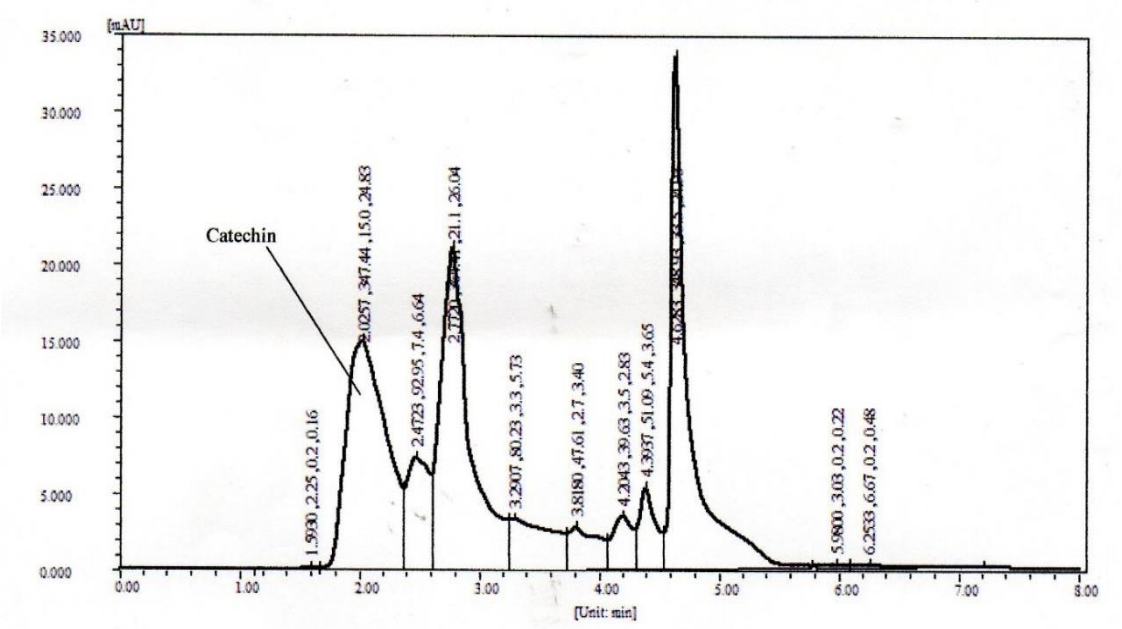

Figure 4: Oyster mushroom spent in ethyl acetate

Using ethyl acetate extraction, oyster mushroom spent exhibited catechin at a retention time of 2.03 minutes, along with several unidentified compounds visible in the graph.

These qualitative results revealed the presence of various bioactive compounds using different solvents for oyster mushroom fruit and spent. Additionally, based on the results above, numerous unknown bioactive compounds were detected in both samples.

Xanthine Oxidase Inhibitory Assay (In vitro)

Pleurotus sp. fruit, mycelia, and spent were subjected to testing for their xanthine oxidase (XO) inhibitory activity. The results indicated that all samples exhibited XO inhibitory activity when measured at 290nm in a spectrophotometer. The fruit of the oyster mushroom demonstrated the highest XO inhibitory activity at 70.55±0.0017%. This was followed by oyster mushroom mycelia at 66.45±0.002% and oyster mushroom spent at 61.59±0.0025%.

Allopurinol, the standard drug for gout, exhibited an XO inhibition rate of 40.1±0.001%. To evaluate the concentration of allopurinol, a purity test was conducted, revealing that the commercially available allopurinol was approximately 33% pure due to the presence of binders and chemical coatings.

These findings highlight the significant XO inhibitory activity of Pleurotus mushrooms, particularly in the fruiting bodies, suggesting their potential as natural alternatives for managing conditions like gout. Additionally, the purity test underscores the importance of assessing the quality and composition of standard drugs used in experimental studies.

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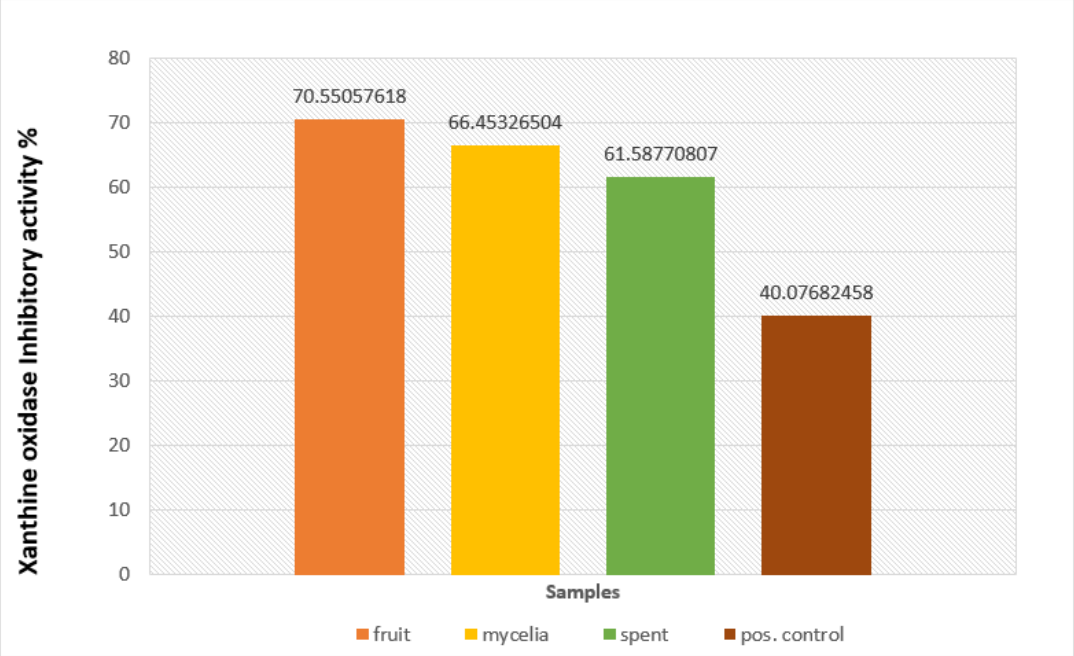

Figure 5: XO inhibitory activities of the three mushroom samples

The three mushroom crude extracts exhibited no significant differences among each other at $p<0.05$. However, the crude extract of oyster mushroom fruit displayed a significant variance compared to allopurinol. This finding corroborates the previous report by Jang et al. (2014), indicating that Pleurotus ostreatus demonstrated XO inhibition at 78.3±0.041% using water extracts.

According to the results, the crude extracts from oyster mushroom fruit showed the highest xanthine oxidase inhibition at 70.55±0.0017%, which was not significantly different from the oyster mushroom mycelia at 66.45±0.002% and oyster mushroom spent at 61.59±0.0025%. However, there was a significant difference observed with Allopurinol, the standard drug for gout, which yielded 40.1±0.001% of XO inhibition.

Furthermore, the positive control drug, Allopurinol of Purinase Brand, was found to be 33% pure in tablet form and contained other drug ingredients such as binders. Binders are a type of excipient, either natural or synthetic, formulated alongside the active ingredient of a medication, holding the ingredients together in a tablet. It ensures the required mechanical strength and volume of tablets with low active doses. Consequently, the purity of the drug influenced the results of the positive control in inhibiting xanthine oxidase in the in-vitro test.

Mycochemical Testing

Various qualitative mycochemical tests were conducted on crude extracts of oyster mushroom fruit, mycelia, and spent, and the results are summarized in Table 1. These tests aimed to determine the types of secondary metabolites present in the samples that could contribute to their antihyperuricemic activity.

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The tests revealed that oyster mushroom fruit contained terpenoids, quinones, coumarin, flavonoids, cardiac glycosides, and tannins, while mushroom spent also contained quinones, coumarins, terpenoids, saponins & sapogenins, and cardiac glycosides. However, the oyster mycelia crude extracts showed negative results in all qualitative mycochemical testing.

Flavonoids such as kaempferol, apigenin, and glycitein have been reported to inhibit xanthine oxidase through competitive inhibition, while tannins are reported to inhibit the enzyme through non-specific binding. Coumarins such as esculetin, umbelliferone, and 7-hydroxy-4-methyl coumarin are reported to inhibit the enzyme through competitive, uncompetitive, and mixed types of inhibition. Other reported inhibitors include polyphenols, methoxylated hydroquinones, and saponins such as ilexsaponin and prosapogenin.

However, terpenoids and steroids have not been reported as inhibitors of this enzyme. Consequently, the phytochemicals that may be responsible for the xanthine oxidase inhibitory activity of C. ovatum include quinones, coumarin, flavonoids, phenols, saponins, and tannins. Flavonoids and coumarins are more concentrated in the oyster mushroom fruit and spent crude extracts, indicating that these mycochemicals are more likely the ones inhibiting the enzyme.

Table 1: Mycochemical profile of the oyster mushroom fruit, mycelia and spent crude extract

MYCOCHEMICAL TEST	FRUIT	MYCELIA	SPENT
i. Plant Acids	-	-	-
ii. Phenols			
1. FeCl3 Test	-	-	-
iii. Tannins & Phenolics			
1. FeCl3 Test	-	-	-
iv. Quinones			
1. H2SO4 Test	+	-	+
v. Coumarins			
1. Alkaline Reagent Test	+	-	+
vi. Flavonoids			
1. Alkaline Reagent Test	+	-	
2. Shinoda Test	-	-	-
vii. Terpenes and Terpenoids			
1. Salkowki's Test (Triterpenes)	-	-	-
2. Salkowki's Test (Terpenoids)	+	-	+
viii. Saponins & Sapogenins			
1. Froth Test	-	-	+
ix. Cardiac Glycosides			
1. Salkowki's Test (H2SO4 Test)	+	-	-
2. Keller-Killiani Test	+	-	+

Table 1 summarized the total flavonoid content in crude extracts of oyster mushroom fruit, mycelia, and spent. The results indicated significant variations in flavonoid levels among the different extracts.

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Oyster mushroom fruit exhibited the highest total flavonoid content at 20.35 mg RE/g extract, denoted by the label "a." This suggests that the fruit extract contained a notably higher concentration of flavonoids compared to the other extracts.

In contrast, oyster mushroom mycelia displayed the lowest total flavonoid content at 9.145 mg RE/g extract, labeled "b." This indicates a lower concentration of flavonoids in the mycelia extract compared to both the fruit and spent extracts. Oyster mushroom spent showed an intermediate total flavonoid content of 17.038 mg RE/g extract, also labeled "a," suggesting a relatively high concentration of flavonoids similar to that of the fruit extract.

These findings suggest that the composition of flavonoids varies significantly among different parts of the oyster mushroom, with the fruit and spent extracts containing higher levels compared to the mycelia extract. This difference in flavonoid content may contribute to variations in the biological activities of the extracts, highlighting the importance of considering the specific mushroom parts when exploring their potential health benefits.

Table 2: Total flavonoid content in of oyster mushroom fruit, mycelia and spent crude extract

Sample	Total flavonoid content (mg RE/g extract)
oyster mushroom fruit	20.35a
oyster mushroom mycelia	9.145b
oyster mushroom spent	17.038a

The total flavonoid content (TFC) was determined using the calibration curve of Rutin and expressed as milligrams of Rutin Equivalent per gram of extract, denoted as mg RE/g extract (Table 2). TFC varied across the samples, with the oyster mushroom fruit exhibiting a TFC of 20.35 mg RE/g extract, which was not significantly different from the TFC of oyster mushroom spent at 17.038 mg RE/g extract.

Flavonoids, well-known antioxidant constituents of plants, possess a broad spectrum of biological and chemical activities, including radical scavenging activity. These compounds have been reported to be responsible for the xanthine oxidase inhibitory activities of plants through their scavenging or chelating activity (Ghimire, 2011).

Acute Oral Toxicity

An in vivo study of the antihyperuricemic activity of oyster mushroom fruit, mycelia, and spent was conducted to determine if it would yield parallel results with its in vitro study. However, before conducting the in vivo study, an acute oral toxicity test was performed to ensure that the extracts themselves were not toxic or harmful to the mice, which would be used as the animal model for the study. The mice were administered a starting dose of 2000 mg/kg, and no mortality or signs of toxicity were observed for 14 days.

According to the guidelines set by the OECD (2001a), the extract belonged to hazard category 5, indicating relatively low acute toxicity hazard, and could be considered safe for consumption by the test animals. Moreover, the LD50, or lethal dose, of mushroom extracts needed to kill 50% of the test animals was estimated to be between 2000 mg/kg and 5000 mg/kg.

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Induction of Hyperuricemia

The intraperitoneal injection of potassium oxonate resulted in an increase in serum uric acid levels up to 2.5 hours, followed by a relatively rapid decrease until 3.5 hours after injection, as shown in Figure 6. This injection significantly elevated uric acid levels from 1 hour to 3.5 hours after induction compared to the uric acid levels of the mice prior to induction. Although potassium oxonate is the most effective method for inducing hyperuricemia in mice (Stavric & Nera, 1978), its effects are time-dependent, and serum uric acid levels eventually return to normal levels after a few hours. Treatment must be administered within a time limit to ensure that the resulting decrease in serum uric acid levels is due to xanthine oxidase inhibition and not solely due to the diminishing effects of potassium oxonate induction.

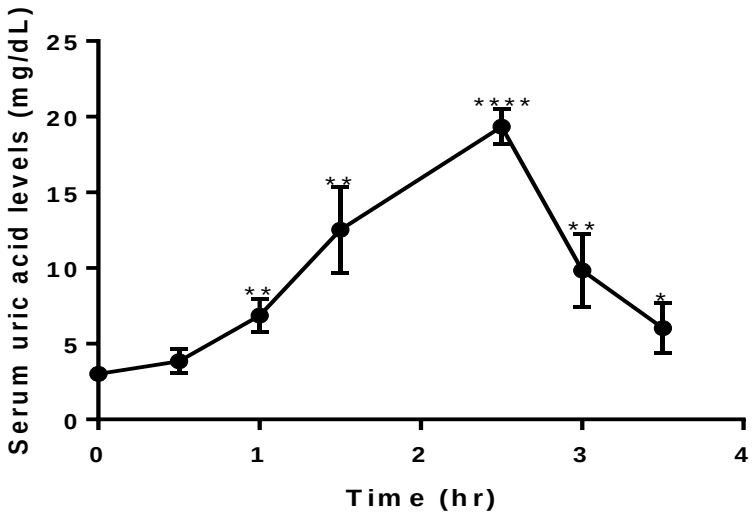

Figure 6: Time-course effect of potassium oxonate on the serum uric acid levels in mice

The in vivo study conducted indicated that oyster mushroom fruit exhibited the highest antihyperuricemic activity, with a mean reduction of 6.68±0.336 mg/dL, as depicted in Figure 4.5. This result was not significantly different from the antihyperuricemic activity observed with oyster mushroom spent, which showed a mean reduction of 7.291±0.29 mg/dL.

The administration of potassium oxonate significantly induced hyperuricemia (12.22±0.239 mg/dL) compared to the normal group (2.123±0.101 mg/dL), with a p-value less than 0.0001. However, oyster mushroom mycelia appeared ineffective in reducing serum uric acid levels, yielding a mean reduction of 11.38±0.137 mg/dL. Notably, a significant reduction in uric acid levels was observed only when administered at the higher dose of 200 mg/kg, indicating that the antihyperuricemic activity of the treatments was dose-dependent. In contrast, Allopurinol, the standard drug, significantly reduced uric acid levels by up to 3.16 mg/dL at a smaller dose of 10 mg/kg ($p<0.0001$). Despite the effective antihyperuricemic activity demonstrated by oyster mushroom fruit and spent, it was not as potent as Allopurinol, as revealed in the in vivo experiment.

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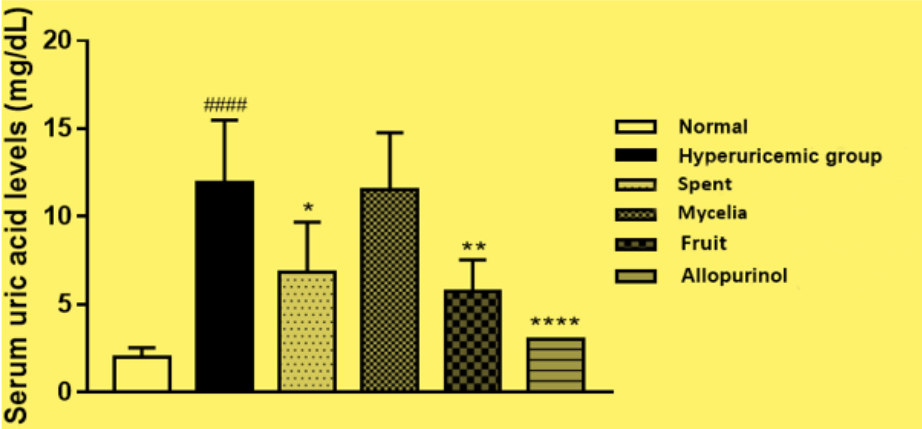

Figure 7: Effects of allopurinol, fruit, mycelia and spent crude extract on serum uric acid levels 1 hour after drug administration on mice with potassium oxonate-induced hyperuricemia. *p<0.05, **p<0.01, ****p<0.0001 when compared to the hyperuricemic group, ####p<0.0001 when compared to normal group

5. CONCLUSION

The qualitative bioactive screening of oyster mushroom fruit and spent revealed the presence of significant xanthine oxidase inhibitory compounds, including catechin, coumarin, and quercetin, confirming their potential antihyperuricemic properties. The extraction process using ethyl acetate and methanol proved effective in capturing a wide range of polar and non-polar bioactive constituents from both mushroom samples. Moreover, the xanthine oxidase inhibitory assay demonstrated considerable activity in all tested samples, with oyster mushroom fruit exhibiting the highest inhibition, followed by mycelia and spent. The results underscore the therapeutic potential of oyster mushrooms in managing conditions like gout and highlight the importance of exploring agricultural by-products for medicinal purposes.

6. RECOMMENDATIONS

- Further Identification of Bioactive Compounds: Given the presence of unidentified bioactive compounds in both mushroom fruit and spent, further analysis and characterization are warranted to elucidate their pharmacological properties and potential synergistic effects.
- Optimization of Extraction Techniques: Continual refinement of extraction methods may enhance the yield and specificity of bioactive compounds, optimizing the antihyperuricemic activity of oyster mushroom extracts.
- In Vivo Studies: While the in vitro assays provided valuable insights into xanthine oxidase inhibition, comprehensive in vivo studies are necessary to evaluate the efficacy and safety of oyster mushroom extracts in animal models, considering factors like dose-response relationships and long-term effects.

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- Exploration of Formulation Strategies: Developing novel formulations, such as encapsulation or nanoformulation, could improve the bioavailability and stability of mushroom extracts, facilitating their incorporation into therapeutic regimens for hyperuricemia management.
- Quality Control of Standard Drugs: The assessment of the purity and composition of standard drugs like allopurinol is crucial for ensuring accurate comparison with natural extracts, highlighting the need for stringent quality control measures in experimental studies.

7. DECLARATION OF INTEREST STATEMENT

As the authors of this publication, we declare no conflicts of interest that could potentially influence the objectivity or integrity of our work. Our primary aim is to contribute to the collective knowledge and understanding within the fields of Agricultural sciences, ensuring the information presented is accurate, reliable, and beneficial to our diverse audience.

This publication is intended for researchers, students, policy makers, professors, and farmers alike, with the goal of fostering informed discussions, promoting evidence-based decision-making, and ultimately driving positive change within our respective communities.

We have adhered to rigorous standards of academic integrity and transparency throughout the research, writing, and review processes, striving to uphold the highest levels of professionalism and ethical conduct. Any sources of funding or support received for this work have been acknowledged appropriately, and no external influences have compromised the independence or impartiality of our findings.

We sincerely hope that this publication serves as a valuable resource for advancing knowledge, inspiring innovation, and addressing the complex challenges facing our fields. Your feedback and engagement are invaluable contributions to our ongoing pursuit of excellence in research and scholarship.

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References

1) Apaya, K. L., & Chichioco-Hernandez, C. L. (2011). Xanthine oxidase inhibition of selected Philippine medicinal plants. Journal of Medicinal Plants Research, 5(2), 289–292.

2) Arellano, F., & Sacristán, J. a. (1993). Allopurinol hypersensitivity syndrome: a review. The Annals of Pharmacotherapy, 27(3), 337–343. http://doi.org/10.1177/106002809302700317

3) Azmi, S. M. N., Jamal, P., & Amid, a. (2012). Xanthine oxidase inhibitory activity from potential Malaysian medicinal plant as remedies for gout. International Food Research Journal, 19(1), 159–165.

4) Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248–254. http://doi.org/10.1016/0003-2697(76)90527-3

5) Brunton, L. L., Lazo, J. S., & Parker, K. L. (2006). Goodman and Gilman's The Pharmacological Basis of Therapeutics. (McGraw-Hill, Ed.) (11th ed.). New York, NY. http://doi.org/10.1213/00000539-198105000-00027

6) Coronel, R. E. (1996). Pili nut Canarium ovatum Engl.: Promoting the conservation and use of underutilized and neglected crops 6. (I. of P. G. and C. P. Research, Ed.). Germany.

7) Cos, P., Ying, L., Calomme, M., Hu, J. P., Cimanga, K., Van Poel, B., … Vanden Berghe, D. (1998). Structure-activity relationship and classification of flavonoids as inhibitors of xanthine oxidase and superoxide scavengers. Journal of Natural Products, 61(1), 71–76. http://doi.org/10.1021/np970237h

8) Cotelle, N., Bernier, J. L., Catteau, J. P., Pommery, J., Wallet, J. C., & Gaydou, E. M. (1996). Antioxidant properties of hydroxy-flavones. Free Radical Biology and Medicine, 20(1), 35–43. http://doi.org/10.1016/0891-5849(95)02014-4

9) Crisostomo, S. (2015). Number of Filipinos suffering from gout hits 1.6 M. The Philippine Star, pp. 1–3. Retrieved from http://www.philstar.com/headlines/2015/03/03/1429462/number-filipinos-suffering-gout-hits-1.6-m

10) Cross, M., Smith, E., Hoy, D., Nolte, S., Ackerman, I., Fransen, M., … March, L. (2014). The global burden of hip and knee osteoarthritis: estimates from the Global Burden of Disease 2010 study. Annals of the Rheumatic Diseases, 73(7), 1323–30. http://doi.org/10.1136/annrheumdis-2013-204763

11) Dalton, D., & Keeling, S. (2011). The Rough Guide to the Philippines (3rd ed.). New Delhi, India: Rough Guides Ltd.

12) De Souza, M. R., De Paula, C. A., Pereira De Resende, M. L., Grabe-Guimarães, A., De Souza Filho, J. D., & Saúde-Guimarães, D. A. (2012). Pharmacological basis for use of Lychnophora trichocarpha in gouty arthritis: Anti-hyperuricemic and anti-inflammatory effects of its extract, fraction and constituents. Journal of Ethnopharmacology, 142(3), 845–850. http://doi.org/10.1016/j.jep.2012.06.012

13) Dianongco, M. L. G., Magbitang, A. D., & Salido, E. O. (2014). Prevalence of Metabolic Syndrome in Filipino Patients with Gout in a Tertiary Hospital, 52(1), 1–4.

14) Edeoga, H. O., Okwu, D. E., & Mbaebie, B. O. (2005). Phytochemical Constituents of Some Nigerian Plants. African Journal of Biotechnology, 4(7), 685–688. http://doi.org/10.4314/br.v8i1.62544

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.11654845

15) Enroth, C., Eger, B. T., Okamoto, K., Nishino, T., Nishino, T., & Pai, E. F. (2000). Crystal structures of bovine milk xanthine dehydrogenase and xanthine oxidase: structure-based mechanism of conversion. Proceedings of the National Academy of Sciences of the United States of America, 97(20), 10723–10728. http://doi.org/10.1073/pnas.97.20.10723

16) Fam, A. G. (2005). Gout: Excess calories, purines, and alcohol intake and beyond. Response to a urate-lowering diet. Journal of Rheumatology, 32(5), 773–777.

17) Ferrari, F. C., Lemos Lima, R. de C., Schimith Ferraz Filha, Z., Barros, C. H., de Paula Michel Araújo, M. C., & Antunes Saúde-Guimarães, D. (2016). Effects of Pimenta pseudocaryophyllus extracts on gout: Anti-inflammatory activity and anti-hyperuricemic effect through xantine oxidase and uricosuric action. Journal of Ethnopharmacology, 180, 37–42. http://doi.org/10.1016/j.jep.2016.01.007

18) Ferraz-Filha, Z. S., de Paula Michel Araujo, M. C., Ferrari, F. C., Andrade, I. P., Dutra, R., & Saude-Guimaraes, D. A. S. (2016). Tabebuia roseoalba : In Vivo Hypouricemic and Anti-inflammatory Effects of Its Ethanolic Extract and Constituents. Journal of Medicinal Plant and Natural Product Research, (7), 591–670. http://doi.org/10.1055/s-0042-105878

19) Fridovich, I. (1965). the Competitive Inhibition of Uricase By Oxonate and By Related. The Journal of Biological Chemistry, 240(6), 2491–2494.

20) Gallegos, R. K. B., Suministrado, D. C., Amongo, R. M. C., & Madlangbayan, M. S. (2013). Some Physical and Mechanical Properties of Pili (Canarium ovatum Engl . cv . Katutubo) Nut as a Function of Nut Moisture Content, 96(1), 66–67.

21) Gosling, A. L., Matisoo-Smith, E., & Merriman, T. R. (2014). Hyperuricaemia in the pacific: Why the elevated serum urate levels? Rheumatology International, 34(6), 743–757. http://doi.org/10.1007/s00296-013-2922-x

22) Hall, I. H., Scoville, J. P., Reynolds, D. J., Simlot, R., & Duncan, P. (1990). Substituted Cyclic Imides as Potential Anti-gout Agents. Journal of Life Sciences, 46, 1923–1927.

23) Harborne, J. B. (1984). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis (2nd ed.). New York, NY: Chapman and Hall.

24) Havlik, J., de la Huebra, R. G., Hejtmankova, K., Fernandez, J., Simonova, J., Melich, M., & Rada, V. (2010). Xanthine oxidase inhibitory properties of Czech medicinal plants. Journal of Ethnopharmacology, 132(2), 461–465. http://doi.org/10.1016/j.jep.2010.08.044

25) Hille, R., & Massey, V. (1981). Tight Binding Inhibitors of Xanthine Oxidase. Pharmacology & Therapeutics, 14, 249–263.

26) Ho, K. Y., Huang, J. S., Tsai, C. C., Lin, T. C., Hsu, Y. F., & Lin, C. C. (1999). Antioxidant Activity of Tannin Components from Vaccinium vitis-idaea L. Journal of Pharmacology and Pharmacotherapeutics, 51, 1075–1078.

27) Kakuda, Y., Jahaniaval, F., Marcone, M. F., Montevirgen, L., Montevirgen, Q., & Umali, O. (2000). Characterization of pili nut (Canarium ovatum) oil: Fatty acid and triacylglycerol composition and physicochemical properties. Journal of the American Oil Chemists Society, 77(9), 991–996. http://doi.org/10.1007/s11746-000-0156-8

28) Kikuchi, T., Watanabe, K., Tochigi, Y., Yamamoto, A., Fukatsu, M., Ezaki, Y., … Akihisa, T. (2012). Melanogenesis Inhibitory Activity of Sesquiterpenes from Canarium ovatum Resin in Mouse B16 Melanoma Cells, 9, 1500–1507.

29) Klippel, J. H., Stone, J. H., Crofford, L. J., & White, P. H. (2008). Primer on the rheumatic diseases. (13th ed.). New York, NY: Springer. http://doi.org/10.1001/jama.299.10.1195-a

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.11654845

30) Kong, L. D., Abliz, Z., Zhou, C. X., Li, L. J., Cheng, C. H. K., & Tan, R. X. (2001). Glycosides and xanthine oxidase inhibitors from Conyza bonariensis. Phytochemistry, 58(4), 645–651. http://doi.org/10.1016/S0031-9422(01)00176-5

31) Kong, L. D., Yang, C., Ge, F., Wang, H. D., & Guo, Y. S. (2004). A Chinese herbal medicine Ermiao wan reduces serum uric acid level and inhibits liver xanthine dehydrogenase and xanthine oxidase in mice. Journal of Ethnopharmacology, 93(2-3), 325–330. http://doi.org/10.1016/j.jep.2004.04.008

32) Krishnan, E., Lienesch, D., & Kwoh, C. K. (2008). Gout in ambulatory care settings in the United States. The Journal of Rheumatology, 35(3), 498–501.

33) Li, E. K. (2004). Gout: A review of its aetiology and treatment. Hong Kong Medical Journal, 10(4), 261–270.

34) Li-Yu, J., Salido, E. O., Manahan, S., Lichauco, J. J., Lorenzo, J. P., Torralba, K. T., … Maceda, L. M. (2007). Philippine Clinical Practice Guidelines for the Management of Gout. Philippine Rheumatology Association.

35) Li-Yu, J. T. (2009). Relationship of Gout and Hyperuricemia in Cardiovascular and Renal Morbidity: 2003 National Nutrition and Health Survey (NNHeS). Philippine Journal of Internal Medicine, 46(6), 279–285. http://doi.org/10.3860/pjim.v46i6.889

36) Mangoff, S. C., & Milner, J. A. (1978). Oxonate-Induced Hyperuricemia and Orotic Aciduria in Mice. Proceedings of the Society for Experimental Biology and Medicine, 115, 110–115.

37) Many, a., Hubel, C. a., & Roberts, J. M. (1996). Hyperuricemia and xanthine oxidase in preeclampsia, revisited. American Journal of Obstetrics and Gynecology, 174(1), 288–291. http://doi.org/10.1016/S0002-9378(96)70410-6

38) Mazzali, M., Hughes, J., Kim, Y. G., Jefferson, J. a, Kang, D. H., Gordon, K. L., … Johnson, R. J. (2001). Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. Hypertension, 38(5), 1101–1106. http://doi.org/10.1161/hy1101.092839

39) Nelson, D. L., & Cox, M. M. (2008). Lehninger Principles of Biochemistry (5th ed.). W.H. Freeman.

40) Nguyen, M. T. T., Awale, S., Tezuka, Y., Ueda, J. Y., Le Tran, Q., & Kadota, S. (2006). Xanthine oxidase inhibitors from the flowers of Chrysanthemum sinense. Planta Medica, 72(1), 46–51. http://doi.org/10.1055/s-2005-873181

41) Nguyen, M. T. T., & Nguyen, N. T. (2011). A new lupane triterpene from Tetracera scandens L ., xanthine oxidase inhibitor. Natural Product Research, 27(1), 61–67. http://doi.org/10.1080/14786419.2011.652960

42) Noro, T., Oda, Y., Miyase, T., Ueno, A., & Fukushima, S. (1983). Xanthine oxidase. Chemical and Pharmaceutical Bulletin, 31(1), 3984=3987.

43) OECD. (2001a). Acute Oral Toxicity – Acute Toxic Class Method. Guideline 423, Adopted 23.03.1996. In: Eleventh Addendum to the OECD Guidelines for the Testing of Chemicals. Organisation for Economic Co-Operation and Development, Paris, (December), 1–14.

44) OECD. (2001b). OECD Series on Testing and Assessment: Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures, (33), 247.

45) Okamoto, K., Eger, B. T., Nishino, T., Pai, E. F., & Nishino, T. (2008). Mechanism of inhibition of xanthine oxidoreductase by allopurinol: crystal structure of reduced bovine milk xanthine oxidoreductase bound with oxipurinol. Nucleosides, Nucleotides & Nucleic Acids, 27(6), 888–893. http://doi.org/10.1080/15257770802146577

46) Owen, P. L., & Johns, T. (1999). Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. Journal of Ethnopharmacology, 64(2), 149–160. http://doi.org/10.1016/S0378-8741(98)00119-6

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.11654845

47) Pham, L. J., & Dumandan, N. G. (2015). Philippine Pili : Composition of the lipid molecular species. Journal of Ethnic Foods, 2(4), 147–153. http://doi.org/10.1016/j.jef.2015.11.001

48) Prasad, P., & Krishnan, E. (2014). Filipino gout: A review. Arthritis Care and Research, 66(3), 337–343. http://doi.org/10.1002/acr.22118

49) Ragasa, C. Y., Torres, O. B., Gutierrez, J. M. P., Kristiansen, H. P. B. C., & Shen, C. C. (2015). Triterpenes and acylglycerols from Canarium ovatum. Journal of Applied Pharmaceutical Science, 5(4), 94–100. http://doi.org/10.7324/JAPS.2015.50416

50) Santos, F., Frota, J., Arruda, B., de Melo, T., da Silva, A. A. D. C. A., Brito, G. A. D. C., … Rao, V. (2012). Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from Protium heptaphyllum in mice. Lipids in Health and Disease, 11(1), 98. http://doi.org/10.1186/1476-511X-11-98

51) Sarawek, S. (2007). Xanthine Oxidase Inhibition and Antioxidant Activity of an Artichoke Leaf Extract (Cynara scolymus L.) and its Compounds, 151.

52) Schlesinger, N. (2008). Hyperuricemia, Gout, and Diet. In L. Coleman (Ed.), Nutrition and Health: Nutrition and Rheumatic Disease (Vol. 300, pp. 169–182). Totowa, NJ: Humana Prese. http://doi.org/10.1001/jama.300.9.1084-b

53) Shakirin, F. H., Azlan, A., Ismail, A., Amom, Z., & Cheng Yuon, L. (2012). Protective effect of pulp oil extracted from canarium odontophyllum Miq. fruit on blood lipids, lipid peroxidation, and antioxidant status in healthy rabbits. Oxidative Medicine and Cellular Longevity, 2012. http://doi.org/10.1155/2012/840973

54) Shintani, H. (2013). Determination of Xanthine Oxidase. Pharmaceutica Analytica Acta, 01(S7). http://doi.org/10.4172/2153-2435.S7-004

55) Skibo, E. B. (1986). Noncompetitive and Irreversible Inhibition of Xanthine Oxidase by Benzimidazole Analogues Acting at the Functional Flavin Adenine Dinucleotide Cofactor. Biochemistry, 25(15)(1986), 4189–4194. http://doi.org/10.1021/bi00363a004

56) Smith, R. D., Essenburg, a. D., & Kaplan, H. R. (1979). The Oxonate Pretreated Rat as A Model for Evaluating Hyperuricemic Effects of Antihypertensive Drugs. Clinical and Experimental Hypertension, 1(4), 487–504. http://doi.org/10.3109/10641967909068620

57) Stavric, B., & Nera, E. a. (1978). Use of the Uricase lnhibited Rat as an Animal Model in Toxicology, 13, 47–74.

58) Sweeney, a. P., Wyllie, S. G., Shalliker, R. a., & Markham, J. L. (2001). Xanthine oxidase inhibitory activity of selected Australian native plants. Journal of Ethnopharmacology, 75(2-3), 273–277. http://doi.org/10.1016/S0378-8741(01)00176-3

59) Thanh, M., Guyen, T. N., Wale, S. A., Ezuka, Y. T., Ran, Q. L. T., Atanabe, H. W., & Adota, S. K. (2004). Xanthine Oxidase Inhibitory Activity of Vietnamese Medicinal Plants, 27(9).

60) Torralba, T. P., & Bayani Sioson, P. S. (1975). The Filipino and gout. Seminars in Arthritis and Rheumatism, 4(4), 307–320. http://doi.org/10.1016/0049-0172(75)90015-3

61) Umali-Stuart, G., & Stuart-Santiago, A. (2015). Philippine Medicinal Plants: Pili. Retrieved May 17, 2016, from http://www.stuartxchange.com/Pili.html

62) Umamaheswari, M., AsokKumar, K., Somasundaram, A., Sivashanmugam, T., Subhadradevi, V., & Ravi, T. K. (2007). Xanthine oxidase inhibitory activity of some Indian medical plants. Journal of Ethnopharmacology, 109(3), 547–551. http://doi.org/10.1016/j.jep.2006.08.020

63) Umamaheswari, M., & Chatterjee, T. (2008). Hypouricemic and xanthine oxidase inhibitory activities of the fractions of Coccinia grandis L. Voigt. Oriental Pharmacy and Experimental Medicine, 7(5), 477–484. http://doi.org/10.3742/OPEM.2008.7.5.477

The Seybold Report
ISSN 1533-9211
DOI: 10.5281/zenodo.11654845

64) Unno, T., Sakane, I., & Kakuda, T. (2000). Inhibition of Xanthine Extract Oxidase by an Aqueous Leaves of Banaba (Lagerstroemia speciosa). Journal of the Japanese Society for Food Science and Technology, 47(9), 740–743. http://doi.org/10.3136/nskkk.47.740

65) Upadhyaya, K. R., Radha, K. S., & Madhyastha, H. K. (2007). Cell cycle regulation and induction of apoptosis by beta-carotene in U937 and HL-60 leukemia cells. Journal of Biochemistry and Molecular Biology, 40(6), 1009–1015.

66) Van Hoorn, D. E. C., Nijveldt, R. J., Van Leeuwen, P. a M., Hofman, Z., M'Rabet, L., De Bont, D. B. a, & Van Norren, K. (2002). Accurate prediction of xanthine oxidase inhibition based on the structure of flavonoids. European Journal of Pharmacology, 451(2), 111–118. http://doi.org/10.1016/S0014-2999(02)02192-1

67) Vázquez, L. H., Palazon, J., Navarro-ocaña, A., Metropolitana, A., Xochimilco, U., & Biológicos, D. S. (2011). The Pentacyclic Triterpenes ?, ?-amyrins: A Review of Sources and Biological Activities. Phytochemicals – A Global Perspective of Their Role in Nutrition and Health, 426, 478–502. http://doi.org/10.5772/27253

68) Voet, D., & Voet, J. G. (2011). Biochemistry (4th ed.). Hooboken, NJ: John Wiley & Sons, Inc.

69) Wang, S. Y., Yang, C. W., Liao, J. W., Zhen, W. W., Chu, F. H., & Chang, S. T. (2008). Essential oil from leaves of Cinnamomum osmophloeum acts as a xanthine oxidase inhibitor and reduces the serum uric acid levels in oxonate-induced mice. Phytomedicine, 15(11), 940–945. http://doi.org/10.1016/j.phymed.2008.06.002

70) Wang, Y., Zhu, J. X., Kong, L. D., Yang, C., Cheng, C. H. K., & Zhang, X. (2004). Administration of procyanidins from grape seeds reduces serum uric acid levels and decreases hepatic xanthine dehydrogenase/oxidase activities in oxonate-treated mice. Basic & Clinical Pharmacology & Toxicology, 94(5), 232–7. http://doi.org/10.1111/j.1742-7843.2004.pto940506.x

71) Yu, Z., Fong, W. P., & Cheng, C. H. K. (2006). The dual actions of morin (3,5,7,2',4'-pentahydroxyflavone) as a hypouricemic agent: uricosuric effect and xanthine oxidase inhibitory activity. The Journal of Pharmacology and Experimental Therapeutics, 316(1), 169–175. http://doi.org/10.1124/jpet.105.092684.mans.

72) Yumita, A., Suganda, A. G., & Sukandar, E. Y. (2013). Xanthine oxidase inhibitory activity of some Indonesian medicinal plants and active fraction of selected plants. International Journal of Pharmacy and Pharmaceutical Sciences, 5(2), 293–296.