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Antioxidant and Xanthine Oxidase Inhibitory Activity of *Sesbania grandiflora* Extract

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ABSTRACT

Xanthine oxidase is a crucial enzyme in the regulation of uric acid, with elevated levels leading to gout and hyperuricemia. To combat these conditions, a synergistic approach using antioxidants and xanthine oxidase inhibitors presents a promising treatment strategy. Given the side effects of synthetic inhibitors, this study explores the natural xanthine oxidase inhibitory and antioxidant properties of Sesbania grandiflora leaves extract. The analysis revealed a significant free radical inhibition rate of 61.59 ± 0.90% at a concentration of 100 µg/mL. The SC_{50} value for the leaves extract was 16.59 ± 0.67 µg/mL, compared to 7.76 ± 0.23 µg/mL for gallic acid, a positive control. The leaves extract also demonstrated notable efficiency in inhibiting uric acid formation, with an IC_{50} value of 0.36 ± 0.341 µg/mL, although this was higher than the 0.01 ± 0.005 µg/mL value for allopurinol. A strong correlation (r = 0.9958) was found between the antioxidant activity and xanthine oxidase inhibition of the extract, underscoring its effectiveness. This study is the first to report the combined effects of xanthine oxidase inhibition and antioxidant activity in Sesbania grandiflora, highlighting its potential as a natural treatment for gout and related diseases.

Keywords: Natural Antioxidants, *Sesbania grandiflora* Extract, Gout, Xanthine oxidase

1. INTRODUCTION

In the modern era, gout and exposure to free radicals have emerged as significant health concerns. Gout, the oldest known joint disease, was first recognized by Hippocrates in the 5th century BC as a condition affecting older men and women (Stapley, 2023). Over the centuries, it has evolved to become a prevalent issue in the 21st century, affecting a broad demographic (Paul et al., 2017). Gout is a form of inflammatory arthritis characterized by recurrent flares of severe joint damage due to the accumulation of uric acid in the body (Cha et al., 2024). Concurrently, the rise in free radical exposure, primarily resulting from unhealthy lifestyles and environmental pollutants, has exacerbated the prevalence of various diseases, including gout. Free radicals are highly reactive molecules that can damage cells, proteins, and DNA, contributing to the development of chronic diseases and accelerated aging (Jomova et al., 2023). The younger generation, in particular, is increasingly susceptible to these conditions due to factors like obesity and exposure to pollutants and radiation.

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The management of gout and free radical-related diseases often involves the use of antioxidants and xanthine oxidase inhibitors (Gawlik-Dziki et al., 2020). Xanthine oxidase is a key enzyme in the regulation of uric acid, and its inhibition is a primary strategy in the treatment of gout (Orhan and Deniz, 2021). Synthetic drugs like allopurinol are commonly used to inhibit xanthine oxidase and manage uric acid levels in the body (Serrano et al., 2020). However, these synthetic inhibitors often come with side effects such as hypersensitivity syndrome, which includes symptoms like fever, rashes, nausea, and hepatic dysfunction (Wong et al., 2014; Francis, 2022). Similarly, synthetic antioxidants, although effective to some extent, have been associated with adverse effects, limiting their long-term use (Lalani et al., 2024). Due to these limitations, there has been a growing interest in exploring natural alternatives. Medicinal plants, in particular, have gained attention for their potential health benefits and lower risk of side effects. Natural antioxidants and xanthine oxidase inhibitors derived from plants can offer a safer and potentially more effective approach to managing gout and protecting against free radical damage. *Sesbania grandiflora*, an herb known for its medicinal properties, has shown promise in this regard.

Sesbania grandiflora, commonly known as 'agathi' or hummingbird tree, is a plant traditionally used in various cultures for its medicinal properties. The leaves of *Sesbania grandiflora* are rich in bioactive compounds, including flavonoids, saponins, and tannins, which have demonstrated significant antioxidant and anti-inflammatory activities. The rationale for choosing *Sesbania grandiflora* for this study is based on its potential to serve as a natural source of xanthine oxidase inhibitors and antioxidants. Unlike synthetic drugs, natural extracts from *Sesbania grandiflora* are less likely to cause severe side effects (Ambastha et al., 2022). This makes it a safer alternative for long-term use in managing gout and preventing free radical-related damage. While there is some existing research on the medicinal properties of *Sesbania grandiflora*, its specific effects on xanthine oxidase inhibition and antioxidant activity in the context of gout treatment remain underexplored.

In this study, xanthine oxidase was extracted from *Sesbania grandiflora* using the ethanol extraction method. The antioxidant activity of the extract was examined using the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay. The xanthine oxidase inhibitory (XOI) activity of the *Sesbania grandiflora* extracts was determined through *in-vitro* analysis. Additionally, the correlation between antioxidant activity and XOI activity was studied using regression analysis. This study aims to fill this gap by providing comprehensive insights into its therapeutic potential. The novelty of this research lies in its pioneering investigation of the synergistic effects of *Sesbania grandiflora* extracts as both xanthine oxidase inhibitors and antioxidants. While *Sesbania grandiflora* has been traditionally recognized for its medicinal properties, this study uniquely focuses on its dual functionality in treating gout and mitigating free radical damage. By systematically analysing the plant's bioactive compounds, this research aims to uncover a natural, effective alternative to synthetic drugs, which are often accompanied by adverse side effects.

2. MATERIALS AND METHODS

2.1 Sample Preparation

The leaves of *Sesbania grandiflora* were thoroughly washed with distilled water to remove contaminants. The leaves were then placed on aluminum foil trays and dried in sunlight. Following this, the leaves were transferred to a vacuum oven at 50 °C for 2 days to ensure complete moisture removal. Once dried, the leaves were ground into a fine powder using a blender. The resulting plant powder was stored in a desiccator with silica gel to maintain low moisture content.

2.2 Plant Extraction

In this extraction method, fresh leaves of *Sesbania grandiflora* were blended to produce a 1-gram

powder sample, which was weighed using an electronic balance. Ethanol (50 mL) was added to the plant powder, and the mixture was thoroughly mixed. The extraction mixture was then refluxed for 4 h, following the method described by Anand et al. (2013). This process was conducted in the dark by covering the flask with aluminum foil. After refluxing, the extracts were filtered through Whatman No. 1 filter paper under reduced pressure. The supernatant was further processed using a rotary evaporator to remove the ethanol and obtain a concentrated, oily extract.

2.3 1,1-Diphenyl -2-Picryl-hydrazyl (DPPH) Radical Scavenging Assay

The antioxidant properties of *Sesbania grandiflora* extract were analysed using the method outlined by Jamal et al. (2016). First, 0.1 mL of extract was added to 3.9 mL of a 0.1 mM methanol solution of DPPH (0.1 mM methanol solution). The solution was then covered with foil and kept in darkness for 1 h. After this period, the absorbance was measured at 517 nm. Pure methanol served as the blank, while the DPPH solution was used as the control. Radical scavenging activity (RSA) was calculated using the following equation:

$$Radical\ scavenging\ activity\ (\%) = \frac{A\ (control) - A\ (sample)}{A\ (control)} \times 100 \qquad (1)$$

2.4 Inhibitory Activity Assay of Xanthine Oxidase

The inhibitory assay of xanthine oxidase activity was tested for the *Sesbania grandiflora* extract and measured using an *in-vitro* analysis described by Wong et al. (2014) with slight modifications. Xanthine was used as the substrate. The test concentrations ranged from 20 µg/mL to 100 µg/mL. The xanthine solution was prepared by dissolving it in a 0.05 M cold sodium phosphate buffer with a pH of 7.5. Additionally, a 150 mM xanthine oxidase solution was prepared by dissolving it in 10 mL of 10 M sodium hydroxide. Then, 2 mL of xanthine oxidase (0.2 units per mL in 0.005 M sodium phosphate buffer, pH 7.5) was prepared. A 1 mL of the *Sesbania grandiflora* leaves extract was mixed with 2.9 mL of the 0.05 M sodium phosphate buffer. This mixture was then combined with 100 µL of xanthine oxidase in the sodium phosphate buffer (pH 7.5) as per Azmi et al. (2012). The solution was pre-incubated at room temperature (25 ˚C). Then, 2 mL of xanthine solution was added as a substrate. The mixture was heated from 25 °C to 90 ˚C for 5 min in a water bath. The absorbance was measured using a UV spectrophotometer (Shimadzu UV-1800, USA) at 295 nm. Allopurinol was prepared at the same concentration as a positive control (Hendriani et al., 2016). For the negative control, plant extract samples were dissolved in 1% dimethyl sulfoxide (DMSO). DMSO was used to increase the reaction chemical production of uric acid to its maximum and to replace the test sample for maximum uric acid formation. Xanthine and sodium phosphate were prepared in the same way as a blank solution and reference compound, and the initial absorbance was obtained using a UV-Vis spectrophotometer (Shimadzu UV-1800, USA). The xanthine oxidase inhibition percentage was calculated using the following equation:

$$Xanthine\ oxidase\ inhibition\ (\%) = \frac{(1\ -\ \beta)}{\alpha} \times\ 100 \qquad (2)$$

where α is xanthine oxidase activity without in any sample extraction, and β is xanthine oxidase activity with the sample extraction.

2.5 Correlation study of antioxidant activity and xanthine oxidase inhibitory (XOI)

The correlation study between the antioxidant activity and XOI activity was conducted by plotting a graph showing the correlation between the concentrations of sample extracts for antioxidant activity and XOI activity. The correlation between the two independent variables was analysed using

correlation and regression analysis in Microsoft Excel. This analysis examined the relationship between the antioxidant activity and xanthine oxidase inhibitory activity of *Sesbania grandiflora* leaves extract.

3. RESULTS AND DISCUSSION

3.1 DPPH Radical Scavenging Assay

The DPPH scavenging activity assay is based on the reduction of the DPPH free radical by antioxidants present in the sample (Hebail et al., 2024). Data were obtained from triplicate trials to ensure the accuracy of the results for both the leaves extract and gallic acid. When an antioxidant reacts with DPPH, the solution's colour becomes lighter, indicating more de-colorization. This de-colorization signifies a higher reducing ability of the sample and, consequently, a greater antioxidant capacity to scavenge free radicals. The results presented in Table 1 demonstrate the percentage inhibition of DPPH at various sample concentrations for both the leaves extract and gallic acid. As the concentration of the sample increases, the percentage of inhibition activity also increases. This indicates a dose-dependent relationship, where higher concentrations of the sample contain more antioxidant compounds capable of neutralizing free radicals. Consequently, the increased presence of these antioxidants enhances the sample's ability to scavenge DPPH radicals, resulting in higher inhibition percentages. This trend suggests that both the leaves extract and gallic acid are effective antioxidants, with their efficacy improving at higher concentrations.

Table 1: Comparison of DPPH scavenging activity between *Sesbania grandiflora* and gallic acid.

Sample (µg/mL)	Leaves Extract (%)	Gallic Acid (%)
20	50.90 ± 0.09	80.74 ± 0.21
40	58.78 ± 0.38	81.86 ± 0.94
60	59.34 ± 0.68	85.92 ± 0.34
80	59.90 ± 0.99	92.11 ± 0.27
100	61.59 ± 0.90	98.98 ± 0.65

Figure 1 illustrates the SC_{50} results of the DPPH radical scavenging assay. As seen in Figure 1, the investigation proceeded with the determination of the dose required to inhibit the DPPH component by 50%. The SC_{50} values for the leaves extract and gallic acid, used as a positive control, were plotted using probit graph analysis. This probit analysis was conducted through regression data analysis using Microsoft Excel. From this regression data, the SC_{50} value was determined. It was observed that the SC_{50} of gallic acid at 16.59 ± 0.67 µg/mL was lower than that of the leaves extract at 7.76 ± 0.23 µg/mL. This indicates that gallic acid required only a small concentration to inhibit 50% of the free radicals. This can be attributed to the higher purity and potency of gallic acid as a known, well-characterized antioxidant, compared to the complex mixture of compounds present in the leaves extract.

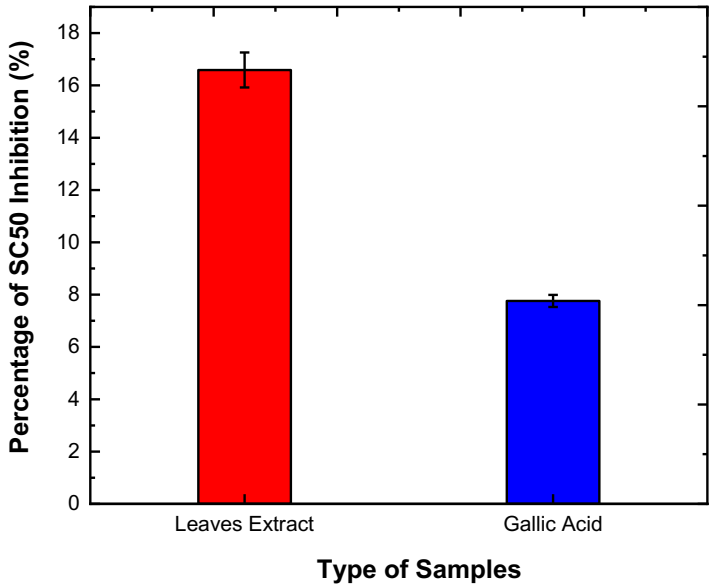

Figure 1: The SC_{50} of DPPH Radical Scavenging assay of leaves extract and gallic acid.

3.2 Inhibitory Activity Assay of Xanthine Oxidase

The xanthine oxidase activity assay was tested using different concentration ranges to determine the effect on xanthine oxidase inhibition activity. The assay involved adding xanthine solution as a substrate. Allopurinol was used as a control in the xanthine oxidase activity assay to provide a comparison with the leaves extract. The increase in inhibition activity with the concentration of both the sample extract and allopurinol, as shown in Table 2, indicates a dose-dependent relationship. This means that higher concentrations of the sample extract and allopurinol are more effective at inhibiting xanthine oxidase activity. This is a typical behaviour observed in enzyme inhibition studies, where increased inhibitor concentrations lead to greater suppression of the enzyme's activity (Chen et al., 2021). The observed trend suggests that both the leaves extract and allopurinol are effective inhibitors of xanthine oxidase, with their inhibitory effects becoming more pronounced at higher concentrations. Figure 2 presents the IC_{50} results of the inhibitory activity assay of xanthine oxidase.

Table 2: The inhibitory activity values of xanthine oxidase for the sample extract and allopurinol.

Sample (µg/mL)	Sample Extract (%)	Allopurinol (%)
20	64.75 ± 0.56	67.13 ± 0.01
40	68.63 ± 0.73	67.38 ± 0.78
60	68.88 ± 0.23	68.25 ± 0.95
80	69.00 ± 001	70.00 ± 0.07
100	70.38 ± 0.02	70.25 ± 0.99

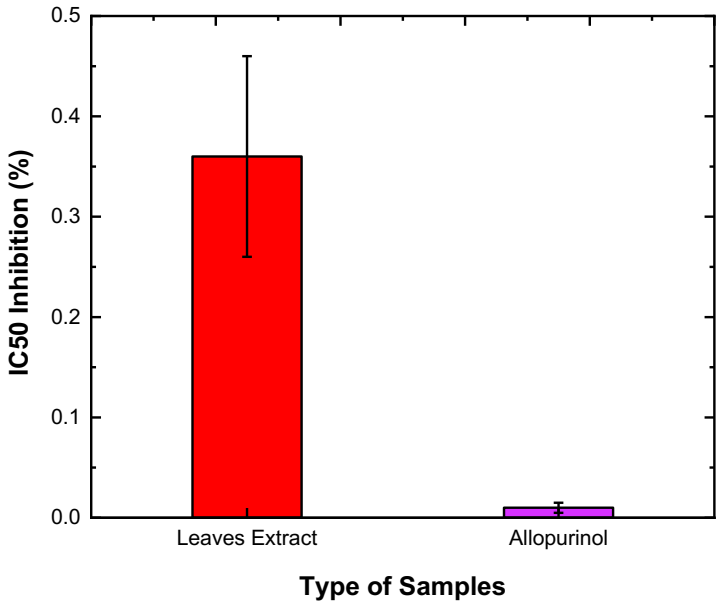

Figure 2: The IC_{50} results of the inhibitory activity assay of xanthine oxidase for the leaves extract and allopurinol.

As seen in Figure 2, the results showed that allopurinol required a lower dose to achieve 50% xanthine oxidase inhibition compared to the leaves extract. The probit analysis indicated that the allopurinol concentration needed to inhibit xanthine oxidase by 50% was 0.01 ± 0.005 µg/mL, which is significantly lower than the concentration required for *Sesbania grandiflora* leaves extract, which was 0.36 ± 0.341 µg/mL. This is likely because allopurinol is a synthetic drug specifically designed to inhibit xanthine oxidase, making it highly effective even at low concentrations. On the other hand, the leaves extract of *Sesbania grandiflora*, although naturally potent, contains a mixture of various compounds, which might not be as specifically targeted or potent in inhibiting xanthine oxidase as allopurinol. Consequently, a higher concentration of the leaves extract is needed to achieve the same level of inhibition. This highlights the superior efficacy of synthetic inhibitors like allopurinol in targeting specific enzymes compared to natural extracts.

3.3 The Correlation Study of Antioxidant Activity and Xanthine Oxidase Inhibitory Activity

The correlation study of *Sesbania grandiflora* leaves extract examined the relationship between antioxidant activity and xanthine oxidase inhibitory properties. This study focused on the response of inhibition activity on free radicals and xanthine oxidase. Figure 3 presents the correlation graph of *Sesbania grandiflora* leaves extract for both DPPH radical scavenging activity assay and xanthine oxidase inhibitory assay.

The correlation study of DPPH radical scavenging and xanthine oxidase inhibitory activity showed effective results, with a strong coefficient close to 1.000, indicating a positive correlation. This strong positive correlation suggests that as the antioxidant activity of *Sesbania grandiflora* leaves extract increases, its ability to inhibit xanthine oxidase also increases (Prabawati et al., 2021). This relationship can be attributed to the fact that antioxidant compounds are known to play significant roles in xanthine oxidase inhibition by interacting at the enzyme's reactive site (Padmalochana et al., 2014). These interactions likely enhance the inhibition of xanthine oxidase, leading to higher antioxidant activity and greater inhibitory effects.

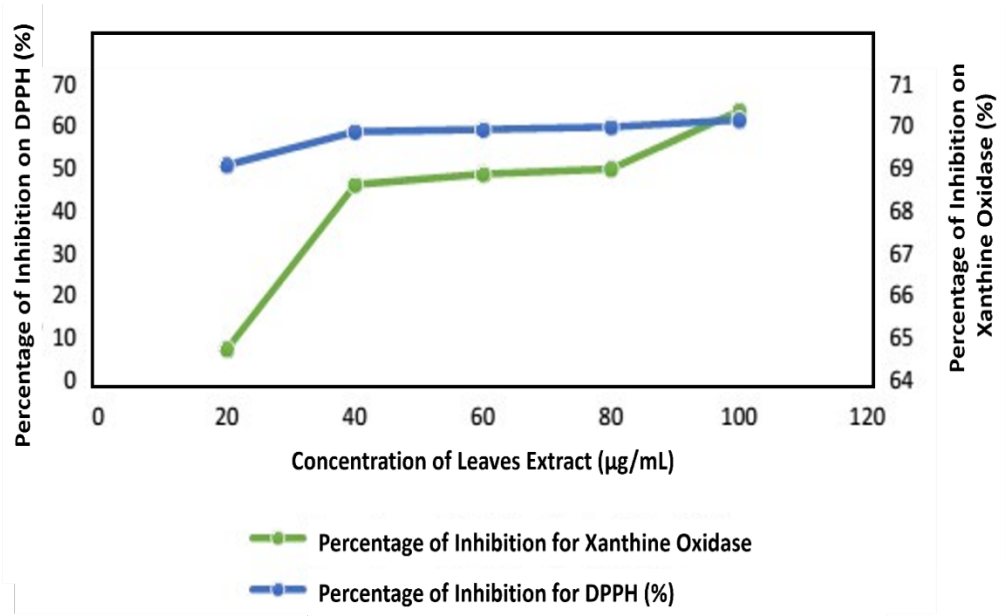

Figure 3: The correlation graph of *Sesbania grandiflora* Extract for both DPPH activity and xanthine oxidase inhibitory assay.

4. CONCLUSION

The *Sesbania grandiflora* extract was analysed for its antioxidant and xanthine oxidase inhibitory properties to determine its potential in treating gout and addressing free radical-related issues. This extract has potential as a xanthine oxidase inhibitor and contains high levels of antioxidants. The analysis of *Sesbania grandiflora* leaves showed a significant free radical inhibition rate of 61.59 ± 0.90% at a sample concentration of 100 µg/mL. Furthermore, the SC_{50} value for the leaves extract was 16.59 ± 0.67 µg/mL, compared to 7.76 ± 0.23 µg/mL for gallic acid, used as a positive control. Additionally, the leaves of *Sesbania grandiflora* were evaluated for their xanthine oxidase inhibitory efficiency in inhibiting uric acid formation. The leaves extract showed an IC_{50} value of 0.36 ± 0.341 µg/mL, which, while higher than the value for allopurinol (0.01 ± 0.005 µg/mL), still indicates significant inhibitory potential. The close IC_{50} value to allopurinol suggests that *Sesbania grandiflora* is a potent and comparable xanthine oxidase inhibitor. The correlation between antioxidant activity and xanthine oxidase inhibitory activity indicates a direct relationship. This correlation labels the extract as a compound with dual properties: antioxidant and xanthine oxidase inhibitory activity. Hence, this herb has significant scientific potential for treating gout and addressing free radical-related issues.

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REFERENCES

Ambastha, S., Kumari, A., Oraon, V., Patnaik, A., & Sharan, L. (2022). Pharmacological review on Sesbania grandiflora (Linn). *International Journal of Botany Studies*, *7*(2), 259-268.

Anand, R., *et al.*, (2013). Comparison of Extraction Techniques for Extraction of Bioactive Molecules from Hypericum perforatum L. *Plant Journal of Chromatographic Science,* 43(10), 530–531.

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.

Cha, Y., Lee, J., Choy, W., Lee, J. S., Lee, H. H., & Chae, D. S. (2024). Pathophysiology and treatment of gout arthritis; including gout arthritis of hip joint: a literature review. *Hip & Pelvis*, *36*(1), 1.

Chen, Y., Yin, Y., & Wang, J. (2021). Recent advance in inhibition of dark fermentative hydrogen production. *International Journal of Hydrogen Energy*, *46*(7), 5053-5073.

Francis, S. (2022). Organic Synthetic Drugs. Bibliotex, Canada. http://rguir.inflibnet.ac.in/bitstream/123456789/16782/1/9781984665997.pdf

Gawlik-Dziki, U., Dziki, L., Anisiewicz, J., Habza-Kowalska, E., Sikora, M., & Dziki, D. (2020). Leaves of white beetroot as a new source of antioxidant and anti-inflammatory compounds. *Plants*, *9*(8), 944.

Hebail, F., Rahouma, K., Muftah, A., Aldroujee, A., & Elghmasi, S. (2024). Estimating Antioxidant and Free Radical Scavenging Activity of Arbutus Pavarii Extracts. *AlQalam Journal of Medical and Applied Sciences*, 65-73.

Hendriani, R., Sukandar, E.Y., Anggadiredja, K., & Sukrasno (2016). In Vitro evaluation of xanthine oxidase inhibitory activity of selected medicinal plants, *International Journal of Pharmaceutical and Clinical Research, 8*(4), 235-238.

Jamal, P., Akbar, I.Z.Y., & Irw, J.I. (2016). Process Development for Maximum Lycopene Production from Selected Fruit Waste and its Antioxidant and Antiradical Activity. *Journal of Food Processing & Technology, 7*, 1-7. https://doi.org/10.4172/2157-7110.1000576

Jomova, K., Raptova, R., Alomar, S. Y., Alwasel, S. H., Nepovimova, E., Kuca, K., & Valko, M. (2023). Reactive oxygen species, toxicity, oxidative stress, and antioxidants: Chronic diseases and aging. *Archives of toxicology*, *97*(10), 2499-2574.

Lalani, A. R., Rastegar-Pouyani, N., Askari, A., Tavajohi, S., Akbari, S., & Jafarzadeh, E. (2024). Food Additives, Benefits, and Side Effects: A Review Article. *Journal of Chemical Health Risks*, *14*(1).

Orhan, I. E., & Deniz, F. S. (2021). Natural products and extracts as xantine oxidase inhibitors-a hope for gout disease?. *Current Pharmaceutical Design*, *27*(2), 143-158.

Paul, B.J., & James, R. (2017). Gout: an Asia-Pacific update. *International Journal of Rheumatic Diseases, 20*(4), 407–416.

Padmalochana, K., & Rajan, M.S.D. (2014). Antimicrobial activity of aqueous, ethanol and acetone extracts of Sesbania grandiflora leaves and its phytochemical characterization, *International Journal of Pharmaceutical Sciences and Research, 5*(12), 957–962.

Prabawati, N.B., Oktavirina, V., Palma, M., & Setyaningsih, W. (2021). Edible flowers: Antioxidant compounds and their functional properties. *Horticulturae*, *7*(4), 66.

Stapley, C. (2023). *A History of Plant Medicine: Western Herbal Medicine from the Ancient Greeks to the Modern Day*. Aeon Books.

Serrano, J. L., Figueiredo, J., Almeida, P., & Silvestre, S. (2020). From xanthine oxidase inhibition to in vivo hypouricemic effect: An integrated overview of in vitro and in vivo studies with focus on natural molecules and analogues. *Evidence-Based Complementary and Alternative Medicine*, *2020*(1), 9531725.

Wong, Y.P., Ng, R.C., Chuah, S.P., Koh, R.Y., Pick, A., & Ling, K. (2014). Antioxidant and Xanthine Oxidase Inhibitory Activities of Swietenia macrophylla and Punica granatum.