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Investigation on Flavanoid Extract From *Annona Squamosa L*. (Sugar Apple) Fruit For Potential Anti-Gout Property: *In Vitro* Studies

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Abstract

Introduction: *Annona squamosa L*. (Sugar Apple) belongs to the Annonaceae family. Different parts of *Annona squamosa L*. have various medicinal properties such as anti-inflammatory, antioxidant, antidiabetic, antimicrobial, antiulcer,and analgesic activity. Objective(s): To determine the antioxidant, anti-inflammatory and anti-gout properties of flavonoid extract of *Annona squamosa L*. fruit in the *in-vitro* and *in-vivo* studies. Methodologies: *Annona squamosa L*. fruit was extracted by maceration process using 70% ethanol solution and evaporated at 40°C. The ethanolic extract of *Annona squamosa L*. fruit was screened for the chemical tests of amino acids, carbohydrates, alkaloids, terpenoids, flavonoids, phenols, and tannins. The antioxidant activity of *Annona squamosa L*. fruit extract undergoes the total phenolic compound (TPC), ferric reducing ability of plasma (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. Xanthine oxidase inhibitory (XOI) assay used for anti-gout property. Results: A 190 g of *Annona squamosa L*. fruit obtains 48.60 g of ethanolic extract. Chemical tests for carbohydrates, amino acids, flavonoids, tannins, and terpenoids have positive results while chemical tests for alkaloids, and phenols have negative results. The total phenolic content (TPC) is 8.106 mg GAE/g of *Annona squamosa L*. fruit.The EC_1 value for the FRAP assay of *Annona squamosa L*. fruit is 2.1307 mg/mL. The IC_{50} values for DPPH and XOI assays of *Annona squamosa L*. fruit are 4.1154 mg/mL and 4.4745 mg/mL, respectively. *Annona squamosa L*. fruit extract shows an increase in antioxidant activities and xanthine oxidase inhibition in a concentration-dependent manner. Conclusion: *Annona squamosa L.*fruit has the potential to be a source of antioxidant, anti-inflammatory, and anti-gout properties.

Keywords: *Annona squamosa L*., Sugar Apple, Antioxidant, Anti-inflammatory, Anti-gout

Introduction

Annona squamosa L. is a multipurpose plant that has several medicinal properties such as anti-inflammatory, antioxidant, antimicrobial, cytotoxic, antiulcer, hepatoprotective, antidiabetic, antilipidemic, antitumor, vasorelaxant, anthelmintic, genotoxic, and analgesic activity (1; 2). The medicinal properties involve bioactive compounds in the different parts of the *Annona squamosa L.* plant as it is used to treat ailments and human diseases. *Annona squamosa L.* is also used traditionally in treating epilepsy, constipation, haemorrhage, dysentery, fever, ulcer, worm infection, and cardiac complications (2).

Gout is the most common inflammatory disease due to hyperuricemia. Hyperuricemia is an elevation in the levelof the serum uric acid in the human body where it reaches a saturated level of 6.8 mg/dL at 37°C and pH 7, forming an inflammatory

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monosodium urate crystal (MSU) in the joints and synovium. Male and female patients with serum uric acid levels higher than 7 mg/dL and 6 mg/dL, respectively are categorised as hyperuricemia(3; 4).

In recent decades, the prevalence of gouty arthritis has been increasing worldwide due to the levitation of risk factors related with the disease, particularly hyperuricemia. Gout and hyperuricemia lead to the levitation of inflammation in the body, resulting in a high risk of complications such as cardiovascular diseases (5). A few of the risk factors of gout include sex, genetic variations, obesity, insulin resistance, medications, and kidney diseases related to the reduction of urate in renal clearance (6; 7).

Nowadays, there are a lot of drugs that have been discovered, developed, and suggested to have the ability to the treatment of gout. Fruits containing anti-inflammatory and anti-hyperuricemia activities might have potential benefits in gout treatment (7). This prompts the concern of discovering natural products that have the potential to treat gouty arthritis. *Annona squamosa L*. has been known as a folk medicine for its antioxidant and anti-inflammatory properties. Hence the study was undertaken to determine the antioxidant, anti-inflammatory and anti-gout properties of *Annona squamosa L*. fruit extract.

Materials and Methods

Chemicals

The chemicals used for this study included, 70% ethanol, Benedict's reagent, Millon's reagent, Mayer's reagent, chloroform, sulphuric acid (H_2SO_4),gallic acid, Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), ascorbic acid, potassium ferricyanide ($K_3[Fe(CN)_6]$), trichloroacetic acid, ferric chloride ($FeCl_3$), disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), *2,2-diphenyl-1-picrylhydrazyl* (DPPH), dimethyl sulfoxide (DMSO), allopurinol, xanthine oxidase, xanthine, hydrochloric acid (HCl), sodium hydroxide (NaOH).

Sources of Annona squamosa L.

The identified *Annona squamosa L*. fruit was confirmed and conducted by a resident botanist at Mini Herbarium, Institute of Bioscience, Universiti Putra Malaysia (UPM), Serdang, Selangor with a voucher no of KM 0091/23.

Preparation of Annona squamosa L. Fruit Extract

A 5 kg of *Annona squamosa L*. fruit was dried in an oven at 50°C for 3 days and ground into a coarse powder. *Annona squamosa L*. fruit extract was prepared by maceration extraction method. The conical flask was filled with 70% ethanol following an extraction fraction ratio of 1:10 (fruit powder: solvent). The maceration process was done for 72 hours at ambient temperature with continuous swirling using an orbital shaker for thorough extraction. *Annona squamosa L*. fruitextract was filtered, evaporated using a rotary vacuum at 45°C evaporator under reduced pressure, and lyophilized to concentrate the extract. *Annona squamosa L*. fruit extract was kept in an airtight container ata temperature of–4°C(8).

Phytochemical Screening

The phytochemical screening was conducted on *Annona squamosa L*. fruit extract by identifying the presence of carbohydrates, alkaloids, flavonoids, amino acids, phenols, tannins, and terpenoids using chemical tests of Benedict's test Mayer's test, alkaline reagent test (9), Millon's test (10), ferric chloride test, ferric chloride-potassium ferricyanide test, and Salkowski's test (11), respectively.

Total Phenolic Content (TPC)

A 1 mL of *Annona squamosa L*. fruit extract is taken for 1 mL and added into a test tube. Next, the Folin-Ciocalteu reagent (FCR) solution and 10% of sodium carbonate (Na_2CO_3) solution are added into the test tube, wrapped with aluminium foil, and heated at 50°C for 5 minutes. The solution mixture is incubated for 10 minutes in a dark

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environment and the absorbance is measured at the wavelength of 415 nm. The blank solution contains the FCR solution and the Na_2CO_3 solution. The standard solution used in the determining the total phenolic content of *Annona squamosa L*. fruit extract is gallic acid. The procedure is carried out in triplicate. TPC of *Annona squamosa L*. fruit extract is expressed in mg gallic acid equivalent (GAE)/g of *Annona squamosa L*. fruit powder (8).

Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric-reducing antioxidant power assay is done to determine the antioxidant activity of *Annona squamosa L*. fruit extract. Different concentrations of *Annona squamosa L*. fruit extract and ascorbic acid are prepared. Ascorbic acid is the reference standard. A 2.5 mL of 0.2 M phosphate buffer (PB) with pH 6.6 and 2.5 mL of 1% $K_3[Fe(CN)_6]$ are added into the test tube and incubated at 50°C for 20 minutes. Next, 2.5 mL of 10% trichloroacetic acid is added and centrifuged for 10 minutes at 3000 rpm. A 2.5 mLaliquot of supernatant from each test tube was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$. The blank solution contains PB, 1% $K_3[Fe(CN)_6]$, 10% trichloroacetic acid, 0.1% $FeCl_3$, and distilled water. The absorbance value was measured at 700 nm(12).A standard curve of 0.1 to 2.0 mM ferrous sulphate heptahydrate was constructed and expressed as mM Fe(II) per gram of dry-weight plant. EC_1 value of *Annona squamosa L*. fruit extract and ascorbic acid were evaluated based on the reading of absorbances equivalent to the theoretical absorbance value of 1 mM of Fe(II) concentration using the corresponding regression equation (13).

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of *Annona squamosa L*. fruit extract is evaluated using the DPPH method. A reaction mixture consisting of *Annona squamosa L*. fruit extract and the DPPH reagent with different concentrations is prepared and incubated in a dark environment. The absorbance of the reaction mixture is measured at the wavelength of 517 nm. The process is performed in triplicate. The reference standard used in the DPPH method is ascorbic acid. The DPPH solution is prepared by dissolving the DPPH powder in the ethanol solution. The DPPH solution and the ethanol solution are used as control and blank, respectively in the procedure. The percentage of the scavenging effect of the sample is calculated using the formula;

$$\text{Scavenging effect (\%)} = (1 - \alpha/\beta) \times 100\%$$

Whereby α is the absorbance of the extractand β is the absorbance of the control. The results are expressed as mean ± SD. IC_{50} value (mg/mL) is the total antioxidant required to reduce the initial DPPH free radicals by 50% and derived from the graph of the percentage of scavenging activity plotted against the various concentrations of *Annona squamosa L*. fruit extract and ascorbic acid, respectively (13).

Xanthine Oxidase Inhibitory Assay (XOI)

The xanthine oxidase activity of *Annona squamosa L*. fruit extract is determined by calculating the formation of uric acid from xanthine. Allopurinol is used as the reference standard in the experiment. *Annona squamosa L*. fruit extract and allopurinol are dissolved and diluted in distilled water and DMSO, respectively to prepare a range of concentrations. A 0.1 mL sample with 1.9 mL of 50 mM PB with pH 7.5 and 0.1 units/mL xanthine oxidase enzyme is pre-incubated at 37°C for 15 minutes.

Next, 0.15 mM xanthine (1 mL) is added and incubated at 37°C for 30 minutes. Next, 0.5 M HCl is added to halt the reaction and the absorbance is measuredat the wavelength of 290 nm. The blank solution and the control used in the experiment are buffers and a solution consisting of xanthine and xanthine oxidase. The percentage of the xanthine oxidase inhibition

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activity (%) is calculated based on the given formula;

Inhibition activity (%) = (1 - α/ β) x 100%

Whereby, α is the absorbance of the extractand β is the absorbance of the control. The inhibition concentration (IC_{50}) that is required to inhibit 50% of the uric acid formation *Annona squamosa L*. fruit extract and allopurinol is evaluated from the standard curve of the inhibition of xanthine oxidase activity (13).

Statistical Analysis

For TPC, FRAP, DPPH, and XOI assays, the results were expressed in mean ± SD(13).

Results and Discussion

Percentage Yield of Annona squamosa L. fruit extract

The percentage yield of 70% ethanolic extraction was 25.58 %. A 190 g of *Annona squamosa L*. fruit powder (Figure 1) extracted in 70% ethanol solution obtained 48.60 g of *Annona squamosa L*. fruit extract and has the appearance of a dark, brown-coloured viscous and semi-solid form as shown in (Figure 2).

Phytochemical Screening

Phytochemical screening was done for *Annona squamosa L*. fruit extract. The chemical tests include identifying the presence of chemical constituents such as carbohydrates, amino acids, alkaloids, flavonoids, phenols, tannins, and terpenoids.

Figure 1: *Annona squamosa L*. Fruit Powder

The chemical test for carbohydrates, amino acids, flavonoids, tannins, and terpenoids has positive results while the chemical test for alkaloids, and phenols has negative results as shown in (Table 1).

Total Phenolic Content (TPC)

The total phenolic content of *Annona squamosa L*. fruit extract is 8.106 mg GAE/g of *Annona squamosa L*. fruit extract. The total phenolic content of *Annona squamosa L*. fruit extract was calculated based on the equation derived from the equationof standard curve for gallic acid which is $y = 0.301x + 0.238$ with the R^2 value of 0.902 as shown in (Figure 3). The presence of phenolic compounds in *Annona squamosa L*. fruit extract gives a potential antioxidant activity.

Based on the previous study, the total phenolic content of ethanol extraction was 13.53 mg GAE/g of *Annona squamosa L*. fruit extract. The plant's

Figure 2: *Annona squamosa L*. Fruit Extract

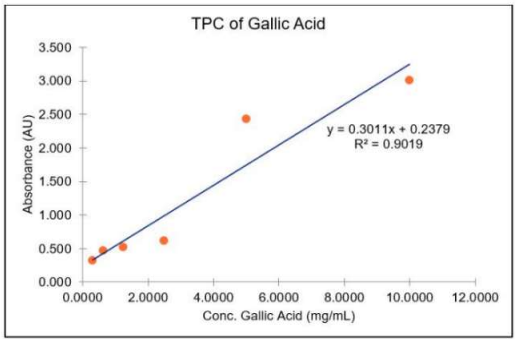

Figure 3: Total Phenolic Content of Gallic Acid

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Table 1: Phytochemical Screening of *Annona squamosa L*. Fruit Extract

Chemical Constituent	Chemical Test	Observation			Inference
		1	2	3	
Carbohydrates	Benedict's Test	+	+	+	Formation of reddish precipitate
Amino Acids	Millon's Test	+	+	+	Formation of white to red precipitate
Alkaloids	Mayer's Test	–	–	–	No reaction
Flavonoids	Alkaline Reagent Test	+	+	+	Formation of yellow to colourless solution
Phenols	Ferric Chloride Test	–	–	–	No reaction
Tannins	Ferric Chloride-Potassium Ferricyanide Test	+	+	+	Formation of dark blue coloured solution
Terpenoids	Salkowski's Test	+	+	+	Formation of reddish-brown coloured interface

Key: (+) = Present, (–) = Absence

Table 2: FRAP Value of Ascorbic Acid and *Annona squamosa L*. Fruit Extract

Conc. Ascorbic Acid (mg/mL)	FRAP Value (mM Fe (II)/g)	Conc. *Annona squamosa L*. Fruit (mg/mL)	FRAP Value (mM Fe (II)/g)
0.31	3.06 ± 0.005	1.56	0.99 ± 0.004
0.63	4.23 ± 0.006	3.13	1.89 ± 0.004
1.25	5.12 ± 0.004	6.25	3.03 ± 0.004
2.50	6.04 ± 0.003	12.50	5.08 ± 0.005
5.00	7.44 ± 0.007	25.00	6.81 ± 0.005
10.00	9.17 ± 0.010	50.00	8.97 ± 0.005

phenolic compoundspresent in the form of polar glycosides allows the plant to be dissolved easily in a polar solvent such as ethanol (8) andgives antioxidant activity where it has redox potential (12).

Ferric Reducing Antioxidant Power Assay (FRAP)

The FRAP values of ascorbic acid and *Annona squamosa L*. fruit extract are listed in (Table 2). The FRAP values of *Annona squamosa L*. fruit extract show an increase in antioxidant activities in a concentration-dependent manner (Figure 4). The reducing power of *Annona squamosa L*. fruit extract on its ability to transfer electrons to the FRAP reagents increases when the FRAP value increases. The EC_1 value of *Annona squamosa L*. fruit extract and ascorbic acid is 2.131 mg/mL and 0.995 mg/mL, respectively.

Ascorbic acid as the reference standard has a significantly smaller EC_1 value than *Annona squamosa L*. fruit extract. The alkaloid compounds present in *Annona squamosa L*. fruit extract act as a reductantagainst FRAP reagents. It has been suggested that phenolic compounds break the radical chain reaction by the donation of hydrogen atomswhich gives the potential of reducing power (13).

2,2-diphenyl-1-picrylhydrazyl (DPPH)

The percentages for radical scavenging activity (RSA) of ascorbic acid

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and *Annona squamosa L*. fruit extract arerecorded as shown in (Table 3). The radical scavenging activity (RSA) of *Annona squamosa L*. fruit extract shows an increase in direct proportion towards the concentration of *Annona squamosa L*. fruit extract (Figure 5). The lower the absorbance taken during the assay, the higher the percentage of RSA for *Annona squamosa L*. fruit extract.The IC_{50} value of *Annona squamosa L*. fruit extract and ascorbic acid is 4.115 mg/mL and 4.918 mg/mL, respectively. In this study, *Annona squamosa L*. fruit extract has a significantly lower IC_{50} value than ascorbic acid.

Annona squamosa L. fruit extract could scavenge the DPPH radicals' odd electron that shows activities of donation of proton which acts as inhibitors for free radicals. This study suggested that *Annona squamosa L*. fruit extract has a high potency of antioxidant activity in a lower concentration as the IC_{50} value of *Annona squamosa L*. fruit extract is lower than the ascorbic acid as the reference standard(13).

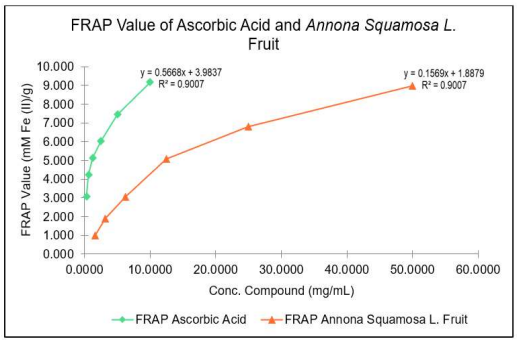

Figure 4: FRAP Value of Ascorbic Acid and *Annona squamosa L*. Fruit Extract, expressed as mM Fe (II)/g of *Annona squamosa L*. Fruit Extract. Values expressed as mean ± SD, n = 3/concentration

Xanthine Oxidase Inhibitory Assay (XOI)

The percentages for the inhibition activity of xanthine oxidase for allopurinol and *Annona squamosa L*. fruit extract are listed in (Table 4). The xanthine oxidase inhibitory (XOI) activity of *Annona squamosa L*. fruit extract demonstrates an increasing concentration-dependent manner of *Annona squamosa L*. fruit extract (Figure 6). The IC_{50} values of *Annona squamosa L*. fruit extract and allopurinol are4.745 mg/mL and 0.154 mg/mL, respectively. The reference standard used is allopurinol have a

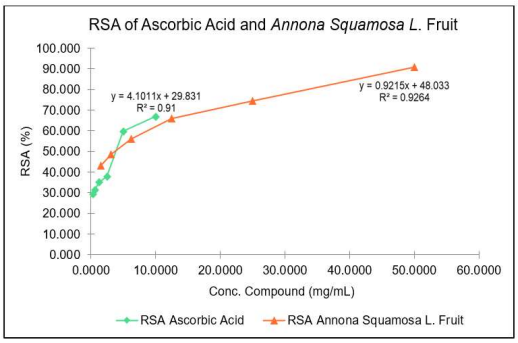

Figure 5: Percentage of Radical Scavenging Activity (RSA) of Ascorbic Acid and *Annona squamosa L*. Fruit Extract. Values expressed as mean ± SD, n = 3/concentration

Table 3: Percentage for RSA of Ascorbic Acid and *Annona squamosa L*. Fruit Extract

Conc. Ascorbic Acid (mg/mL)	RSA (%)	Conc. *Annona squamosa L*. Fruit (mg/mL)	RSA (%)
0.31	29.10 ± 0.004	1.56	42.92 ± 0.005
0.63	31.30 ± 0.005	3.13	48.92 ± 0.004
1.25	34.86 ± 0.004	6.25	56.12 ± 0.003
2.50	37.90 ± 0.003	12.50	65.94 ± 0.004
5.00	59.73 ± 0.004	25.00	74.40 ± 0.004
10.00	66.84 ± 0.007	50.00	90.90 ± 0.005

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Table 4: Percentage for XOI of Allopurinol and *Annona squamosa L*. Fruit Extract			
Conc. Allopurinol (mg/mL)	XOI (%)	Conc. *Annona squamosa L*. Fruit (mg/mL)	XOI (%)
0.03	36.97 ± 0.003	0.31	42.92 ± 0.005
0.06	45.14 ± 0.004	0.63	48.92 ± 0.004
0.13	49.81 ± 0.003	1.25	56.12 ± 0.003
0.25	58.76 ± 0.002	2.50	65.94 ± 0.004
0.50	72.76 ± 0.002	5.00	74.40 ± 0.004
1.00	87.16 ± 0.002	10.00	90.90 ± 0.005

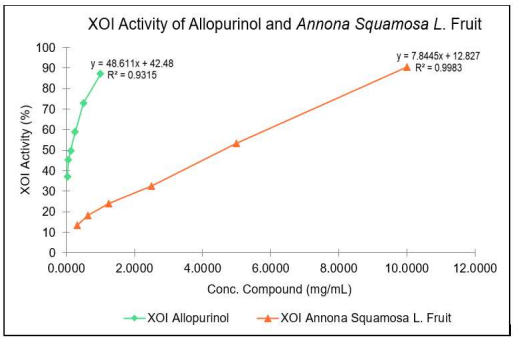

Figure 6: Percentage of Xanthine Oxidase Inhibitory (XOI) Activity of Allopurinol and *Annona squamosa L*. Fruit Extract. Values expressed as mean ± SD, n = 3/concentration

significantly lower IC_{50} value than *Annona squamosa L*. fruit extract.

This study proposed that *Annona squamosa L*. fruit extract has the potential to be a xanthine oxidase inhibitor and an anti-gout agent. A previous study shows*Annona squamosa L*. fruit ethanolic extract has 64.88%xanthine oxidase inhibition power (8). A xanthine oxidase inhibitor possesses the ability to inhibit xanthine oxidase in the pathway of hydroxylation of hypoxanthine to xanthine to uric acid. *Annona squamosa L*. fruit extract inhibits the generation of superoxide anion ($O_{2\text{-}}\bullet$) and hydrogen peroxide (H_2O_2) where both are involved in the catalysation of substrate in the purine metabolic pathway when xanthine dehydrogenase is converted to xanthine oxidase (13).

Conclusion

In this study, *Annona squamosa L*. (sugar apple) fruit has the potential to be a source of antioxidants. The percentage yield of *Annona squamosa L*. fruit extract is 25.58 %. In phytochemical screening,chemical tests for carbohydrates, amino acids, flavonoids, tannins, and terpenoids show positive results. The total phenolic content of *Annona squamosa L*. fruit extract is 8.106 mg GAE/g of *Annona squamosa L*. fruit extract. The EC_1 value of *Annona squamosa L*. fruit extract is 2.131 mg/mL. The IC_{50} values of *Annona squamosa L*. fruit extract in DPPH and XOI assay are 4.115 mg/mL and 4.745 mg/mL, respectively. This study has confirmed the antioxidant properties of *Annona squamosa L*. fruit extract.

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References

1 Gajalakshmi, S., Divya, R., Deepika, V. D., Mythili, S., &Sathiavelu, A. (2011). Pharmacological Activities of Annona squamosa : A Review. International Journal of Pharmaceutical Sciences Review and Research, 10(2), 24–29.

2 Adesanwo, J. K., Akinloye, A. A., Otemuyiwa, I. O., & Akinpelu, D. A. (2020, October). Chemical Characteristics and Biological Activities of Annona squamosa

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10.5530/ctbp.2024.3s.7

Fruit Pod and Seed Extracts. Journal of Exploratory Research in Pharmacology, 6(1), 5–15.

3 Dehlin, M., Jacobsson, L., & Roddy, E. (2020, June). Global epidemiology of gout: prevalence, incidence, treatment patterns and risk factors. Nature Reviews Rheumatology, 16(7), 380–390.

4 Patil, T., Soni, A., & Acharya, S. (2021, September). A brief review on in vivo models for Gouty Arthritis. Metabolism Open, 11, 100100.

5 Scanu, A., Luisetto, R., Ramonda, R., Spinella, P., Sfriso, P., Galozzi, P., & Oliviero, F. (2022, October). Anti-Inflammatory and Hypouricemic Effect of Bioactive Compounds: Molecular Evidence and Potential Application in the Management of Gout. Current Issues in Molecular Biology, 44(11), 5173–5190.

6 Murdoch, R., Barry, M. J., Choi, H. K., Hernandez, D., Johnsen, B., Labrador, M., Reid, S., Singh, J. A., Terkeltaub, R., Mellado, J. V., &Dalbeth, N. (2021, April). Gout, Hyperuricaemia and Crystal-Associated Disease Network (G-CAN) common language definition of gout. Rheumatic & Musculoskeletal Diseases, 7(2), e001623.

7 Abdul Jalil, A. A., Syed Putera, S. S. P. A., Nor Afendi, N. S. F., Nor Azlan, A. Y. H., &Maarof, M. (2022, November). Potential Effect of Nutritional Fruits on Gout Therapy – A Review. Asian Journal of Medicine and Health Sciences, 5(2), 2–24.

8 Yunita, Y., Santoso, A., &Subandi, S. (2021, May). Xanthine oxidase inhibitory activity and identification of flavonoid in ethanol extract of sugar apple fruit (Annona squamosa L.). International Conference on Life Sciences and Technology (ICoLiST 2020) | AIP Conference Proceeding, 2353, 030068-1–030068-8.

9 Shaikh, J. R., & Patil, M. K. (2020). Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies, 8(2), 603–608.

10 Dhrumi, S., Pooja, S., Sonali, M., Charmi, P., Zalak R, R., & Meenu S, S. (2022, May). Scientific Tools and Techniques for Qualitative and Quantitative Analysis of Bacterial Proteins. Acta Scientific Microbiology, 5(5), 152–160.

11 Kancherla, N., Dhakshinamoothi, A., Chitra, K., &Komaram, R. B. (2019). Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of Cayratia auriculata (In Vitro). Maedica - A Journal of Clinical Medicine, 14(4), 350–356.

12 Jafri, S. A. A., Khalid, Z. M., Khan, M. Z., &Jogezai, N. (2022, January). Evaluation of phytochemical and antioxidant potential of various extracts from traditionally used medicinal plants of Pakistan. Open Chemistry, 20(1), 1337–1356.

13 Li, L. S., Chiroma, S. M., Hashim, T., Adam, S. K., Mohd Moklas, M. A., Yusuf, Z., & Abdul Rahman, S. (2020, June). Antioxidant and anti-inflammatory properties of Erythroxylumcuneatum alkaloid leaf extract. Heliyon, 6(6), E04141.