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Mechanism Based Screening for Development of Polyherbal Antigout Formulation

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ABSTRACT
Gout, a debilitating and recurrent form of arthritis, has garnered increased attention due to its rising prevalence. Many natural remedies have been historically employed to manage gout, yet their mechanisms of action remain uncertain. This research paper aims to shed light on the therapeutic advantages and biochemical mechanisms of various phytochemicals derived from selected plant sources for the treatment of gout. The study examined the interaction of standard drugs with the XO receptor (2E1Q) and found that Standard drugs showed lower binding affinities to the XO receptor (2E1Q) than phytoconstituents. Maceration and decoction extracts contained phenolic and flavonoid compounds, notably in ethanol-water maceration. XO enzyme was isolated from cow milk, with a crude enzyme concentration of 7.10 mg protein/mL and a reaction rate of 3.1095×10^3 µmol min^{-1} mL^{-1}. Individual herbs displayed better inhibition activity than standard drugs, especially maceration ethanolic and water extracts. A combination of papaya, long pepper, and curry leaves exhibited superior inhibition. The formulation "Arishta" was prepared, suggesting a potential XO inhibition-based approach for gout management. The study not only validates the longstanding folklore claims associated with Indian medicinal plants but also unveils their immense potential as rich sources of bioactive compounds for both medicinal and pharmaceutical purposes. These findings underscore the significance of further exploration and utilization of these plant-derived compounds, opening doors to promising avenues in the fields of medicine and herbal research.

1. INTRODUCTION:

Globally, gout is a major issue, but there have been significant developments in its management in recent years. In 2021, the market for gout therapeutics was estimated to be worth USD 2.78 billion, and in the following 15 years, it is predicted to rise at a CAGR of 13.7%. Gout is the type of arthritis. The term gout is used to describe a variety of conditions characterized by hyperuricemia. Inflammatory joint illness and increased blood levels of uric acid, which deposit uric acid crystals mostly in joints and kidneys, are hallmarks of these conditions. This deposition eventually leads to painful swelling, arthritis, and urate nephrolithiasis.

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Gout can cause discomfort in a variety of places on the body, including: Membranes that surround tendon, Tendon sheaths, Cushions approximating sacs between bones and other tissues, Bursae, Joints of mostly toes and all body joints, Elevated uric acid levels lead stones in kidney.

Hyperuricemia is defined when the plasma uric acid concentration is above 7 mg/dl in male adults, or 6 mg/dl in female adults. Only 10% of people with hyperuricemia develop gout. About 0.12% to 0.19% of the population in India has gout. The presence of oestrogen hormone aids in urate clearance, which is why the incidence is higher in men aged 50 and over but lower in premenopausal women. Common location for gout is the first metatarsophalangeal joint, the large toe. The first attack may occur at a different location, including the ankle, knee, or hand, or instep of the foot, in about one third of patients.

The most typical clinical signs include acute synovitis, persistent erosive, deformation arthritis, tophi and nephrolithiasis. Uric acid is produced through the purine metabolism and can be found in many different foods and in human tissue. Monosodium urate (MSU) crystals develop in tissue because of hyperuricemia (serum uric acid >7mg/dL), which is underlying cause of gout. Hyperuricemia occurs when there is a discrepancy between production and excretion of urates, such as overproduction or under excretion.

Numerous allopathic medications are available to address gout, such as Allopurinol (Aloprim, Zyloprim), which diminishes uric acid production; Colchicine (Colcrys, Gloperba, Mitigare), renowned for its anti-inflammatory properties; Febuxostat (Uloric), which also curtails uric acid production; Indomethacin (Indocin, Tivorbex), a potent NSAID pain reliever; Lesinurad (Zurampic), facilitating uric acid elimination through urine; Pegloticase (Krystexxa), specialized in breaking down uric acid; Probenecid, which aids the kidneys in excreting uric acid; and Steroids (corticosteroids) renowned for their anti-inflammatory action. However, these drugs may entail certain side effects. For instance, Allopurinol can lead to fever, rash, hepatitis, and kidney issues, while Febuxostat may result in rash, nausea, reduced liver function, and an elevated risk of heart-related complications.

In contrast to the potential side effects associated with allopathic medications, Ayurveda offers a holistic and natural approach to managing gout, emphasizing the restoration of the body's balance and addressing the root causes of the condition. Complementary and alternative medicine, particularly herbal medicine, plays a pivotal role in this approach. The utilization of herbal remedies for the treatment and management of gout symptoms and related complications is gaining popularity. Traditional gout therapies involve the use of a diverse array of plants, including *Gossypium herbaceum, Hibiscus sabdariffa, Tinospora cordifolia, Ficus carica, Caryophyllus aromaticus, Piper longum, Piper nigrum, Withania somnifera,* and numerous others. While many of these herbs have long-standing traditional usage, recent research has shed light on their mechanisms of action. Several of these plants and their constituents exert anti-gout effects through various pathways, encompassing uricosuric activity, inhibition of XO, anti-inflammatory actions, and antioxidant properties. This multifaceted approach within herbal medicine showcases its potential as a promising avenue for addressing the complexities of gout management.

The elusive mechanisms underlying the effectiveness of various natural remedies in managing gout remain uncertain. Recognizing and highlighting the therapeutic benefits and biochemical actions of distinct phytochemicals derived from selected plants for gout treatment hold significant promise. Leveraging synergistic effects, combination therapy can enhance the potency of gout medications. Complex cellular mechanisms, chiefly involving inflammasomes and toll-like receptors linked to tyrosine, underlie the potent inflammatory response incited by monosodium urate crystals—ultimately stemming from uric acid, the final oxidation product of purine. The enzymatic conversion of hypoxanthine to xanthine and subsequently to uric acid, catalyzed by XO, plays a pivotal role. Given these insights, the development of a mechanism-based polyherbal formulation, delivering therapeutic benefits with minimal side effects, remains a compelling avenue for exploration.

In response to the growing awareness and preference for alternative treatments, it is imperative to subject a range of ancient Indian Ayurveda, Unani, and other medicinal plants to systematic screening and scientific evaluation. This endeavor aims to yield enhanced, safer therapeutic options with reduced side effects. The objectives of this study encompass virtual screening of phytoconstituents for potential modes of action, the meticulous preparation and assessment of extracts employing diverse solvents, as well as the development and evaluation of polyherbal formulations. The overarching goal is to maintain serum uric acid levels within a normal range and prevent joint damage attributable to hyperuricemia.

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MATERIAL AND METHODS:

Chemical and reagent:

All reagents used in this study were of analytical grade and purchased from reputable college local dealers.

Molecular docking studies:

The study employed the ligand-protein docking method to investigate potential interactions. A literature review identified Indian medicinal plants containing XO Enzymes. Protein structures were retrieved from the Protein Data Bank, specifically focusing on the 3D Crystal Structure of Human Xanthine Oxidoreductase mutant, Glu803Val enzyme (PDB: 2E1Q), in complex with their enzyme inhibitors. Subsequently, the selected protein structures underwent a thorough preparation process, involving the removal of water structures and optimization of ionization and tautomeric positions of amino acid residues. Quality assessment of protein structures was performed using Ramachandran Plots. In the protein-ligand docking method, phytochemicals sourced from Indian medicinal plants served as ligands. The SDF file format of these phytochemical ligands was obtained from PubChem. The in-silico study focused on the inhibition of XO enzymes, utilizing a total of 118 phytochemicals.

Collection of Material:

The plant materials including Papaya leaves, curry leaves, and shewga leaves, Hadjod stem powder and long pepper powder were taken from local market in Kolhapur.

Maceration Technique:

The maceration process involved extracting the chosen five plants, namely *Cissus quandrangularis, Carica papaya, Moringa oleifera, Murraya koenigii, and Piper nigrum,* using a 1-gram powdered sample in a 25 ml beaker. To this, 10 ml of ethanol was added. The beakers were intermittently agitated while allowing them to soak for 48 hours. Subsequently, the filtrate was separated and collected after the extraction process.

Decoction Technique:

The decoction method was employed, utilizing water as the solvent. Decoction was carried out for five selected plants, specifically *Cissus quandrangularis, Carica papaya, Moringa oleifera, Murraya koenigii, and Piper nigrum*, in which a 5-gram powdered sample of each selected plant was placed in a 100ml beaker, and 50 ml of water was added. The mixture was boiled at 100°C for 30 minutes. Subsequently, the filtrate was separated and collected after the decoction process.

Determination of Phenolic Content:

In the determination of total phenolic content, Gallic acid served as the reference standard, and the methodology was adapted from A. Duraiswamy et al. Several crucial steps were followed to achieve accurate results. Initially, a 7.5% W/V Na2CO3 solution was prepared by carefully weighing 7 grams of sodium carbonate (Na2CO3) and dissolving it in 100 mL of water. Simultaneously, the Folin-Ciocalteu (F.C) reagent was diluted with an equal volume of water. For the herbal drug extracts, both maceration and decoction methods were employed separately, each resulting in 100µg/mL solutions. To assess the phenolic content, 1 mL from each of these extracts was mixed with 0.5 mL of the prepared Folin-Ciocalteu reagent. Additionally, 1.5 mL of the 7.5% Na2CO3 solution was added, and the total volume was adjusted with water. Subsequent incubation in darkness for 30 minutes led to the formation of a distinct dark blue color. The absorbance of the resulting solution was then measured at 760nm, facilitating the determination of total phenolic content in the herbal samples.

Determination of Flavonoid Content:

The quantification of total flavonoid content in the samples was carried out with quercetin serving as the reference standard, employing a methodology adapted from A. Duraiswamy et al. Firstly, a 10% W/V aluminum chloride (AlCl3) solution was prepared by dissolving 1 gram of AlCl3 in 10 mL of distilled water. Additionally, the preparation of nitric acid (NANO2) was mentioned, although the details were not provided. Furthermore, a 1 M NaOH solution was created by dissolving 1 gram of sodium hydroxide pellets in 20 mL of water, yielding a 1N NaOH solution. In each experimental flask, 1 mL of the 100µg/mL concentration of maceration and decoction extracts from the selected herbal drugs was introduced. Subsequently, 0.5 mL of the 10% AlCl3 solution and 2 mL of the prepared 1M NaOH solution were added. The volume was adjusted to 10 mL with methanol, and the mixture was incubated in darkness for 30 minutes. Following incubation, the absorbance was measured at 415nm, facilitating the determination of the total flavonoid content in the analyzed samples.

In vitro Assay:

Isolation of Xanthine Oxidase Enzyme:

A 1000 mL of fresh cow's milk were collected and subjected to centrifugation at 2500 rpm for 45 minutes to separate the cream. The cream was then divided into five portions, each containing 250 mL, and mixed with a 0.2 M 1 mM dibasic sodium phosphate solution, stirred for 90 minutes, and ethylene diamine tetraacetic acid was added. Following this, the suspension underwent another round of centrifugation at 2500 rpm for 30 minutes,

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yielding 245 mL of subnatant. Subsequently, 15% of the subnatant was gradually mixed with 37 mL of butanol, and ammonium sulfate was incrementally added to reach 25% saturation. This mixture was poured gently into the subnatant (282 mL) and left for an hour. The entire concoction was then subjected to centrifugation at 2500 rpm for 20 minutes, yielding 260 mL of supernatant. Ammonium sulfate was slowly added to reach 80% saturation while continuously stirring the supernatant for 30 minutes, followed by overnight standing. The resultant brown liquid was left to stand overnight once more, resulting in the precipitation of XO. The compound of this precipitate was then collected.

Enzyme Activity Assessment of Xanthine Oxidase:
Initially, 1.9 mL of a pH 7.3 monobasic sodium phosphate solution was prepared. Subsequently, 1 mL of a 0.15 mM xanthine solution was introduced into a test tube and thoroughly mixed. To this xanthine solution, 0.1 mL of crude enzyme solution was added, and the mixture was gently inverted. After allowing the mixture to stand for 20 minutes, its absorbance was measured at 293 nm using a UV-visible spectrophotometer. For the blank sample, 0.1 mL of distilled water and enzyme solution were employed as references in the spectrophotometric analysis. This systematic approach facilitated the evaluation of XO enzyme activity.

Gross Quantification:
The Bovine Serum Albumin (BSA) Stock Solution was prepared by dissolving 50 mg of BSA in 1 mL of water. To achieve the desired dilution, an appropriate amount of diluent was added. Subsequently, 4 mL of Biuret reagent was introduced into the solution. The resulting mixture was incubated at room temperature, and its absorbance was quantified at 540 nm.

Effect of pH on XO catalyzed reaction:
In a test tube, a solution of 1.9 mL monobasic sodium phosphate with a pH of 7.3 was introduced. Subsequently, 1 mL of a 0.15 mM xanthine solution was added and thoroughly mixed. To this mixture, 0.1 mL of the crude enzyme solution was carefully incorporated, and the contents were gently inverted. The resulting blend was allowed to stand undisturbed for 20 minutes, following which its absorbance was measured at 293 nm using a UV-visible spectrophotometer. As a reference, a blank sample consisting of 0.1 mL distilled water and enzyme solution was utilized. Furthermore, the described procedure was repeated across a range of pH values, specifically pH 5.8, 6.3, 6.8, 7.3, 7.8, and 8.8, to comprehensively assess the enzymatic activity under varying pH conditions.

Effect of Temperature on XO Catalyzed Reaction:
To investigate the impact of temperature on the XO crystal, initially, a pH 7.3 monobasic sodium phosphate solution (1.9 mL) was prepared. Subsequently, 1 mL of a 0.15 mM xanthine solution was introduced into a test tube and meticulously mixed. Next, 0.1 mL of the crude enzyme solution was added to the mixture, followed by gentle inversion to ensure thorough mixing. The resultant blend was allowed to stand undisturbed for duration of 20 minutes. Following this incubation period, the absorbance was measured at 293 nm using a UV-visible spectrophotometer. To establish a baseline, a blank sample was prepared, consisting of 0.1 mL of distilled water and enzyme solution. This systematic procedure was then replicated at varying temperatures, specifically at 15, 20, 25, 30, and 35 °C, to comprehensively assess the temperature-dependent behavior of the XO crystal.

Inhibitory Potential of Maceration and Decoction Extracts from Selected Herbs on Xanthine Oxidase Activity:
In this study, the examination of inhibitory activity for extracts obtained from selected herbs through maceration (E/E:W) and decoction techniques on XO was conducted. The procedure commenced by extracting individual plant samples using the designated maceration and decoction methods. Simultaneously, Allupurinol, a standard reference compound, was prepared in various dilutions ranging from 20 to 100µg/mL, with ethanol serving as the diluent. These dilutions were then introduced into separate test tubes containing 0.9 mL of monobasic sodium phosphate buffer with a pH adjusted to 7.3. Subsequently, 1 mL of the XO enzyme solution was added to each test tube, ensuring thorough mixing, followed by a 30-minute incubation period. After incubation, 1 mL of a 0.15 mM xanthine solution was introduced into each test tube, with meticulous mixing, and the mixtures were left undisturbed for an additional 20 minutes. In parallel, a blank sample was prepared by substituting the XO enzyme solution with 0.1 mL of distilled water. Absorbance measurements were then conducted at 293 nm utilizing a UV spectrophotometer, with Allupurinol serving as the standard reference to calculate the inhibition percentage of XO activity for each respective plant extract. This rigorous procedure allowed for the comprehensive exploration of the potential antigout properties of the herbal extracts under investigation. % Inhibition of XO activity by Maceration and Decoction extracts was calculated by equation.

$$y = \frac{x - x_1}{x} \times 100$$

Where,
x= XO activity (without extract)

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x1=XO activity (with extract)
y= Percent inhibition

Development of Polyherbal Formulation:
The development of the antigout formulation incorporated five plant-based components: *Piper Longum, Carica Papaya, Murraya Koenigii, Cassia Quadrangularis,* and *Moringa Oleifera*. This Arishta formulation boasts an extended shelf life, attributed to the presence of alcohol, which serves as a natural preservative.

Preparation Methods of Arishta Formulation:
The process commenced with the utilization of crude drugs for the Arishta formulation, which were then transferred to a fermentation vessel. A solution comprising sugar, honey, and jaggery was meticulously prepared and added to the vessel. The addition of Dhataki Pushp (*Woodfordia Fructicosa*) initiated the fermentation process. Subsequently, the earthen lid was securely closed, and the edges were sealed with a cloth smeared with clay. The fermentation took place under constant temperature conditions, ranging from 1 to 3 months, within a dark environment with minimal air circulation. Following fermentation, the Arishta was meticulously filtered, and the fluid was carefully decanted. Finally, the prepared Arishta was bottled and securely sealed for storage.

RESULT:

The Ramachandran plot, a critical tool for analyzing protein structures, provides valuable insights into the conformational quality of amino acid residues. Based on the dataset at hand, a comprehensive distribution was observed within the plot. A significant majority of residues (87.8%) are concentrated in the most favored regions (A, B, L) as shown in Fig 1, indicative of prevalent and favorable backbone angles. Additionally, approximately 11.0% of residues are positioned within the additional allowed regions (a, b, l, p), maintaining acceptable angles. Moreover, the analysis identifies three end-residues, excluding Glycine and Proline, totaling 104, and an additional 73 Proline residues. Among the 1307 residues examined, the Ramachandran plot underscores the importance of a high-quality protein model having over 90% of its residues located within the most favored regions. This observation aligns with our evaluation of 118 structures that meet specific resolution and R-factor criteria, highlighting the significance of conformational fidelity in protein structure analysis.

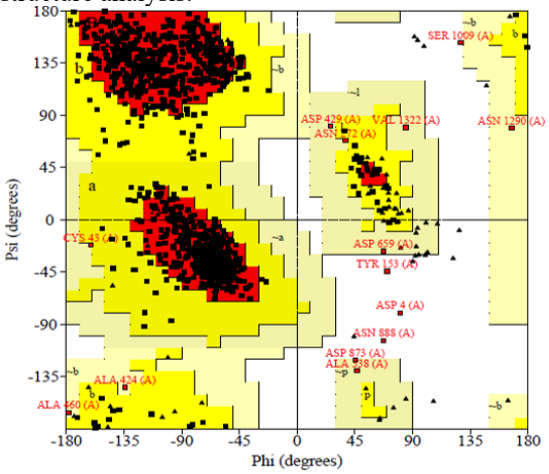


Figure 1: Ramachandran plot

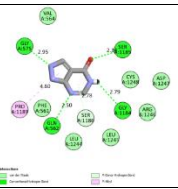
Table 1. Binding affinity of the standard drug

Sr. no.	Name of Standard Drug	Binding Affinity
1.	Allupurinol	-6.5
2.	Fabuxostat	-7.8

Table 2. Phytoconstituent of Interaction on the XO receptor (2E1Q) with their binding affinity and binding residue:

Sr. No.	Herb	Phytoconstituent	Binding Affinity
1.	*Moringa oleifera* (Shevgya leaves)	Astragalin	-9.8
		Chlorogenic_acid	-9.8
2.	Cassia quadrangularis (Hadjod)	Taraxerol	-10.7
		Cyanidin-3-rhamnoglucoside	-10.6
3.	Murraya Koeniigi	Mahanimbinol	-10.3
		Mahanine	-10.4
		Murrayakonine_A	-11.6
		Murrayanol	-10
4.	Long pepper	Aristololactam	-10.4
		Mussaenosidic_acid	-10.9
5.	Carica Papaya	Lutein	-10.2

Table 3: Phytochemical Interaction on the XO receptor with good binding affinity with 2D models

Phytoconstituent	Binding affinity	3D interaction of phytochemicals with 2E1Q receptor protein
Allopurinol	-6.5	

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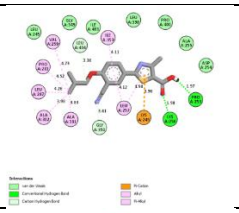
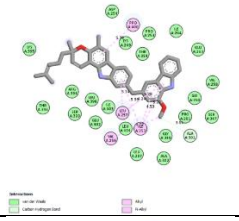
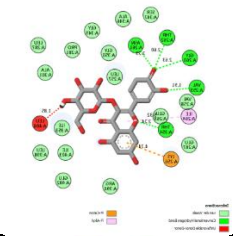
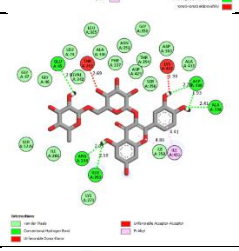
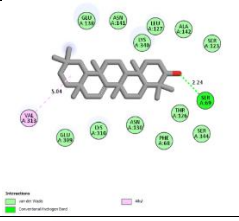
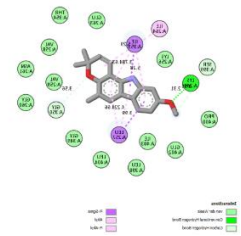
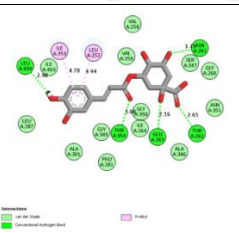
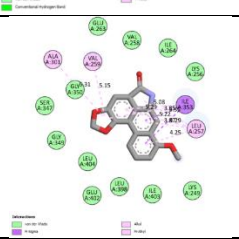
Fabuxostat	-7.8	
Murrayaonine A	-11.4	
Quercetin-3-Glycoside	-8.1	
Rutin	-9.7	
Taraxerol	-10.7	
Murrayanol	-10	
Chlorogenic acid	-9.8	
Aristolatam	-10.4	

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Determination of phenolic content:

Phenolic compounds are renowned for their anti-inflammatory and antioxidant characteristics, making them valuable components in the management of gout symptoms. The assessment of phenolic content within these extracts serves as a foundational step in evaluating the potential efficacy of these plant extracts for gout management. The table 1 outlines the phenolic content levels in the selected plant extracts, shedding light on their potential contributions to gout treatment:

Table 4: Total phenolic content using both maceration and decoction extract of each selected herbal drug.

Sr No.	Plant extract	Total Phenolic contentequivalent to Gallic acid GA/g
a.	**Decoction**	
1	*Cissus quandrangulari* (Hadjod stem)	37.5 mg GAE/g DW
2	*Carica papaya* (Papaya leaves)	14 mg GAE/g DW
3	*Moringa oleifera* (shewga leaves)	9 mg GAE/g DW
4	*Murraya koenigii* (curry leaves)	123 mg GAE/g DW
5	*Piper nigrum* (long pepper)	58.5 mg GAE/g DW
b.	**Maceration (Ethanol)**	
1.	*Cissus quandrangulari* (Hadjod stem)	60.26mg QE/g DW
2.	*Carica papaya* (Papaya leaves)	37.93mg QE/g DW
3.	*Moringa oleifera* (shewga leaves)	23.26 mg QE/g DW
4	*Murraya koenigii (curry leaves)*	68.26mg QE/g DW
5	*Piper nigrum (long pepper)*	35.13mg QE/g DW
c.	**Maceration (ethanol:water)**	
1.	*Cissus quandrangulari* (Hadjod stem) (Ethanolic)	84.66 mg QE/g DW
2.	*Carica papaya* (Papaya leaves) (Ethanolic)	63.6mg QE/g DW
3.	*Moringa oleifera* (shewga leaves) (Ethanolic)	55mg QE/g DW
4	*Murraya koenigii (curry leaves)*	78.93mg QE/g DW
5	*Piper nigrum (long pepper)*	75.86mg QE/g DW

Determination of total flavonoid content:

Flavonoids hold significance in gout management due to their recognized anti-inflammatory and antioxidant properties. These compounds may help alleviate the inflammation and oxidative stress associated with gout, providing relief from pain and discomfort during attacks. Additionally, some flavonoids exhibit the potential to inhibit XO, a key enzyme in uric acid production. This inhibition may contribute to lower uric acid levels, a critical factor in gout development. By including plant extracts rich in flavonoids in gout treatment strategies, researchers aim to harness their therapeutic potential. The table presented below outlines the flavonoid content levels in the selected plant extracts, shedding light on their potential contributions to gout treatment:

Table 5: Estimation of Total Flavonoid content

Sr. No.	Plant extract (Maceration anddecoction)	Total Flavonoid content equivalentto Rutin
1	*Carica Papaya* [decoction extract]	84.66 mg RE/g DW
2	*Murraya koenigii* [maceration Extract E/W]	63.6mg RE/g DW
3	*Murraya koenigii* [decoction extract]	55mg RE/g DW
4	*Piper nigrum* [maceration extract E/w]	78.93mg RE/g DW
5	*Piper nigrum* [decoction extract]	75.86mg RE/g DW
6	*Cissus quandrangulari* [maceration extract E/W]	60.26mg RE/g DW
7	*Cissus quandrangulari* [Decoction extract]	37.93mg RE/g DW
8	*Moringa oleifera* [Maceration extract E/W]	23.26 mg RE/g DW
9	*Moringa oleifera* [Decoction extract]	68.26mg RE/g DW

Isolation process of Xanthine Oxidase from cow milk:

The isolation of XO from cow milk was achieved using the Ammonium Sulphate Precipitation Method. After standing overnight and undergoing a subsequent centrifugation step at 2500 rpm for 30 minutes, a brown precipitate containing 1 mg of enzyme was obtained. The protein content of the crude XO was determined to be 7.10 mg protein mL^{-1} at 540 nm. Furthermore, the XO catalyzed reaction exhibited an absorbance rate of 3.1095×10^3 µmol min^{-1} mL^{-1}. These results signify the successful isolation and quantification of XO from cow milk, serving as a crucial step in further research and analysis.

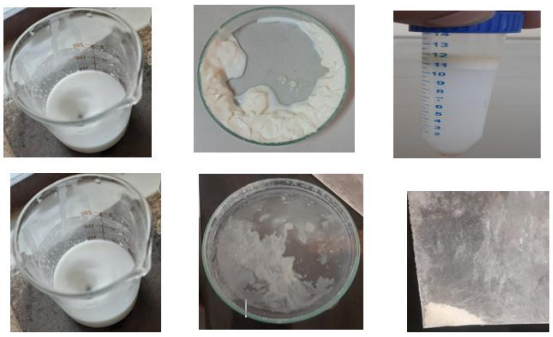

Figure 2: Steps of isolation of XO enzyme from cream to crude enzyme

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Effect of pH on XO catalyzed reaction:
The study examined the impact of varying pH levels on the XO catalyzed reaction. The findings revealed a notable trend: as the pH increased from 5.8 to 7.3, the XO activity exhibited a steady increase. However, when the pH levels were further elevated, there was a discernible decline in XO activity. Consequently, pH 7.3 was identified as the optimal pH level for this enzymatic reaction.

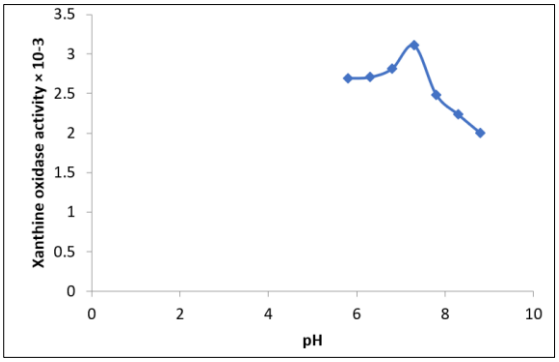

Figure 3: Relationship between Solution pH and XO Activity

Effect of temperature on XO catalyzed reaction:
The investigation focused on the influence of temperature on the XO catalyzed reaction. The results demonstrated a clear pattern: as the temperature increased from 15 to 25 °C, XO activity exhibited a progressive rise. However, beyond this point, further temperature elevation resulted in a decline in XO activity. Consequently, a temperature of 25 °C was determined as the optimal temperature for this enzymatic reaction.

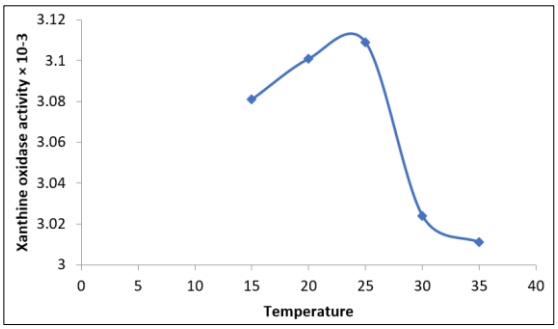

Figure 4: Relationship between the temperature and Xanthine Oxidase Activity

Estimation of Antigout Activity:
The assessment of antigout activity involved the examination of both individual plant extracts and polyherbal formulations, utilizing the XO inhibitory method. Extracts were prepared using two different solvents: ethanol and a 50:50 ethanol/water mixture. Allupurinol served as the reference standard for evaluating antigout activity. It exhibited the highest inhibitory activity at a concentration of 80 µg/mL, achieving a remarkable 75.1% inhibition rate. Over the concentration range of 20 to 100 µg/mL, the inhibitory activity of Allupurinol steadily increased, ranging from 62.27% to 75.1%.

Table 6: Xanthine Oxidase Activity of Allupurinol

Sr. No.	Concentration (µg/mL)	% inhibition
1	20	62.27
2	40	70
3	60	70.6
4	80	75.1

Table 7: Xanthine Oxidase Activity of Maceration and decoction extracts

Sr. No.	Plant Extractname	ExtractConc. (µg/mL)	Inhibitory activity XO Result of Maceration and Decoction extract 273 nm		
			% Inhibition of maceration extract (E)	% Inhibition of maceration Extract (E/W)	% Inhibition of Decoction Extract
1.	*Cissus quandrangularis* (Hadjod stem)	20	39.18	67.54	18.54
		40	69.63	65.63	31.72
		60	65.8	56	40.18
		80	70.72	67.7	52.18
2.	*Carica papaya* (Papaya leaves)	20	80	70.18	52.36
		40	80	69	54.4
		60	79	69.18	53
		80	74.8	65	63
3.	*Moringa oleifera* (shewga leaves)	20	69	63	40.18
		40	34	64	43.18
		60	4.45	62	45.63
		80	67	60	63.18
4.	Piper nigrum (long pepper)	20	61	63	44.5
		40	63	59	55.99
		60	64	67	54.56
		80	71	77	53.02
5.	Murraya koenigii (curry leaves)	20	68	82	30.45
		40	89.2	81	39.54
		60	65	77.3	45.27
		80	70	67	50

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The study results highlight the significant inhibitory activity exhibited by the herbal extracts when prepared with the 50:50 ethanol/water mixture. In continuation of this research, various combinations of these extracts were tested within the same mixture, and their impact on XO Activity was assessed. Detailed findings from these additional studies are presented in the table below:

Table 8: Xanthine Oxidase Activity Result of polyherbal drug extract Maceration extraction:

Sr. No.	Plant Extract Name	ExtractConc. (µg/mL)	Inhibitory activity XO Result of Maceration extract 273 nm	
			Absorbance Of Polyherbal drug	% Inhibition of maceration Extract (E)
1.	*Cissus quandrangularis* (Hadjod stem) + *Moringa oleifera* (shewga leaves)	20	0.338	69.7
		40	0.333	69.2
		60	0.322	64.2
		80	0.299	72
2.	*Carica papaya* (Papaya leaves) + Murraya koenigii (curry leaves) + Piper nigrum (long pepper)	20	0.341	69
		40	0.339	69.18
		60	0.293	73.3
		80	0.261	76.2

Evaluation of Arishta formulation:

The evaluation of the Arishta formulation for color, taste, odor, sugar content, pH, solid content, alcohol content, and *in vitro* assay is crucial for comprehensive quality control and efficacy assessment. It ensures that the formulation consistently meets standards of palatability, safety, and therapeutic effectiveness. Additionally, these parameters help confirm regulatory compliance and product stability, offering assurance of quality and reliability for gout management. Detailed outcomes of the developed formulation are presented in the table below, offering valuable insights into its characteristics and performance.

Table 9: Evaluation of Arishta formulation

Sr. No.	Evaluation parameter	Result of evaluation
1.	Color	Brown
2.	Taste	Sweet/sour
3.	Odour	Sweet-scented
4.	Sugar Content %	3.6%
5.	pH	4
6.	Solid Content %	14%w/v
7.	Alcohol Content %	12 %v/v
8.	*In vitro* assay (80µg/mL)	73.3%

DISCUSSION:

The focus of this research revolves around the inhibition of XO enzymes, as these enzymes play a pivotal role in the conversion of hypoxanthine to xanthine and, ultimately, xanthine to uric acid, contributing to hyperuricemia. The inhibition of these enzymes holds the potential to control or prevent hyperuricemia effectively. To investigate this, PyRx was employed for molecular docking, allowing the analysis of approximately 118 ligands with the XO (2E1Q) receptor protein. The binding conformations between the ligands and the receptor protein were evaluated using PyRx 0.8 Autodock Vina.

The selection of herbal plants was guided by their phytochemical interactions with the relevant proteins, as well as the dock scores derived from an extensive in silico research analysis. The study involved the extraction of specific components from five herbal medications through both maceration and decoction methods. Subsequently, the phenolic and flavonoid contents of the extracts were individually analyzed. The results revealed that all maceration and decoction extracts from the chosen herbal medications contained the complete flavonoid content.

The XO enzyme was isolated from cow milk, with the crude enzyme exhibiting a protein concentration of 7.10 mg/mL at 540 nm. Separate maceration and extraction procedures were carried out during the decoction and extraction processes. The anti-gout activity was assessed using XO activity, with Allupurinol serving as the standard for comparison with various herbal extracts. Notably, the maceration extracts exhibited a higher percentage of XO activity compared to the decoction extracts of specific medications.

Furthermore, both maceration and decoction extracts underwent evaluation for their individual in vitro XO inhibitory activities. These in vitro results have paved the way for the development of an improved formulation. Among the plants selected for the formulation, Carica Papaya, Murraya koenigi, and Piper longum exhibited superior activity in the maceration procedure, signifying their potential in the context of gout management.

CONCLUSION:

In conclusion, the comprehensive evaluation of research data highlights several significant findings. Through in-silico studies, Murrayaonine-A in curry leaves was identified with an impressive binding affinity, demonstrating potential in the context of gout management. The investigation involved the use of both ethanol and ethanol-water solvents for maceration, as well as the decoction extraction

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method, for five herbal medications. Among these, maceration extracts from five herbal medicines demonstrated superior antigout efficacy when compared to decoction counterparts. Furthermore, a comprehensive assessment of XO inhibitory activity of herbal extracts, including polyherbal drug extracts, was conducted. The maceration extract of Carica papaya exhibited a higher percentage of XO activity compared to the standard drug Allupurinol in in vitro tests, underscoring promising roles in antigout action.

This study has expanded the understanding of the anti-gout potential of Arishta (liquid) formulations, with the highest XO inhibition observed. These findings provide a solid foundation for future research including exploring the synergistic effects of combining herbal extracts to develop more potent anti-gout formulations and conducting clinical trials to validate the efficacy and safety of these natural remedies. Additionally, investigating the long-term effects and patient outcomes of such herbal treatments can contribute to the development of effective and sustainable gout management strategies.

CONFLICT OF INTEREST:

The authors declare that there are no conflicts of interest.

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