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Assessment of Anti Arthritic Properties of Selected Compounds Targeting Inflammatory Pathways

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Abstract

Arthritis is a highly prevalent global condition that encompasses many subtypes. Chiefly osteoarthritis and rheumatoid arthritis occurrence and risk is strongly influenced by age, sex, genetics and environment. Arthritis epidemiology is characterized by high prevalence, particularly in older adults and women and increasing trends worldwide, driven by aging and modifiable risk factors. Improvements in treatment with conventional drugs have led to reduced mortality but an increased number living with disability. Conventional DMARD's drugs target arthritis to reduce inflammation, prevent joint damage but they are now seldom used due to their adverse effect. Anti-inflammatory diets rich in omega 3- fatty acids, fruits, vegetables and whole grains appear to lessen symptoms and improve function. The present study was designed to investigate the anti-arthritic activity of selected compounds- Hydroxychloroquine, Gond (Tragacanth gum), Cocoa, and Calcium using in vitro models. The selected compounds were authenticated and subjected to preliminary phytochemical screening. The in vitro anti-arthritic activity was evaluated using protein denaturation inhibition and Human Red Blood Cell (HRBC) membrane stabilization assays. Diclofenac sodium was used as the standard reference drug. All tested compounds showed statistically significant ($p<0.001$) inhibition of protein denaturation and protection of HRBC membranes. Gond showed the highest inhibition of protein denaturation (85.71% at 1000 µg/ml), followed by HCQ, Calcium, and Cocoa. In membrane stabilization, HCQ showed maximum protection (89.47%), followed by Gond, Cocoa, and Calcium. The study concludes that the selected compounds exhibit promising anti-arthritic activity in vitro. Gond and HCQ demonstrated superior effects, indicating their potential as effective agents for arthritis management.

Keywords: Anti-arthritic activity, protein denaturation, membrane stabilization, Rheumatoid arthritis, Hydroxychloroquine, cocoa, Gond, calcium.

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INTRODUCTION

Arthritis is a medical term for disorders involving joint inflammation and damage. More than 100 types of arthritis and associated disorders exist. Among these, osteoarthritis and rheumatoid arthritis are the greatest ones. The majority of joint diseases impact synovial joints. One type of arthritis has symptoms that are different from those of another [1].

The symptoms of arthritis, an autoimmune disease, include pain, swelling, and inflexibility. It originates from the Greek term "disease of the joints." It is described as acute or chronic joint inflammation that frequently coexists with structural damage and pain [2] It affects people in various demographic and geographic contexts and has a significantglobal impact on public health. The prevalence of RA varies throughout the world, while it is frequently higher in industrialized countries. Numerous factors, such as genetics, insufficient reporting in some parts of the globe, and vulnerability to environmental risk factors, can be attributed to this variation in frequency. According to estimates, RA affects between 0.5 and 1% of adults worldwide, making it one of the most common conditions [3]. In addition to the hyperplasia of synovial cells, arthritis causes an inflammatory riposte. As a result, excessive synovial fluid buildup in the joints results in the development of synovial cell sheets that inflame the joint sites [4]. As a result fatigue, malaise fever, multiple joint pain, prolonged morning stiffness, and other extra-articular manifestations are some of its symptoms [5, 6]. Discomfort, swelling, and ultimately the degradation of

bone and cartilage, resulting in joint damage, are the three stages of the progression of RA in the inflammatory synovium. Patients may experience a range of clinical symptoms, from mild pain and swelling to more severe conditions, including contractures, muscle atrophy, and full or partial joint immobility [7, 8]. This inflammation is a cause of denaturation of proteinsin conditions like arthritis [9]. Chemical mediators released by injured tissue and migratory cells cause inflammation [10]. It also causes the release of lysosomal enzymes, which leads to lipid peroxidation of membranes, contributing to specific pathological conditions, including rheumatoid arthritis. These enzymes' extracellular activity is thought to be connected to either acute or chronic inflammation [11]. Arthritis is treated with medications, physical & occupational therapy. Many types of arthritis are progressive. Reducing joint pain and inflammation, optimizing joint function, and avoiding joint deformity and destruction are the objectives of RA treatment. Treatment regimens include weight-bearing exercises, pharmaceutical combinations, and teaching patients about rest and disease [12]. Early treatments included salicylate and aspirin used since the early 20th century for symptomatic relief of inflammation and pain. A major break theory was the introduction of disease modifying anti rheumatic drugs (DMARD's) beginning in the mid 20th century. In the late 1990's biologic DMARD's targeting specific immune pathways such as TNF inhibitors entanercept, adalimumab etc have gained importance.

Over all the progression of anti arthritis drugs followed from symptoms relief to modify disease course, transforming RA management to prevent disability and improve quality of life. Continued research has focused on identifying more targeted therapies with enhanced efficacy and reduced toxicity. We investigated the natural products for the presence of anti-inflammatory properties for an alternative therapy to DMARD's [13].

MATERIAL AND METHODS

1 Sample Collection

Compounds were obtained from an Organic Mandy, Kondapur. The compounds had been recognizedas well as authenticated by Dr.Madhava Shetty, botanist, Department of Botany, S.V. University, Tirupati. Selected compounds are Hydroxychloroquine, Gond, Cocoapowder, Calcium

Hydroxychloroquine(HCQ)

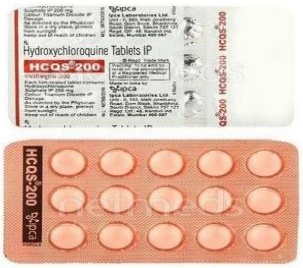

Fig.1 Hydroxychloroquine

Hydroxychloroquine, an analog of chloroquine, was initially marketed as an antimalarial drug for the treatment of malaria but was later replaced by more effective agents. However, it was subsequently identified as a disease-modifying antirheumatic drug (DMARD) and has since been used to treat autoimmune diseases [14].

Gond

Fig.2 Gond

Gond Katira, also known as Tragacanth gum, is a natural gum obtained from the sap of several species of the Astragalus genus belonging to the Leguminosae family. Gond Katira is known for its anti-inflammatory properties, making it a traditional remedy for joint pain and inflammation. Its cooling properties also make it a popular ingredient in refreshing beverages and desserts, especially in hot climates [15].

Cocoa Powder

Fig.3 Cocoa Powder

Cocoa powder, derived from the seeds of Theobroma cacao of the Sterculiaceae family, is commonly known as cocoa beans. The primary constituents of cocoa beans include theobromine and cocoa butter, along with smaller amounts of caffeine, volatile compounds that contribute to its flavor, and polyphenols. The stimulant and diuretic effects of cocoa are mainly attributed to theobromine, while its nutritive properties make it a popular ingredient in both foods and beverages. Cocoa butter is widely used as a base in pharmaceutical suppositories. Additionally, cocoa is rich in phytochemicals, particularly polyphenols, which exhibit both anti-inflammatory and antioxidant properties [16].

Calcium

Fig.4 Calcium Power

Calcium and Inflammation

Calcium, a key component in both inflammation and inflammatory diseases, primarily exists in the form of Ca^{2+}. It is believed that during an inflammatory episode, mast cells release and metabolize inflammatory mediators such as histamine, prostaglandins, and leukotrienes, processes that are closely associated with Ca^{2+} dynamics [17].

During the inflammatory response, Ca^{2+} can modulate synovial inflammation in rheumatoid arthritis (RA) patients and regulate the metabolism of arachidonic acid in T cells [18]. Furthermore, during the development of RA, Ca^{2+} mediates the infiltration of multiple immune cells, resulting in an uncontrolled inflammatory response [19]. These findings suggest that Ca^{2+} itself plays an essential role in the initiation and regulation of inflammation, particularly in RA [20].

Phytochemical Analysis

To identify the active constituents of the selected compounds-such as tannins, saponins, terpenoids, anthraquinones, alkaloids, flavonoids, carotenoids, and D-galacturonic acid a-preliminary qualitative phytochemical screening was conducted.

Protein Denaturation Assay

A 5 mL reaction mixture was prepared containing 0.2 mL egg albumin, 2.8 mL phosphate buffer solution (PBS) (pH 6.4), and 2 mL of the test solution at concentrations of 200, 400, 600, and 1000 µg/mL. The control solution contained distilled water instead of the test sample (used in all in vitro anti-arthritic assays).

After 15 minutes of incubation at 37°C, the mixtures were heated at 70°C for 5 minutes. Once cooled to room temperature, the absorbance of each sample was measured at 620 nm. The percentage inhibition of protein denaturation was calculated, and all tests were performed in triplicate using the following formula [21]:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test Sample}) \times 100}{\text{Absorbance of Control}}$$

Human Red Blood Cell (HRBC) Membrane Stabilization Assay

For this assay, 3 mL of blood was collected from a healthy volunteer who had not taken any NSAIDs in the previous two weeks. The blood was mixed with Alsever's solution and centrifuged at 3000 rpm for 15 minutes to separate the packed cells. The cells were then washed three times with isosaline solution (0.85% w/v NaCl, pH 7.2). A 10% (v/v) packed cell suspension was prepared using isosaline and used immediately.

The test solution contained 0.5 mL of the sample at various concentrations (as used in the protein denaturation assay), 2 mL of hypotonic saline solution (0.36% w/v NaCl), 1 mL PBS (pH 7.2), and 0.5 mL of the blood suspension. The mixtures were incubated at 37°C for 30 minutes, followed by centrifugation at 3000 rpm.

The absorbance of the supernatant was measured at 570 nm. Each test was performed in triplicate, and the mean value was calculated. The percentage stabilization of HRBC membranes was then determined [22].

RESULTS

The biochemical analyses of the selected samples represent variation in secondary metabolites (Phenols, flavanoids, tannins, alkaloids, coumarins, terpenoids etc.) the phytochemical regime of the plant is presented in table 01.

Table 01: Summary of Phytochemical Analysis of Selected Compounds.

Phytochemicals	Cocoa	Gond
Alkaloids	+	-
Flavonoids	+	-
Steroids	+	-
Tannins	+	-
Saponins	+	-
Terpenoids	+	-
Coumarins	+	-
Xanthoproteins	+	-
Carotenoids	-	-
Quinones	-	-
Anthraquinones	-	-
D-galacturonicacid	-	+
D-glucuronicacid	-	+

(+) represents the presence and (–) represents the absence of constituents.

Table 02: Percentage Inhibition by the Protein Denaturation Method

Conc (µg/ml)	Diclofenac sodium	Hydroxychloroquine	Gond	Cocoa	Calcium
200	11.11±0.0203**	4.54±0.0115**	58.92±0.0203**	21.42±0.0115**	35.29±0.0176**
400	54.16±0.0186**	13.63±0.0115**	64.28±0.012**	50 ± 0.012**	58.82±0.0176**
600	58.33±0.0145**	34.84±0.0145**	67.85±0.012**	52.38±0.0033**	67.64±0.0058**
1000	90.27±0.012**	89.39±0.0088**	85.71±0.012**	64.28±0.0058**	76.47±0.0145**

Student's t-test: Extremely statistically significant ($p < 0.001$)

Table 03: Percentage Protection by Membrane Stabilization Method

Conc (µg/ml)	**Diclofenac sodium**	**Hydroxychloroquine**	**Gond**	**Cocoa**	**Calcium**
200	48.27±0.012**	71.92±0.024**	45.23±0.0203**	48.57±0.0088**	44.44±0.0067**
400	58.62±0.0173**	77.19±0.024**	59.52±0.0219**	54.28±0.0115**	50±0.0058**
600	70.68±0.0176**	84.21±0.012**	64.28±0.024**	57.14±0.0145**	52.77±0.0088**
1000	79.31±0.0145**	89.47±0.0058**	80.95±0.0088**	65.71±0.0153**	63.88±0.012**

Extremely statistically significant ($p < 0.001$)

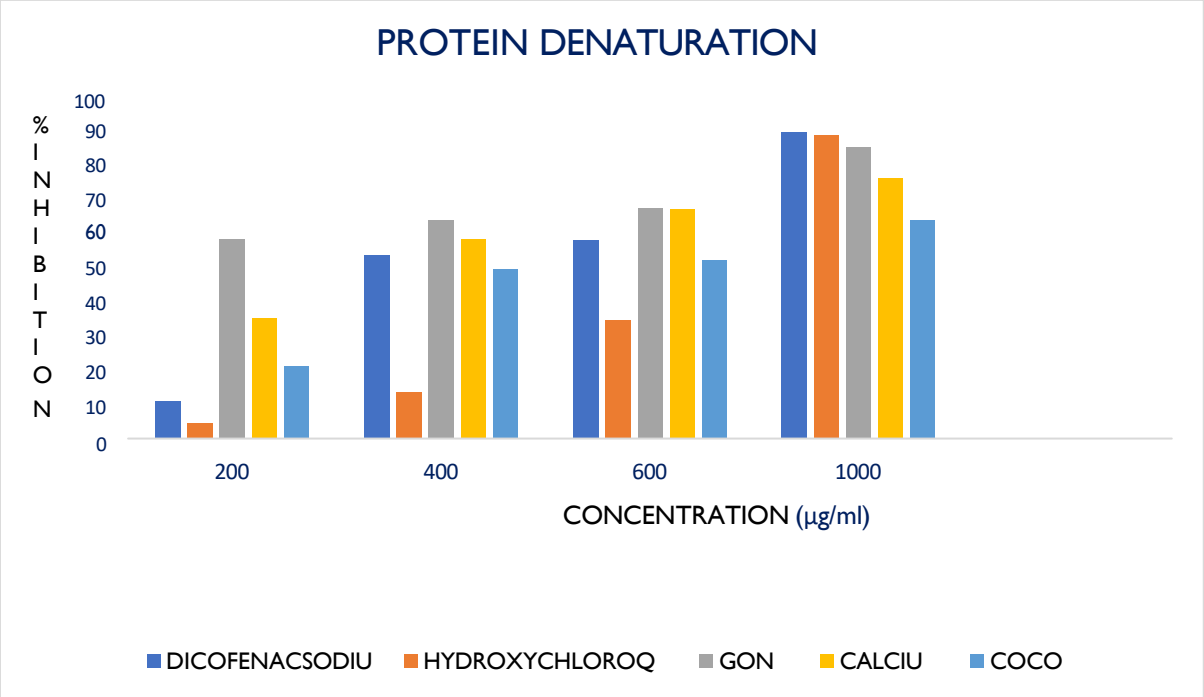

Figure 5. Percentage Inhibition of Protein Denaturation

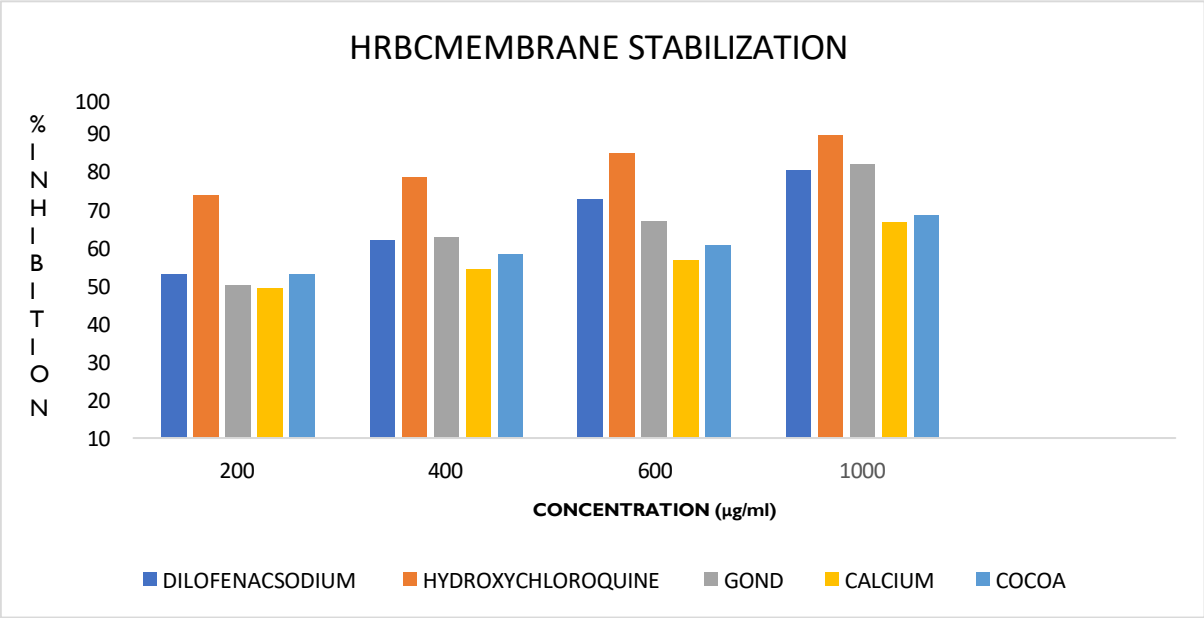

Figure 06: Percentage Protection of HRBC Membrane Stabilization

DISCUSSION

Arthritis affects people of all ages, races, sexes, and geographical locations, and it encompasses over 100 different types, including juvenile arthritis, rheumatoid arthritis (RA), psoriatic arthritis, gout, ankylosing spondylitis, and osteoarthritis, the latter being primarily degenerative.

RA is a chronic inflammatory autoimmune disease characterized by inflammation of the joints and synovial membranes, leading to pain, cartilage degradation, and bone distortion. Protein denaturation, which occurs under extreme conditions such as heat and chemical exposure, can adversely affect joints, synovial membranes, and cartilage. Although the mechanism of action of NSAIDs was proposed by Mizushima [23] before the discovery of their inhibitory effect on cyclooxygenase [24], it can be concluded that protection against protein denaturation, which occurs during inflammatory processes, may contribute significantly to the anti-rheumatic effects of NSAIDs.

The lysosomal membrane and human red blood cell (HRBC) membrane share structural similarities; therefore, stabilization of HRBC membranes may reflect the stabilization potential of lysosomal membranes. By inhibiting the release of lysosomal constituents from activated neutrophils-such as proteases and bactericidal enzymes-lysosomal membrane stabilization can prevent further tissue inflammation and damage. Accordingly, HRBC membrane stabilization, particularly against hypotonicity-induced lysis, is a widely used in vitro measure of anti-inflammatory activity [25].

Protein denaturation is considered one of the key contributors to the pathogenesis of RA. Therefore, the protein denaturation assay was used to evaluate the anti-arthritic potential of selected compounds at concentrations of 200, 400, 600, and 1000 µg/mL. Hydroxychloroquine showed significant inhibition of protein denaturation, with % inhibition values of 4.54%, 13.63%, 34.84%, and 89.39% at 200, 400, 600, and 1000 µg/mL, respectively. Gond exhibited very good activity, with % inhibition of 58.92%, 64.28%, 67.85%, and 85.71% at the same concentrations. Cocoa demonstrated effective inhibition, with % inhibition of 21.42%, 50%, 52.38%, and 64.28%. Calcium also showed good results, with % inhibition of 35.29%, 58.82%, 67.64%, and 76.47%. Diclofenac sodium, used as the standard, exhibited % inhibition of 11.11%, 54.16%, 58.33%, and 90.27%. The HRBC membrane stabilization assay was performed to evaluate the membrane-protective effects of the selected compounds in comparison with Diclofenac sodium. The integrity of the cell membrane is essential for cell viability. Exposure to harmful agents, including hypotonic solutions, can cause membrane lysis, hemolysis, and oxidative damage to hemoglobin. Membrane-stabilizing substances can protect cells from such damage, and therefore this assay serves as an indicator of anti-inflammatory potential.

The results of HRBC membrane stabilization were as follows:

- Hydroxychloroquine: % protection = 71.92%, 77.19%, 84.21%, 89.47% (200, 400, 600, 1000 µg/mL)
- Gond: % protection = 45.23%, 59.52%, 64.28%, 80.95%
- Cocoa: % protection = 48.57%, 54.28%, 57.14%, 65.71%
- Calcium: % protection = 44.44%, 50%, 52.77%, 63.88%
- Diclofenac sodium (standard): % protection = 48.27%, 58.62%, 70.68%, 89.47%

These results demonstrate that Hydroxychloroquine, Gond, Cocoa, and Calcium possess significant anti-arthritic and membrane-stabilizing activities,

comparable in some cases to the standard Diclofenac sodium, supporting their potential use in mitigating inflammatory responses associated with RA.

CONCLUSION

Inflammation is a natural protective response against tissue damage caused by heat, injury, swelling, or microbial agents, and it plays a critical role in tissue repair. Inflammatory diseases, including various forms of rheumatoid disorders, are among the leading causes of morbidity worldwide, often referred to as the "king of human miseries." Standard treatments for rheumatoid arthritis (RA), such as non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, primarily provide symptomatic relief but do not address underlying pathological mechanisms like protein denaturation or membrane instability. Prolonged use of NSAIDs, including Diclofenac sodium, can lead to side effects such as renal insufficiency and gastroduodenal complications due to cyclooxygenase inhibition and reduced prostaglandin levels. In this study, the anti-arthritic potential of natural and pharmacological compounds-Hydroxychloroquine (HCQ), Gond, Cocoa, and Calcium-was evaluated using the protein denaturation and HRBC membrane stabilization assays. HCQ modulates the immune system and reduces inflammation, Gond possesses traditional anti-inflammatory and soothing properties, Cocoa inhibits pro-inflammatory cytokines and enzymes through its flavonoids, and Calcium supports bone density and mineralization. The results demonstrated that all four compounds were more effective than Diclofenac sodium in inhibiting albumin denaturation and stabilizing HRBC membranes, suggesting that they may serve as promising alternatives or complementary agents for managing arthritis and other inflammatory conditions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT

Not applicable.

ETHICAL STATEMENT

Not applicable.

AUTHOR CONTRIBUTION CONCEPT AND DESIGN

Dr. Sireesha Kalva, Data collection: Md Afreen Sulthana, Analysis: Nikhitha Korra, Writing: Tejasri Oruganti.

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