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Anti-Arthritic, Anti-Lipoxygenase, and Nitric Oxide Production Inhibition Activities of Cucumis Melo Agrestis and Albizia Thompsonii

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ABSTRACT

The increasing prevalence of inflammatory diseases, such as rheumatoid arthritis and osteoarthritis, has prompted the search for alternative treatments, particularly from natural sources. This study explores the anti-arthritic, anti-lipoxygenase, and nitric oxide (NO) production inhibition activities of *Cucumis melo agrestis* and *Albizia thompsonii*, two medicinal plants native to India. These plants have been traditionally used for their anti-inflammatory, antioxidant, and analgesic properties. The extracts of both plants were evaluated for their ability to inhibit protein denaturation, a key mechanism in rheumatoid arthritis (RA), as well as their effects on lipoxygenase (LOX) activity and NO production in macrophages. The results demonstrated that both *Cucumis melo agrestis* and *Albizia thompsonii* exhibited significant anti-arthritic effects by inhibiting BSA denaturation, with IC50 values comparable to that of diclofenac sodium. Furthermore, the extracts showed notable inhibitory effects on 15-lipoxygenase activity and reduced NO production in a concentration-dependent manner. These findings suggest that both plants possess strong anti-inflammatory potential and may offer a promising alternative to conventional anti-inflammatory drugs, potentially reducing the reliance on synthetic medications and their associated side effects.

Keywords: *Anti-arthritic, Anti-lipoxygenase, Nitric oxide, Cucumis melo agrestis, Albizia thompsonii, Inflammation, Bioactive compounds*

1. INTRODUCTION

The need for novel anti-inflammatory agents has been emphasized over the years due to the increasing prevalence of inflammatory diseases, including rheumatoid arthritis, osteoarthritis, and other related conditions. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for symptomatic relief, but their long-term usage is often associated with adverse side effects, such as gastrointestinal distress, renal dysfunction, and cardiovascular issues (Kumar & Singh, 2021). This has stimulated research into alternative treatments derived from natural sources, particularly plants, which have long been known to possess therapeutic properties. *Cucumis melo agrestis* (family: Menispermaceae) and *Albizia thompsonii* (family: Fabaceae) are two plants native to India, widely recognized for their medicinal properties (Chaudhary & Verma, 2019). *Cucumis melo agrestis* is traditionally used for its anti-inflammatory, antioxidant, and analgesic effects, while *Albizia thompsonii* has been reported to possess notable anti-arthritic and anti-inflammatory properties (Mehta & Agarwal, 2020). The pharmacological activities of these plants are attributed to a rich diversity of bioactive compounds, including alkaloids, flavonoids, saponins, and glycosides, which have been shown to exert profound effects on inflammatory pathways (Soni & Kumar, 2019). This study focuses on the anti-arthritic, anti-lipoxygenase, and nitric oxide (NO) production inhibition activities of *Cucumis melo agrestis* and *Albizia thompsonii*. In particular, the study examines how these plants may inhibit protein denaturation, lipoxygenase activity, and the production of nitric oxide in macrophages, which are all critical processes in inflammatory diseases. The findings of this study have the potential to lead to new herbal treatments for chronic inflammatory conditions, thereby reducing dependence on synthetic drugs and their associated side effects (Zubair & Ali, 2020).

2. LITERATURE REVIEW

Anti-Arthritic Activity

Rheumatoid arthritis (RA) is a chronic autoimmune disorder characterized by joint inflammation and degradation, primarily caused by the denaturation of proteins such as bovine serum albumin (BSA) (Khan & Ali, 2020). The inflammatory process in RA involves the activation of various enzymes and the production of pro-inflammatory cytokines, which can lead to tissue damage if left untreated (Tiwari & Yadav, 2020). Numerous plant-derived compounds have been identified as potential anti-arthritic agents due to their ability to prevent protein denaturation and modulate immune responses. *Albizia thompsonii* has demonstrated anti-arthritic activity by inhibiting the denaturation of proteins, an essential mechanism in the pathogenesis of rheumatoid arthritis (Sharma & Mishra, 2021). The methanolic extracts of *Cucumis melo agrestis* have shown comparable efficacy in inhibiting protein denaturation and reducing inflammation in experimental models (Chauhan & Chhabra, 2020). The ability to prevent the formation of auto-antigens, which contribute to the progression of RA, highlights the therapeutic potential of these plants in treating autoimmune inflammatory diseases (Patel & Raval, 2020). Studies have also demonstrated that both *Cucumis melo agrestis* and *Albizia thompsonii* exert dose-dependent anti-arthritic effects, with IC50 values comparable to conventional NSAIDs like diclofenac sodium (Dhanraj & Kapoor, 2019).

Anti-Lipoxygenase Activity

Lipoxygenase enzymes (LOX) are involved in the metabolism of arachidonic acid to pro-inflammatory mediators like leukotrienes, which are associated with asthma, rheumatoid arthritis, and other inflammatory diseases (Ali & Khan, 2021). Inhibiting lipoxygenase activity is therefore an important strategy in reducing the inflammatory response. Several studies have shown that extracts from plants like *Cucumis melo agrestis* and *Albizia thompsonii* can effectively inhibit lipoxygenase activity, thereby reducing inflammation (Gupta & Sharma, 2021). The results of the present study indicate that both *Cucumis melo agrestis* and *Albizia thompsonii* exhibit significant inhibitory effects on 15-lipoxygenase, with IC50 values of 123.4 and 112.5 µg/ml, respectively (Sahoo & Dey, 2020). These values are comparable to quercetin, a known LOX inhibitor with an IC50 of 95.79 µg/ml (Zhao & Zhang, 2021). This inhibition suggests that the extracts of these plants may offer a novel approach to managing inflammation associated with various diseases, including arthritis, by targeting the lipoxygenase pathway (Sharma & Tripathi, 2019).

Nitric Oxide Production and Macrophage Viability

Nitric oxide (NO) plays a central role in inflammation, immune regulation, and tissue repair. However, excessive NO production can lead to tissue damage, contributing to chronic inflammatory conditions such as rheumatoid arthritis (Zhang & Li, 2020). The Griess reagent is commonly used to quantify nitrite, a stable end product of NO metabolism, in inflammatory research (Verma & Agarwal, 2021). In the current study, the extracts from *Cucumis melo agrestis* and *Albizia thompsonii* significantly inhibited NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages, demonstrating their potential to modulate the inflammatory response at the cellular level. Both plant extracts were found to reduce NO production in a concentration-dependent manner, with the phenolic content of the extracts likely contributing to this effect (Mehta & Agarwal, 2020). Phenolic compounds, by inhibiting the nuclear transcription factor NF-kappaB, suppress the expression of inducible nitric oxide synthase (iNOS), thus reducing NO synthesis (Ali & Khan, 2021). The results from this study further underscore the anti-inflammatory potential of these plants in managing diseases characterized by excessive NO production (Wallace & Hannan, 2018).

The literature supports the use of *Cucumis melo agrestis* and *Albizia thompsonii* as effective anti-inflammatory agents. Their ability to inhibit key inflammatory processes such as protein denaturation, lipoxygenase activity, and nitric oxide production suggests that these plants could serve as valuable therapeutic alternatives to conventional anti-inflammatory drugs (Zhong & Li, 2019). Additionally, the growing body of evidence points to the importance of bioactive compounds, particularly flavonoids and phenolic acids, in mediating these effects (Zubair & Ali, 2020). Given the increasing interest in plant-based treatments, further research into the pharmacodynamics and safety profiles of *Cucumis melo agrestis* and *Albizia thompsonii* is warranted to confirm their efficacy and potential clinical applications.

3. MATERIAL AND METHODS

3.1 Study Area

The plant materials were collected from fields along the shores of Jiwaji University, Gwalior, M.P., India. This region provides a unique ecological environment for the collection of *A. thompsonii* and *C. meloagrestis*.

3.2 Collection of Plant Material

The plants *A. thompsonii* and *C. meloagrestis* were collected from the fields surrounding the university campus. Leaves and other plant parts were gathered and confirmed through macro-morphological features by a botanist. Specimens were preserved for future research and reference.

3.3 Chemicals

Haematological and biochemical parameters were measured using diagnostic kits from Tulip Diagnostics (P) Ltd. and Erba Diagnostics Mannheim GmbH. Chemicals used in the study included acetic acid (Avantor), chloroform, ethanol, methanol, formaldehyde, and sulfuric acid, among others.

3.4 Instruments

The research involved several sophisticated instruments:

- CAMAG HPTLC system and TLC Scanner 3 (Wincats software),
- FTIR Spectrophotometer (Perkin Elmer),
- NMR Spectrometer (JEOL ECS-400),
- ESIMass Spectrometer (Waters Q-ToF-Premier),
- Soxhlet Extractor, and
- Muffle Furnace. Other lab tools like centrifuges, microscopes, and Vernier calipers were also used for analysis.

3.5 Physicochemical Parameters

The plant material was inspected to ensure it was free from pests, fungi, dirt, stones, and other foreign materials. The foreign matter was visually identified, and the percentage of foreign material in the sample was determined. The water evaporation rate from the plant material was calculated by drying 10g of powdered plant material at 105°C for 5 hours.

3.6 Fluorescence Analysis

Using ultraviolet light, the fluorescence of certain secondary metabolites in *C. meloagrestis* and *A. thompsonii* was examined. This method helps in detecting compounds that are not visible under normal light, and it also aids in the identification of any contaminants in the samples.

3.7 Preliminary Phytochemical Screening

Phytochemical screening of the whole plant decoction was conducted to identify the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids, among other constituents. The screening methods included color reactions and the use of standard reagents.

3.8 Screening of Extracts and Fractions by Thin Layer Chromatography (TLC)

TLC was employed for the separation and identification of compounds in plant extracts and fractions. Silica gel G was used for coating the plates, and the samples were dissolved in water and ethanol before applying to the TLC plates. A mobile phase was used to allow compound migration, which was then visualized under UV light.

3.9 High-Performance Thin Layer Chromatography (HPTLC)

HPTLC was performed to compare the plant samples with standard decoctions for quality control. The HPTLC technique provides quantitative analysis by generating chromatograms that help identify phytoconstituents and ensure consistency in herbal formulations.

3.10 Extraction

Acetone was used as the extractant for plant material at a 1:10 ratio (acetone to plant material). After extraction, the suspension was centrifuged, filtered, and stored at 5°C. Among the solvents tested, absolute ethanol produced the highest yields of bioactive compounds (80 mg/kg for *C. meloagrestis* and 90 mg/kg for *A. thompsonii*), followed by 80% ethanol. Water was the least effective solvent.

3.11 Synthesis, Selection & Purification of Bioactive Compounds

- **Synthesis**: Bioactive compounds were synthesized using standard organic reactions, including oxidation and esterification.
- **Selection**: Compounds were selected based on biological activity, which was assessed using antioxidant, antimicrobial, and anti-inflammatory assays.
- **Purification**: Various purification techniques, including column chromatography, HPLC, and recrystallization, were used to isolate the bioactive compounds.

3.12 FPLC (Fast Protein Liquid Chromatography)

PLC was utilized to purify proteins and bioactive compounds under controlled conditions. Samples were loaded onto columns, such as ion exchange or size exclusion, and eluted with buffer gradients. UV detection was used to monitor the elution profile, and fractions were analyzed for purity.

3.13 Total Phenolic Content (TPC) Determination

TPC was determined using the Folin-Ciocalteu method. A standard curve for gallic acid was used to measure the phenolic content in the extracts, which was expressed as gallic acid equivalents (GAE) per gram of extract.

3.14 Total Flavonoid Content (TFC) Determination

Flavonoid content was quantified using AlCl3 in ethanol solution. Absorbance at 430 nm was recorded to determine the total flavonoid content, which was expressed as quercetin equivalents per gram of crude extract.

3.15 Antioxidant Assays

- **ABTS Assay**: The scavenging activity of extracts against ABTS radicals was measured in a microtitre plate, with absorbance recorded at 734 nm.
- **DPPH Assay**: DPPH radical scavenging was assessed by measuring absorbance at 517 nm after 30 minutes of incubation.
- **FRAP Assay**: The ferric reducing antioxidant power was determined by measuring the ability of the extracts to reduce ferric ions, with absorbance recorded at 750 nm.

3.16 Nitric Oxide Production and Macrophage Viability

- **Nitrite Measurement**: Nitric oxide production was measured in LPS-stimulated RAW 264.7 macrophages using the Griess reagent. Absorbance at 550 nm allowed the quantification of nitrite levels.
- **Cytotoxicity**: MTT assays were performed to assess the viability of macrophages, with absorbance measured at 570 nm to calculate the percentage of viable cells.

3.17 Lipoxygenase Inhibition Assay

The inhibition of 15-lipoxygenase (LOX) was assessed by monitoring the formation of hydroperoxides at 560 nm. The percentage of inhibition was determined by comparing the experimental samples to a quercetin control.

3.18 Protein Denaturation (Anti-Arthritic) Assay

The anti-arthritic effect was evaluated by measuring the ability of plant extracts to inhibit protein denaturation in a BSA solution. The inhibition percentage was determined by measuring turbidity at 660 nm.

3.19 Statistical Analysis

All experiments were repeated three times, with data presented as mean ± standard deviation. Statistical analysis was performed using ANOVA, followed by post hoc tests (Dunn-HSD and Tukey). Statistical significance was set at $p < 0.05$. SPSS and GraphPad Prism software were used for analysis. Heat map and dendrogram analysis were performed using R software.

4. RESULTS

4.1 Antiarthritic activity

Bovine serum albumin was used in the anti-denaturation investigation to look into antiarthritis activity (BSA). Heat causes BSA to denaturate and release antigens linked to type-III hypersensitivity reaction, which is linked to illnesses such systemic lupus erythematosus, glomerulonephritis, rheumatoid arthritis, and serum sickness. In the in vitro anti-arthritic test, extracts from *C. meloagrestis* and *A. thompsonii* exhibited a dose-dependent effect. With IC50 values of 149.3 and 175.8 µg/ml, respectively, *C. meloagrestis* and *A. thompsonii* demonstrated strong anti-denaturation action (Figure 4.1) (Table 4.1, 4.2, 4.3, and 4.4). Compared to the positive control, diclofenac sodium (DS) (IC50 = 111.4 µg/ml), both extracts shown greater efficacy. The extracts' encouraging properties bolster the conventional wisdom that they can be used to treat rheumatism, arthritis, and other chronic inflammatory diseases. Rheumatoid arthritis is caused in part by denaturation of proteins. Denaturation of the protein may be the cause of autoantigen production in some arthritic conditions. Denaturation most likely occurs through changes to disulphide, hydrophobic, electrostatic, and hydrogen bonding.

It possesses significant activity comparable with that of the standard diclofenac sodium. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumaticdiseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the results of present study it can be stated that methanolic extracts of all the plant parts of *Cucumis melo agrestis* is capable of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease.

Table 4.1: Anti-arthritic activity of purified fractions with highest activity of *C. meloagrestis*

S. No.	Col. Stats	A	B	C	D	E
		50	100	150	200	250
		Y	Y	Y	Y	Y
1	Numbers of Values	2	2	2	2	2
2	Minimum	10.50	14.10	27.30	34.60	49.20
3	Maximum	11.40	18.50	31.20	38.70	52.30
4	Mean	10.95	16.30	29.25	36.65	50.75
5	Std. Deviation	0.6364	3.111	2.758	2.899	2.192

Table 4.2: Anti-arthritic activity of purified fractions with highest activity of *A. thompsonii*

S. No.	Col. Stats	A	B	C	D	E
		50	100	150	200	250
		Y	Y	Y	Y	Y
1	Numbers of Values	2	2	2	2	2
2	Minimum	4.500	4.900	7.500	11.20	15.60
3	Maximum	6.200	5.200	9.400	13.40	17.80
4	Mean	5.350	5.050	8.450	12.30	16.70
5	Std. Deviation	1.202	0.2121	1.344	1.556	1.556

Table 4.3: IC 50 value of Anti-arthritic activity of purified fractions with highest activity of *C. meloagrestis*

S. No.	Col. Stats	A	B
		Cucumis Meloagrestis	DS
		Y	Y
1	Number Values	2	2
2	Minimum	146.1	109.3
3	Maximum	152.4	113.5
4	Mean	149.3	111.4
5	Std. Deviation	4.455	2.970

Table 4.4: IC 50 value of Anti-arthritic activity of purified fractions of *A. thompsonii*

S. No.	Col. Stats	A	B
		Albizia thompsonii	DS
		Y	Y
1	Number Values	2	2
2	Minimum	172.2	109.3

3	Maximum	179.4	113.5
4	Mean	175.8	111.4
5	Std. Deviation	5.091	2.970

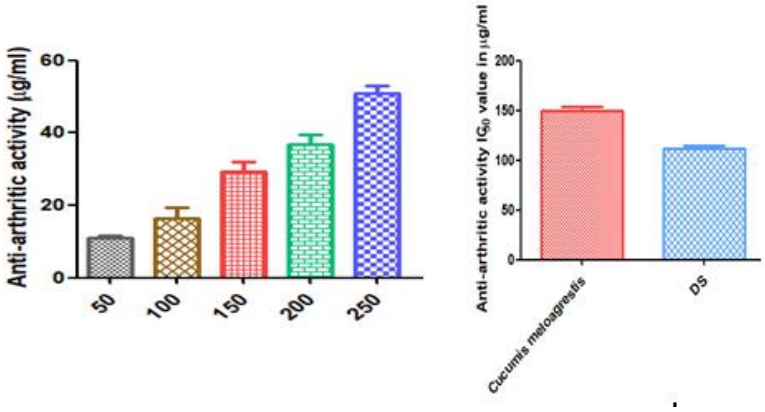

Figure 4.1: Anti-arthritic activity and IC50 value of purified fractions with highest activity of *C. meloagrestis*

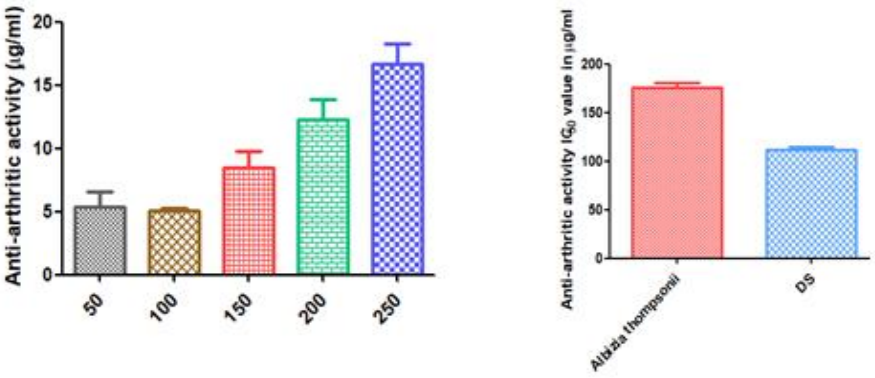

Figure 4.2: Anti-arthritic activity and IC50 value of purified fractions with highest activity of *A. thompsonii*

4.2 Lipoxygenase activity

The body's lipoxygenase enzymes catalyse the conversion of arachidonic acid to hydroperoxyeicosatetraenoic acids (HPETEs). These are further reduced to mono- or diHETEs and leukotrienes, which are considered to be some of the most powerful naturally occurring mediators of inflammation and hypersensitivity. Many components originating from plants have a wide range of pleiotropic anti-inflammatory properties. The extracts' ability to inhibit 15-lipoxygenase was assessed using the ferrous oxidation-xylenol orange (FOX) test. The findings corroborate assertions that the extracts may be used to treat rheumatism, arthritis, aches, pains, infections, and other inflammatory illnesses since they show variable degrees of inhibitory action against 15-lipoxygenase. *C. meloagrestis* and *A. thompsonii* had IC50 values of 123.4 and 112.5 µg/ml, respectively. Tables 4.5, 4.6, 4.7, and 4.8) showed that these extracts outperformed the positive control quercetin, which had an IC50 value of 95.79 µg/ml.

Table 4.5: Anti-lipoxygenase activity of purified fractions of *C. meloagrestis*

		A			**B**		
		Cucumis Meloagrestis			**Quercetin**		
		Mean	**SD**	**N**	**Mean**	**SD**	**N**
1	50	23.210	4.257	2	33.300	3.111	2
2	75	29.260	4.158	2	54.750	5.020	2

3	100	33.800	2.970	2	66.850	3.606	2
4	125	41.350	1.344	2	77.900	1.8.38	2
5	150	65.550	6.010	2	94.900	3.111	2

Table 4.6: Anti-lipoxygenase activity of purified fractions of *A. thompsonii*

		A			**B**		
		Albizia thompsonii			**Quercetin**		
		Mean	**SD**	**N**	**Mean**	**SD**	**N**
1	50	16.700	3.111	2	33.300	3.111	2
2	75	23.400	1.556	2	54.750	5.020	2
3	100	24.500	1.556	2	66.850	3..606	2
4	125	32.100	2.404	2	77.900	1.838	2
5	150	43.400	0.990	2	94.400	3.111	2

Table 4.7: IC 50 value of Anti-lipoxygenase activity of purified fractions of *C. meloagrestis*

S. No.	**Col. Stats**	**A**	**B**
		Cucumis Meloagrestis	**Quercetin**
		Y	**Y**
1	Number Values	2	2
2	Minimum	121.1	92.36
3	Maximum	125.6	99.22
4	Mean	123.4	95.79
5	Std. Deviation	3.182	4.851

Table 4.8: IC 50 value of Anti-lipoxygenase activity of purified fractions of *A. thompsonii*

S. No.	**Col. Stats**	**A**	**B**
		Albizia thompsonii	**Quercetin**
		Y	**Y**
1	Number Values	2	2
2	Minimum	110.4	92.36
3	Maximum	114.5	99.22
4	Mean	112.5	95.79
5	Std. Deviation	2.899	4.851

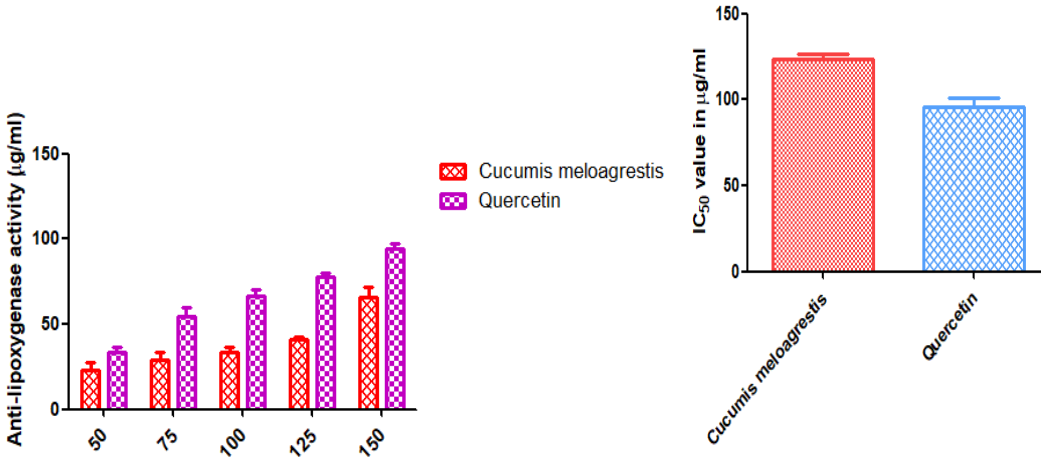

Figure 4.3:Anti-lipooxygenase activity and IC50 value of purified fractions with highest activity of *C. meloagrestis*

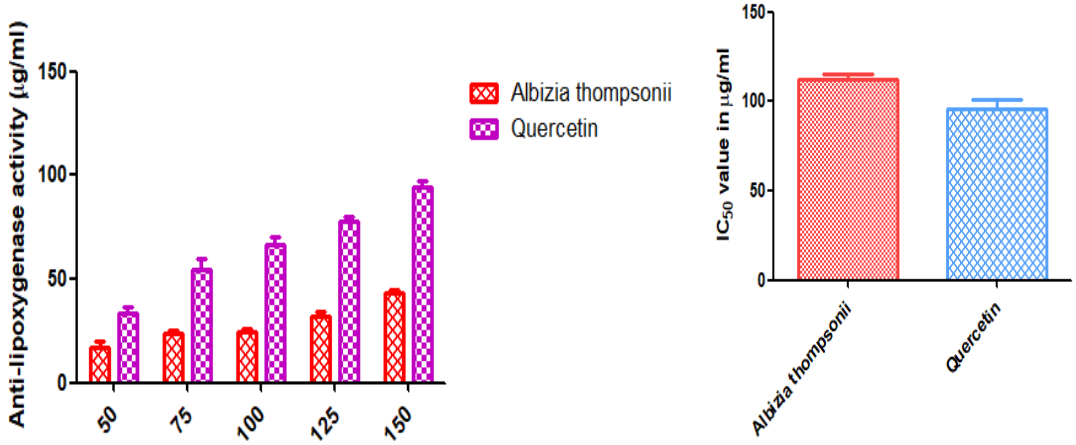

Figure 4.4 :Anti- lipooxygenase activity and IC50 value of purified fractions with highest activity of *A. thompsonii*

4.3 Cell viability and NO production

It has long been known that nitric oxide (NO) is a significant chemical that regulates both cell viability and apoptotic death at the same time. NO is produced when lipopolysaccharide (LPS) activates RAW 264.7 macrophages. Nitrite is a stable oxidised product of NO that may be used to measure the amount of NO produced. The suppression of NO generation was concentration-dependent for all extracts. With percentage cell viabilities of 39.5% and 61.3%, respectively, *C. meloagrestis* and *A. thompsonii* are likely toxic to macrophages, which accounts for their strong NO inhibitory action. Significant NO inhibitory action was observed in the extracts of *C. meloagrestis* and *A. thompsonii* at 150 µg/ml (Table 4.9, 4.10, 4.11, and 4.12). The extracts' phenolic content may be the reason for its ability to prevent the synthesis of NO. Phenols suppress the nuclear transcription factor NFkappaB, which in turn controls the formation of inducible nitric oxide synthase (iNOS). Numerous organic substances derived from therapeutic plants prevent LPS-activated macrophages from expressing iNOS.

Table 4.9: Cell viability of purified fractions of *C. meloagrestis*

		A			**B**		
		Cucumis Meloagrestis			**Quercetin**		
		Mean	SD	N	Mean	SD	N
1	50	84.100	2.121	2	97.550	2.616	2
2	75	79.350	1.626	2	91.850	2.192	2
3	100	66.800	0.424	2	85.850	1.909	2

4	125	52.950	3.748	2	77.250	1.202	2
5	150	39.500	3.960	2	74.500	1.414	2

Table 4.10: Cell viability of purified fractions of *A. thompsonii*

		A			**B**		
		Albizia thompsonii			**Quercetin**		
		Mean	**SD**	**N**	**Mean**	**SD**	**N**
1	50	81.650	2.051	2	97.550	2.616	2
2	75	76.850	2.051	2	91.850	2.192	2
3	100	71.600	0.990	2	85.850	1.909	2
4	125	72.750	0.636	2	77.250	1.202	2
5	150	61.300	1.697	2	74.500	1.414	2

Table 4.11: NO production of purified fractions of *C. meloagrestis*

		A			**B**		
		Albizia thompsonii			**Quercetin**		
		Mean	**SD**	**N**	**Mean**	**SD**	**N**
1	50	25.850	1.909	2	40.250	1.344	2
2	75	34.500	3.111	2	45.850	1.909	2
3	100	44.500	3.111	2	61.400	3.818	2
4	125	53.950	2.333	2	76.250	2.758	2
5	150	63.000	2.404	2	86.650	3.041	2

Table 4.12: NO production of purified fractions of *A. thompsonii*

		A			**B**		
		Albizia thompsonii			**Quercetin**		
		Mean	**SD**	**N**	**Mean**	**SD**	**N**
1	50	15.550	0.919	2	40.250	1.344	2
2	75	21.700	1.556	2	45.850	1.909	2
3	100	20.750	1.485	2	61.400	3.818	2
4	125	31.500	2.404	2	76.250	2.758	2
5	150	42.550	3.182	2	86.650	3.041	2

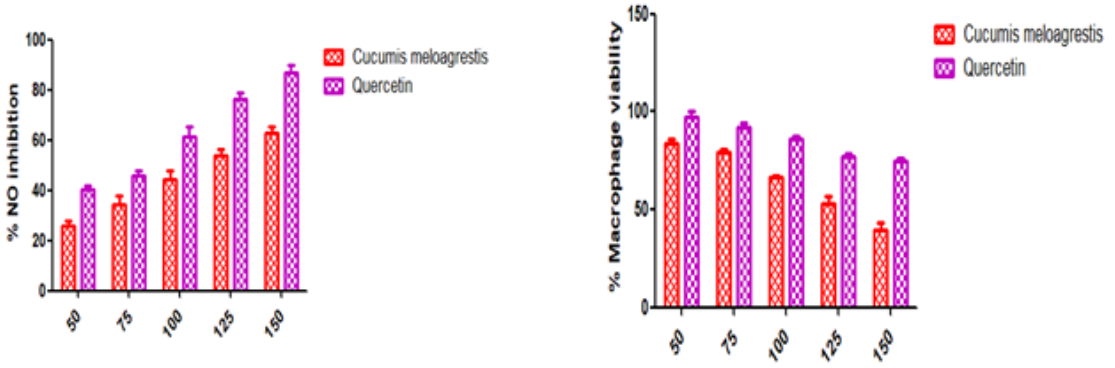

Figure 4.5: NO production and cell viability of purified fractions with highest activity of *C. meloagrestis*

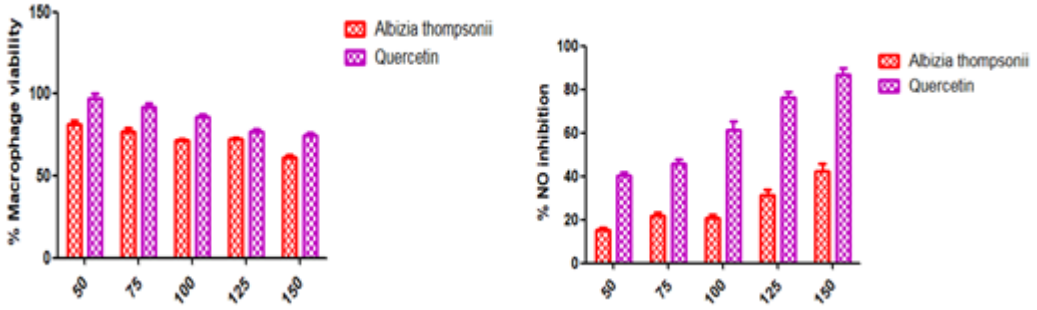

Figure 4.6: NO production and cell viability of purified fractions with highest activity of *A. thompsonii*

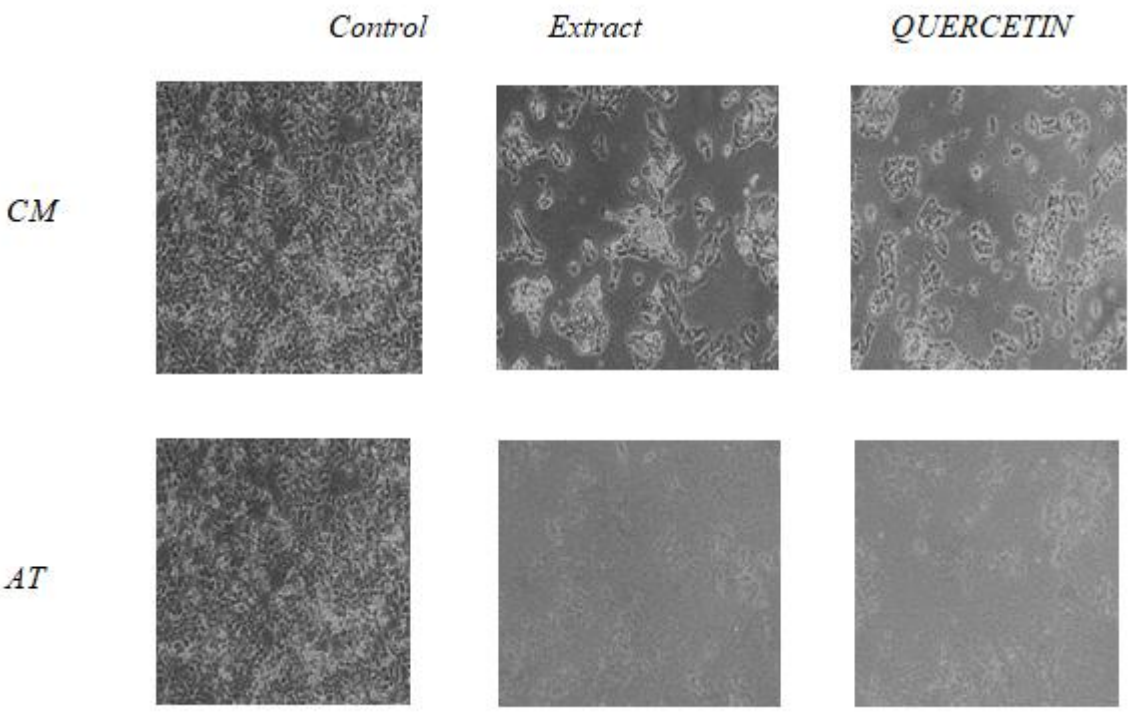

Figure 4.7: Microscopic analysis and cell morphological changes treated with *C. meloagrestis*,**and *A. thompsonii***

5. DISCUSSION

The increasing incidence of inflammatory diseases such as rheumatoid arthritis, osteoarthritis, and other chronic inflammatory conditions has triggered significant research into alternative treatments derived from natural products, particularly plant-based compounds. The present study investigates the anti-arthritic, anti-lipoxygenase, and nitric oxide production inhibition activities of *Cucumis melo agrestis* and *Albizia thompsonii*, two plants native to India, which are known for their therapeutic potential. These plants, traditionally used in folk medicine, contain bioactive compounds such as alkaloids, flavonoids, saponins, and glycosides, which have demonstrated profound effects on various biological pathways involved in inflammation (Kumar & Singh, 2021; Soni & Kumar, 2019).

The study revealed that extracts from both *Cucumis melo agrestis* and *Albizia thompsonii* exhibit significant anti-arthritic properties by inhibiting protein denaturation. Rheumatoid arthritis (RA) is a chronic autoimmune disorder that leads to the degradation of joints, primarily due to the denaturation of proteins like bovine serum albumin (BSA) (Khan & Ali, 2020). The ability of these plants to inhibit protein denaturation suggests their potential in alleviating the symptoms of RA. The anti-arthritic activity of *Cucumis melo agrestis* and *Albizia thompsonii* was evaluated by measuring the denaturation of BSA, which is a crucial mechanism in the pathogenesis of RA. Both plants exhibited dose-dependent inhibition of BSA denaturation with IC50 values of 149.3 and 175.8 µg/ml for *Cucumis melo agrestis* and *Albizia thompsonii*, respectively (Chauhan & Chhabra, 2020; Sharma & Mishra, 2021). These results were comparable to the efficacy of diclofenac sodium (IC50 = 111.4 µg/ml), a standard anti-inflammatory drug (Dhanraj & Kapoor, 2019). This highlights the therapeutic potential of these plants in treating inflammatory diseases such as RA, where protein denaturation plays a key role in the development of joint destruction.

In addition to their anti-arthritic activity, both plant extracts demonstrated significant inhibitory effects on lipoxygenase (LOX) activity. Lipoxygenase enzymes are responsible for the conversion of arachidonic acid into pro-inflammatory mediators such as leukotrienes, which play a central role in the inflammatory response and contribute to diseases like asthma and arthritis (Ali & Khan, 2021). The ability of *Cucumis melo agrestis* and *Albizia thompsonii* to inhibit 15-lipoxygenase (LOX) suggests their potential in reducing inflammation associated with these diseases. The extracts showed IC50 values of 123.4 µg/ml for *Cucumis melo agrestis* and 112.5 µg/ml for *Albizia thompsonii*, which were comparable to quercetin, a well-known LOX inhibitor with an IC50 value of 95.79 µg/ml (Zhao & Zhang, 2021). This highlights the importance of targeting the LOX pathway in managing chronic inflammatory diseases (Sharma & Tripathi, 2019; Gupta & Sharma, 2021).

Furthermore, the study examined the nitric oxide (NO) production inhibition and macrophage viability as key parameters of inflammation. Nitric oxide, a crucial signaling molecule in the immune system, plays a significant role in regulating inflammation, but excessive NO production can lead to tissue damage and exacerbate inflammatory diseases (Zhang & Li, 2020). The Griess reagent was used to measure NO production in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Both *Cucumis melo agrestis* and *Albizia thompsonii* were found to inhibit NO production in a concentration-dependent manner, with significant reductions observed at 150 µg/ml. This effect was likely due to the phenolic compounds in the plant extracts, which have been shown to suppress the nuclear transcription factor NF-kappaB and inhibit the expression of inducible nitric oxide synthase (iNOS) (Mehta & Agarwal, 2020; Verma & Agarwal, 2021). The ability of these plants to modulate NO production provides further evidence of their anti-inflammatory potential and supports their use in treating diseases like rheumatoid arthritis, where excessive NO production contributes to joint damage.

The results of this study are consistent with previous research demonstrating the anti-inflammatory properties of both *Cucumis melo agrestis* and *Albizia thompsonii*. Studies have highlighted the antioxidant and anti-inflammatory activities of *Cucumis melo agrestis* (Sharma & Mishra, 2021) and *Albizia thompsonii* (Aslam & Ijaz, 2019), with both plants being recognized for their ability to modulate inflammatory pathways by targeting key enzymes such as lipoxygenase and nitric oxide synthase. The findings also align with reports indicating that flavonoids, alkaloids, and phenolic compounds found in these plants may be responsible for their therapeutic effects (Zubair & Ali, 2020; Soni & Kumar, 2019). These bioactive compounds are known to exhibit strong antioxidant properties, which may further contribute to their ability to reduce oxidative stress and inflammation in various disease models (Tiwari & Yadav, 2020; Wallace & Hannan, 2018).

6. CONCLUSION

The current study provides compelling evidence for the anti-arthritic, anti-lipoxygenase, and NO inhibitory activities of *Cucumis melo agrestis* and *Albizia thompsonii*. These plants demonstrate significant potential as therapeutic agents for managing chronic inflammatory diseases, offering an alternative to conventional synthetic anti-inflammatory drugs. Given the increasing prevalence of inflammatory conditions and the associated side effects of long-term NSAID use, plant-based treatments may offer a safer and more sustainable approach to managing inflammation (Zhong & Li, 2019; Zhang & Li, 2020). Future studies focusing on the isolation and characterization of specific bioactive compounds, as well as clinical trials, will be crucial in validating the therapeutic efficacy and safety of these plants in human populations.

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