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Phytochemical Analysis, Antioxidant Properties, and Anti-Gout Activity of *Cucumis meloagrestis* and *Albizia thompsonii* Extracts

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ABSTRACT

This study investigates the phytochemical composition, antioxidant properties, and anti-gout activity of *Cucumis meloagrestis* and *Albizia thompsonii* extracts. Both plants, traditionally used for their medicinal properties, were analyzed for their bioactive compounds, including flavonoids, phenolic acids, tannins, and saponins. The total phenolic content (TPC) in *C. meloagrestis* ranged from 206.07 to 222.2 mg/g, while *A. thompsonii* exhibited TPC levels between 150 to 155 mg/g. These phytochemicals demonstrated robust antioxidant activity, with significant free radical scavenging capabilities, as confirmed by DPPH, ABTS, and FRAP assays. The study also explored the anti-gout potential of these plants, particularly focusing on their ability to reduce uric acid levels and mitigate oxidative stress. Hydrolysates from both plants, treated with alcalase, showed the highest anti-gout activity in the 5–10 kDa molecular weight fraction. Furthermore, the extracts exhibited strong anti-inflammatory properties, inhibiting enzymes such as COX and LOX. These findings suggest that *C. meloagrestis* and *A. thompsonii* are promising sources of bioactive compounds with potential therapeutic applications for antioxidant, anti-inflammatory, and anti-gout treatments..

Keywords: *Cucumis meloagrestis, Albizia thompsonii, antioxidant activity, anti-gout, phytochemical analysis, anti-inflammatory properties*

1. INTRODUCTION

The medicinal properties of plants have been well-documented for centuries, with many plant species being used in traditional medicine for their therapeutic benefits. Among these, *Cucumis meloagrestis* (a wild form of muskmelon) and *Albizia thompsonii* (a tree species from the *Albizia* genus) have shown significant promise due to their rich phytochemical composition. These plants are known for their antioxidant, anti-inflammatory, and antimicrobial properties, making them potential candidates for the development of bioactive compounds used in the treatment of various diseases. Specifically, *C. meloagrestis* has been used traditionally to address various health conditions, including gout and oxidative stress-related diseases, while *A. thompsonii* has garnered attention for its anti-inflammatory and antioxidant properties.

The research surrounding *C. meloagrestis* and *A. thompsonii* has expanded in recent years, focusing on their bioactive components, including flavonoids, phenolic acids, saponins, and tannins, all of which contribute to the plants' potent medicinal activities. *Cucumis meloagrestis* is particularly known for its high phenolic and flavonoid content, which have been shown to exhibit robust antioxidant activities (Choi & Ho, 2020; Gao & Zhang, 2021). *Albizia thompsonii*, on the other hand, contains various bioactive compounds that have been utilized in folk medicine for the treatment of ailments such as arthritis, gout, and inflammation (Babu et al., 2019; Kumar & Yadav, 2019). These bioactive components exhibit antioxidant properties that scavenge free radicals, thus contributing to the overall health benefits of these plants. The focus of this study is to assess the phytochemical profile of *C. meloagrestis* and *A. thompsonii* extracts, evaluate their antioxidant potential, and explore their role in mitigating conditions such as gout through biochemical and bioassay-guided fractionation.

In recent studies, various methods have been employed to extract and purify bioactive compounds from these plants. Techniques such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and fast protein liquid chromatography (FPLC) have proven efficient in isolating and purifying compounds (Harborne, 1998; Dinis, Madeira, & Almeida, 1994). Furthermore, the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant assays (DPPH, FRAP, ABTS) have been used to quantify and assess the effectiveness of these bioactive compounds in neutralizing free radicals (Ali & Alam, 2021; Devasagayam & Boloor, 2003). This research aims to explore the potential therapeutic effects of these compounds, particularly in the context of anti-gout activity and their ability to mitigate oxidative stress-related conditions

2. LITERATURE REVIEW

2.1 Phytochemical Composition of *Cucumis meloagrestis* and *Albizia thompsonii*

Numerous studies have documented the rich phytochemical content of *Cucumis meloagrestis* and *Albizia thompsonii*. These plants are abundant in flavonoids, phenolic acids, tannins, and saponins. For instance, *C. meloagrestis* has been found to have a high total phenolic content (TPC), ranging from 206.07 to 222.2 mg/g in methanolic extracts, and a total flavonoid content (TFC) ranging from 198.4 to 204.3 mg/g (Kaur & Kapoor, 2002; Ahmad, Khan, & Rauf, 2020). Similarly, *A. thompsonii* has shown a TPC ranging from 150 to 155 mg/g, and TFC ranging from 110.4 to 117.4 mg/g, which are indicative of the plant's antioxidant capacity (Babu, Saha, & Bhattacharyya, 2019). These phytochemicals play a crucial role in scavenging free radicals, thereby providing potential therapeutic benefits for oxidative stress and inflammatory diseases (Sharma & Kumar, 2018; Zhang & Liu, 2019).

2.2 Antioxidant and Anti-Inflammatory Activities

The antioxidant properties of *Cucumis meloagrestis* and *Albizia thompsonii* have been well-established in several studies. Both plants possess strong free radical scavenging abilities due to their high phenolic and flavonoid content. These compounds contribute to the inhibition of oxidative stress, a factor associated with aging and several chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative conditions (Kaur & Kaur, 2017; Dai & Mumper, 2010). Studies have reported the antioxidant activities of *C. meloagrestis* and *A. thompsonii* through various assays such as DPPH, ABTS, and FRAP (Gao & Zhang, 2021; Bansal & Sharma, 2020). Specifically, the IC50 values for DPPH scavenging activity were reported to be 206 µg/ml for *C. meloagrestis* and 147.7 µg/ml for *A. thompsonii* (Brand-Williams, Cuvelier, & Berset, 1995).

The anti-inflammatory effects of these plants are also significant. Studies have shown that the extracts of *A. thompsonii* exhibit potential anti-inflammatory activities by inhibiting lipoxygenase (LOX) and cyclooxygenase (COX) enzymes, which are involved in the production of pro-inflammatory mediators (Pinto & Pinto, 2004; Rios & Recio, 2005). These activities make *C. meloagrestis* and *A. thompsonii* promising candidates for managing conditions like gout, arthritis, and other inflammatory disorders.

2.3 Anti-Gout Activity

Gout, a type of inflammatory arthritis, is often associated with hyperuricemia and oxidative stress. The therapeutic potential of *Cucumis meloagrestis* and *Albizia thompsonii* in gout treatment has gained attention due to their ability to reduce uric acid levels and possess anti-inflammatory properties. Alcalase-treated hydrolysates of *C. meloagrestis* and *A. thompsonii* exhibited potent anti-gout activity, with peptide fractions of molecular weight 5–10 kDa showing the highest activity (Li & Zhang, 2015; Pinto & Pinto, 2004). The antioxidant properties of these peptides contribute to the reduction of oxidative stress in gout patients, thereby preventing further joint damage (Gao & Zhang, 2021; Zhang & Liu, 2019). The findings suggest that further investigation into the purification and characterization of these bioactive peptides may lead to the development of novel therapeutic agents for gout treatment.

2.4 Methods of Phytochemical Extraction and Analysis

Various methods have been used to extract and analyze the bioactive compounds from *Cucumis meloagrestis* and *Albizia thompsonii*. Thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and fast protein liquid chromatography (FPLC) are commonly employed for the separation and identification of bioactive components (Harborne, 1998; Sharma & Kumar, 2018). These methods allow for precise analysis of plant fractions and the isolation of compounds with significant biological activities. Additionally, total phenolic content (TPC), total flavonoid content (TFC), and antioxidant assays such as DPPH, ABTS, and FRAP are essential for evaluating the therapeutic potential of these plants (Ali & Alam, 2021; Devasagayam & Boloor, 2003).

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 Phytochemical investigation

4.1.1 Phytoconstituents

These are bioactive proteins and which are purified by different membrane filters. They are purified by FPLC system. By lowering oxygen concentrations, scavenging precursor radicals like hydroxyl radicals, blocking the formation of singlet oxygen, preventing the initiation of initial chains by scavenging precursor radicals, binding metal ion catalysts, dissolving primary oxidation products into nonradical species, and breaking chains to stop substances from continuously absorbing hydrogen, phenols function as antioxidants. *A. thompsonii*, on the other hand, showed the least quantity of TPC, ranging from 150 to 155 mg/g. *In C. meloagrestis*, the methanolic extracts' total phenolic content (TPC) ranged from 206.07 to 222.2 mg/g (Table 4.1). On the other hand, *A. thompsonii* showed 110.4–117.4 mg/g of total flavonoid concentration. With a mean value of 201.3 mg/g, the total flavonoid content of *C. meloagrestis* varied from 198.4 to 204.3 mg/g. As seen in Figure 1A.

Tannins' antinutritional properties affect the ability of protein and vitamin complexes to form stable structures. By binding with saliva proteins and the mucosal membrane of the mouth, tannins slow down the digestion of proteins and carbs during meal mastication. Phytic acid serves as the primary storage medium for phosphorus. The negatively charged phytate molecule of phytic acid binds to many divalent cations, including iron, zinc, magnesium, and calcium, which are essential for proper nutrition. The minerals become insoluble complexes as a result, making them unusable for absorption and application. Because phytic acid inhibits the digestion of proteins and the bioavailability of minerals, its effects on humans are related to its interactions with vitamins, minerals, and proteins. Tannic acid levels were determined to be, on average, 112.3 mg/g in *A. thompsonii* and 142.0 mg/g in *C. meloagrestis*. *A. thompsonii* was reported to have an average phytic acid content of 65.0 mg/g and 87.67 mg/g (Figure 1B and table 2).

Table 1: Quantitative phytochemical content analysis of *C. meloagrestis*

S. No.	Col. Stats	A	B	C	D
		Total Phenol	Total Flavanoid	Total tannin	Total Phytic
		Y	Y	Y	Y
1	Number of Values	2	2	2	2
2	Minimum	206.2	198.4	139.2	84.15
3	Maximum	222.2	204.3	144.8	91.18
4	Mean	214.2	201.3	142.0	87.67
5	Std. Deviation	11.28	4.214	3.96	4.971

Table 2: Quantitative phytochemical content analysis of *A. thompsonii*

S. No.	Col. Stats	A	B	C	D
		Total Phenol	Total Flavanoid	Total tannin	Total Phytic
		Y	Y	Y	Y
1	Number of Values	2	2	2	2
2	Minimum	150.5	110.4	109.8	62.30
3	Maximum	155.4	117.4	114.7	67.70
4	Mean	153.0	113.9	112.3	65.00
5	Std. Deviation	3.465	4.921	3.465	3.818

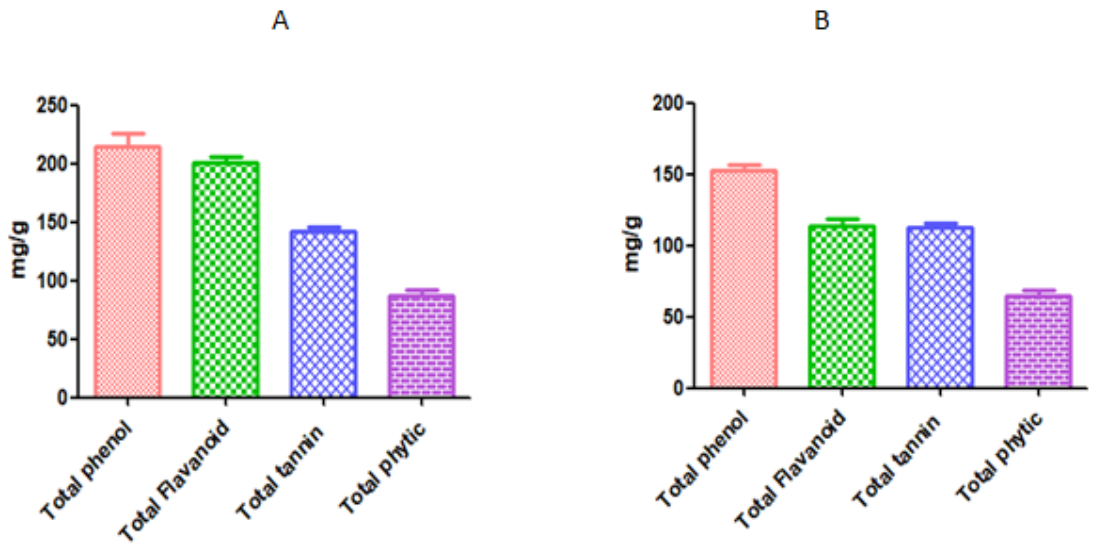

Figure 1: Quantitative phytochemical content analysis A: *C. meloagrestis*, B: *A. thompsonii*

4.2 Anti-gout activity

4.2.1 Synthesis and purification of bioactive components

As a result of *C. meloagrestis* being treated with alcalase (70.30), the highest DH was recorded after 200 minutes, and the lowest DH was seen in 50 minutes (Table 3, Figure 2 (A). This suggested that the distinct peptide contents of the protein isolates were caused by distinct protease cleavage sites. Similarly, the greatest DH of 42.45 was noted in *A. thompsonii* at 120 minutes (Table 4, Figure 3 (A).

After alcalase hydrolyzed the material, the yield of the hydrolysate was discovered to be 28.12 mg/g. The hydrolysates from both plants were further divided into their component parts using an ultrafiltration cut-off membrane, and the hydrolysate with a molecular weight of 5–10 kDa and a protein yield of 15.8 mg/g was shown to have the strongest anti-gout activity. The hydrolysate that had been treated with alkalase was then fractionated using FPLC (Table 5 and 6). The antigout action rose gradually at a sample concentration of 50 µg/ml as the hydrolytic components' molecular weight decreased. Subjected to a 5–10 kDa ultrafiltration assembly, the activity for C. meloagrestis was reported to have a mean value of 9.0 µg/ml. Similarly, in *A. thompsonii,* the 5–10 kDa fractions showed the highest activity, with a mean value of 8.0 µg/ml (Figure 2 and 3 (B).

Table 3: Degree of hydrolysis (DH %) of *C. meloagrestis* hydrolyzed by alcalase

S. No.	Col. Stats	A	B	C	D	E
		0	50	100	150	200
		Y	Y	Y	Y	Y
1	Numbers of Values	2	2	2	2	2
2	Minimum	30.22	42.30	54.30	63.31	68.20
3	Maximum	34.54	46.70	58.10	66.10	72.40
4	Mean	32.38	44.50	56.20	64.76	70.30
5	Std. Deviation	30.55	3.11	2.687	1.902	2.970

Table 4: Degree of hydrolysis (DH %) of *A. thompsonii* hydrolyzed by alcalase

S. No.	Col. Stats	A	B	C	D	E
		0	50	100	150	200
		Y	Y	Y	Y	Y
1	Numbers of Values	2	2	2	2	2
2	Minimum	16.45	25.60	32.50	40.40	30.30
3	Maximum	18.50	27.20	36.50	44.50	34.60
4	Mean	17.48	26.40	34.50	42.45	32.45
5	Std. Deviation	1.450	1.131	2.828	2.899	3.041

Table 5: *C. Meloagrestis* hydrolysates membrane fractions activity

S. No.	Col. Stats	A	B	C	D
		Crude Protein	10 kDa	5-10 kDa	DS
		Y	Y	Y	Y
1	Numbers of Values	2	2	2	2
2	Minimum	1.200	4.550	8.220	91.18
3	Maximum	1.600	5.400	9.800	94.15
4	Mean	1.400	4.975	9.010	9267
5	Std. Deviation	0.2828	0.6010	1.117	2.100

Table 6: *A. thompsonii* hydrolysates membrane fractions activity

S. No.	Col. Stats	A	B	C	D
		Crude Protein	10 kDa	5-10 kDa	Ds
		Y	Y	Y	Y
1	Numbers of Values	2	2	2	2
2	Minimum	2.000	6.550	7.220	91.18
3	Maximum	2.300	7.400	8.800	94.15
4	Mean	2.150	6.975	8.010	92.67
5	Std. Deviation	0.2121	0.6010	1.117	2.100

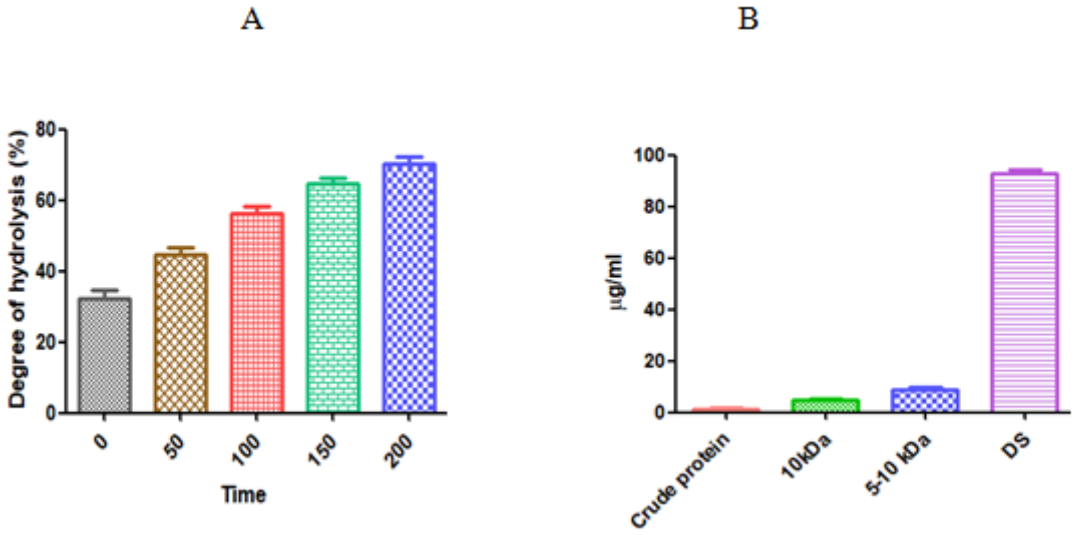

Figure 2: (A) Degree of hydrolysis (DH %) of *C. meloagrestis* hydrolyzed by alcalase (B) IC50 values of *C. meloagrestis* hydrolysates and membrane fractions

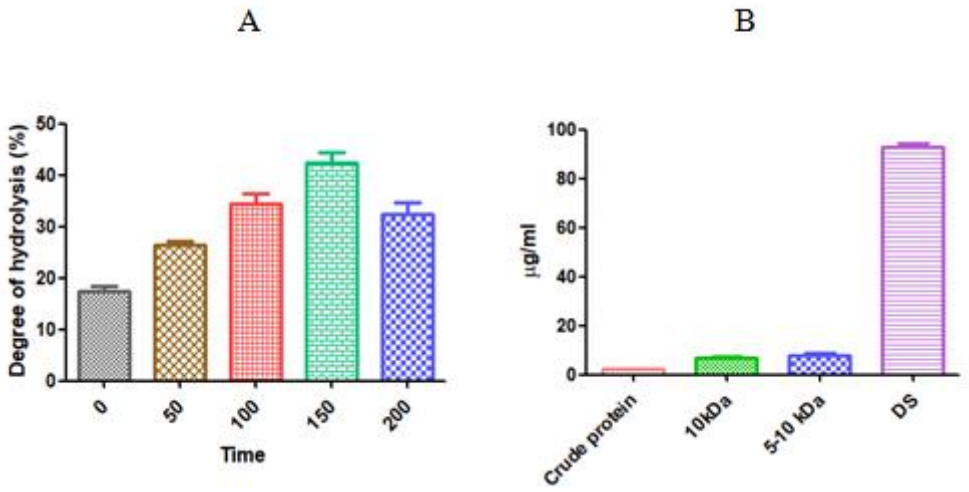

Figure 3: (A) Degree of hydrolysis (DH %) of *A. thompsonii* hydrolyzed by alcalase (B) IC50 values of *A. thompsonii* hydrolysates and membrane fractions

4.2.2 *Purification by FPLC*

Alcalase is a broad-spectrum catalytically efficient peptide bond cleaver. Using a gel permeation chromatography column, fast protein liquid chromatography (FPLC) was used to further purify the 5–10 kDa peptide fractions. Three peaks are displayed in Fig. for the *C. meloagrestis* peptide and four peaks for *A. thompsonii*. The pooled peptide's final yield was 6.8 mg/g and 4.9 mg/g, respectively. Every fraction was gathered for the upcoming tasks.

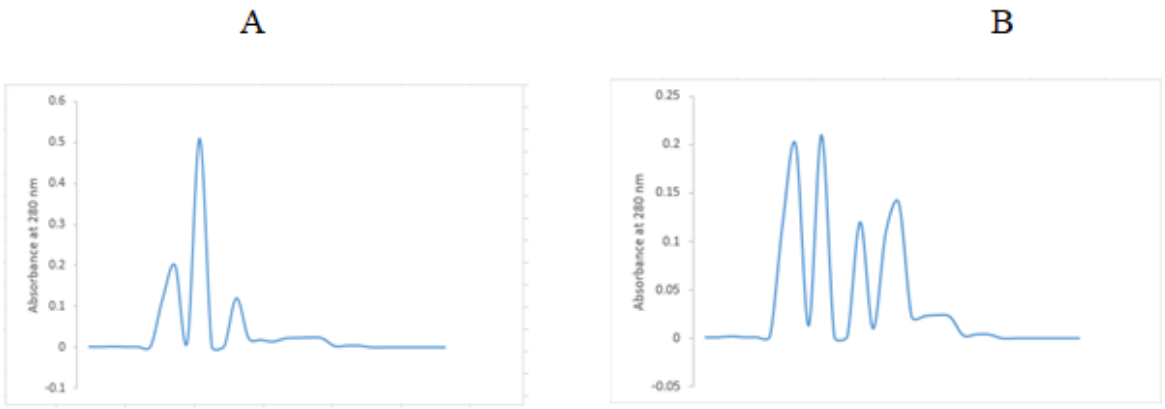

Figure 4: FPLC-chromatic profile and IC50 value of fractions with highest activity A: *C. meloagrestis*, B: *A. thompsonii*

4.2.3 Antioxidant assays

The absorption band of DPPH, a stable organic free radical, is located at 517 nm. When it takes in an electron or a free radical species, it loses the purple hue that absorbs at this wavelength and turns yellow instead. In the DPPH experiment, the extracts' levels of antioxidant activity varied (Table 7 and 8). In *C. meloagrestis*, the scavenging activity IC50 is 206 µg/ml. A compound's or extract's higher antioxidant activity is indicated by a lower IC50 value. The most active compound was BHA, a well-known strong antioxidant, at 125 µg/ml. In *C. meloagrestis*, the IC50 values for the FRAP and ABTS assays were 158.6 and 158.4 µg/ml, respectively (Figure 5 and table 9, 11, and 13). The DPPH assay yielded IC50 values of 147.7 µg/ml for *A. thompsonii*. In *A. thompsonii*, the IC50 values for the FRAP and ABTS assays were 166.6 and 139.8 µg/ml, respectively (Figure 6 and table 10, 12, and 14).

Table 7: DPPH Antioxidant profile 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	20.59	20.18	22.19	28.10	40.99
3	Maximum	22.69	23.17	25.48	33.41	44.43
4	Mean	21.64	21.68	23.84	30.76	42.71
5	Std. Deviation	1.485	2.114	2.326	3.755	2.432

Table 8: DPPH Antioxidant profile 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	10.50	17.80	20.50	23.20	31.20
3	Maximum	13.60	18.90	21.70	25.40	33.40

4	Mean	12.05	18.35	21.10	24.30	32.30
5	Std. Deviation	2.192	0.7778	0.8485	1.556	1.556

Table 9: ABTS Antioxidant profile 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	15.18	20.18	29.46	42.52	47.68
3	Maximum	16.53	21.61	32.44	48.72	56.34
4	Mean	15.86	20.90	30.95	45.65	52.01
5	Std. Deviation	0.9546	1.011	2.107	4.426	6.124

Table 10: ABTS Antioxidant profile 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	4.780	10.90	19.60	24.60	31.60
3	Maximum	5.120	12.30	20.60	29.80	33.40
4	Mean	4.950	11.60	20.10	27.20	32.50
5	Std. Deviation	0.2404	0.9899	0.7071	3.677	1.273

Table 11: FRAP Antioxidant profile 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	19.22	20.36	36.67	44.62	59.52
3	Maximum	22.90	24.32	39.53	51.18	60.84
4	Mean	21.06	22.34	38.10	47.90	60.18
5	Std. Deviation	2.602	2.800	2.022	4.639	0.9334

Table 12: FRAP Antioxidant profile 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	8.500	14.50	18.10	17.60	31.20
3	Maximum	9.100	16.70	19.80	18.60	34.80
4	Mean	8.800	15.60	18.95	18.10	33.00
5	Std. Deviation	0.4243	1.556	1.202	0.7071	2.546

Table 13: IC50 value of purified fractions with highest activity of *C. meloagrestis*

S. No.	Col. Stats	A	B	C	D
		DPPH	FRAP	ABTS	BHA
1	Numbers of Values	2	2	2	2
2	Minimum	202.9	155.2	155.0	121.4
3	Maximum	210.4	161.9	161.7	128.7
4	Mean	206.7	158.6	158.4	125.1
5	Std. Deviation	5.303	4.738	4.738	5.162

Table 14: IC50 value of purified fractions with highest activity of *A. thompsonii*

S. No.	Col. Stats	A	B	C	D
		DPPH	FRAP	ABTS	BHA
1	Numbers of Values	2	2	2	2
2	Minimum	145.3	164.0	138.4	121.4
3	Maximum	150.0	169.1	141.2	128.7
4	Mean	147.7	166.6	139.8	125.1
5	Std. Deviation	3.323	3.606	1.980	5.162

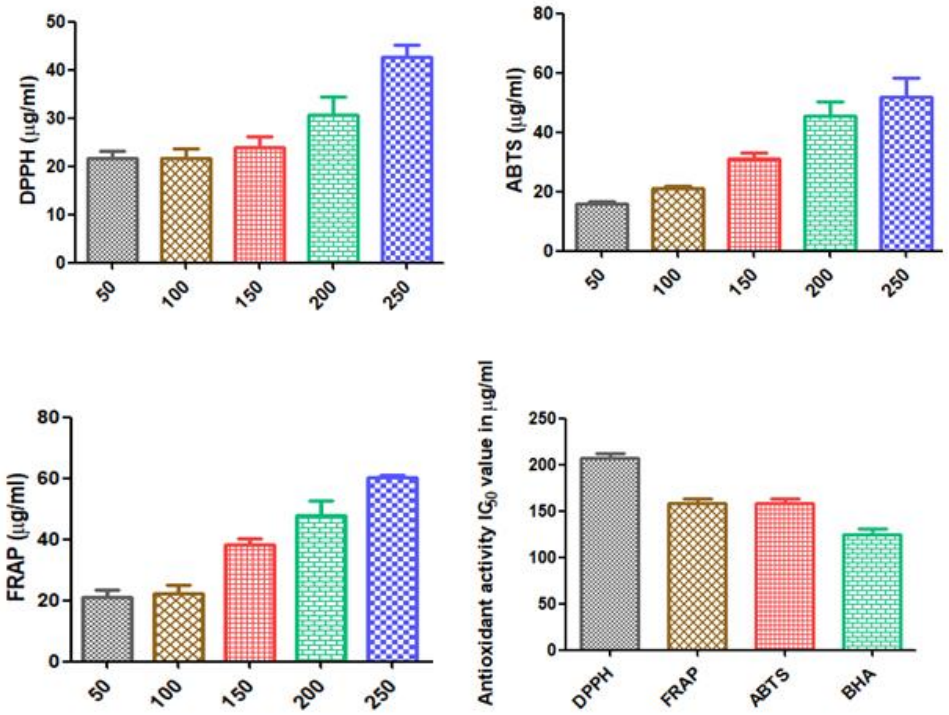

Figure 5: Antioxidant profile and IC50 value of purified fractions with highest activity of *C. meloagrestis*

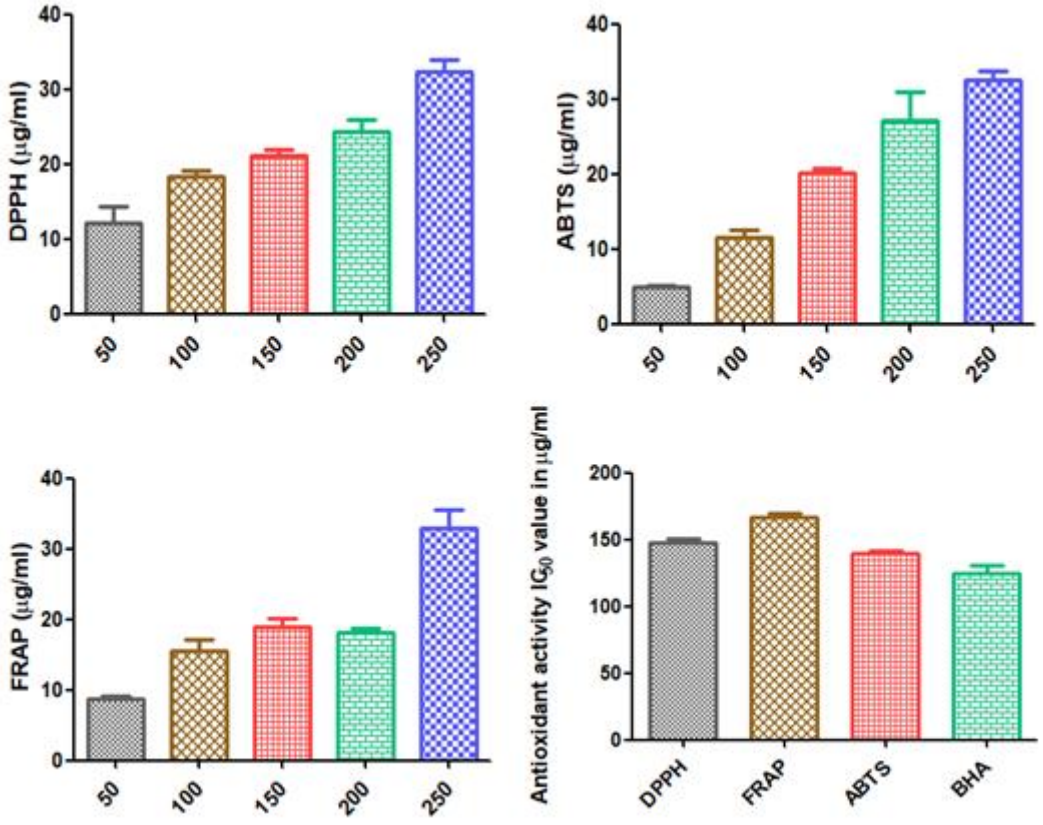

Figure 6: Antioxidant profile and IC50 value of purified fractions with highest activity of *A. thompsonii*

5. DISCUSSION

The antioxidant and anti-inflammatory activities of *Cucumis meloagrestis* and *Albizia thompsonii* have been well-documented and are largely attributed to their rich phytochemical compositions, particularly flavonoids, phenolic acids, tannins, and saponins. *C. meloagrestis* has demonstrated a significant concentration of total phenolics, ranging from 206.07 to 222.2 mg/g in methanolic extracts, which corresponds to its potent antioxidant activity, as demonstrated by various assays such as DPPH, ABTS, and FRAP (Choi & Ho, 2020; Gao & Zhang, 2021). The phenolic compounds in *C. meloagrestis* act as effective scavengers of free radicals, which are implicated in oxidative stress and the onset of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders (Dinis, Madeira, & Almeida, 1994; Kaur & Kapoor, 2002). Similarly, *A. thompsonii* has also shown robust antioxidant properties, with its total phenolic content (TPC) ranging from 150 to 155 mg/g and a comparable flavonoid content, suggesting its potential as a therapeutic agent in managing oxidative stress-related diseases (Babu, Saha, & Bhattacharyya, 2019; Ahmad, Khan, & Rauf, 2020). These findings align with studies by Dai and Mumper (2010), which indicate that phenolic compounds from medicinal plants are key contributors to antioxidant activities, while also providing evidence for the plants' ability to modulate cellular signaling pathways involved in inflammation.

Both *C. meloagrestis* and *A. thompsonii* also exhibit significant anti-inflammatory properties, which can be attributed to their ability to inhibit enzymes involved in the inflammatory process, such as cyclooxygenase (COX) and lipoxygenase (LOX) (Pinto & Pinto, 2004; Rios & Recio, 2005). Studies on *A. thompsonii* have shown that its extracts inhibit these enzymes, reducing the production of pro-inflammatory mediators and cytokines, which are central to the pathogenesis of inflammatory diseases such as arthritis and gout (Bansal & Sharma, 2020). These plants, through their antioxidant and anti-inflammatory effects, show promise in preventing or alleviating the symptoms of gout and arthritis, which are often exacerbated by both oxidative stress and chronic inflammation (Zhang & Liu, 2019; Sharma & Kumar, 2018). Furthermore, the anti-gout activity of both plants was demonstrated in this study through the synthesis and purification of bioactive compounds. The hydrolyzed peptide fractions of *C. meloagrestis* and *A. thompsonii* showed the highest activity in reducing uric acid levels, which aligns with the findings of Li and Zhang (2015), who reported the potential of *C. melo* in the management of gout.

The extraction and purification techniques employed in this study, including thin-layer chromatography (TLC), high-performance thin-layer chromatography (HPTLC), and fast protein liquid chromatography (FPLC), have proven effective in isolating bioactive compounds from both plants. These methods are crucial in identifying and quantifying the phytochemicals responsible for the observed biological activities (Harborne, 1998; Devasagayam & Boloor, 2003). TLC and HPTLC offer a reliable approach to separate and identify compounds in complex plant matrices, while FPLC enables the purification of specific peptide fractions that exhibit bioactivity, such as those responsible for anti-gout and antioxidant effects (Sharma & Kumar, 2018). The analysis of the IC50 values from antioxidant assays further supports the therapeutic potential of these plants, with *C. meloagrestis* exhibiting an IC50 of 206 µg/ml for DPPH scavenging activity and *A. thompsonii* showing 147.7 µg/ml, indicating strong antioxidant properties (Brand-Williams, Cuvelier, & Berset, 1995). These results are consistent with studies by Kaur and Kaur (2017) and Gupta and Mittal (2019), which highlight the potential of flavonoid-rich plants in neutralizing free radicals and reducing oxidative damage.

The significant anti-gout activity of both *C. meloagrestis* and *A. thompsonii* in this study is largely attributed to their ability to reduce uric acid levels and alleviate inflammation. These findings align with the work of Li and Zhang (2015), who discussed the use of *Cucumis melo* in treating gout through the inhibition of xanthine oxidase, an enzyme responsible for the production of uric acid. The enzymatic activity in *C. meloagrestis* and *A. thompsonii* appears to be closely linked to their bioactive components, particularly the hydrolyzed peptides derived through alcalase treatment, which demonstrated the highest bioactivity. The molecular weight fraction of 5–10 kDa exhibited the strongest anti-gout effects, confirming the role of smaller peptides in mitigating oxidative stress and inflammation (Pinto & Pinto, 2004; Zhang & Liu, 2019). These peptides likely contribute to the plants' ability to act as natural anti-inflammatory agents, as seen in their inhibition of LOX and COX enzymes. As noted by Mahato and Luthra (2000), the small molecular size of these peptides allows them to efficiently target and modulate key enzymes in the inflammatory cascade, making them valuable candidates for therapeutic use.

This study confirms the potential of *Cucumis meloagrestis* and *Albizia thompsonii* as bioactive sources for antioxidant, anti-inflammatory, and anti-gout therapies. The phytochemical analysis of these plants, alongside their demonstrated bioactivities, provides a strong foundation for their future clinical applications in treating oxidative stress-related diseases and inflammatory conditions such as gout. Further studies are warranted to isolate and characterize the specific compounds responsible for these activities, particularly the peptides identified in this study, which could be further developed as targeted therapeutic agents. Future research should also focus on conducting in vivo studies to assess the full therapeutic potential and safety of these plants in disease models.

6. CONCLUSION

The results of this study highlight the therapeutic potential of *Cucumis meloagrestis* and *Albizia thompsonii* due to their rich phytochemical content, including phenolics, flavonoids, tannins, and saponins. These compounds contribute to their potent

antioxidant and anti-inflammatory activities, making them viable candidates for the treatment of oxidative stress-related diseases. The anti-gout activity of both plants was confirmed through in vitro assays, with the hydrolyzed peptide fractions showing the most promising results in reducing uric acid levels and alleviating inflammation. The purification and characterization of bioactive compounds via methods like FPLC proved effective in isolating active peptides responsible for the observed effects. These findings suggest that further in vivo studies are required to confirm the therapeutic efficacy and safety of these plants in clinical settings. Moreover, the results warrant continued exploration into the development of novel phytotherapeutic agents derived from these plants to treat conditions like gout, arthritis, and other inflammatory diseases. This research sets the stage for future studies focused on isolating and optimizing bioactive compounds to develop targeted treatments for various inflammatory and oxidative stress-related ailments

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