RESEARCH ARTICLE

Unveiling the Therapeutic Potential of *Basella alba*: A Comprehensive Investigation into Its Anti-arthritic Properties, Phytochemical Profile, and Molecular Docking Insights

Jithin Mathew[1*], Shreeja M[1], Swathi AR[1], K Ragesh[2]

[1]*Department of Pharmacology, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Mangalore, Karnataka, India.*

[2]*Department of Pharmaceutical Analysis, Westfort College of Pharmacy, Thrissur, Kerala, India.*

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ABSTRACT

The study investigated the therapeutic efficacy of *Basella alba*, a traditional medicinal plant, against rheumatoid arthritis. Comprehensive analyses, including phytochemical screening, physicochemical evaluation, molecular docking, and antioxidant assays, were conducted. The physicochemical properties, such as ash and extractive values, indicated the high quality and purity of the plant material. Molecular docking studies showed that active compounds in *B. alba* exhibited strong binding affinities to key proteins involved in rheumatoid arthritis pathogenesis, suggesting potential inhibitory activity. Antioxidant capabilities were assessed through DPPH and nitric oxide scavenging assays. Phytochemical evaluation confirmed the presence of alkaloids, flavonoids, steroids, and triterpenoids. The secondary metabolites revealed significant mineral content (12% ash value), low insoluble mineral residues (1% acid-insoluble ash value), and high levels of hydrophilic (23.32% water-soluble extractives) and lipophilic compounds (8.48% alcohol-soluble extractives). Linoleic acid, palmitic acid, and cis-13-octadecanoic acid exhibited the highest binding affinities to the COX II receptor in molecular docking studies, with linoleic acid showing the strongest interaction. The DPPH assay showed a rise in antioxidant activity with increasing concentration, reaching 71.34% suppression at 100 µg/mL. The nitric oxide scavenging assay showed concentration-dependent antioxidant activity, reaching 64.20% inhibition at 100 µg/ml. The results suggest that the ethanolic extract of *B. alba* has significant antioxidant and anti-arthritic potential.

Keywords: Rheumatoid arthritis, Physicochemical evaluation, Binding affinity, Linoleic acid.

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INTRODUCTION

Rheumatoid arthritis (RA) is a multifaceted immune-mediated syndrome marked by persistent inflammation in the joints. It involves a combination of genetic predisposition, environmental factors, and immune system dysregulation. In RA, the immune system erroneously targets the synovium (joint lining), causing inflammation, pain, swelling, and ultimately leading to joint damage and deformity if not treated.[1] RA can also impact other organs, resulting in extra-articular manifestations such as inflammation of the eyes, lungs, heart, and blood vessels. Recent advancements in the diagnosis and treatment of RA, including the introduction of rheumatism-modifying therapeutic agents, have substantially enhanced patient outcomes.[2] These medications encompass conventional synthetic, biologic, and targeted synthetic therapies. Antioxidants are essential for protecting against free radicals, which are implicated in aging and degenerative conditions such as cancer, cardiovascular diseases, and cognitive dysfunction. The excessive production of free radicals can be exacerbated by pollution, illness, medication, stress, and exercise, emphasizing the need for antioxidants in therapeutic regimens.[3] Standard arthritis treatments, however, are often associated with serious side effects, leading to an interest in natural remedies. Herbal therapies, known for their anti-inflammatory and antioxidant activities, offer a complementary approach with fewer side effects. Herbs like *Curcuma longa*, *Zingiber officinale*, and *Rosmarinus officinalis* have shown potential in reducing inflammation and tissue damage.[4] Evaluating traditional medicines is essential, and one such plant is *Basella alba*, also known as Malabar spinach. This plant, rich in essential

**Author for Correspondence: jithinmathew051@gmail.com*

nutrients and potent antioxidants, has various therapeutic properties, including anti-inflammatory, wound healing and many more.[5]

This study aims to address the holistic treatment approach required for rheumatoid arthritis (RA) by investigating the potential of *B. alba*, a natural plant source. Through a combination of *In-silico* predictions and empirical evidence from phytochemical and antioxidant studies, a thorough understanding of *B. alba's* therapeutic benefits for RA patients is sought. Notably, *B. alba*, abundant in essential nutrients and potent antioxidants, exhibits significant anti-inflammatory and antioxidant properties that could mitigate inflammation and tissue damage in RA.[6] This research emphasizes *B. alba's* potential as a complementary treatment with fewer adverse effects compared to standard medications, presenting a promising natural alternative for RA management. Importantly, despite its potential, there is a lack of scientific studies evaluating the anti-arthritic activity of *B. alba's* ethanolic extract. Consequently, this study seeks to explore the pharmacological, computational, and pharmacognostic aspects, examining the effects of the ethanolic extract of *B. alba* on rheumatoid Arthritis (RA) and oxidative stress.[7]

MATERIALS AND METHODS

Extraction of Herbal material

The plant leaves of *B. alba* were collected, cleaned, and then shade-dried over a period of 15 days. After drying, the foliage was ground into a coarse powder using a mechanical grinder. This powder underwent cold maceration in ethanol for 7 days. Following maceration, the mixture was filtered through muslin cloth, and the resulting filtrate was left to air-dry. The alcoholic extract was then concentrated by simple distillation, and the distilled alcohol was used for additional extraction. The remaining residue from the distillation was further concentrated and evaporated in a water bath under controlled temperature conditions.[8]

Preliminary qualitative phytochemical analysis

The ethanolic extract of *B. alba* underwent qualitative tests to identify different phytoconstituents such as alakloids, glycosides, proteins, triterpenoids, saponins, steroids, flavanoids.[9, 10]

Physicochemical assessment of *B. alba* leaf characteristics

Quantification of Water-soluble extract content in B. alba leaves

Four grams of powder derived from dried leaves were submerged in 100 mL of water for one hour and thoroughly blended. The mixture was subsequently heated to 100°C in a water bath and then filtered. The filtrate was transferred to a pre-weighed porcelain dish and dried at 105°C until the moisture was completely removed.[11]

Quantification of Alcohol-soluble extract content in B. alba leaves

Four grams of ground substance were soaked in 100 mL of alcohol with constant agitation and left to stand for 16 hours. The solution was then strained, and the effluent was evaporated in a pre-weighed porcelain dish before being dried at 105°C.[12]

Assessment of Ash content in *B. alba* leaves

Analysis of total ash content in B. alba leaves

Two grams of powdered leaf were transferred to a pre-weighed silica crucible. The powder was then incinerated in a muffle furnace at temperatures between 500 and 600°C until only carbon-free ash remained. The percentage of ash was determined relative to the initial weight of the powdered residue.

Analysis of residue insoluble in acid content in B. alba leaves

The ash derived from the total ash was heated with 25 mL of 1 N HCl for 5 minutes and then sifted through an appropriate filtering medium for ash analysis to capture the insoluble residue. The filter paper with the residue was transferred to a pre-weighed silica crucible and burned at 650°C in a muffle furnace until all carbon was eliminated. The fraction of ash that remained insolvable in acid was calculated relative to the starting weight of the desiccated powder.[13]

Docking simulation studies

The analysis was conducted using the Glide module within the Schrödinger 2020-4 suite, specifically utilizing Maestro 12.3. Crystal structures of the target protein with adequate resolution were retrieved from the PDB database.[14]

Protein preparation

The study was performed using the "Protein Preparation Wizard" within the Schrödinger Glide suite. This process included pre-processing, optimizing the protein structure, removing water molecules, and performing energy minimization steps.

Ligand preparation

Preparation of all the ligands was done prior to docking by using "LigPrep module" from Schrodinger by producing tautomeric and low energy ionization states where pH range is 7.4 ± 0.

Generation of Receptor grids and Docking process

The process involved creating a grid using Schrodinger's Glide module to represent receptor characteristics, which are crucial for identifying interactions with ligands. Docking at extra precision provided insights into binding affinity assessed through G scores and docking scores.[15,16]

Antioxidant Studies

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

Solutions of *B. alba* and ascorbic acid were prepared in ethanol at concentrations extending from 20 to 100 µg/mL for evaluating their free radical scavenging activities. DPPH was then introduced into these solutions, followed by 30-minute incubation in darkness, and absorbance measurements were taken at 517 nm against a control sample.[17, 18]

Assessment of nitric oxide scavenging assay

A 10 mM solution of sodium nitroprusside in phosphate-buffered saline (pH 7.4) was prepared and mixed with various concentrations of the sample (ranging from 20–100 µg/mL). The solution was incubated at 25°C for 150 minutes. Following incubation, Griess reagent was introduced, and the absorbance was recorded at 546 nm, with ethanol used as the control.[19]

RESULTS

Preliminary phytochemical profiling of ethanolic extract of *B. alba* (EEBA) leaves

The phytochemical analysis of the ethanolic extract from *B. alba* leaves indicated the occurrence of various phytoconstituents like alkaloids, proteins, steroids, triterpenoids, flavonoids.

Physicochemical assesment of ethanolic extract of *B. alba* leaves.

Ash value

The ash residue of *B. alba* leaves, reflecting the inorganic constituents, measured at 12%, indicates a substantial mineral composition derived from soil absorption during growth, enriching the plant's nutritional content. Moreover, the acid-insoluble ash value of 1% signifies the presence of non-volatile residues like silica, highlighting the plant's mineral diversity.

Extractive value

The extractive values offer a glimpse into the phytochemical makeup of *B. alba* leaves. With a water-soluble extractive value of 23.32%, the leaves exhibit a wealth of hydrophilic compounds such as sugars, organic acids, and flavonoids, easily dissolved in water. Conversely, the alcohol-soluble extractive value of 8.48% indicates the presence of lipophilic compounds like alkaloids, terpenoids, and fatty acids, soluble in alcohol detailed in Table 1.

Molecular Docking study

Molecular docking of the constituents of *B. alba* was performed against the target protein 5IKQ (COX II). The phytoconstituents were positioned into the interaction groove of the COX II receptor, along with the interaction strength was expressed in terms of the docking score as shown in Table 2. All derivatives exhibited binding free energies ranging from -3.383 to -0.892 kcal/mol. Linoleic acid, palmitic acid, and cis-13-octadecenoic acid showed the highest binding energies with scores of -3.383, -3.217, and -2.553 kcal/mol, respectively. Notably, linoleic acid displayed the highest binding free energy of -3.383 kcal/mol, with hydrogen bonding interactions observed with ARG 376 and GLN 374. The docking scores for these compounds are detailed in Table 2.

Antioxidant studies

Evaluation of DPPH free radical scavenging activity in EEBA extract

The antioxidant activity of the ethanolic extract of *B. alba* (EEBA) was evaluated using the DPPH radical scavenging assay. The extract showed scavenging effectiveness proportional to the concentration of DPPH radicals, with percentages of 39.88, 45.95, 57.58, 62.86, and 71.34% at concentrations ranging from 20 to 100 **μg/m**L (Table 3). The IC_{50} value, which indicates the concentration needed to scavenge 50% of the DPPH radicals, was found to be 49.08 μg/ml. In comparison, ascorbic acid, the standard antioxidant, demonstrated scavenging percentages from 42.79 to 97.92% at similar concentrations, with an IC_{50}

Table 1: Physicochemical analysis of Ash and Extractive values of ethanolic extract of *B. alba* leaves

Name of the test	*Ethanolic content (%)*
Ash value	
Total ash value	12 %
Acids insoluble ash value	1 %
Extractable value	
Alcohol soluble extractive value	8.48 %
Water soluble extractive value	23.32 %

Table 2: Docking scores of bioactives from *B. alba* against COX II

S. No.	*PubChem ID*	*Compound name*	*Docking score (kcal/mol)*
1	5280450	Linoleic acid	-3.383
2	985	Palmitic acid	-3.217
3	5312441	cis-13-Octadecanoic acid	-2.553
4	445639	Oleic acid	-2.508
5	54722209	1-(+)-Ascorbic acid 2,6 dihexadecanoate	-1.89
6	31405	Phenol, 2,6-bis(1,1-dimethylethyl)-d	-1.808
7	13849	Pentadecanoic acid	-0.892

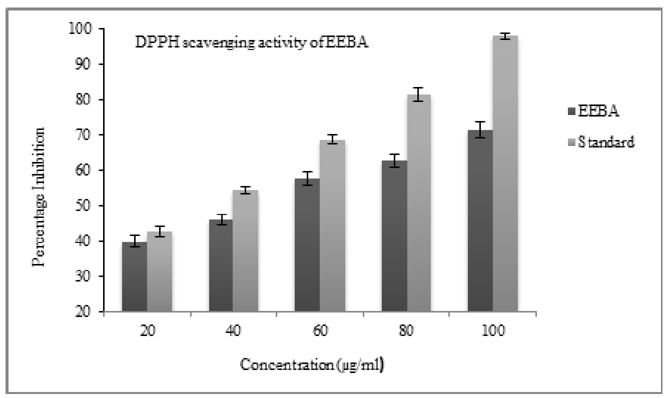

Figure 1: Antioxidant activity of ethanolic extract of B. alba in DPPH radical scavenging assay

Table 3: Antioxidant efficacy of *B. alba* ethanolic extract via DPPH assay

Sample	*Concentration (μg/ml)*	*Percentage DPPH radical scavengingactivity*	*IC_{50} (μg/mL)*
EEBA	20	39.88 ± 1.6302	
	40	45.95 ± 1.4790	49.08
	60	57.58 ± 1.8824	
	80	62.86 ± 1.8711	
	100	71.34 ± 2.3260	
	20	42.79 ± 1.4822	
Standard	40	54.25 ± 1.0104	31.98
(Ascorbic acid)	60	68.68 ± 1.1465	
	80	81.46 ± 2.0350	
	100	97.92 ± 0.9852	

(n=3), Values were expressed as Mean ± SD value of 31.98 μg/mL. The percentage inhibition of DPPH radical scavenging assay is mentioned in Figure 1.

Evaluation of Nitric oxide (NO) free radical scavenging activity in EEBA extract

The nitric oxide scavenging activity of the ethanolic extract of *B. alba* (EEBA) was assessed using a spectrophotometric assay. EEBA exhibited a dose-dependent inhibition of nitric oxide radicals, with scavenging percentages ranging from 34.15 to 64.20% at concentrations between 20 and 100 μg/mL (Table 4). The IC_{50} value, indicating the concentration required to scavenge 50% of the nitric oxide radicals, was found to be 61.73 μg/mL. In comparison, ascorbic acid, the standard antioxidant, demonstrated scavenging percentages from 39.60 to 95.80% at corresponding concentrations, with an IC_{50} value of 39.59 μg/mL (Figure 2).

DISCUSSION

The evaluation of ethanolic extract of *B. alba* (EEBA) revealed notable phytochemical properties. It showed a substantial presence of inorganic components indicated by the total ash value, while the acid-insoluble ash value highlighted minimal non-soluble impurities. The extract also demonstrated significant solubility in both alcohol and water, with a notably higher water-soluble extractive value compared to alcohol-soluble. These findings collectively suggest that the extract contains a diverse range of organic and inorganic compounds, showing a preference for water solubility.

Table 4: Effect of ethanolic extract of *B. alba* (EEBA) on nitric oxide radical scavenging

Sample	*Concentration (μg/ml)*	*Percentage nitric oxide scavenging activity*	*IC50 (μg/mL)*
EEBA	20	34.15 ± 1.5742	61.73
	40	42.70 ± 1.5701	
	60	49.41 ± 1.5376	
	80	59.64 ± 2.0400	
	100	64.20 ± 0.8177	
Standard (Ascorbic acid)	20	39.60 ± 1.5534	39.59
	40	46.80 ± 0.9808	
	60	64.06 ± 1.0261	
	80	76.07 ± 1.0512	
	100	95.80 ± 1.5708	

(n = 3), Values were expressed as Mean ± SD

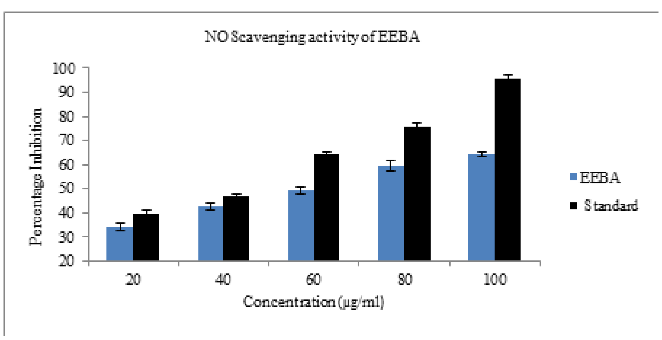

Figure 2: Scavenging activity of nitric oxide radicals by ethanolic extract of *B. alba* (EEBA)

Docking scores for the analyzed compounds against the COX II receptor varied, indicating diverse interaction levels. Linoleic acid and palmitic acid exhibited the highest affinity with more negative scores, suggesting strong binding potential. In contrast, cis-13-octadecanoic acid, oleic acid, and 1-(+)-ascorbic acid 2,6-dihexadecanoate showed moderate affinity, while phenol and pentadecanoic acid demonstrated lower affinity. These results highlight linoleic acid and palmitic acid as promising candidates for further investigation, potentially offering significant anti-rheumatoid activity against the target receptor.

The ethanolic extract of *B. alba* (EEBA) exhibited dose-dependent activities in scavenging both DPPH radicals and nitric oxide radicals. For DPPH radical scavenging, the highest activity was observed at 100 μg/mL, showing significant antioxidant potential but slightly lower potency compared to ascorbic acid. Similarly, in nitric oxide scavenging, the extract exhibited notable activity, particularly at higher concentrations, though slightly less potent than the standard. These results suggest that EEBA holds promise as a natural antioxidant, potentially beneficial in conditions involving oxidative stress related to rheumatoid arthritis.

CONCLUSION

The comprehensive study on *B. alba* has highlighted its therapeutic potential through various analyses. Phytochemical analysis identified alkaloids, flavonoids, steroids, and triterpenoids, all recognized for their health benefits. Physicochemical evaluations indicated satisfactory ash and extractive values, indicating high purity and concentration of active compounds in the plant extract. Molecular docking studies provided insights into how these bioactive compounds interact with COX II, supporting its traditional medicinal use, especially in managing inflammation associated with conditions like rheumatoid arthritis. *In-vitro* antioxidant assessments, including DPPH and nitric oxide scavenging assays, confirmed *B. alba's* strong antioxidant capabilities, crucial in combating oxidative stress, particularly relevant in rheumatoid arthritis. Overall, *B. alba* emerges as a promising natural remedy with antioxidant and anti-rheumatoid properties. This study paves the way for future research, suggesting the extract could be beneficial for inflammatory and oxidative stress-related conditions, such as rheumatoid arthritis.

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