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Research Article

Unveiling The Antioxidant and Antimigraine Efficacy of Biophytum Sensitivum: A Multi-Modal Investigation Involving Serotonin Modulation and Molecular Docking

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ABSTRACT

The present study investigates the Biophytum sensitivum methanolic extract for its phytochemical profile, antioxidant potential, and antimigraine activity, integrating both in vitro and in silico approaches. A preliminary phytochemical screening confirmed the presence of key secondary metabolites, notably flavonoids, alkaloids, phenolics, and saponins. The total flavonoid content (TFC) was quantitatively estimated using aluminum chloride colorimetric assay, revealing a significant concentration of flavonoid constituents, which are known contributors to pharmacological bioactivity. Antioxidant activity was evaluated via hydrogen peroxide scavenging and nitric oxide radical inhibition assays, both of which demonstrated dose-dependent free radical neutralization, highlighting the extract's redox-balancing capacity. The antimigraine potential was assessed through inhibition of serotonin release from blood platelets, a pivotal mechanism in migraine pathophysiology. The extract showed notable inhibitory effects, suggesting serotonergic modulation. Furthermore, molecular docking studies were performed to explore the binding affinity of major phytoconstituents with serotonin receptor subtypes and other migraine-relevant targets, providing molecular insight into the observed bioactivity. Collectively, these findings substantiate the traditional use of B. sensitivum and support its potential development as a natural therapeutic agent for migraine management, mediated through antioxidant and serotonergic mechanisms.

INTRODUCTION

Migraine is a complex neurological disorder characterized by recurrent episodes of moderate to

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severe headaches, often accompanied by symptoms such as nausea, sensitivity to light and sound, and visual disturbances [1,2,3]. This debilitating condition significantly impacts the quality of life for millions of individuals worldwide. Some of the patients experience a more specific disturbance prior to headache, called 'aura' which usually has visual disturbance. Migraine attacks are episodic and resolve with time. Associated symptoms such as nausea vomiting and increased sensitivity to light (photophobia) and sound (phonophobia) occur during the headache phase [4]. The molecular pathophysiology of migraine is not fully understood and this has made the treatment and/or management of migraine difficult. Currently available drugs such as ergotamine and its derivatives, several synthetic drugs like NSAIDS, 5HT receptor agonists (Triptans), 5 HT2 receptor antagonists and even antiemetics [5,6] are associated with various side effects and the treatment is based on trial and error method. Due to several drawbacks associated with the conventional drug therapy in migraine treatment, there has been a wide research to find out natural products which could be effectively used for the treatment of migraine. Plants such as *Tanacetum parthenium* [7] *Petasites hybridus*, [8] and others have been studied for relief of migraine. *Biophytum sensitivum* (L.) DC. (Syn. Biophytum petersianum Klotzch), an important medicinal plant is used in traditional medicine by many people in Asia, Africa and Pacific islands especially in Indian medicine [9,10]. The reported beneficial effects of *Biophytum sensitivum* include anti-inflammatory [11] and antidiabetic [12] effects. A polysaccharide isolated from *Biophytum sensitivum* has been found to enhance complement fixation [13]. Amentoflavone, one of the constituents of *Biophytum sensitivum* has been shown to inhibit Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) catalyzed prostaglandin biosynthesis [14]. However, no study on the antimigraine activity of *Biophytum sensitivum* has been reported. In order to verify the anecdotal claims that *Biophytum sensitivum* has numerous phytochemical benefits, we have investigated the antimigraine activity of this plant.

MATERIALS AND METHODS

Drugs & chemicals: Ascorbic acid was obtained from Basic Nutrition Co., Ltd. and serotonin was obtained from Otto Chemie Pvt Ltd. Chemicals and reagents used were of analytical grade.

Preparation of herbal extract: 200 g of powdered leaves of *Biophytum sensitivum* was weighed and taken in a 500 mL round bottomed flask. To this 400 mL of methanol was added then the round bottomed flask was attached to a condenser and distilled at temperature of 30 °C for 48 hours. The extract obtained was air dried in an evaporating dish till constant weight was obtained.

Phytochemical analysis: The phytochemical screening of methanolic extract of Biophytum sensitivum was performed as per the standard procedure described in Khandelwal [15].

Total Flavonoid content of AEBS by $AlCl_3$ method:

100 uL of plant extract is taken in different test tubes. and add 400ul of methanol to dilute. add 100 uL $AlCl_3$ in all samples. Add 100 ul of 1M sodium acetate to each test tube. Stir quickly. Incubate the sample at room temperature in dark for 45mins. Blank is prepared with methanol. And standard is prepared with quercetin dissolved in methanol (1mg/ml stock solution). Absorbance of all test samples and standards was measured at 450nm [16].

Antioxidant activity

Hydrogen peroxide scavenging assay:

For varying test and standard concentrations, the absorbance at 230 nm was measured. A phosphate buffer is made with a 40 mM H_20_2 solution. 0.6 ml of an H_20_2 solution with different standard values (10-50 g/mL) and tent concentrations was added to this phosphate buffer (40 mM). The blank is an H_20_2 free phosphate buffer. The following formula was used to get the percentage of inhibition. H_2O_2 scavenging percentage = [(Ac-At)/Ac] ×100 Whereas At represents the absorbance of the samples or standards, Ac represents the absorbance of the control [17].

Nitric Oxide Scavenging assay :

We assessed the absorbance at 546 nm for different test and standard concentrations. Standard Ascorbic acid and 100µl of plant extract were taken in duplicate. To the extracts in test tubes, 3ml of sodium nitroprusside (10MM) was added. At room temperature, the incubation period lasts 150 minutes. Fill each test tube with 3ml of Griess reagent (1%Sulphanilamide, 2% H3Po4 and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride). The blank will be a test tube containing only phosphate-buffered saline. The percentage of inhibition was calculated using the formula below.

Calculate the scavenging activity (%) using the following equation -

Scavenging activity % = [(Ac-At)/ Ac] ×100 [18]

Antimigraine activity

Platelet serotonin release inhibition assay:

Optical density is determined using UV Visible spectrophotometer at 270 nm. Fresh blood is collected from a volunteer and take 9ml of this blood, 6 µl of serotonin and 1 ml of 10% of extract is taken accordingly. Centrifuge this mixture at 2000 rpm for 10 mins. Supernatant is discarded and sediment is again centrifuged at 6000 rpm for 10 mins. Now, supernatant and platelet aggregation is measured.

% of serotonin release was calculated as - optical density of sample-blank/blank ×100

5% sodium citrate - take 5g of sodium citrate and solubilise it in 100 ml water or 1 g in 20 ml water

Buffer (PH = 7.4) of 20 ml [19].

***In silico* analysis**

Binding score evaluation using AutoDock Vina tool:

Molecular Docking

Molecular docking is a computational technique used in structural biology, bioinformatics, and drug discovery to predict how two molecules, typically a small ligand and a larger protein interact with each other. The primary goal of molecular docking is to predict the preferred orientation and conformation of the ligand when bound to the target protein [20].

Selection of CGRP protein and preparation of ligands:

Models of the CGRP protein based on crystallography or homology were retrieved from pertinent databases, including the Protein Data Bank. Chem3D Pro 12.0 was used to optimize the 3D structures of Cupressuflavone and Amentoflavone, whose chemical structures were retrieved from the PubChem database. The selection of the particular PDB ID 6ZHO for the migraine-related CGRP protein is based on a number of important considerations. An accurate depiction of the protein's conformation, including

crucial areas involved in ligand binding and receptor activation, is guaranteed by PDB ID 6ZHO, a high-resolution crystal structure of the CGRP receptor [21].

Molecular docking setup and validation:

Using AutoDockTool v1.5.6, the binding interactions between Amentoflavone, Cupressuflavone, and the CGRP receptor PDBID:6ZHO were examined. Given the flexibility of both the ligand and the receptor, the grid parameters were carefully selected to incorporate the ligand-binding site. The proteins used in this work were created, and the tautomeric states and stabilization of amino acid residue ionization were used to optimize the modified protein. Using this method, hydrogen was added and water molecules were eliminated. A PDB file was made in order to preserve the altered protein structure for docking investigations [22].

RESULTS

Preliminary Phytochemical Analysis:

Phytochemical screening of the alcoholic extract of *Biophytum sensitivum* (AEBS) revealed the presence of carbohydrates, steroids, saponin glycosides, flavonoids, alkaloids, lignins, phenols, aminoacids.

Table 1: Preliminary phytochemical analysis of AEBS

Phytoconstituents	Test	Result
Carbohydrates	Fehlings test	+
Steroids	Salkowski test	+
Saponin glycosides	Foam test	+
Flavonoids	Sulphuric acid test	+
Alkaloids	Dragendorff's test	+
Lignins	Weisner test	+
Phenols	FeCl3 test	+

Total Flavonoid content by AlCl3 method:

Table 2: Total flavonoid content of AEBS

Sample	Total flavonoid content in µg/100 g of extract (in QE)
AEBS	9.49

Antioxidant Activity

Hydrogen peroxide (H_2O_2) scavenging assay:

The output of the assay for scavenging hydrogen peroxide radicals were analysed and were portrayed in table and figure.

Table 3: H_2O_2 radical scavenging potency of AEBS

S. No	Compound	(Concentration) (µg/ml)	% inhibition (mean ± SEM)	IC50 Values (µg/ml)
1.	Ascorbic acid	10	19.46 ± 0.37	25.13
		20	42.29 ± 1.42	
		30	63.95 ± 1.02	
		40	79.98 ± 2.09	
		50	90.24 ± 1.62	

2.	AEBS	10	12.14 ± 2.35	28.70
		20	28.45 ± 1.96	
		30	54.70 ± 2.26	
		40	83.79 ± 1.20	
		50	89.95 ± 1.31	

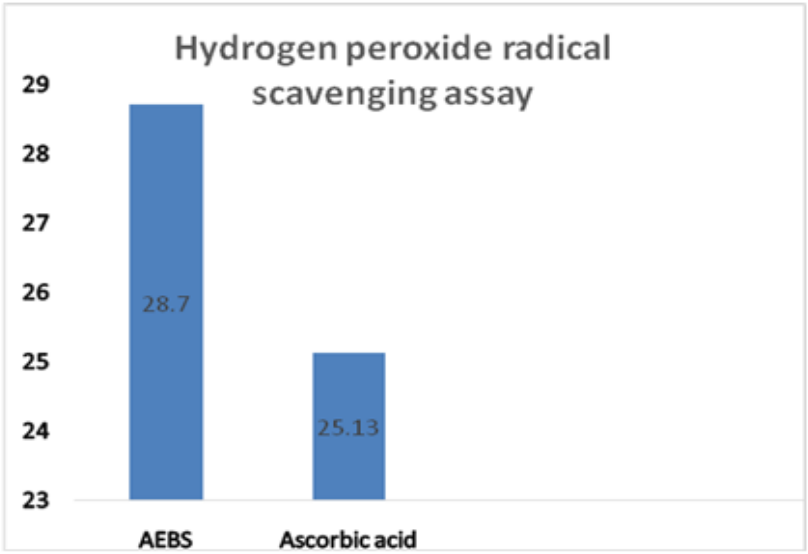


Figure 1: Graphical illustration of the Hydrogen peroxide radical scavenging assay's IC50 Values for AEBS and Ascorbic acid.

The H_2O_2 scavenging assay was considered to determine the antioxidant potency of AEBS. With increasing dose, AEBS displayed a greater percentage reduction of hydrogen peroxide radicals, and its 50% radical inhibitory concentration came out to be 25.13 µg/ml (Table 1). The AEBS's potential was on equivalent levels with that of the ascorbic acid, and its 50% radical inhibitory concentration came out to be 28.70 µg/ml.

Nitric Oxide Scavenging assay:

The results of the assay for scavenging nitric oxide radicals were analysed and were shown in table and figure.

Table 4: Nitric oxide radical scavenging activity of AEBS

S. No	Compound	Concentration (µg/ml)	% inhibition (mean ± SEM)	IC50 Values (µg/ml)
1.	Ascorbic acid	10	18.43 ± 0.45	26.44
		20	20.40 ± 1.81	
		30	60.67 ± 1.74	
		40	75.99 ± 2.74	
		50	90.11 ± 1.32	
2.	AEBS	10	10.16 ± 1.83	29.69
		20	25.08 ± 2.27	
		30	51.81 ± 1.26	
		40	73.14 ± 0.59	
		50	85.87 ± 1.02	

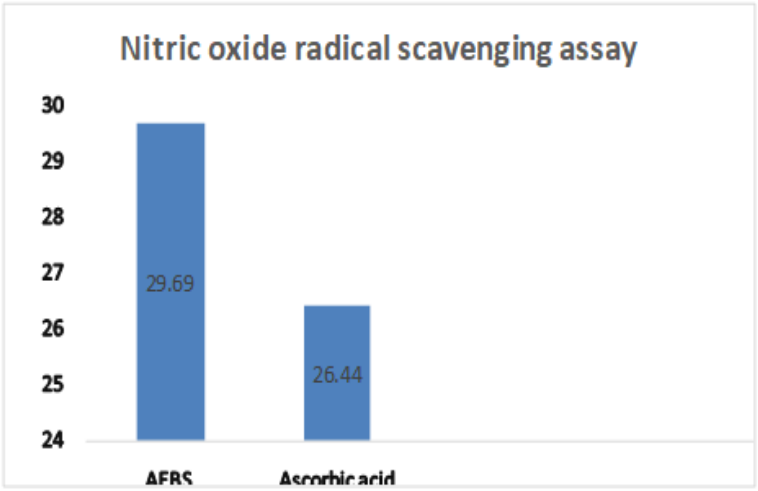


Figure 2: Graphical illustration of the Nitric oxide radical scavenging assay's IC50 Values for AEBS and Ascorbic acid.

The antioxidant capacity of alcoholic leaf extract from *Biophytum sensitivum* was examined using the Nitric oxide radical scavenging assay. Increased doses of AEBS caused a greater percentage of superoxide radicals to be suppressed, and its 50% radical inhibitory concentration was found to be 26.44 µg/ml (Table 2). The AEBS's 50% radical inhibitory concentration was found to be comparable to that of ascorbic acid (26.44 µg/ml).

Antimigraine Activity

Table 5: Effect of alcoholic extract of Biophytum *sensitivum* in Platelet serotonin release inhibition assay

S. No	Compound	Concentration (µg/ml)	% inhibition (mean ± SEM)	IC50 Values (µg/ml)
1.	AEBS	100	72.13 ± 1.93	69.12
		50	41.52 ± 0.82	
		25	19.33 ± 1.39	
2.	Ascorbic acid	100	88.61 ± 0.74	41.93
		50	59.12 ± 1.28	
		25	28.74 ± 0.93	

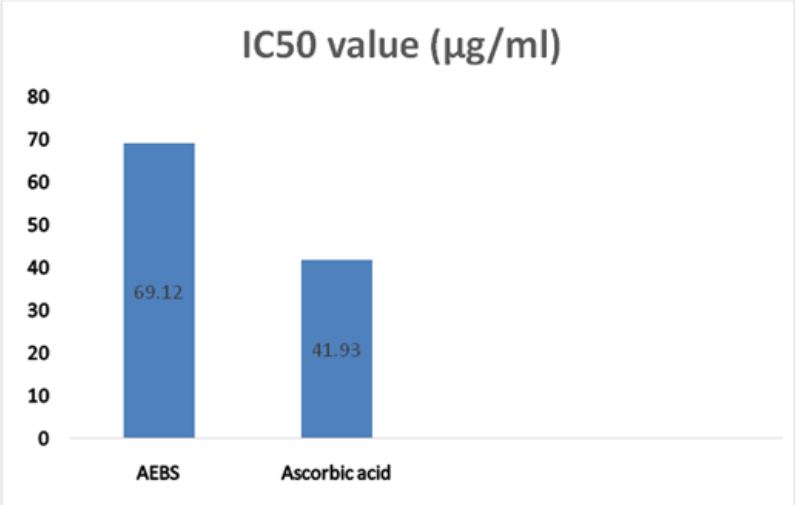


Figure 3: Values for AEBS and Ascorbic acid in Platelet serotonin release inhibition assay

The antimigraine activity of alcoholic leaf extract from *Biophytum sensitivum* was examined using the Platelet serotonin release inhibition assay.

Binding score evaluation using Auto Dock Vina tool

IC50 value (µg/ml)

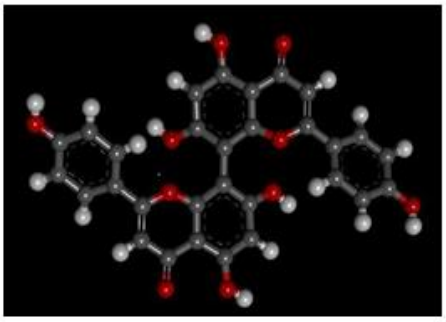


Figure 4: 3D structure of Cupressuflavone

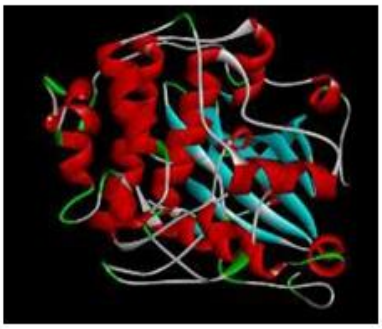


Figure 5: 3D structure of CGRP0

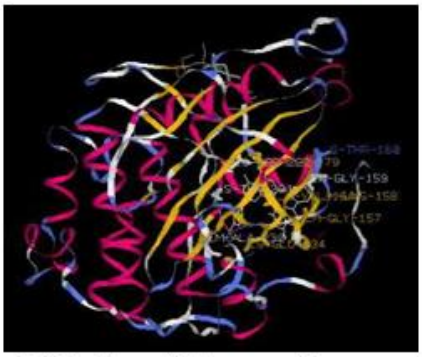


Figure 6: Binding of Cuprresoflavone with CGRP

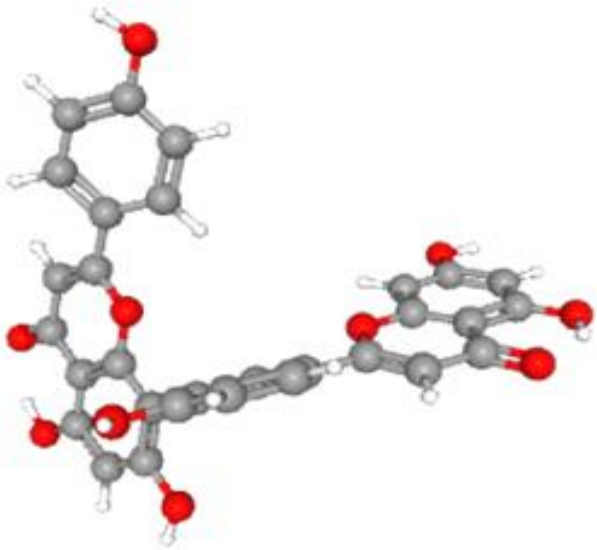


Figure 11: 3D structure of Amentoflavone

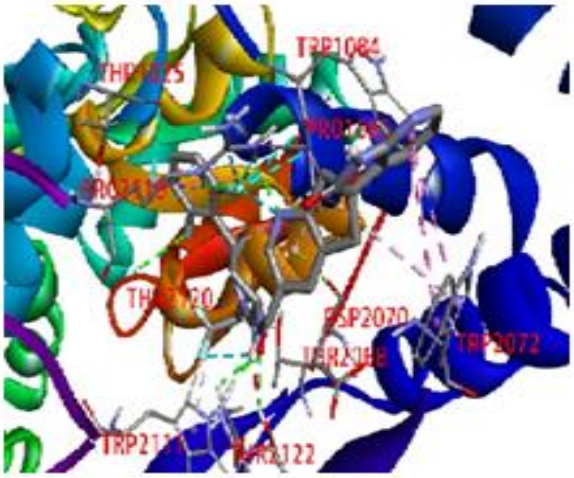


Figure 12.: Interaction between Amentoflavone and CGRP

DISCUSSION

Hydrogen peroxide occurs normally at low focus levels noticeable all-around air, water, human body, plants, microorganisms and food. H2O2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH) that can start lipid peroxidation and cause DNA damage. Alcoholic extract of Biophytumsensitivum effectively scavenged hydrogen peroxide which might be credited to the presence of phenolic groups that could donate electrons to hydrogen peroxide, subsequently neutralizing it into water [23]. Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, and are involved in the regulation of various physiological processes, including inflammation. Excessive production and release of NO is associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthase (NOS), which metabolizes arginine to citrulline with the formation of NO via five-electron oxidative reaction. These compounds are responsible for altering the structural and functional behavior of many cellular components. The alcoholic extract of Biophytumsensitivum shows significant nitric oxide scavenging activity in a dose dependent manner. This can be attributed to the presence of phytoconstituents like flavonoids, alkaloids and phenols.[24] During migraine attacks, increased release of serotonin from the storage granules of platelets and high amount of 5-HT metabolites in the urine samples have been reported. In view of this, the effect of alcoholic extract of Biophytumsensitivum on platelet serotonin release was conducted in human blood. The present results revealed the modulatory nature of AEBS on 5-HT release from storage granules of platelets. The results suggest that AEBS has a significant inhibitory role in serotonin release from platelets. These findings may also contribute towards the prophylactic antimigraine mechanism of AEBS [25]. The docking assessment, utilizing molecular docking simulations, provides a theoretical foundation for understanding how Cupressuflavone and Amentoflavone interacts with the CGRP receptor (Mounika G et al., 2021). The formation of hydrogen bonds with specific amino acid residues such as LYS2103, ARG2119, THR2120, and THR2122 in CGRP, as evidenced by the docking results, suggests a potential mechanism for Cupressuflavone and Amentoflavone's activity. The favourable binding energy strengthens the hypothesis that Cupressuflavone (-10.03 kcal/mol)

and Amentoflavone (-10.53 kcal/mol) could have a significant ability to interact with CGRP [26].

CONCLUSION

In conclusion, the comprehensive array of assessment conducted in this research affords a thorough understanding of Cupressuflavone and Amentoflavone's potential as therapeutic agents in the context of migraine management. The combined findings contribute valuable insights into their potential interactions with the CGRP receptor. The study shows that compounds have strong affinity with CGRP and that they possibly possess antimigraine activity. Further, isolation of active ingredients is necessary to know the active principle responsible for Antimigraine activity and to study mechanism of the active principle. The future scope of the work would be the isolation of the ingredient responsible for the activity, can be converted to formulation for preclinical studies.

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