Advances in Bioresearch
Adv. Biores., Vol 15 (1) January 2024: 407-410

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.15.1.407410

Advances in Bioresearch

SHORT COMMUNICATION

In-vitro and *In-silico* Evaluation Study of Piperine Extracted from Root and Stem of Wild *Piper nigrum* L.

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ABSTRACT
Wild Piper nigrum belonging to the family Piperaceae is found distributed in the Western Ghats in India. The extracts of Piper species have shown to enhance the bioavailability of few drugs through their Phytochemical study. The phytochemicals extracted from the root and stem of wild Piper nigrum have not been subjected to antimicrobial and antiviral activity. Hence an attempt has been made in the current study to study its properties. Piperine was extracted from the collected roots and stem of wild Piper nigrum from the Agumbe forest of Karnataka. The phytochemical constituent, Piperine extracted was evaluated for its in vitro antimicrobial activity using the agar filter paper disc diffusion method and agar well diffusion method. All 5 bacteria namely Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhi and Staphylococcus aureus and 5 fungi namely Aspergillus niger, Aspergillus flavus, Penicillium spp, Drechslera spp and Fusarium spp were used as test organisms during the assay. The results revealed significant and noteworthy antibacterial activity compared to its antifungal activity. Piperine exhibited maximum activity against Pseudomonas aeruginosa with an inhibition zone of 28mm and Fusarium spp exhibited an inhibition zone of 12mm. In-silico ADMET study using OSIRIS software also revealed the non-toxic property of Piperine with a drug likeliness of 0.22784. Hence it is worth attempting to study the effects of this isolated phytochemical constituent on as many bacteria and fungi as possible so that the potency of the compound could be fully understood and applied in drug development.
Keywords- *Piper nigrum L., Piperine, Antimicrobial assay, ADMET.*

Received 24.11. 2023 Revised 01.12.2023 Accepted 11.01.2024

How to cite this article:
Mangala K J. *In-vitro* and *In-silico* Evaluation Study of Piperine Extracted from Root and Stem of Wild *Piper nigrum* L. Adv. Biores., Vol 15 (2) March 2024: 402-406.

INTRODUCTION

Piperaceae is one of the largest family with Genus *Piper.* It is known to be widely spread in both the tropical and subtropical regions all around the world. The Indian subcontinent alone has been reported to contain one hundred and eight species of *Piper* [1]. Of the 108 species reported, 26 species belong to the Western Ghats and surrounding peninsular and coastal regions [2-4]. Most of the species that belong to the genus Piper are known to contain rich aromatic constituents [5]. The study on the phytochemical constituents obtained from Piper species are known to improve the bio-availability of many drugs [1]. The origin of *Piper nigrum*, which is a rich source of bioactive compounds is the Western Ghats region. It is also known as 'Black Pepper' with medicinal and commercial importance.

The Wild Black Pepper grows almost to a height of 10 m climbing with support on the trunks of the trees. Fruits obtained from Black pepper are mainly used as spice besides being used in Ayurveda, Siddha and Unani medicine for providing remedies to help proper digestion, to increase appetite, and as a remedy for cough, cold, toothache and other diseases. One of the most common ayurvedic preparation known as 'Trikatu' contains black pepper, long pepper and ginger and is used for treating many diseases [7]. The antimutagenic properties of Bell pepper (*Capsicum annum*) and Black pepper (*Piper nigrum*) were revealed in one the study on *Drosophila melanogaster* [6].

Even though much work has been carried out in the field of pharmacology, chemistry and medicine on *Piper nigrum*, not much work has been done on the bioactive compounds of Wild Black pepper, especially on biological activities [2] Hence in the present study we have designed a method to isolate the bioactive compounds from wild *Piper nigrum* and to study their *in-vitro* antimicrobial and *in-silico* antiviral activities. Any drug candidate should be subjected to a toxicity test before being given a marketplace because most of drugs fail at this particular stage [3]. Therefore, there is a need for predicting the ADMET properties of the drug candidates using different methods either by *in vitro* or *in silico* [8, 9]. *In silico* method involves the use of software like the admet-SAR tool [10] for screening and studying ADME properties for phytocompounds.

MATERIAL AND METHODS

Piperine was one of the important organic constituents extracted in the present study. Dried roots and stem of wild *Piper nigrum* were used for the extraction. The root and stem used in the extraction were collected from forests of Agumbe in Karnataka. The material was cleaned by washing and later dried at 40^0 C in a hot air oven. Then the material was powdered and used for extraction purpose.

Extraction of piperine alkaloid from root and stem was carried out in a soxhlet extractor. 200g of root powder and stem powder was taken in a soxhlet extractor. Extraction was carried out by using 900ml o petroleum ether as a solvent for 5-6 hours by maintaining the temperature at 60-80^0C. The content was then filtered and the filtrate was crystallised using rotavapour. After 2-3 days rectangular shaped crystals of piperine were observed under the microscope.

Bacterial and Fungal strains

Bacterial strains such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi* and *Bacillus subtilis* were used as test organisms for antibacterial assay. Fungal strains such as *Fusarium spp, Aspergillus flavus, Drechslera spp, Penicillium spp* and *Aspergillus niger* were used as test organisms for antifungal assay.

Antimicrobial assay

Piperine that was extracted using *P.nigrum* root and stem was then tested for its antibacterial and antifungal activity by dissolving in ethanol. Agar diffusion assay (Bocker, 1980) was followed in the present study.

Readymade antibiotic filter paper discs was used for the study that was procured from Himedia Private Ltd., Mumbai. Streptomycin discs (10µg/disc) were employed as the positive control. Later Piperine extract with 500µg concentration was used for antibacterial assay. Filter paper discs were immersed in the extracts of Piperine and then mounted on the surface of petriplates containing nutrient agar medium. Th petriplates were incubated for 24 hours at 37^0C. After 24 hours of incubation, the petriplates were observed and the zone of inhibition was measured and recorded.

For the antifungal assay, both agar filter paper disc diffusion method as well as agar well diffusion method was employed. In this method, Potato Dextrose agar medium and Piperine with a concentration of 500µg was used along with the standard antifungal agent, Actidione (10µg/10ml). For the agar well diffusion method, wells were bored and then 0.1 ml of both standard and test solutions were placed into different wells. Later the petriplates were kept in an incubator for 48 hours at 28^0C. After incubation, the petriplates were observed for the results and activity was recorded.

In-silico ADMET study

We predicted the absorption, distribution, metabolism, excretion, and toxicity (ADMET) of Piperine for its drug-likeliness property via *in silico* methods. The OSIRIS software was used to perform an *in silico* prediction. Piperine molecule was drawn by opening the OSIRIS software and studied for its property. The drug-likeness and toxicity of the compound was determined.

RESULTS

Antimicrobial assay and *in silico* ADMET studies of Piperine revealed the following results.

Results of antibacterial activity

The results showed very good antibacterial activity of Piperine when compared to streptomycin. *Pseudomonas aeruginosa* was found to be more sensitive to Piperine extract of root and stem (Plate 1 and Plate 2) followed by *Bacillus subtilis, Salmonella typhi, E. coli* and *Staphylococcus aureus* with different inhibition zone as tabulated in Table 1.

Table 1. Antibacterial activity of Piperine extract of root and stem.

	Diameter of zone of inhibition (in mm)		
Test Organisms	Piperine from Root (500µg)	Piperine from Stem (500 µg)	Streptomycin (10µg)
Pseudomonas aeruginosa	28	22	28
Bacillus subtilis	25	20	23
Salmonella typhi	19	20	22
E. coli	12	11	17
Staphylococcus aureus	11	12	16

Results of antifungal activity

The antifungal activity showed good inhibition of *Fusarium spp* (Plate 3 and Plate 4) by the Piperine extract of root and stem followed by *Penicillium spp, Aspergillus flavus, Aspergillus niger* and *Drechslera spp* with different inhibition zone as tabulated in Table 2.

Table 2. Antifungal activity of Piperine extract of root and stem.

	The diameter of zone of inhibition (in mm)		
Test Organisms	Piperine from Root (500µg)	Piperine from Stem (500 µg)	Actidione (10µg)
Fusarium spp	12	7	19
Penicillium spp	9	7	18
Aspergillus flavus	7	No activity	16
Aspergillus niger	No activity	No activity	16
Drechslera spp	No activity	No activity	11

Results of *in-silico* ADMET study

ADMET study performed using OSIRIS software are shown in Table 3

Table 3. Drug likeliness of Piperine

Molecule Name	Drug likeness	Mutagenic	Tumorigenic	Reproductive Effective	Irritant
Piperine	0.22784	None	None	None	None

DISCUSSION

Even though many antibiotics are known to be widely used in medicine there has been always a search for a variety of antimicrobials derived from plants. Antimicrobials derived from plants are always of much importance and are better options due to their safety and also in controlling bacterial and fungal infections effectively without causing many side effects. In the present study even though Piperine was considered to be a good antibacterial agent but it was not found to be so effective as an antifungal agent [11-16]. The Piperine extract obtained from both root and stem showed almost very similar activity.

Of all the five bacteria used in the study for testing the activity of Piperine, all were found to be sensitive. Among all the five sensitive bacteria, *Pseudomonas aeruginosa* (Gram-negative) showed maximum sensitivity and placed at first place followed by *Bacillus subtilis* (Gram-positive), *Salmonella typhi* (Gram-negative), *E. coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive).

Piperine showed good activity and was hence more effective against *Pseudomonas aeruginosa* and slightly less effective against *Staphylococcus aureus.* It was noteworthy that all five bacteria showed significant antibacterial activity. The antifungal activity also showed maximum inhibition with *Fusarium spp with* Piperine. This was followed by *Penicillium spp.* The study revealed poor inhibition of Piperine with *Aspergillus flavus, Aspergillus niger and Drechslera spp.* Piperine was hence found to be showing more effective antibacterial activity than as antifungal activity.

Thus from our study it was found that Piperine was more potential and could be to be used as an effective antibacterial agent, antifungal agent and also non-toxic to cells with a drug likeliness of 0.22784 by *in-silico* ADMET study. Furthermore, studies can also be designed to synthesize these compounds or the chemically modified compounds of these molecules and could be used for medical purposes.

CONFLICT OF INTERESTS

There is no conflict of interest in the present study.

ETHICAL CONSIDERATIONS

The present study didn't involve animals and hence did not require approval by the ethical committee.

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