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ORIGINAL ARTICLE **OPEN ACCESS**

In Vitro Anti-HIV Activity of *Tabernaemontana divaricata* (L.) R. Br. Ex Roem and Schult with Docking Study

Ankita A. Sawant[1*], Bhavana U. Jain[2], Yasmin H. Momin[3], Deepa S. Yadav[3], Harshad Popat Khade[3], Komal. V. Dombale[1], Safiya A. Jali[1], Snehal S. Patil[4], Monali M. Shewale[4], Rupali Prakash Lade[5]

[1]PG Student, Department of Pharmaceutical Chemistry, Appasaheb Birnale College of pharmacy, Sangli. (MS)- 416416, India.

[2]Assistant professor, Department of Pharmaceutical Chemistry, Appasaheb Birnale College of pharmacy, Sangli. (MS)- 416416, India.

[3] Department of Pharmaceutical Chemistry, Annasaheb Dange College of Pharmacy Ashta. (MS)- 416416, India.

[4]Assistant professor, Department of Pharmaceutical Chemistry, Krishna Institute of Pharmacy, Karad. (MS)- 416416, India.

[5]Assistant professor, Department of Pharmaceutical Chemistry, Women's College of Pharmacy, Peth Vadgaon, Kolhapur (MS), India.

*Correspondent author: ankisawant7075@gmail.com

ABSTRACT

Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult., a plant with a history of traditional medicinal use, is of increasing interest for its potential antiviral properties. This study investigated the in vitro anti-HIV activity of different extracts from *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. and conducted molecular docking analyses to explore the potential mechanisms underlying this activity. Ethanol and aqueous extracts of the leaves of this plant were prepared and evaluated for their anti-HIV activity using an enzyme pepsin activity inhibition assay. The extracts exhibited significant dose-dependent anti-HIV effects, with the ethanol extract of leaves demonstrating the highest inhibitory activity against HIV-1 infection. The study conducted molecular docking studies on phytochemical compounds from *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. against key viral targets, including HIV-1 protease. Results showed strong binding affinities between compounds and viral proteins, indicating their potential as anti-HIV agents.

Keyword: AIDS, Anti-HIV, Docking Study, HIV-1 Protease, *Tabernaemontana Divaricata* (L.) R. Br. ex Roem. and Schult.

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INTRODUCTION

Plants have been used as medicines since ancient times and there is still a significant market for these plants today. Their reliance on conventional medicine for basic medical care [1] and their importance in contemporary society are both increasing constantly. Naturally occurring chemicals are regarded to be safer, more readily biodegradable, and less likely to develop drug resistance than synthetic molecules. 25% of all medications are produced using medicinal plants in industrialized countries, whereas closer to 80% are produced in impoverished countries [2].

Tabernaemontana divaricata (L.) R. Br. ex Roem. and Schult, a member of the Apocynaceae family, is a common garden plant in Southeast Asia and other tropical countries [3]. The pinwheel flower is scientifically known as *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. This little evergreen shrub has leaves that grow to a maximum length of 6 inches and a height of 5 to 6 feet. At the ends of the stems, little clusters of five-petal white pinwheel flowers arise [4]. It includes many phytoconstituents that cause illness [5]. Human immunodeficiency virus (HIV) is the name of the virus that causes HIV infection and can lead to AIDS, the most severe form of the sickness. All HIV patients are advised to get antiretroviral medication (ART), which lowers blood virus loads [6]. A wide range of organic compounds including proteins, alkaloids, flavonoids, lignans, and others, have been shown to block certain enzymes and proteins necessary for the HIV life cycle, such as reverse transcription, viral entry, the integrase or protease. Examining anti-HIV compounds in natural products may be a more effective way to develop medications. It reportedly includes many alkaloids with a variety of pharmacological properties [7]. It was recently

established that in an in vitro experiment, *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. ethanolic extracts inhibited more than aqueous extracts.

MATERIAL AND METHODS

Plant collection and authentication:

The leaves of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. were picked at Vita, Maharashtra, India, in October 2022. The Department of Botany at Kasturbai Walchand College in Sangli identified and verified the plant.

Plant material drying and powdering:

The freshly obtained leaves were cleaned. The plant's fresh leaves were ground into a coarse powder using a mixer and passed through filter number 40 after being shade dried for about two weeks, stored at 25 °C.

Extract preparation [8-10]

After being turned from dry leaves into coarse powder. extraction was carried out using two different methods, namely

Aqueous Extract

25 grammes of coarse powder of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. leaves were taken in the breaker. Add chloroform and distilled water. placed for 7 days with an intermittent stirring filter. Filter paper and filtrate were stored at 4°C until further use. Pharmacological examinations and phytochemical investigations are performed.

Soxhlet Extraction Method

Herbal drugs are extracted using proper medium and Soxhlet apparatus for medicinal extracts with lower to higher solvent polarity. 25 grammes of powder of dried fresh leaves processed with petroleum ether (60–80) at 50^{o}C for 2 days (fat and pigment components removed). The treated powder was further processed with chloroform at 51^{o}C for 2 days (to remove chlorophyll). The treated powder was further processed with ethanol at 68^{o}C for 2 days. Dried, concentrated extracts were used to study, isolate, and investigate the pharmacological effects of plants.

Phytochemical screening [11, 12]

Aqueous and ethanolic leaf extracts prepared from fresh and dried leaves were examined for their phytochemical composition. It will be able to observe the traditional conclusions and recognize a number of components by adding the appropriate reagent solutions.

Pharmacological Screening

In vitro Anti-HIV Activity

Chemical required

Enzyme Pepsin, Hemoglobin, Pepstatin, Sodium Acetate trihydrates, Sodium Chloride, Tri Chloro Acetic acid. Acetate buffer was prepared by 50 Mm Na Acetate tri hydrate and 0.1 M NaCl with pH- 3.5.

Instruments required

UV visible Spectrophotometer - Shimadzu, Remi for 8000 rpm, Micropipette 5-40 µl, 40-200 µl, 200-1000 µl, Eppendorff (1.75ml), electronic balance.

iii. Enzyme Pepsin activity inhibition Assay: -

Given that both Pepsin and HIV-1 protease are members of the same Aspartate enzyme family, they exhibit similar proteolytic properties. HIV-1 protease is one of the main enzymes in the HIV-1 life cycle. In this work, this enzyme was utilized to test the anti-HIV activity of the aqueous and ethanol extracts in place of the HIV-1 protease.

Procedure [13]

For this test, 500µl of the reaction mixture was filled with 50µg of pepsin, 800µg of haemoglobin, and ethanol and aqueous extracts. After 20 minutes of 37^{0}C incubation, 700 µl of 5% TCA were added to the mixture to terminate the reaction. The supernatant was then removed after being centrifuged at 14000 g for 5 min. At 280 nm, optical density (OD) was spectrophotometrically measured. Positive and negative controls, as well as the sample, each had their own set of blanks. Following the foregoing method, enzyme and substrate were taken as a negative control, and pepstatin, a well-known inhibitor of HIV-protease, was taken as a positive control. This test provides repeatable findings since each sample was obtained in three times. Percentage of inhibition was calculated by using a formula.

Inhibition (%) = [(OD of negative control - OD of sample) /OD of negative control] ×100

To get the most effective enzyme activity, a number of evaluates were carried out

Table 1. Parameter

Parameter	Optimum range
pH	2- 4
Incubation period	30 min.
Reaction volume	1000 µL
Incubation temperature	37^0C
Centrifugation	14000 rpm.

Isolation and Identification of Phytoconstituents

Chemical isolation

An ethanolic extract of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. leaves were used for the chemical isolation. Filter the filtrate, then dry it out using evaporation. At a pH of 5, dry ethanolic extract dissolves with tartaric acid (10 g of tartaric acid to 100 ml of distilled water). After that, separate the acidic layer from the ethyl acetate layer by extracting ethyl acetate that has been saturated with water. With Na_2CO_3, the acidic layer is made alkaline to pH 11, which is subsequently extracted with ethyl acetate. Separate the aqueous and ethyl acetate layers once more. Alkaloids were found in the ethyl acetate layer.

Spectroscopic analysis [14-15]

Ultraviolet spectroscopy:

UV-visible analysis was performed on the isolated component. At A.B.C.P., Sangli, the UV analysis was finished using a UV spectrophotometer (JASCO V- 730). An isolated compound was combined with ethanol to create a sample with a concentration of 10 g/ml.

ii. Infrared spectroscopy:

One of the most effective analytical methods for revealing chemical structure is infrared spectroscopy. Identification of functional groups in a compounds structure is made easier by IR. Using concentration and desiccation, the column chromatography-isolated compound was dried. A KBr press was used to create a pellet after the dry chemical was triturated with KBr. The study, which used FTIR from the Jasco corporation, was carried out at the Appasaheb Birnale College of Pharmacy in Sangli.

Docking Study

A method called docking is used for establishing the desired position of a specific molecule with respect to other molecules when a therapeutic molecule and a target are bound together to form a stable complex[16]. Using a docking examination, the isolated molecule anti-HIV activity was identified. The Appasaheb Birnale College of Pharmacy in Sangli conducted a docking investigation.

Software used: VLifeMDS

Drug molecule: Taberdines L

Targeted receptor[17]: HIV-1 Protease (PDB ID: 3NU3)

RESULT AND DISCUSSION

Physicochemical Screening:

Table 2. Physicochemical evaluation

Sr. No.	Phytochemical test	Aqueous Extract	Ethanol Extract
1.	Alkaloids	+ve	+ve
2.	Flavonoids	+ve	+ve
3.	Tannins and phenolic compound	+ve	+ve
4.	Carbohydrate	+ve	+ve
5.	Glycoside	+ve	+ve
6.	protein	+ve	+ve

Pharmacological Screening

In vitro Anti-HIV activity

Table No.3- Putative anti-HIV activity of Ethanolic and Aqueous of Extracts

Sr.no	Compound	Concentration	Reading 1	Reading 2	Reading 3	Mean	% inhibition
1.	Control	-	0.058	0.048	0.066	0.057	
2.	Pepstatin (std)	100 µg/ml	0.007	0.006	0.005	0.006	89.47
3.	Aqueous	1mg/ml	0.879	0.789	0.712	0.793	78.12
4.	Ethanolic	1mg/ml	0.949	0.890	0.788	0.875	85.73

Spectroscopic analysis: [18-20]

Ultraviolet spectroscopy:

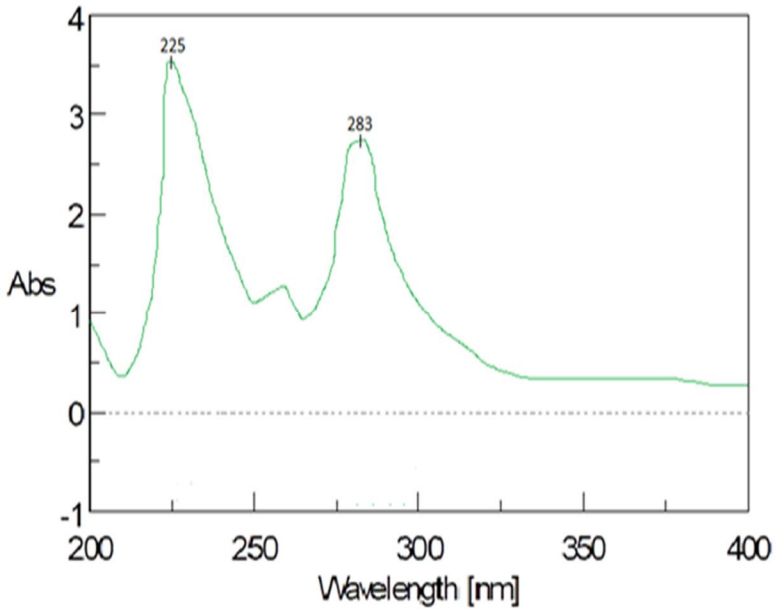

Fig. 1. UV of isolated component

The sample's maximum absorbance was measured at 225 nm (3.54) and 283 nm (2.81) for an isolated component made from *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. These values show that there are Taberdines L in the sample.

Infrared Spectroscopy: [21-23]

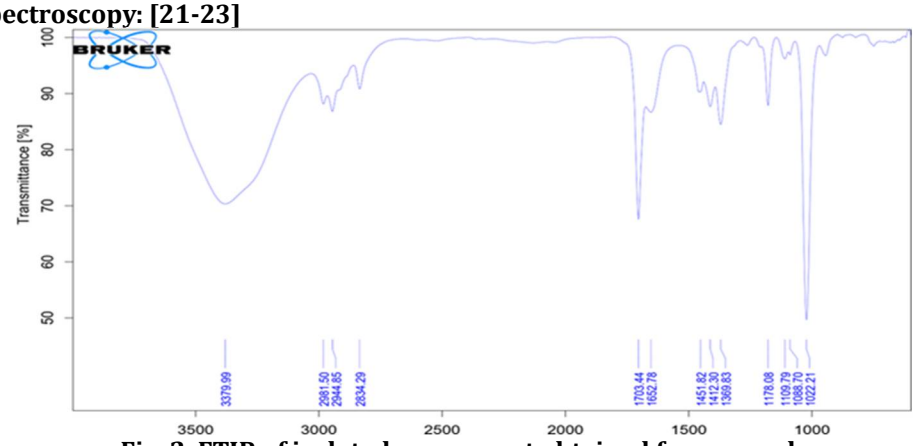

Fig. 2. FTIR of isolated component obtained from sample

Table 4. FTIR of isolated component from sample

Sr.no.	Peak value (cm^{-1})	Functional group	Assignment
1.	3379.99	Secondary amine	N-H stretch
2.	2981.50, 2944.85 & 2834.29	Aromatic C-H	C-H stretch
3.	1451.82 & 1412.30	Aromatic C=C	C=C stretch
4.	1369.83	Nitro group	N-O stretch
5.	1178.08 & 1109.79	Amine C-N	C-N stretch
6.	1088.70 & 1022.21	C-O	C-O stretch

According to the FTIR spectrum shown above, the functional group of standard Taberdines L is almost the same when compared to a reference sample and an isolated component. Therefore, Taberdines L may exist based on the IR spectrum.

Docking [24-25]

Anti-HIV Evaluation

Table 4. Amino Acid with Type of Interaction for Anti-HIV Activity

Sr.no.	Amino Acid	Type of interaction
1.	LEU23A	Hydrophobic Interaction
		Van Der Waal Interaction
2.	ASP25A	Van Der Waal Interaction
3.	GLY49A	Van Der Waal Interaction
4.	ILE50A	Van Der Waal Interaction
5.	ILE84A	Hydrophobic Interaction
		Van Der Waal Interaction
6.	GLY127B	Hydrophobic Interaction
		Van Der Waal Interaction
7.	ALA128B	Van Der Waal Interaction
8.	GLY149B	Hydrophobic Interaction
		Van Der Waal Interaction
9.	ILE150B	Hydrophobic Interaction
		Van Der Waal Interaction

Table 6. Result of GA docking for 3NU3

Receptor	Drug/ Ligand	Docking Score
3NU3	Taberdines L	-5.138906

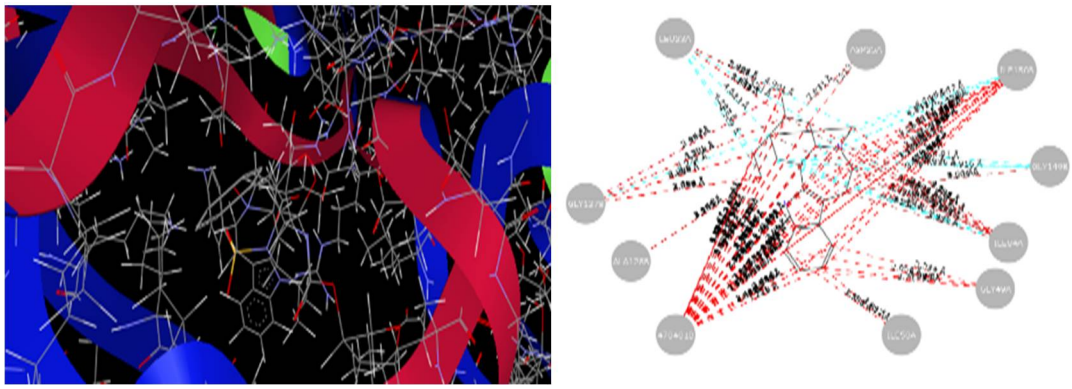

Fig.No.3 – 3D and 2D representations of docking for anti-HIV activity

CONCLUSION

Phytochemical analysis of the leaves of *Tabernaemontana divaricata* (L.) R. Br. ex Roem. and Schult. revealed the presence of alkaloids, flavonoids, tannins and phenolic compounds, carbohydrate, glycosides, and protein. Aqueous and ethanolic extracts both showed promise as HIV inhibitors. Ethanolic extract exhibits more anti-HIV activity than Aqueous. Chemical separation was used to separate Taberdines L from an ethanolic extract; its presence was then confirmed utilizing spectroscopic analysis (UV, IR) techniques. Isolated component interacts better with receptor based on docking score.

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