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Short Communication

Screening of *Phyllanthus Niruri* Leaf Phytoconstituents by *Insilico* and *in vivo* Methods for Anticonvulsant Activity

Panjugula Manisha, Kusampudi Preethi Alekhya, Likitha Lakshmi Rajyam, Harishma T, Pallavi G, and Konda Swathi*

Department of Pharmacy, Institute of Pharmaceutical TechnologyIndia

***Corresponding author**

Konda Swathi, Department of Pharmacy, Associate Professor, Institute of Pharmaceutical Technology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati 517101, Andhra Pradesh, India

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Keywords

- Anti-convulsant Activity; Phyllanthus Niruri; Estradiol; Maximal Electroshock-Convulsant Method; Syrup Formulation

Abstract

Background: In the present study, we evaluated anticonvulsant activity of Phyllanthus niruri leaves powder by Insilico and in vivo methods. A molecular docking investigation of leaf components with certain proteins associated with diseases was carried out using Schrodinger Maestro (v13.0) software.

Materials and Methods: Higher scores were also seen in the molecular docking data when compared to typical medications that are easily accessible. Invivo activity was carried out by 1 method i.e., Maximal Electroshock-Induced Convulsions.

Results and Discussion: The animals were then treated with 100mg/kg body weight i.p of Estradiol syrup formulation. According to the findings of the acute toxicity trials, estradiol was not harmful in nature on 300mg/kg concentration by showing 80% cell viability. The number of viable animals tends to decrease as the concentration of Estradiol increases.

Conclusion: Phyllanthus niruri leaves powder exhibited anticonvulsant activity in Maximal Electroshock Induced convulsion method.

ABBREVIATIONS

Mg: Milligram; Kg: Kilogram; GP: Glycoprotein; BBB: Blood brain barrier; GI: Gastro Intestine; TPSA: Total Polar Surface Area.

INTRODUCTION

Chanka piedra (Phyllanthus niruri Linnaus) plant belongs to the Euphorbiaceae family. It is widespread throughout the world's tropical and subtropical nations. This is an annual herb that is common along India's coast. It has a very limited lifespan and has been employed in the Indian ayurvedic system for over 2000 years. The 600–700 species of the Phyllanthus genus, which includes the common field weed P. niruri, differ just a little from one another [1].

Over 450 million people worldwide, according to WHO, have at some point in their life dealt with mental, neurological, or behavioral difficulties [2]. As estimated 50 million people worldwide suffer from epilepsy with countries with lower middle incomes accounting for higher than 85% of instances [3,4]. 221 taxa from 53 plant families were included as epilepsy therapies nine of the most significant 16th and 17th century herbal treatments in Europe [5].

A typical neurological symptom of epilepsy, which is brought on by excessive and hypersynchronous electrical discharges in the brain, is recurrent, unprovoked seizures. There are numerous recognized epilepsy syndromes, each of which affects the neurological system differently and in which seizures are important phenotypic components [6]. The episodic high-frequency impulse firing of a group of brain neurons, also known as focus, is linked to seizures. A local abnormal discharge that begins elsewhere in the brain may later spread there [7].

Insilico studies

ADMET properties: ADMET profiles are calculated [8], to provide a quick evaluation of a compound's in vivo behaviour that can be used to assess its potential for use as a drug. The physicochemical properties, including the octanol/water partition coefficient (LogP), topological polar surface area (TPSA), rotatable bond count (RB), molecular hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and molecular weight (MW), were also determined using the SwissADME webserver [9].

Molinspiration: A web-based programme called Molinspiration is used to get data on variables like Milogp, TPSA (Total Polar Surface Area), the amount of rotatable bonds, drug likeness data of molecules, and drug bioactivity predictions [10].

Mlogp: It is a significant measure utilized in rational design to evaluate both molecular hydrophobicity and good permeability across the cell membrane.

Total Polar Surface Area (TPSA): It is an incredibly accurate predictor of drug transport properties including intestinal absorption, bioavailability, BBB penetration, etc. and is directly related to a molecule's hydrogen bond potential.

Drug likeness: Drug similarity scores were computed by considering the amount of rotatable bonds, molecular weight, and heavy element count, number of hydrogen acceptors, hydrogen donors, and volume.

Molecular docking studies [11, 12]

Using a programme named Schrodinger, docking studies for anti-Parkinson's and antioxidant activities are conducted. (Version 13.0). Less energy is needed to bind the target, the higher the negative number. Effective substances bond with less force.

MATERIALS AND METHODS

Phyllanthus niruri methanolic extract

Materials: Phyllanthus niruri leaves, methanol, Soxhlet apparatus, Wattman filter paper, Heating mantle, round bottom flask.

Plant Material: Phyllanthus niruri leaves were gathered, dried, and ground into a fine powder. The range of 45-212m (8%), 212-600m (35%), 600m-1.18mm (43%) and 1.18-3.35 (14%) is the range of the particle size distribution (%w/w) obtained by sieving.

Solvent Extraction [13]: A Whatman 25 mm by 100 mm cellulose thimble was filled with a sample of leaves weighing 25 grammes. 250ml of 90% ethanol was used as the solvent for the traditional Soxhlet procedure. The heating power was changed to two [2], cycles per hour in order to finish six [6], cycles of extraction in three hours while keeping the mantle's temperature below 60°C. The acquired crude extract solutions were concentrated, and then it was left to air dry at room temperature. In order to reduce component damage, higher temperatures were avoided. Prior to gravimetric weighing to assess the yield, all extracts were maintained at room temperature.

$$\%\text{yield} = \frac{\text{Amount of crude extract obtained}}{\text{Total amount of powder taken}} \times 100$$

Preliminary evaluation of Phyllanthus Niruri [14]

Test for Alkaloids

Dragondroff'sTest: Dragendroff's reagent was added to the 2ml test solution. The presence of alkaloids is indicated by a reddish-brown precipitate.

Test for Glycosides

Legal's Test: Pyridine and alkaline sodium nitroprusside were added to 2ml of test solution to produce a blood red hue.

Test for Flavonoids

Shinoda Test: A few pieces of magnesium ribbon were added to the 2 ml test solution, and then drops of conc. H2SO4 were added to it. The coloring appears pink scarlet or crimson red.

Test for Tannins: Ferric chloride test: In order to give the 2 ml test solution a blue-green hue, ferric chloride was added.

Test for Proteins and Amino Acids

Millon's test: 2 ml of the test solution are mixed with Millon's reagent, which forms a white precipitate that turns red when heated.

Test for Steroids

Liebermann-Burchard Test: The test solution was mixed with 3–4 drops of acetic anhydride, boiled, cooled, and 3 drops of concentrated H2SO4. At the intersection of the two layers, a brown ring develops. The presence of steroids is indicated by the upper layer turning green.

Test for Triterpenoids

Salkowski Test: A little amount of concentrated H_2SO_4 (3ml) and 2ml of chloroform were added to the test solution and thoroughly mixed. Triterpenoids are present because of the reddish-brown colour that is present.

In vivo Activity

Acute Toxicity Studies [15]: Materials: 0.1% Sodium CMC.

Test compound Animals: Male wistar rats

Method: The Wistar rats utilised in this investigation weighed between 150 and 250 g when they were adults and healthy. After an overnight fast, each group's animals are divided into one. The test chemical was administered orally in dosages of 10, 30, 100, 300, and 1000 mg/kg in CMC solution with 0.1% sodium. The only substance given to the animals in the control group was vehicle (0.1% sodium CMC). The animals were monitored for 48 hours after receiving the test drug to note any mortality.

In vivo Anticonvulsant Activity

Maximal Electroshock - Induced Convulsion [16]: The effectiveness of the test formulation's anticonvulsant was examined using an electro-convulsometer. Six groups of healthy male Wistar rats, each weighing 150–250g, were divided after a 24-hour fast. The test formulation, suspended in sterile saline, was administered intravenously at a dose of 100 mg/kg body weight. The only treatment given to the animals in the control group was vehicle (normal saline). The test started 30 minutes after the intravenous injection. Corneal electrodes were used to deliver electrical current to the brain in order to maximize seizures. The stimulus parameter for mice was a 200 ml pulse of 50 mA given at 60 Hz. The anticonvulsant's efficiency was compared to that of the reference drug, pheytoin (25 mg/kg), by the elimination of the tonic extensor spasm in the hind limbs.

FORMULATION [17]

Preparation of Estradiol syrup Formulation ingredients (Table 1).

Formulation methodology: Take 66.66gm of sucrose and add 30ml of distilled water to it and place them on the heating mechanical stirrer with the temperature set of 60°C for 30minutes. After vigorous stirring add 16.6mg of estradiol and glycerin to the above solution and again stir it on the stirrer for 10minutes. Now add flavoring agent vanillin and colouring agent amaranth red and add remaining 70ml of distilled water and again set for stirring for 10ml. Now filter the 100ml solution through filter paper for any particulate matter and transfer it into a clean and neat amber colour bottle.

RESULTS AND DISCUSSION

Soxhlet Extraction

The yield of the extract after Soxhlet extraction was 3.72% (w/w), and the extracted amount was 0.93 gm.

Preliminary Phytochemical Studies

The test revealed the presence of phytochemical constituents are discussed in the Table 2.

The leaf phytoconstituents of Phyllanthus niruri plant were screened for Insilico docking studies and Invivo studies.

Table 1: List of Formulation Ingredients and its Role

Ingredients	Quantity	Role
Estradiol	16.6mg	Steroid
Sucrose	66.66gm	Preservative
Sodium benzoate	2mg	Preservative
Glycerin	1ml	Sweetening agent
Vanillin	1ml	Flavoring agent
Amaranth red	1ml	Coloring agent
Distilled water	100ml	-

As per literature review the dose of Estradiol was taken as 16.6 mg

Table 2: Phytochemical analysis of leaf extract of Phyllanthus niruri roots

S. No	Phytoconstituents	Test	Observation	Report
1	Carbohydrates	Molisch test	Purple ring	Present
2	Glycosides	Legal's test	Blood red colour	Absent
3	Alkaloids	Dragandroff's test	Reddish brown ppt	Present
4	Flavanoids	Shinoda test	Crimson red	Present
5	Steroids	Liebermann-Burchard test	Green colour	Present
6	Triterpenoids	Salkowski test	Yellow colour	Present
7	Tannins	Ferric chloride test	Blue green colour	Present
8	Protein and Amino acids	Millon's test	Red colour	Absent

The leaf phytoconstituents of Phyllanthus niruri plant were screened for Insilico docking studies and Invivo studies

Insilico Studies

Molinspiration (Table 3 and 4)

A higher bioactivity score indicates a higher likelihood of being more active. Compounds with a bioactive score more than 0.00 are highly active, while those with a score between -0.50 and 0.00 are moderately active, and those with a score below -0.50 are inactive (Table 5).

The yellow portion (yolk) is for high probability of brain penetration, and the white part is for high probability of passive absorption by the gastrointestinal tract. Yolk and white regions are not incompatible. Additionally, the points are coloured red if they are projected to not be a P-gp substrate (PGP) and blue if they are predicted to be actively effluxed by P-gp (PGP+) (Figure 1-6, Table 6-11).

CONCLUSION

The aforementioned data lead us to the inference that the phytoconstituents found in Phyllanthus niruri leaves showed potential for anticonvulsant activity when tested in vivo. We also support more comprehensive ADMET and molecular docking studies, which demonstrated Estradiol's potent anticonvulsant properties. This study may offer fresh ideas for the creation of detailed pharmacological and toxicological studies as well as for the eventual patenting of the developed formulation.

Table 3: Physicochemical properties of Phyllanthus niruri leaf phytoconstituents

Compound	Total polar surface area	No of atoms	M.Wt	nON	nOH NH	No of violation s	No of rotatable bonds	Volume
Nirurin	245.29	47	664.66	15	9	3	8	571.60
Nor securine	29.54	15	203.24	3	0	0	0	182.22
Phyllanthin	55.40	30	418.53	6	0	0	13	409.59
Nirphyllin	84.87	32	448.51	8	1	0	12	415.99
Niranthin	64.64	31	432.51	7	0	0	12	407.97
Estradiol	40.46	20	272.39	2	2	0	0	268.74
Ricinoleic acid	57.53	21	298.47	3	2	1	15	326.88

Table 4: Prediction of the bioactivity of plant components in leaves

Compound	MI bioactivity score	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Nirurin	2021.03	-0.18	-0.94	-0.71	-0.44	-0.07	-0.14
Nor securine	2021.03	0.03	-0.18	-0.46	-0.41	-0.29	0.06
Phyllanthin	2021.03	-0.02	-0.06	-0.08	0.01	0.00	-0.05
Nirphyllin	2021.03	-0.02	-0.08	-0.14	-0.05	-0.03	-0.02
Niranthin	2021.03	-0.03	-0.12	-0.19	-0.09	-0.05	-0.09
Estradiol	2021.03	0.18	0.20	-0.36	0.95	-0.02	0.81
Ricinoleic acid	2021.03	0.38	0.27	-0.02	0.52	0.30	0.53

A higher bioactivity score indicates a higher likelihood of being more active. Compounds with a bioactive score more than 0.00 are highly active, while those with a score between -0.50 and 0.00 are moderately active, and those with a score below -0.50 are inactive.

Table 5: Swiss ADME data of leaf phytoconstituents

Compound	Physiochemical properties				Lipophilicity	Pharmacokinetics			Lipinski rule	BA score	Lead likeness
	N rotb	H-acceptors	H-donors	TPA (A^0)	mlogp	GI	BBB	P-gp			
Nirurin	8	15	9	245.29	-2.35	Low	No	Yes	No	0.17	No
Norsecurin	0	3	0	29.54	1.68	High	Yes	No	Yes	0.55	No
Phyllanthin	13	6	0	55.38	2.43	High	Yes	Yes	Yes	0.55	No
Nirphyllin	12	8	1	84.84	1.39	High	Yes	No	Yes	0.55	No
Niranthin	12	7	0	64.61	1.91	High	No	No	Yes	0.55	No
Estradiol	0	2	2	40.46	3.53	High	Yes	Yes	Yes	0.55	No
Ricinolenic acid	15	3	2	57.53	3.69	High	Yes	Yes	Yes	0.85	No

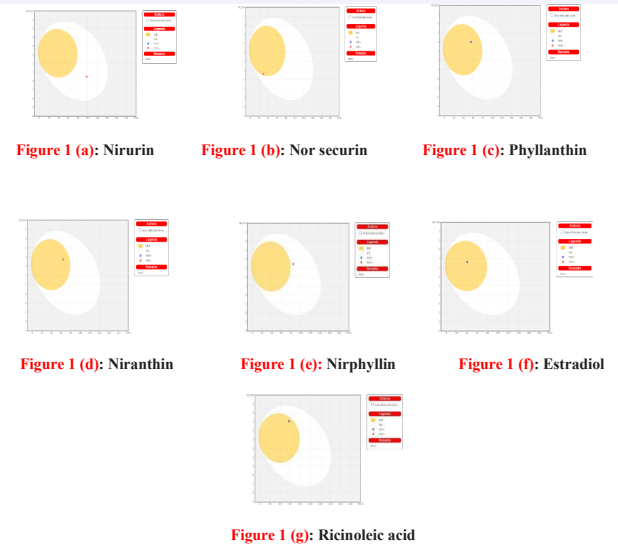

Figure 1 Boiled egg representation of Phyllanthus niruri leaf phytoconstituent

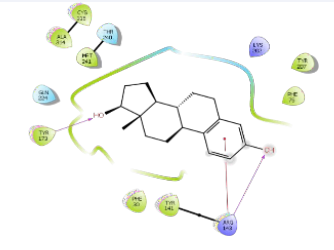

Figure 2 Interaction of Estradiol with interacting amino acid residues of protein 2A1H protein.

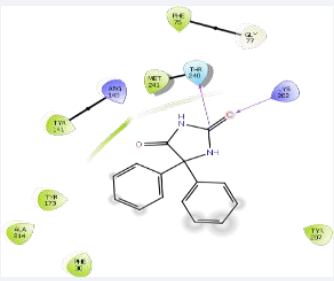

Figure 3 Interaction of Phenytoin with interacting amino acid residues of 2A1H

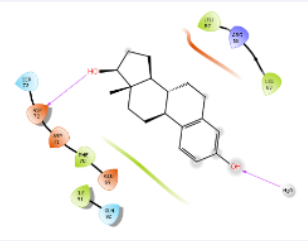

Figure 4 Interaction of Estradiol with interacting amino acid residues of 3F8E protein

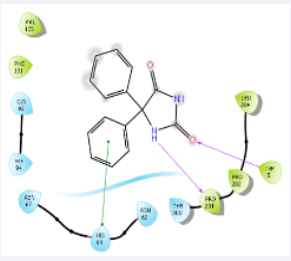

Figure 5 Interaction of Phenytoin with interacting amino acid residues of 3F8E protein

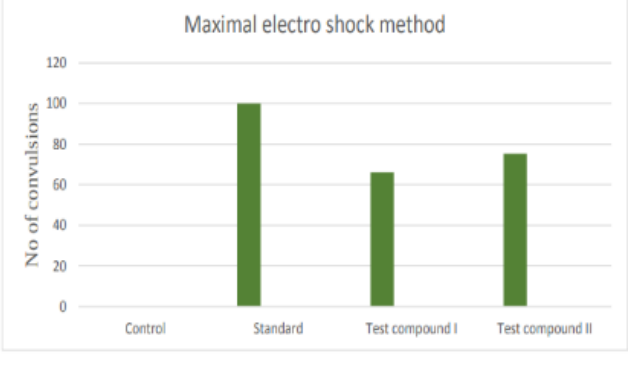

Figure 6 Graphical representation of Maximal electroshock method

Table 6: Docking studies of some leaves phytoconstituents showing anticonvulsant activity

S.No	Compound	Glide score	
		2A1H	3F8E
1	Nirurin	-4.420	-4.406
2	Nor securin	-4.392	-4.036
3	Phyllanthin	-3.369	-3.477
4	Niranthin	-4.395	-3.725
5	Nirphyllin	-3.782	-3.709
6	Estradiol	-4.937	-4.748
7	Ricinoleic acid	-0.202	-0.490
8	Phenytoin	-4.362	-4.740

Table 7: The interactions between the ligands that received the best scores in docking tests and the 2A1H protein are shown below.

Protein	Ligand	Amino acid interaction	Type of interaction	Binding affinity
2A1H	Estradiol	TYR:173	OH Conventional H-Bonding	-4.937
		ARG:143	OH Conventional H- Bonding	
		ARG:143	Pi cation	
2A1H	Phenytoin	THR:240	C Conventional H-Bonding	-4.362
		LYS:202	O conventional H-Bonding	

Table 8: The interactions between the ligands that received the best scores in docking tests and the 3F8E protein are shown below.

Protein	Ligand	Amino acid interaction	Type of interaction	Binding affinity
3F8E	Estradiol	ASP:72	OH Conventional H- Bonding	-4.748
3F8E	Phenytoin	PRO:201	NH Conventional H- Bonding	-4.740
		HS:64	Pi-Pi staking	
		TRP:5	O Conventional H-Bonding	

Table 9: Phytochemical parameters of developed Estradiol syrup

Physicochemical parameters									
S.NO	Colour	Odour	Taste	pH	Specific gravity (wt/ml)	Density (wt/ml)	Viscosity (Centi pose)	Crystallization	Turbidity
Trail I	Pale pink	Characteristic odour	Sweet	6.42	1.102g/ml	0.182	0.65	NF	No
Trail II	Dark pink	Characteristic odour	Sweet	6.58	1.22 g/ml	0.22	0.71	NF	No

Table: 10: Trail I: Stability studies through Physicochemical Parameters of developed syrup formulation

Sample	Time duration	Temperature (°C)	Physicochemical parameters								
			Colour	Odour	Taste	pH	Density (wt/ml) at 25°C	Specific gravity (g/ml)	Viscosity (Centipose)	Crystallization	Turbidity
1A	24hrs	4°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
1B		Room temperature	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
1C		47°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
2A	48hrs	4°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
2B		Room temperature	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
2C		47°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
3A	72hrs	4°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
3B		Room temperature	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO
3C		47°C	NC	NC	NC	6.42	0.182	1.102	0.65	NF	NO

Table 11: Trail II: Stability studies through Physicochemical Parameters of developed syrup formulation

Sample	Time duration	Temperature (°C)	Physicochemical parameters								
			Colour	Odour	Taste	pH	Density (wt/ml) at 25°C	Specific gravity (g/ml)	Viscosity (Centipose)	Crystallization	Turbidity
1A	24hrs	4°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
1B		Room temperature	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
1C		47°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
2A	48hrs	4°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
2B		Room temperature	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
2C		47°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
3A	72hrs	4°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
3B		Room temperature	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO
3C		47°C	NC	NC	NC	6.58	0.214	1.22	0.719	NF	NO

Table 12: Dose dependent acute toxicity studies

DOSE (mg/kg)	TOXIC SYMPTOMS & IRRATABILITY
10	-ve
50	-ve
100	-ve
300	-ve
1000	Found irritability

Selected dose was 100mg/kg b.w (oral)

Table 13: Anticonvulsant by Maximal electroshock method

Treatment	Dose	MES induced Convulsions
Control	100mg/kg	0
Phenytoin (Standard)	25mg/kg	100
Test compound I (Estradiol syrup formulation)	100mg/kg	66.1±0.141
Test compound II (P. niruri leaves extract)	300mg/kg	75.2±0.338

Data is presented as a mean ± SEM (n = 6). Experimental group was compared with control
*p<0.0001, considered significant. Experimental group was compared with standard
**p<0.0001, considered significant.

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Conflict of Interest Statement

The authors claim that the study presented in this paper was not impacted by any financial or personal conflicts that they are aware of.

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