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CURRENT PERSPECTIVES ON THE PHYTOCHEMICAL, PHARMACOLOGICAL, ETHNOBOTANICAL, AND MOLECULAR DOCKING PROFILE OF *PHYLLANTHUS NIRURI* WITH SPECIAL REFERENCE TO THE MECHANISMS OF HEPATOPROTECTION

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ABSTRACT

Phyllanthus niruri L., "Stone-Breaker" or "Bhumyamalaki", holds a colossal position in the traditional medicinal system of the world. This robust annual herb is globally adaptable to tropical and subtropical zones and maintains a large pool of active metabolites even when grown in nutrient deficient soils. Anatomically, it is characterised by a unique phyllanthoid branching architecture. Phytochemical profiles reveal that the plant is a major repository of diverse bioactive compounds including marker lignans (phyllanthin and hypophyllanthin), flavonoids (quercetin), tannins (corilagin), alkaloids and terpenoids. Recent pharmacological studies validate its traditional uses with potent antiurolithic activities by modulating the kinetics of calcium oxalate crystals to inhibit their adherence to the renal tissue. Furthermore, it exhibits strong hepatoprotective, antiviral and antifibrotic effects by reducing oxidative stress, inhibiting Hepatitis B replication and suppressing pro-inflammatory pathways such as NF-κB. In silico molecular docking validates *P. niruri* as a biological resource of high value for targeted clinical interventions.

KEYWORDS: *Phyllanthus niruri*, hepatoprotective, molecular docking, phyllanthin, geographical distribution.

INTRODUCTION

Phyllanthus niruri L. (Family: Phyllanthaceae) is a small erect, annual herb which `holds a monumental status across traditional medicine systems worldwide.[1] This medicinal plant is widely known in Indian Ayurveda as 'Bhumyamalaki' and is also often mentioned in Traditional Chinese Medicine (TCM) and South American indigenous folk practices. Due to its wide spectrum of therapeutic activity, this plant has gained enormous clinical attention.[2, 3] Popularly referred to as 'Stone-Breaker' ('Quebra-Pedras' in Brazil), it has been a traditional remedy for urolithiasis, specific liver disorders and viral outbreaks for centuries.[4] Over the last few decades, ethnopharmacological validating frameworks have been actively trying to match traditional applications with evidence-based molecular targets. *P. niruri* has demonstrated powerful therapeutic mechanisms targeting intricate metabolic cascades, showing anti-hepatotoxic, anti-hyperglycemic, anti-viral, anti-inflammatory and immunomodulatory interventions.[5]

Geographical distribution

The biogeography of the genus *Phyllanthus* is extremely large, with cosmopolitan adaptation profiles across tropical and subtropical zones. *P.niruri* is widely distributed in warm regions and grows in maritime and coastal borders, rainforest ecosystems and riverine basins throughout Tropical Africa, Americas, Asia and Oceania.[7, 8] The herb is a highly adaptive wild proliferator in the Indian subcontinent. It occurs naturally in rural and agricultural landscapes in states such as Jharkhand, Bihar, Chhattisgarh and several southern coastal provinces.[1] Its vegetative cycle is strongly linked to seasonal macro-climates; the plant emerges just after the first monsoonal showers in June, becomes fully mature in fruit between late July and August, and stays in wild fields until the middle of midwinter.[1, 9] The plant exhibits a profound ecological resilience to establish itself in nutrient-depleted soils, riverbeds, road margins and highly disturbed settings, establishing itself as a hardy weed, while conserving its biological reservoir of high-value active metabolites.[1, 10]

Description of the genus

Phyllanthus is one of the largest and most structurally diverse genera in the family Phyllanthaceae (formerly classified as a part of Euphorbiaceae) consisting of more than 700–800 distinct species, classified in 11 complicated sub-generic groups.[7, 8] Within the genus,

structural and anatomical variations are profound, from small annual prostrate herbs, highly specialized climbers, robust shrubs to large arborescent trees.[8] A key taxonomic character of most sub-genera is the presence of 'phyllanthoid branching', a unique morphological feature where the main vertical axes are vegetative with scale-like leaves (cataphylls), while the lateral branches mimic compound leaves, carrying true, beautifully arranged green foliage and supporting the reproductive flower arrangements.[11] The main traditional formulations used all over Southeast Asia, Brazil, India and China are built on specific herb groups within the subgenera Kirganelia, Cicca and Phyllanthus.[8] This is especially the case for highly evaluated therapeutic sister species, such as *Phyllanthus amarus, Phyllanthus urinaria, Phyllanthus fraternus* and the heavy-canopy tree *Phyllanthus emblica* (Indian Gooseberry).[1, 12, 13] Precise molecular barcoding and classical taxonomy prevent accidental substitution among these species in commercial drug production because their secondary metabolite expressions and specific therapeutic margins differ significantly.[1, 14]

Morphology of Phyllanthus niruri

Phyllanthus niruri is visually described as an annual, erect, completely glabrous herb with an average height profile between 30 to 60 cm.[1] The structures of the vertical stem are characterized by a clean smooth surface and are often branched just at the immediate base, establishing an elegant, bushy architecture.[1,15] The foliage is of a peculiar structural organization, which often confuses non-botanists into believing that it is a pinnately compound structure. Regarding its leaf architecture, the leaves are numerous, subsessile, distichous and closely arranged along the horizontal lateral branches in a distinct overlapping (imbricating) fashion.[1] For dimensions, single leaf blades are elliptic-oblong to obtuse with entirely smooth margins and fine acutely pointed stipules protecting the leaf-stem junction.[1, 16] Looking at the floral system, the species is monoecious, that is, separate male and female floral organs are developed independently on the same single plant specimen.[1] For its reproductive elements, flowers are very small, yellow white with bases tinged with a little red, in axillary clusters, male flowers in groups of 1 to 3, female flowers strictly solitary.[1] They have six distinct tepals/sepals surrounding a small anther assembly of about 0.25–0.4 mm.[1, 17] Finally, for capsules and seeds, the fruit capsules are strongly compressed/oblate, stramineous in texture, with fine reticulate veining patterns, a payload of tiny seeds and a total diameter of approximately 3 mm.[1, 17]

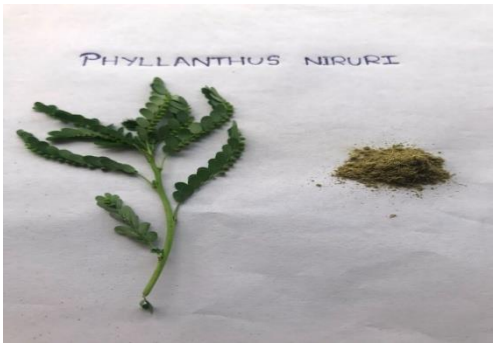

Fig 1: The whole morphology of *Phyllanthus niruri.*

Anatomical Features of Phyllanthus niruri

Leaf Anatomy (Lamina and Midrib)

Transverse sections of the leaf lamina show an internal dorsiventral (bifacial) organization delimited by a uniseriate epidermis of regular, thin-walled parenchymatous cells covered by a definite, smooth protective cuticle.[1] Stomatal distribution is predominantly hypostomatic, characterized by paracytic (parallel-celled) complexes that occur more frequently on the lower epidermis.[1] The internal mesophyll is distinctly differentiated into two separate zones. For the palisade layer, it is located on the upper adaxial side, comprising tightly packed, vertically elongated parenchymatous cells, which are abundant in chloroplasts.[1,42] Regarding the spongy layer, it is positioned on the lower abaxial side, featuring loosely arranged, isodiametric parenchyma cells with prominent intercellular spaces.[42] Finally, the central midrib region contains a localized collateral vascular bundle, surrounded by a parenchymatous bundle sheath. The cortical zones are filled with non-glandular trichomes, calcium oxalate crystals (prisms or druses) and specialized secretory structures such as laticifers.[42]

Stem Anatomy

The internal cross-section of the stem of *P. niruri* shows a typical dicotyledonous arrangement. Regarding the epidermis and hypodermis, the boundary of the plant is a single-layered epidermis of compact cells with cuticle, and immediately beneath this is a collenchymatous hypodermis of 2 to 3 layers of cells providing early mechanical support.[1] Looking at the cortex, the main cortex is made up of multiple layers of loosely arranged thin-walled parenchymatous cells with prominent intercellular spaces.[41] For the vascular architecture, the vascular tissue is a continuous closed tube, where a thin outer band of phloem elements is separated from the deeply lignified, radially arranged xylem vessels by a narrow, active cambial strip.[1, 41] Finally, focusing on the pith, the center of the stem is filled

with a large, wide pith of thin-walled parenchymatous cells, often containing calcium oxalate crystals for storage.[41]

Root Anatomy

In mature plants the underground system forms a reinforced secondary arrangement. Looking at the periderm, the periphery is bounded by a thin periderm, made up of a few layers of flattened, brown cork cells (phelloderm).[43] For the cortex, the underlying cortical zone is relatively narrow and compressed, made up of closely packed, secondary parenchymatous cells.[43] Finally, regarding the vascular elements, the stele is dominated by a large medullary core of secondary xylem vessels and thick-walled xylem fibers with narrow, uniseriate or biseriate medullary rays distributed throughout.[43, 44] The xylem core is highly lignified and surrounded by a narrow peripheral band of secondary phloem elements which facilitates efficient transport of nutrients.[44]

Phytochemical reviews

The remarkable pharmacological versatility of *P. niruri* is directly related to its uniquely rich, highly dense chemical blueprint. The detailed qualitative, quantitative and Gas Chromatography-Mass Spectrometry (GC-MS) profiles reveal that the leaves, fruits and stems are major storage sinks for various classes of active secondary metabolites.[5] Quantitative screening of leaf extracts showed significant output of active molecules with average total alkaloid content of 81.00 ± 0.57 mg/g, total phenol concentration of 110.00 ± 1.00 mg GAE/g and total flavonoid content of 7.43 ± 0.40 mg RU/g of extract.[5] These isolated compounds are part of the following major groups of structural categories. For lignans, the most important isolated active molecules are phyllanthin and hypophyllanthin.[4] These compound matrices are used as major chemical markers for the species, exhibiting structural symmetry that guides powerful hepatoprotective and cell stabilizing profiles.[18] Other variants isolated are neolignans, norlignans and sesquineolignans.[7] Focusing on flavonoids, the plant contains broad flavonoid complexes, mainly quercetin, rutin, iso quercitrin, astragalin and kaempferol.[1,19] These molecules have high free radical scavenging potentials because of their heavily hydroxylated phenolic rings.[6] Regarding tannins and polyphenols, the plant exhibits high density polyphenolic strings, including massive arrays of hydrolyzable tannins, such as corilagin, geraniin, gallic acid, ellagic acid and chebulagic acid.[1,20] Corilagin is highly regarded for its ability to block specific cellular growth cascades.[1] Looking at alkaloids, they present certain heterocyclic nitrogen compounds like

isoquinoline, securinega type structures and norsecurinine.[5, 21] These basic compounds show selective affinity to microbial cell membranes and cellular receptors.[5] Finally, for terpenoids and sterols, the major compounds are lupeol, beta-sitosterol, phytol, oleanolic acid and specific diepoxy-hexadecane derivatives.[4, 5, 22] These lipophilic fractions play a key role in the modulation of tissue inflammatory pathways and in the stabilization of membrane structures.[5]

Structure of representative compounds

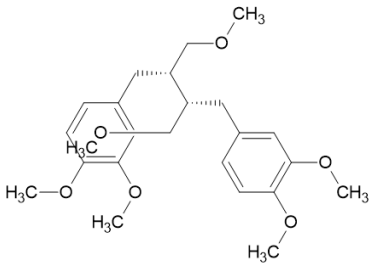

Fig 2:- Phyllanthin.

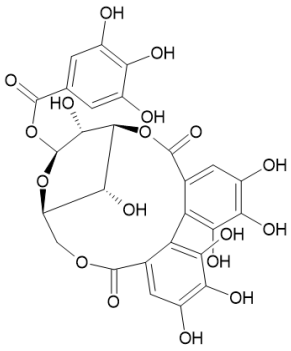

Fig 3:- Corilagin.

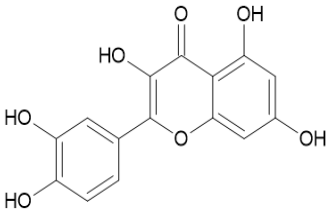

Fig 4:- Quercetin.

Pharmacological properties

Modern pharmacological evaluations have subjected crude aqueous, methanolic, and ethanolic extracts of *P. niruri*, as well as its purified isolated compounds, to rigorous in vitro and in vivo models. These studies validate many of its traditional applications.

Intervention for Nephrolithiasis (Antiurolithic Activity)

P. niruri is true to its historical name ‘stone breaker’ and is an effective therapeutic agent against urolithiasis.[4] In vitro crystallization models using human urine samples from healthy cohorts show that the presence of *P. niruri* extract changes the core physical dynamics of stone formation significantly.[4, 23] It does not prevent initial precipitation but causes calcium oxalate (CaOx) crystals to develop as much smaller particles and encourages the formation of Calcium Oxalate Dihydrate (COD) structures rather than Calcium Oxalate Monohydrate (COM).[4] This modification is clinically relevant because COD crystals have dramatically lower adhesion properties, and they cannot stick to renal tubular epithelial cells.[4, 24] In vivo rat models of induced bladder calculi, regular oral administration of *P. niruri* decoctions has been shown to limit the growth of stones, reduce the excretion of urinary crystallization promoters such as calcium, and induce profound smooth muscle ureteral relaxation.[4, 25] This

mechanical relaxation helps in clearing stone fragments and aiding painless passage of the urinary tract.[4]

Hepatoprotective and Antiviral Effects

The modern clinical fame of *P. niruri* was first driven by its extraordinary effectiveness against Hepatitis B virus (HBV) and chemically induced liver injury.[2, 8] The active marker lignans, phyllanthin and hypophyllanthin, and some hydrolyzable tannins are powerful hepatoprotective agents.[18, 20] Mechanistically, these compounds inhibit lipid peroxidation of the hepatocellular membranes, maintain internal cellular glutathione stores, and restore elevated serum biomarkers including Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) after exposure to extreme toxins such as carbon tetrachloride (CCl_4) or paracetamol.[26, 27] In terms of anti-viral performance, the extract inhibits HBV DNA polymerase, prevents the binding of Hepatitis B surface antigen (HBsAg) to its target receptors, and disrupts intracellular viral replication cycles, without damaging healthy parenchymal tissue.[8, 28]

Indepth Review of Hepatoprotective Mechanisms

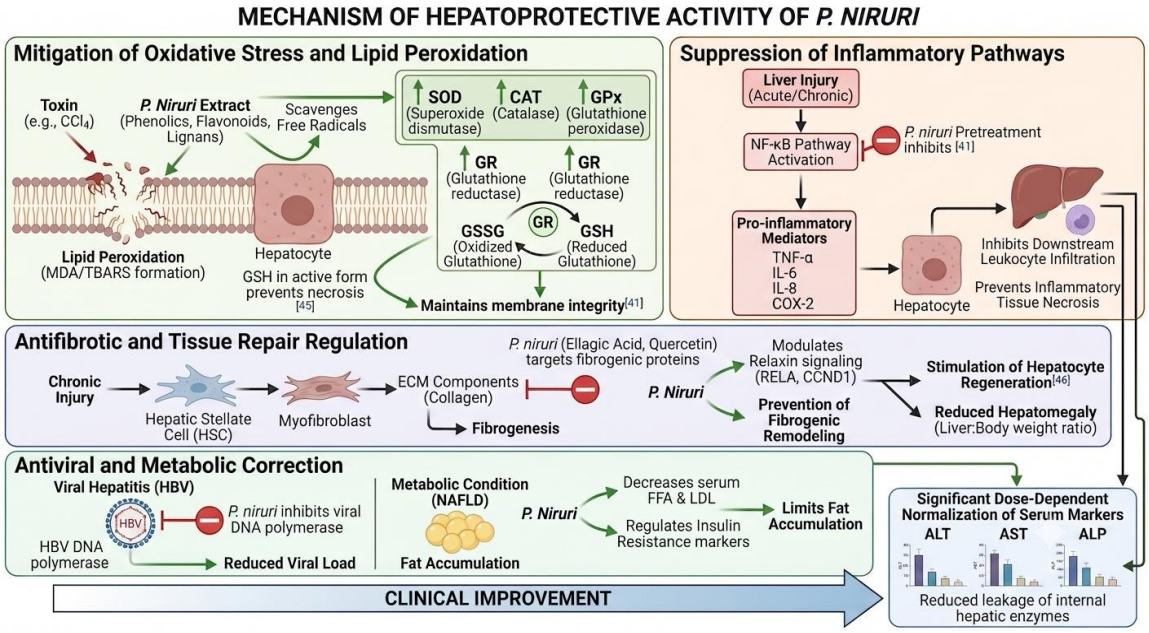

Fig. 5- A Schematic diagram represents mechanism of hepatoprotective action for *Phyllanthus niruri.*

Mitigation of Oxidative Stress and Lipid Peroxidation: The main mechanism of *P. niruri* in protecting the liver is its potent antioxidant activity, which is largely due to its high content of phenolics, flavonoids and lignans (e.g., phyllanthin and hypophyllanthin).[1, 44] *P. niruri*

significantly decreases lipid peroxidation (measured by malondialdehyde or TBARS formation) and maintains the structural integrity of the hepatocyte plasma membrane under the influence of hepatic toxins. It upregulates and restores the baseline activities of essential endogenous antioxidant enzymes, including.[41, 44] Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GSP), Glutathione reductase (GR), This restorative process keeps intracellular glutathione (GSH) in its active, reduced form and efficiently scavenges toxic free radicals before they cause cell necrosis.[41, 44]

Suppression of Inflammatory Pathways: In response to chronic or acute liver injury, a profound cascade of pro-inflammatory signaling molecules is released. Extracts of *P. niruri* given as a pretreatment efficiently down-regulate the expression of key nuclear transcription factors and inflammatory mediators.[44] It inhibits the nuclear factor kappa B (NF-κB) pathway, which directly results in a significant reduction in the expression of pro-inflammatory cytokines including:Tumor necrosis factor-alpha (TNF-α), Interleukin-6 (IL-6), Interleukin-8 (IL-8), Cyclooxygenase-2 (COX-2), This herb suppresses this chemical signal loop to inhibit downstream leukocyte infiltration and prevent inflammatory tissue necrosis within the hepatic lobules.[44]

Antifibrotic and Tissue Repair Regulation: Chronic injury results in the conversion of hepatic stellate cells (HSCs) to myofibroblasts, which causes an excessive accumulation of extracellular matrix (ECM) components (fibrogenesis). Molecular docking and modern network pharmacology analyses have shown that the bioactive compounds present in *P. niruri* (e.g., ellagic acid and quercetin) directly target structural proteins involved in fibrogenesis.[45] The herb modulates the relaxin signaling pathway and targets important proteins such as RELA and CCND1.[45] This results in prevention of fibrogenic remodeling, reduction in liver:body weight ratios (hepatomegaly) and stimulation of true hepatocyte regeneration.[45, 46]

Antiviral and Metabolic Correction: In viral hepatitis, *P. niruri* inhibits Hepatitis B virus (HBV) replication by interfering with endogenous viral DNA polymerase activity, thereby reducing the viral load and protecting the adjacent tissue.[1] Mechanistically, it also limits fat accumulation in metabolic conditions such as Non-Alcoholic Fatty Liver Disease (NAFLD) by decreasing serum free fatty acids, low-density lipoproteins (LDL), and regulating insulin resistance markers.[46] These cellular protections are directly responsible for clinical improvement by reducing the leakage of internal hepatic enzymes and leading to a significant

dose-dependent normalization of serum markers including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP).[41, 44, 46]

Anti-Proliferative and Anticancer Activity

In vitro screening in several human malignant cell lines, including HT29 (colorectal carcinoma), HepG2 (hepatocellular carcinoma), MeWo (skin melanoma), A549 (lung carcinoma) and MCF-7 (breast cancer), demonstrates that extracts of *P. niruri* are highly selective anti-proliferative agents.[1] The plant is rich in polyphenols and tannins, especially in corilagin, which causes cell cycle arrest at the G2/M checkpoint and induces cell death (apoptosis) via activation pathways of caspase-3 and caspase-9.[1, 29] Moreover, corilagin inhibits tumor invasion, migration, and tissue adhesion by blocking the transforming growth factor β1 (TGF-β1)/Akt/extracellular signal-regulated kinase (ERK)/Smad signaling pathways.[1,30] The administration of hydroalcoholic extracts in animal models of skin carcinogenesis was able to reduce papilloma yields, showing a strong chemopreventive potential.[1, 31]

Anti-Microbial and Anti-Fungal Action

The extract has shown strong bactericidal and fungicidal activities against Gram-positive and Gram-negative pathogenic strains.[6, 32] Studies to test its efficacy against oral pathogens such as Enterococcus faecalis, Porphyromonas gingivalis, and Treponema denticola show a significant Zone of Inhibition (ZOI).[33] This antimicrobial activity is attributed to the combined action of saponins, flavonoids and tannins, that damage the integrity of the microbial cell wall and inhibit the internal energy metabolism.[5, 33] Methanolic fractions also exhibited very high activity against common test organisms like Bacillus cereus and Escherichia coli.[6]

Antioxidant, Anti-Inflammatory and Analgesic Effects

The total phenolics and flavonoids concentrations in *P. niruri* are high enough to inhibit free radicals as shown by DPPH, ABTS and Ferric Reducing Antioxidant Power (FRAP) assays.[6] The ethyl acetate and methanolic fractions were highly efficient in scavenging reactive oxygen species (ROS).[6, 34] This antioxidant activity is closely related to its anti-inflammatory properties. Purified phytocompounds like phytol, vitamin E and 4-methoxy-2,3,6-trimethylphenol inhibit the production of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and cyclooxygenase-2 (COX-2) enzyme activity.[5, 35] Animal pain models have shown

a marked reduction of nociceptive responses, which supports its traditional use for joint pain, colic and inflammatory diseases.[6, 36]

Anti-Plasmodial and Anti-Diabetic Activities

Extracts of *P. niruri* and related species have shown considerable anti-plasmodial activity against both chloroquine-sensitive and chloroquine-resistant strains of Plasmodium falciparum and Plasmodium berghei.[7, 37] This activity helps to mitigate the high oxidative stress and severe anemia associated with malaria infections.[7] The flavonoids and tannins of the plant have been reported to be potent inhibitors of α-glucosidase and α-amylase enzymes and thus act as regulators of metabolism to control hyperglycemia,[3, 38] It protects pancreatic beta-cells against oxidative damage in diabetic animal models, improves insulin sensitivity and helps restore normal fasting blood glucose profiles.[3, 39, 40]

***Insilico* molecular Docking studies**

The hepatoprotective potential of natural alkaloids phyllanthin against a panel of seven targeted liver associated therapeutic protein targets (PDB IDs: 8BHE, 5LDO, 2R3X, 3NXU, 4IQK, 2C6U and 2H14) was assessed and validated by an in silico molecular docking study by CB-DOCK2. The binding affinities, orientation and molecular interactions of these phytochemical ligands in the catalytic pockets of enzymes were systematically compared with standard synthetic hepatoprotective drugs namely Ursodeoxycholic acid, N-Acetylcysteine, Metdoxine & Bifendate used as hepatoprotective. In this context, the present comparative study aims toclarify the exact molecular mechanisms, receptor binding preferences and structural efficacy of phyllanthin in alleviating hepatic stress and improving liver function by comparing docking scores and hydrogen bonding profiles of the natural compounds with the established synthetic controls.

Table No-1: -Binding affinity for phytochemicals with target enzymes.

Target Enzymes	Binding affinity (KJ/Mol)				
	phyllanthin	Ursodeoxycholic Acid	N-Acetylcysteine	Metadoxine	Bifendate
8BHE	-7.8	-9.5	-5.0	-5.5	-7.6
5LDO	-6.0	-7.1	-4.8	-5.7	-7.0
2R3X	-8.1	-9.7	-4.9	-5.8	-7.3
3NXU	-7.1	-9.2	-4.8	-5.5	-6.7
4IQK	-7.6	-9.7	-4.8	-6.4	-7.6
2C6U	-5.1	-7.9	-4.3	-5.4	-5.8
2HI4	-8.1	-7.1	-4.9	-6.6	-7.6

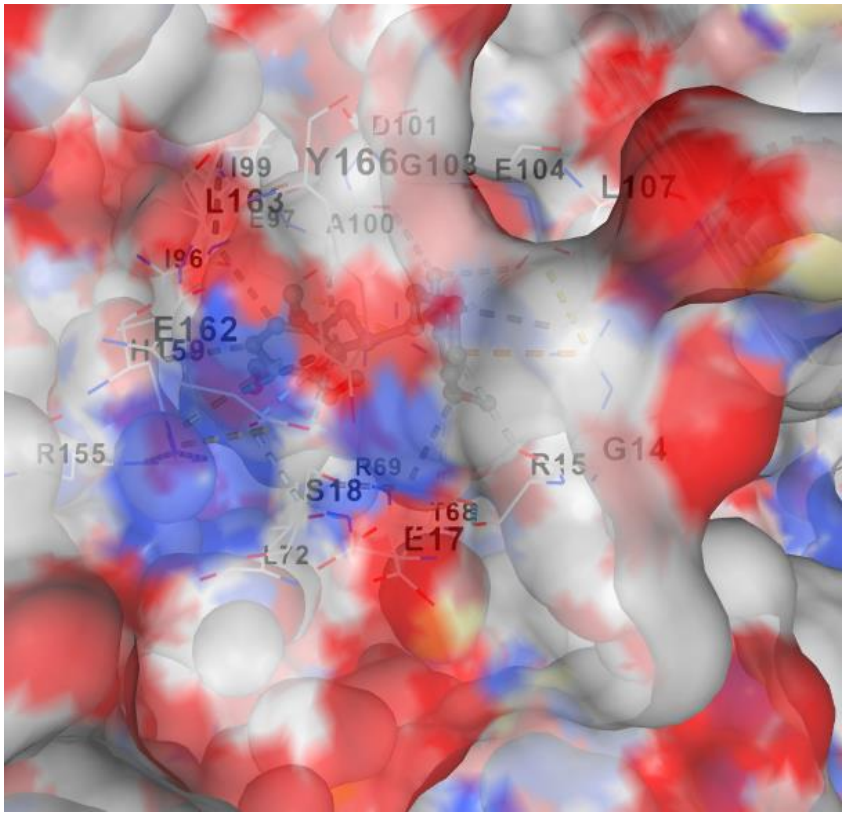

Fig. 6: Binding affinity of phyllanthin in 8BHE Target enzymes.

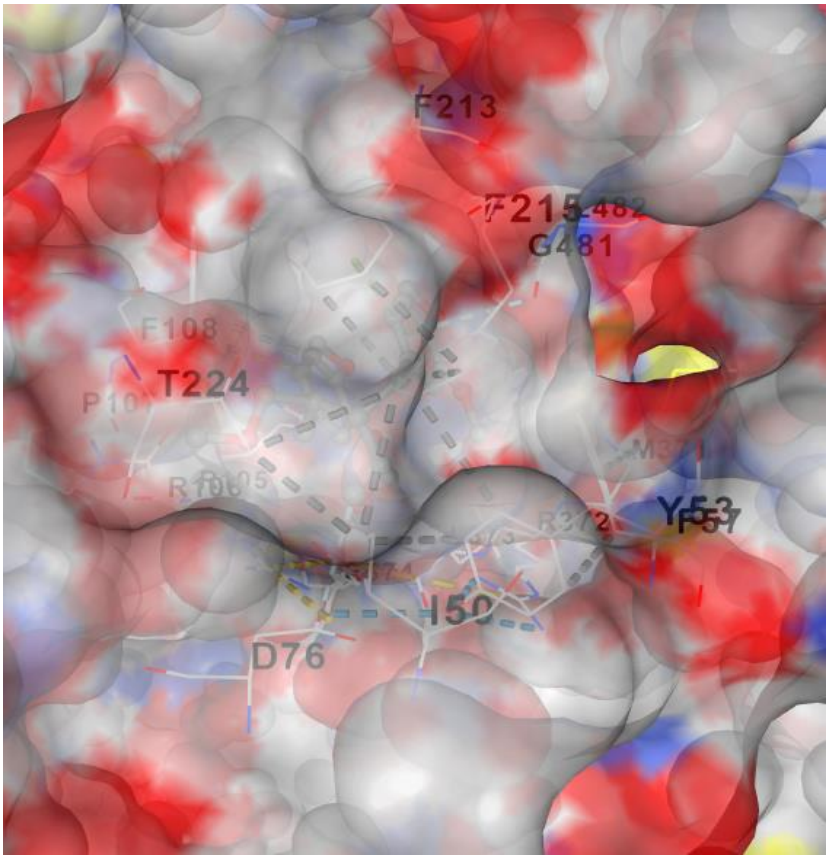

Fig. 7: Binding affinity of phyllanthin in 3XNU Target enzymes.

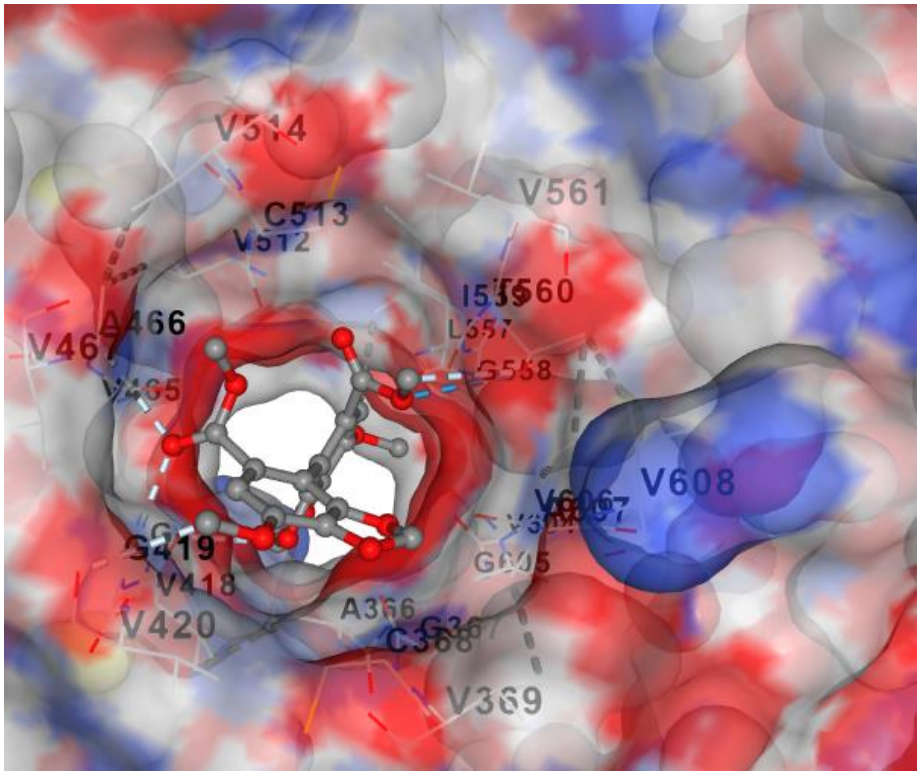

Fig. 8: Binding affinity of bifendate in 4IQK Target enzymes.

The comparative molecular docking analysis of the target compounds against seven key metabolic and therapeutic enzymes (8BHE, 5LDO, 2R3X, 3NXU, 4IQK, 2C6U, and 2HI4) showed distinct variations in the binding affinities expressed as Gibbs free energy (ΔG, kJ/Mol). Among the ligands evaluated, Ursodeoxycholic Acid exhibited the strongest inhibitory profile by showing the lowest binding energy for most of the target proteins. The highest binding intensities were observed for 2R3X and 4IQK (both at -9.7 kJ/Mol). This suggests a very stable thermodynamic interaction, which can be due to the favorable hydrophobic interactions and hydrogen bonding in the active sites. Phyllanthin and the standard drug Bifendate demonstrated high binding potential, particularly with 2HI4 and 2R3X proteins, with a binding affinity of -8.1 kJ/Mol for phyllanthin. On the other hand, N-Acetylcysteine showed the weakest binding profile across the entire panel, with affinities closely clustered between -4.3 kJ/Mol and -5.0 kJ/Mol, indicating much weaker intermolecular interactions with these particular target enzymes. Overall, these in silico results demonstrate that Ursodeoxycholic Acid and phyllanthin are potential candidates with high therapeutic targeting efficiency compared to the conventional standards such as Metadoxine and Bifendate.

CONCLUSION

In conclusion, *Phyllanthus niruri* is a highly adaptable and pharmacologically versatile medicinal plant that successfully bridges the gap between traditional folk practices and evidence-based molecular medicine. It is structurally and anatomically resilient, thriving in a diverse variety of ecosystems while hosting a dense high-value reservoir of secondary metabolites. Qualitative, quantitative and in silico studies confirm its multi-target therapeutic efficacy is due to its diverse chemical arsenal, mainly comprising of lignans, flavonoids, tannins, polyphenols and alkaloids. Its historical reputation as a "stonebreaker" has been validated by rigorous pharmacological testing, which shows that it can modify the dynamics of calcium oxalate crystallization and cause ureteral relaxation to allow for painless passage of stones. At the same time, it exerts powerful hepatoprotective and antiviral mechanisms, reducing oxidative stress, inhibiting damaging pro-inflammatory signaling pathways (TNF-α, IL-6 and NF-κB), preventing fibrogenic tissue remodeling and limiting replication of viral hepatitis. Apart from the anti-proliferative, antimicrobial, anti-plasmodial and anti-diabetic actions documented, *P. niruri* shows an enormous potential as a complete therapeutic agent. Molecular barcoding and reliable taxonomy will remain important for future development to maintain quality control and avoid accidental substitution and to guarantee that the full clinical and commercial potential of this powerful herb is realized safely.

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