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HEDERA NEPALENSIS: PHYTOCHEMISTRY AND PHARMACOLOGICAL ACTIVITIES

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ABSTRACT: This review explores the phytochemistry and pharmacological activities of *Hedera nepalensis*, commonly known as Chinese or Himalayan ivy. It contains various beneficial compounds such as saponins, phenolics, polyacetylenes, and essential oil. *H. nepalensis* shows strong antioxidant effects, fights bacteria, fungi, viruses (like influenza A and SARS-CoV-2), and even has anti-cancer properties. It helps reduce blood sugar levels and is used for diabetes management by blocking an enzyme called DPP-4. The plant also protects the liver, helps wounds to heal faster, and is effective against parasites. *H. nepalensis* is used to treat respiratory problems, joint pain, infections, and skin conditions in traditional medicine. Modern studies confirm these traditional uses and reveal new possibilities for its compounds to be used in medicines. In conclusion, *H. nepalensis* is a valuable plant with a wide range of health benefits. More research is needed to fully understand its potential, improve extraction methods, and develop safe, effective medicines from it.

INTRODUCTION: The family Araliaceae consists of approximately 70 genera and 700 species of flowering plants, including 15 species of the genus Hedera [1]. This genus mostly shows its presence in areas such as Asia, China, North Europe, and North America; also, significant presence can be observed in Northern Pakistan [2]. The species selected in the present study is *Hedera nepalensis* (synonym: *Hedera helix*), is used for treating rheumatism, lung infections, and fever [3]. *H. nepalensis* has been reported to contain flavonoids, tannins, steroids, terpenoids, and glycosides [1]. It possess antihelmintic, molluscicidal, antileishmanial, antifungal, spasmolytic, and sedative effects [4-6].

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According to previous studies, berries and leaves are used to cure indolent abscesses and ulcers and are diaphoretic, cathartic, and stimulating. Literature has documented *H. nepalensis* as a natural folk remedy (China), especially for managing diabetes [7, 8]. Studies have shown that *H. nepalensis* extracts have significant inhibitory effects on dipeptidyl peptidase-4 (DPP-4) [8]. Prominent antiviral effects were observed when utilizing a 30% ethanol extract of *H. helix*. Multiple studies have demonstrated that commercially available dry extracts of *H. nepalensis* are both safe and efficacious for treating respiratory issues in adults and children alike [9]. Additionally, research has shown that a 95% ethanol extract of *H. nepalensis* inhibited proliferation in the A549 human non-small-cell lung cancer cell line [10].

Phytochemistry:

Saponins: Saponins found in plant (**Table 1)** are of triterpene structure such as hederacosides B, C, and D; α-hederin, β-hederin and δ-hederin [11].

The contents of hederasaponin C and *α*-hederin were determined using HPLC-UV. Their percentages ranged from 0.40-4.01% and 0.21-0.54% based on absolute dry mass, respectively[12]. *α*-hederin and hederacoside Care one of the most frequently isolated triterpene saponins [13].

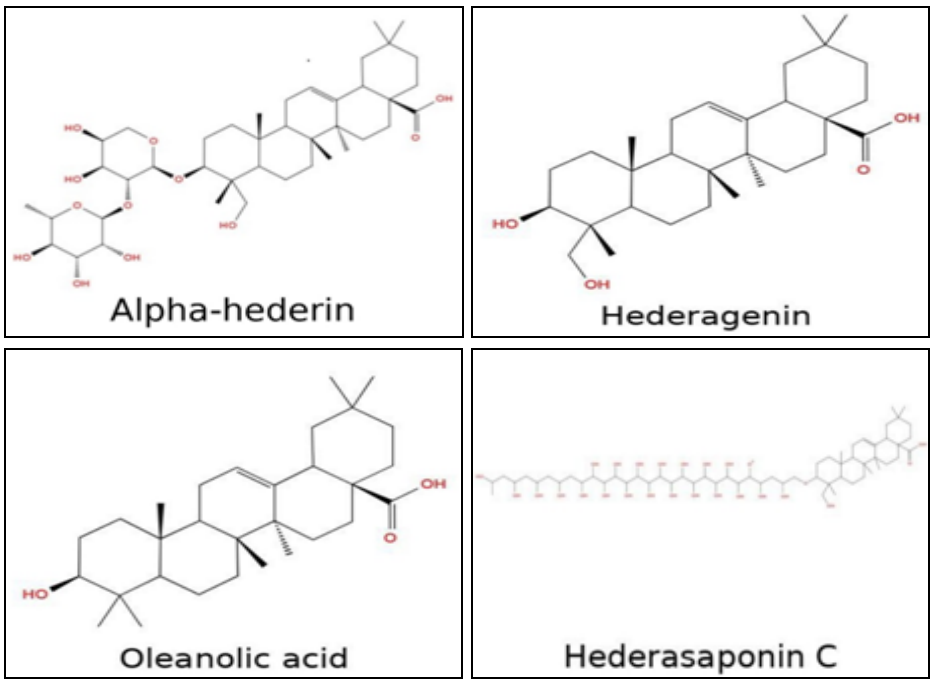

FIG. 1: CHEMICAL STRUCTURES OF TRITERPENE SAPONINS

Phenolic Compounds: The phenolic components of many *Hedera* species are poorly understood and only a small number of species have been thoroughly examined. HPLC and LC-MS were used to identify rutin and chlorogenic acid in *H. nepalensis* leaves and stem extract [14]. Catechin and caffeic acid are detected using HPLC-DAD in the aqueous and ethyl acetate extract, in a study intended to identify antioxidant components from the aerial part of plant. The highest concentration of phenolics found in the ethyl acetate extract [15].

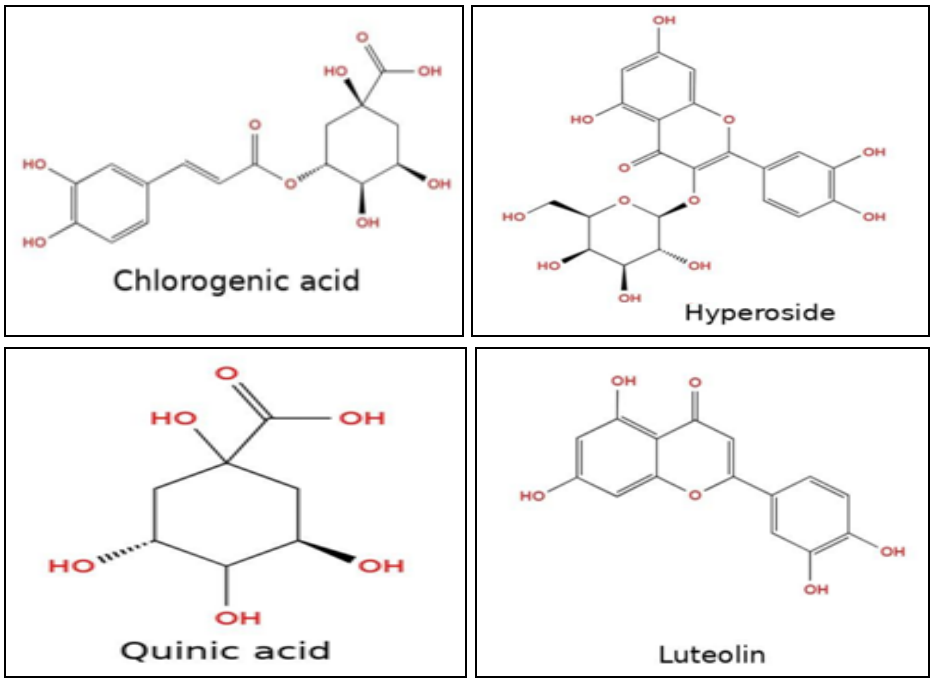

FIG. 2: CHEMICAL STRUCTURE OF PHENOLIC AND FLAVONOID COMPOUNDS

Polyacetelenes: Polyacetylenes have only been identified in a few plant families such as Apiaceae and Araliaceae. They are of interest to pharmacologists and plant pathologists owing to their antifungal and growth-inhibiting qualities [16]. Interestingly, falcarinol and didehydrofalcarinol, which are found in both species, are the main allergens in ivy that have irritating qualities and are moderate sensitizers [17]. Falcarinol, 11, 12 dehydrofalcarinol, didehydrofalcarinol, and falcarinone are among the polyacetylenes identified in the stems, leaves, fruits androots of *H. nepalensis* [18-20].

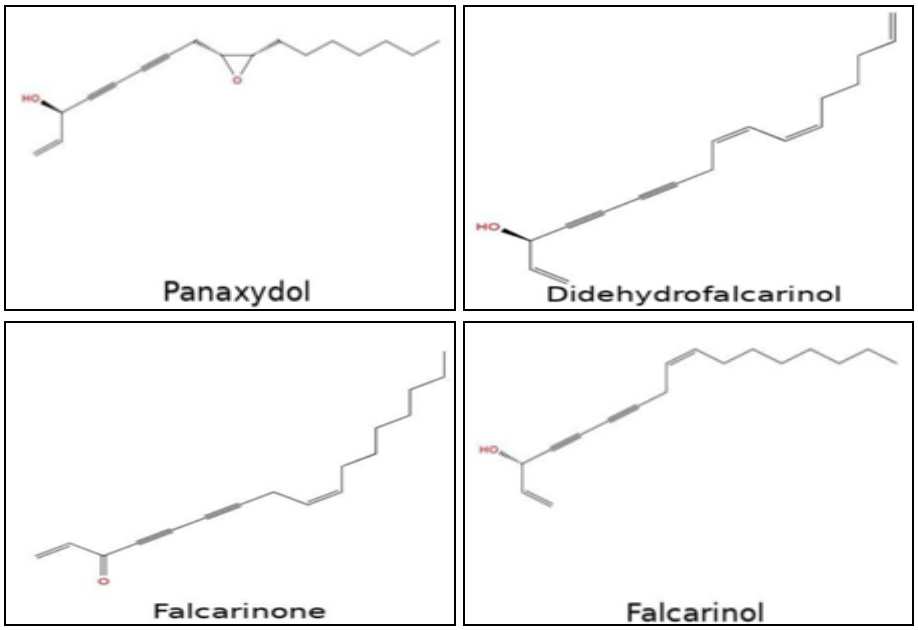

FIG. 3: CHEMICAL STRUCTURE OF POLYACETYLENES

Volatile constituents: Twenty-one chemicals were discovered by GC/MS analysis of the volatile oil that was hydro-distilled from *H. nepalensis* var. sinensis. The primary components of the oil were phthalic diisobutyl ester, caryophyllene oxide, sclareolide, spathulenol, *β*-caryophyllene, and *α*-caryophyllene (humulene) [21]. The most abundant compounds were limonene, *β*-pinene, sabinene, *β*-caryophyllene, germacrene D, and *α*-pinene, with concentrations ranging from 15.85 to 10.18% [22].

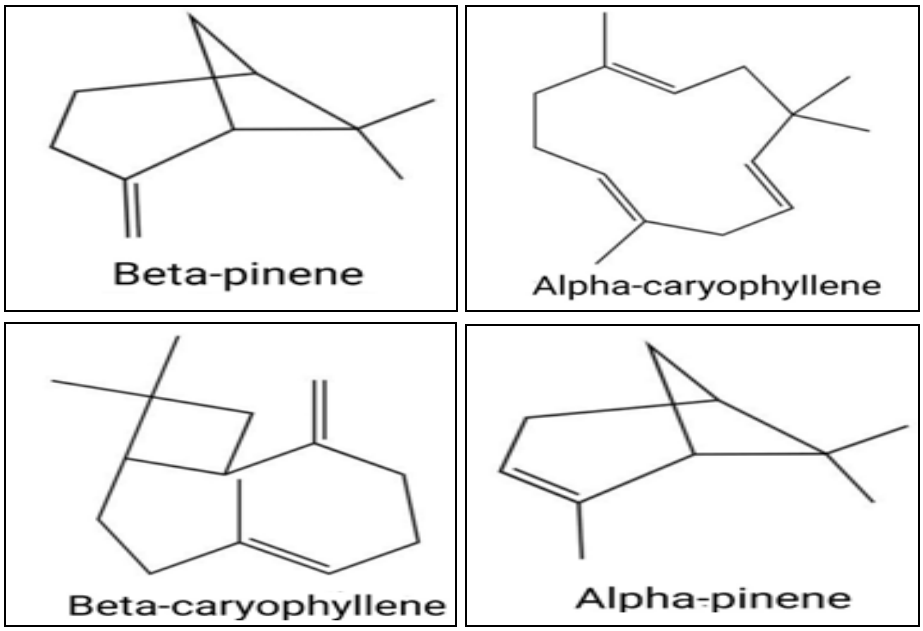

FIG. 4: CHEMICAL STRUCTURE OF VOLATILE CONSTITUENTS

Miscellaneous Compounds: Petroselinic, oleic, cis-vaccenic, and palmitoleic acids are fatty acid found in *H. helix*. Although cis-vaccenic and palmitoleic acids were accumulated in the pericarp, petroselinic acid was mostly found in the seeds [18]. TLC, HPLC, and GC-MS were used to separate, identify, and estimate the quantity of free amino acids in *H. helix*. Aspartic acid, phenylalanine, proline, leucine, isoleucine, glycine, valine, and tyrosine were found in the results. Proline was the most prevalent [23]. An Egyptian study team identified the alkaloid emetine in four Egyptian varieties of helix, including helix var. baltica, hibernica, margata, and erecta. This is the only report on the identification of alkaloids from the Genus Hedera [24]. Cholesterol, campesterol, stigmasterol, *β*-sitosterol, and *α*-spinasterolare representative forms of sterol in *H. helix* [18, 24].

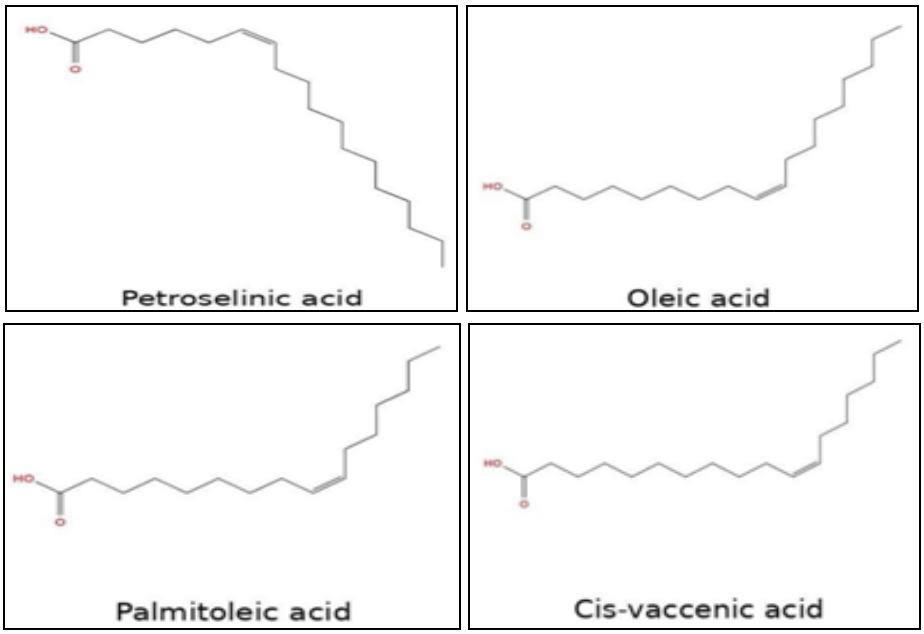

FIG. 5(A): CHEMICAL STRUCTURE OF MISCELLANEOUS COMPOUNDS

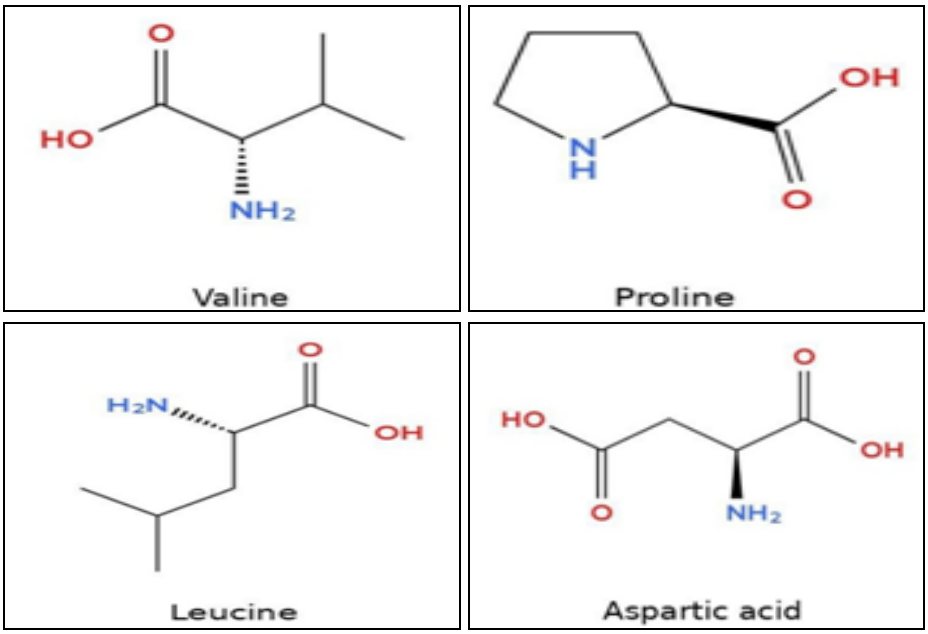

FIG. 5(B): CHEMICAL STRUCTURE OF MISCELLANEOUS COMPOUNDS

TABLE 1: PHYTOCHEMICAL COMPOUNDS OF *HEDERA NEPALENSIS* AERIAL PARTS

Compound type	Identified compounds
Saponins	HN-saponinsA,B,D1,D2,E,F,H,I,K,M,N,P; hederagenin 3-O-*α*-L-arabinopyranosyl 28-O-*β*-D-glucopyranosyl ester, hederagenin 28-O-*α*-L-rhamnopyranosyl-(1-4)-O-*β*-D-glucopyranosyl-(1-6)-O-*β*-

	D-glucopyranosyl ester, pulsatillasaponin, Lupeol [25-27]
Phenolic Acids	Quinic acid, neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, catechin, caffeic acid [14, 15]
Flavonoids	Rutin, hyperoside, 3,3′,4′,7-tetrahydroxyflavone, luteolin [14]

Pharmacological Activity:

Antioxidant Activity: The antioxidant properties of *H. nepalensis* and its compounds were studied extensively using various methods. Tests on *α*-hederin and hederasaponin C showed they could neutralize free radicals like DPPH, superoxide anions, and hydrogen peroxide, while also demonstrating metal-binding abilities [28]. Its antioxidant potential was evaluated using techniques such as ORAC, TEAC, DPPH bleaching, and others. Results revealed that leaves collected in winter had the highest antioxidant activity, followed by summer leaves, while flowers and fruits showed the lowest activity [29].

Scientists investigated the antioxidant capabilities of crude extract, ethyl acetate fraction, and aqueous fraction derived from *H. nepalensis*. Their findings revealed that these compounds exhibited the ability to neutralize free radicals in proportion to their concentration and offered protection against free radical-induced DNA damage. Among the tested samples, the ethyl acetate and aqueous fractions demonstrated the most potent antioxidant effects [1]. The methanolic extract of *H. nepalensis* was tested using the TBARS, DPPH radical-scavenging, ABTS radical-scavenging, and DNA protection assays. The results identified rutin and chlorogenic acid as the two main phenolic antioxidants responsible for the activity [14]. The antioxidant activity, along with the total flavonoid and phenolic contents, was examined in the crude extract of *H. nepalensis* and its fractions (n-hexane, ethyl acetate, and aqueous extracts). Using the phosphomolybdenum technique, the results showed that the ethyl acetate fraction had the highest overall antioxidant activity and reducing power, followed by the aqueous fraction, n-hexane fraction, and crude extract [15].

Cytotoxic Activity: The study found that the methanolic extract of *H. helix* was toxic to brine shrimp, mainly due to its phenolic compounds, while the saponins in the extract were inactive. Researchers tested three saponins from the plant-*α*-hederin, hederagenin, and hederacoside C-on cervical tumor and normal cells. Among these, *α*-hederin was the most effective at slowing tumor cell growth, hederagenin showed moderate effects, and hederacoside C had no impact. This suggests that *α*-hederin has potential as an anti-cancer agent [30]. Researchers examined the potential breast cancer-fighting properties of hederacolchiside A1 through both *in-vitro* and *in-vivo* studies. The compound demonstrated significant effects on various cell lines, particularly MCF-7 breast cells [31]. Additionally, the study assessed the anticancer properties of the methanolic extract derived from leaves and stems. This evaluation employed three methods: potato disc, radish seed phytotoxicity, and brine shrimp cytotoxicity tests. The findings revealed that the extract displayed considerable activity across all three assays [5].

Antimicrobial activity: The *in-vitro* antifungal activity of triterpenoid saponins from *H. helix*, including hederagenin, *α*-hederin, *δ*-hederin, and hederasaponin C, was tested using the agar dilution method. Monodesmosidic hederagenin derivatives showed broad efficacy against dermatophytes and *Candida* strains, with *C. glabrata* being the most susceptible. The most active component was found to be *α*-hederin [32]. Preparations derived from *H. helix* were evaluated for their antimicrobial properties against several bacterial species, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*. Among the tested extracts, those obtained using methanol and ethyl acetate demonstrated the highest efficacy in suppressing the growth of these bacterial strains. The high concentration of hederacoside C in *H. helix* leaves was identified as the main factor responsible for their strong antibacterial activity against 23 bacterial species from 22 genera (both Gram-positive and Gram-negative), as well as *Candida albicans* [24, 33].

In a study using the agar disc diffusion method, the 70% alcoholic leaf extract of *H. helix* demonstrated strong antibacterial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Proteus vulgaris*, *Campylobacter jejuni*, and *Candida albicans*, outperforming azithromycin and amphotericin [34]. Additionally, the flowers, fruits, and leaves showed antifungal activity against plant

pathogens like *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, and others, with fluconazole used as a comparison in the agar dilution test. The plant's phenolic content was credited for these effects [35-36]. In one study, the methanol-water (80:20) extract of *H. nepalensis* aerial parts showed no antibacterial activity against six bacterial strains (*Shigella flexneri*, *P. aeruginosa*, *E. coli*, *Salmonella typhi*, *S. aureus*, and *Staphylococcus methicillin*). In another study, the plant's crude methanolic extract and its fractions were tested for antibacterial and antifungal properties. The chloroform fraction showed 60% antibacterial activity against *S. aureus*, while other fractions and the methanolic extract displayed moderate to low antibacterial effects. However, the extract did not show any antifungal activity against *Rhizopus stolonifer*, *Fusarium oxysporum*, *Penicillium notatum*, *A. niger*, *A. flavus*, or *Trichoderma harzianum* [37].

Antiviral Activity: The antiviral properties of *H. helix* extract and its isolated compound, hederasaponin B, against Enterovirus 71 (EV71), a primary cause of hand, foot, and mouth diseases was studied. Efficacy was evaluated on EV71 sub-genotypes C3 and C4a in Vero cells using western blot analysis and cytopathic effect (CPE) reduction assays. Both hederasaponin B and a 30% ethanol extract of plant containing hederasaponin B showed significant antiviral activity. Additionally, hederasaponin B was found to reduce the expression of the viral VP2 protein, suggesting the inhibition of viral capsid protein synthesis [38]. A study examined the antiviral effects of *H. helix* against influenza A/PR/8 (PR8) virus. When orally administered with ivy extract, the antiviral activity of oseltamivir was significantly enhanced. Compared to oseltamivir alone, co-administration of the hederasaponin Frich fraction of ivy extract reduced pulmonary inflammation in PR8-infected rats. The treatment also decreased the infiltration of inflammatory cells into the bronchial alveoli of PR8-infected mice, along with a decline in tumor necrosis factor-alpha and chemokine (C-C motif) ligand 2 levels [39]. The extract from *H. helix* leaves was found to directly reduce SARS-CoV-2 *in-vitro* infection [40].

Anticancer Activity: Two main anticancer compounds from *H. nepalensis*: pulsatillasaponin A and hederagenin 3-O-α-L-arabinopyranoside were found to kill lung cancer cells (A549) and stop their growth in a dose-dependent manner. Further tests on cancer cells (MCF7, MDAMB231, and HeLa) showed that lupeol, along with *H. nepalensis* extract and its fractions, reduced cell growth by more than 50%. Lupeol was particularly effective against breast cancer cells, and both the n-hexane and ethyl acetate fractions, as well as pure lupeol, showed strong potential for cancer prevention [15, 41].

Antidiabetic Activity: Both normal and alloxan-induced diabetic rabbits showed hypoglycemic effects from the aqueous and methanolic extracts of *H. nepalensis* leaves, which markedly reduced blood glucose levels. *H. nepalensis* and its separated component were tested for their *in vitro* inhibitory effects on dipeptidyl peptidase 4 (DPP-4), a crucial target for the treatment of diabetes.

Strong DPP-4 inhibitory activity was retained by the crude extract, particularly when fractionated with ethyl acetate or n-hexane. The potential antidiabetic benefits of ethanolic extracts of leaves and stems were investigated. Whereas the stem extract had no signifant impact, the leaf extract dramatically lowered blood glucose levels. An adjuvant treatment for diabetic wounds and hyperglycemia may be provided by the leaf extract [42].

Anti-parasitic Activity: Monodesmosides such as α- and δ-hederin showed moderate anti-trypanosomal activity during *in-vitro* research against *Trypanosoma bruceibrucei*. Among this, α-hederin was the most effective, with a minimum inhibitory concentration (MIC) of 25 µg/ml. In contrast, bidesmosides such as hederacoside C and D, showed no activity at doses up to 100 µg/ml [43]. In an *in-vitro* study, the saponin complex 60% (CS 60), containing hederasaponin C, hederasaponin B, and phenolic compounds (rutin, caffeic acid, and chlorogenic acid), and the purified saponin complex 90% (CSP 90), containing primarily hederasaponin C and B, were tested for anthelminthic activity against the trematodes *Fasciola hepatica* and *Dicrocoelium spp.* After a 24-hour exposure, α-hederin was effective in killing both trematodes at concentrations of 0.005 and 0.001 mg/ml [24].

Molluscicidal Activity: Aqueous extracts of *H. nepalensis* leaves and fruits were tested on three snail species collected from Nigeria such as *Physaacuta*, *Bulinus sp.*, and *Biomphalaria pfeifferi*. Both extracts showed high mortality rates and were effective in killing all tested snails tested [44]. An experiment showed that hederacoside F and *α*-hederin were more effective than hederacoside C in killing *Mycobecterium tuberculata*, *P. corneus var. rubra* and *Planorbis corneus*.

Wound Healing Activity: *H. nepalensis* saponins, including hederacosides and *α*-hederin, support wound healing by enhancing collagen bundle thickness and organization, likely due to their antioxidant properties [29].

Hepatoprotective Activity: *H. nepalensis* extract showed hepatoprotective effects by reducing liver enzymes, oxidative stress markers, and improving antioxidant enzyme activity in models of CCl_4 and acetaminophen-induced liver damage [45]. The ethanolic extract of *H. nepalensis* leaves improved liver and kidney function by increasing albumin and total proteins, while reducing bilirubin, globulin, creatinine, and key enzymes (ALP, GGT, ALT, AST). In contrast, the stem extract showed no significant effects.

Antimycobacterial Activity: The percentage of growth inhibition was recorded for each suspension of AgNPs (silver nanoparticles) at varying concentrations. At 30 µL, the *H. nepalensis* showed 47.67±0.33 growth inhibition percentage, whereas it was found to have growth inhibition 97.33±0.31 at 40 µL concentration. The concentration of AgNPs was directly correlated with the growth inhibition %. The biosynthesized AgNPs have promising potential for the development of anti-TB nanomedicines [46].

CONCLUSION: *Hedera* species, particularly *H. nepalensis* demonstrate significant potential as sources of bioactive compounds with diverse pharmacological activities. These plants contain a wide array of phytoconstituents, including triterpene saponins, phenolic compounds, polyacetylenes, and volatile oils. The most prominent compounds identified are *α*-hederin, hederacoside C, and hederasaponin B. These findings highlight the therapeutic potential of Hedera species, especially *H. nepalensis*, in various medical applications. However, further research is needed to fully elucidate the mechanisms of action, optimize extraction methods, and develop standardized formulations for potential clinical use. The diverse pharmacological activities and rich phytochemical profile of this plant make it promising candidate for future drug discovery and development efforts.

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