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A Review Article on the Phytochemical Investigation and Anti-Cancer Activity of Vitex Negundo

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ABSTRACT

The ethanolic extract of Vitex trifolia has been investigated for its potential antitumor activity against Dalton's ascites lymphoma (DAL) in a murine model. In the study, tumor-bearing mice were administered the extract orally at a dose of 400 mg/kg body weight for 14 consecutive days, with 5-fluorouracil (20 mg/kg) serving as the standard anticancer drug. The antitumor effect was evaluated by assessing body weight changes, packed cell volume (PCV), viable tumor cell count, lifespan, and hematological parameters, including red blood cell (RBC) and white blood cell (WBC) counts. The results demonstrated that treatment with the ethanolic extract significantly reduced body weight gain, PCV, and viable tumor cell count. Additionally, the extract prolonged the lifespan of tumor-bearing mice and improved hematological parameters toward normal levels. These changes were statistically significant compared with the control group ($p < 0.01$), indicating a protective effect against tumor-associated alterations. Overall, the findings suggest that V. trifolia possesses promising antitumor potential and may serve as a source of bioactive compounds for further anticancer research. However, additional studies are required to identify its active constituents, elucidate the underlying mechanisms, and establish its safety and therapeutic efficacy through further preclinical and clinical investigations.

Keywords: Vitex trifolia, Antitumor activity, Dalton's ascites lymphoma, Ethanolic extract, Anticancer potential.

Introduction

Cancer is a heterogeneous group of diseases characterized by uncontrolled cell proliferation, impaired cell death, and the potential for invasion and metastasis. Tumours are broadly classified as benign or malignant, with malignant tumours capable of invading surrounding tissues and spreading to distant organs through the blood or lymphatic system. Cancer classification is based on the tissue or organ of origin and the type of cell involved, encompassing numerous distinct malignancies. The incidence and progression of cancer are influenced by multiple factors, including age, genetic predisposition, lifestyle, dietary habits, and environmental exposures. Cancer continues to pose a substantial public health burden in India, emphasizing the need for effective prevention, early diagnosis, and improved therapeutic strategies.

Despite significant advances in cancer management, conventional treatments, including chemotherapy and radiotherapy, are often associated with adverse effects, damage to normal tissues, and the development of therapeutic resistance. These limitations have intensified research into novel anticancer agents with improved efficacy, selectivity, and safety profiles. Natural products, particularly medicinal plants and their bioactive phytoconstituents, represent valuable sources of potential anticancer compounds. Various plant-derived molecules have demonstrated promising cytotoxic, antiproliferative, antioxidant, and apoptosis-inducing properties in experimental studies. Therefore, systematic phytochemical investigation and pharmacological evaluation of medicinal plants may contribute to the identification of novel therapeutic candidates. Further mechanistic, preclinical, and clinical studies are essential to establish their safety, efficacy, and potential applications in cancer treatment.

Botanical Description and Taxonomical Classification

A large shrub or sometimes a small slender tree; bark thin, grey; branchlets quadrangular, whitish with a fine tomentum. Leaves 3-5 foliate; leaflets lancoelate, acute, the terminal leaflet 5-10 by 1.6-3.2 cm. with a petiole 1-1.3 cm. long, the lateral leaflets smaller with a very short petiole, all nearly glaborous above, covered with a fine white tomentum beneath, base acute; common petioles 2.5-3.8 cm long.3-5 Flowersin pedunculate branched tomentose cymes, opposite along the quadrangular tomentose rachis of a large terminal often compound pyramidal panicle (axillary peduncles in the upper axils sometimes present); bracts 1.5- 2.5 mm long, lancoelatecaduceus. Calyx 3 mm long, white tomentose; teeth triangular, 0.8- 1mm long. Ovary glaborous; style glaborous; stigma forked. Drupe less than 6 mm diameter, black when ripe.3, 5, 7, 8 The plant has pungent, bitter, acrid taste; heating, astringent, stomachic, anthelmintic; promotes the growth of hair; useful in disease of the eye, consumption, inflammation, leucoderma, enlargement of the spleen, bronchitis, asthma, biliousness, painful teething of children. The root is an antidote to snake venom. The root is considered tonic, febrifuge and expectorant,5,6,7 otalgia, arthritis, dyspepsia, colic, rheumatism, leprosy, verminosis, flatulence, dysentery, urinary disorders, wounds, ulcers, bronchitis, cough, malarial fever, haemorrhoids, dysmenorrhoea, leprosy, skin diseases and general debility. The plant is reported to have expectorant,

carminative, digestive, anodyne, antiseptic, alterant, antipyretic, diuretic, emmenagogue, depurative, rejuvenating, ophthalmic, vulnerary and tonic.7 The leaves are aromatic, tonic and vermifuge.5,6A decoction of Nirgundi leaves is given with the addition of long pepper in catarrhal fever with heaviness of head and dullness of hearing. A pillow stuffed with the leaves of Nirgundi is placed under the head for relief of headache. The juice of the leaves is said to have the property of removing foetid discharges and worms from ulcers.3,5,6 The flowers are useful in diarrhoea, cholera, fever, haemorrhages, hepatopathy and cardiac disorders. Leaves and bark are useful in scorpion stings, seeds are considered useful in eye diseases in form of anjan.7 Tincture of root bark in 1 to 2 dr. doses is recommended in cases of irritable bladder and of rheumatism. Powdered root is prescribed for piles as a demulcent for dysentery. Root is used in dyspepsia, colic, rheumatism, worms, boils and leprosy.3, 6 The leaves are discutient and are useful in dispersing swelling of joints from acute rheumatism and of the testes from suppressed gonorrhoea. The dried fruit acts as a vermifuge. 5, 6Fruit is nervine, cephalic and emmenagogue; dried fruits acts as a vermifuge; flowers are cool and astringent.6 Special Characters Branchlets have prominent auxiliary spines and leaflets posses numerous minute glad dots. The fragrant white flowers contain many stamens. Fruits resemble wood apple. The tender shoots and leaves are quite brittle.

Pharmacognostical Characteristics of Vitex Negundo

The pharmacognostic evaluation of Vitex negundo leaves revealed important microscopic and quantitative characteristics, including a stomatal index of approximately 17.65, vein-islet number of 16.1, and palisade ratio of 1.16. Physicochemical analysis showed a total ash value of 5.20–6.4%, acid-insoluble ash of 1.06%, loss on drying of 3.1–6.50%, water-soluble extractive of 11.5%, and alcohol-soluble extractive of 11.5–26.2%. These parameters are useful for the identification, quality assessment, and standardization of the plant material. Phytochemical investigations have reported several bioactive constituents, including flavonoids such as vitexin, orientin, luteolin, apigenin, quercetin, and kaempferol; iridoids such as aucubin, agnuside, and negundoside; and terpenoids such as vitexilactone and vitetrifolin D. Additional constituents include lignans, alkaloids, and essential oil components, including linalool, α-pinene, limonene, and 1,8-cineole. The diverse phytochemical profile of V. negundo supports its pharmacological potential and highlights the importance of further investigation into its bioactive constituents and possible therapeutic applications.

Phytochemical screening of Vitex Negundo

Phytochemical investigations of Vitex negundo leaves have employed preliminary qualitative screening, chromatographic techniques, and antioxidant assays to characterize their chemical composition and biological potential. Preliminary phytochemical screening of the hydroalcoholic extract was conducted using standard qualitative tests to investigate the presence of flavonoids, tannins, saponins, terpenoids, sterols, steroids, reducing sugars, and alkaloids. These analyses relied on characteristic colour changes, precipitate formation, and froth development. Thin-layer chromatography (TLC) using silica gel GF254 plates and a chloroform–ethyl acetate–acetic acid solvent system (5:5:0.1) was employed to separate and identify phytochemical constituents by comparison with reference standards. Furthermore, gas chromatography–mass spectrometry (GC–MS) analysis of the hexane extract was performed using an HP-5MS column and helium as the carrier gas to characterize its volatile and other chromatographically detectable constituents. The antioxidant potential of the extract was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay at concentrations ranging from 12.5 to 200 µg/mL. Following incubation in the dark for 30 minutes, absorbance was measured at 517 nm, with all measurements performed in triplicate. Collectively, these analytical approaches provide a foundation for phytochemical characterization and preliminary assessment of antioxidant activity in V. negundo. However, the identification of specific bioactive compounds and their potential pharmacological relevance requires further confirmatory investigation.

Major Phytochemical Constituents of Vitex Negundo

Phytochemical investigations of Vitex negundo have revealed a diverse range of secondary metabolites, including flavonoids, lignans, terpenoids, iridoids, alkaloids, stilbenes, and essential oil constituents. The leaves contain flavonoids such as casticin, chryso-splenol, vitexin, and vitexicarpin, along with polymethoxylated flavones and the iridoid glycoside agnuside. Phytochemical studies of the seeds have identified lignans, including negundins and vitedonin, as well as triterpenoids such as betulinic acid and ursolic acid, the alkaloid vitrafalal, and the diterpene vitedoin. Further investigations have isolated lignan derivatives, including vitedoamine A, and several iridoid glycosides derived from mussaenosidic acid. The roots have also been reported to contain sesquiterpenes, flavone glycosides, iridoid glycosides, aucubin, and stilbenes. In addition, the leaves contain antioxidant constituents, including flavonoids, vitamin C, and carotenoids, which may contribute to the modulation of oxidative stress. Tannins and triterpenoids have also attracted interest for their potential anti-inflammatory and biological activities. Notably, bioassay-guided fractionation of the chloroform-soluble leaf extract yielded vitexicarpin, which exhibited cytotoxic activity against human cancer cell lines. These findings highlight the phytochemical diversity of V. negundo and support further investigation of its constituents as potential sources of anticancer and other pharmacologically active agents. However, their specific mechanisms, selectivity, and therapeutic efficacy require further validation.

Anticancer Potential of Vitex Negundo

Vitex negundo has attracted considerable research interest as a potential source of plant-derived anticancer agents owing to its diverse phytochemical composition, particularly flavonoids and phenolic compounds. Experimental studies have reported that extracts and isolated constituents of the plant exhibit antiproliferative and cytotoxic effects against various cancer cell lines, potentially through apoptosis induction and cell-cycle regulation. Among

the identified phytoconstituents, artemetin, vitexicarpin, and penduletin have demonstrated anticancer activity associated with increased reactive oxygen species (ROS) generation, DNA damage, and activation of caspase-mediated apoptotic pathways. Other flavonoids, including luteolin, kaempferol, and quercetin, have been investigated for their potential to modulate cancer-related signaling pathways involved in cellular proliferation and survival. Luteolin has also been associated with the regulation of CDK1/Cyclin B signaling in breast cancer stem cells, while modulation of the PI3K/AKT/mTOR pathway represents a potential mechanism underlying the anticancer effects of flavonoids. Furthermore, the root-derived fraction VN-Root-EA-P3 has exhibited cytotoxic and genotoxic effects against lung cancer cells, with comparatively lower toxicity reported in normal human dermal fibroblasts. These findings indicate that V. negundo may represent a promising source of bioactive compounds for anticancer drug discovery. Nevertheless, the available evidence is predominantly experimental, and further studies are required to establish the molecular mechanisms, cancer-cell selectivity, pharmacokinetic properties, safety, and therapeutic efficacy of these compounds through appropriate preclinical and clinical investigations.

Anticancer Mechanism of Vitex Negundo

The anticancer potential of Vitex negundo is attributed to its flavonoid-rich phytochemical profile, which may influence multiple cellular signaling pathways involved in tumor development and progression. Among these, the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin (PI3K/AKT/mTOR) pathway plays a crucial role in regulating cell proliferation, survival, metabolism, and protein synthesis. Dysregulation of this pathway is frequently associated with several malignancies, including breast, lung, colon, liver, and prostate cancers. Flavonoids such as quercetin, kaempferol, luteolin, apigenin, naringenin, and vitexin have been investigated for their potential to modulate cancer-associated signaling pathways and promote apoptosis. In addition, V. negundo extracts have been proposed to influence nuclear factor kappa B (NF-κB) signaling, thereby potentially reducing inflammation-associated cellular survival. Other proposed mechanisms include cell-cycle arrest, inhibition of angiogenesis, suppression of tumor cell proliferation, and modulation of oxidative stress.

The PI3K/AKT/mTOR signaling cascade also interacts with ribosomal protein S6 kinase (p70S6K) and Unc-51-like autophagy-activating kinase (ULK), linking cellular growth and protein synthesis with autophagy regulation. Modulation of these interconnected pathways may contribute to the observed anticancer effects of plant-derived flavonoids. However, the specific contribution of V. negundo extracts and individual constituents to these mechanisms requires further experimental validation. Their therapeutic potential is also limited by challenges such as poor solubility, low bioavailability, and rapid metabolism. Therefore, further mechanistic, pharmacokinetic, toxicological, and clinical studies are necessary to establish their efficacy and safety as potential anticancer agents.

Role of Individual Phytoconstituents of Vitex Negundo associated with Anticancer Properties

Vitex negundo contains several phytoconstituents with promising anticancer potential, particularly flavonoids, triterpenoids, and lignans. Flavonoids such as artemetin, vitexicarpin (casticin), and penduletin have demonstrated antiproliferative effects, apoptosis induction, and cell-cycle arrest in experimental cancer models. Quercetin, kaempferol, and luteolin may also modulate the PI3K/AKT/mTOR signaling pathway, thereby inhibiting cancer cell proliferation and survival. Additionally, the root-derived flavanone fraction VN-Root-EA-P3 has exhibited cytotoxic activity against A549 lung adenocarcinoma cells. Triterpenoids, including ursolic acid, oleanolic acid, and betulinic acid, have been investigated for their potential to induce apoptosis, while lignans such as negundin A and negundin B possess promising biological activities. Overall, these compounds highlight the potential of V. negundo as a source of anticancer agents. However, further studies are necessary to establish their mechanisms of action, safety, selectivity, and therapeutic efficacy.

Safety and Toxicological Evaluation

Toxicological studies of Vitex negundo have generally indicated a favourable safety profile in experimental animal models. Acute oral toxicity studies have reported an LD_{50} value greater than 5000 mg/kg for certain extracts, suggesting relatively low acute toxicity under the tested conditions. Studies evaluating the essential oil have also reported low acute dermal toxicity, with an LD_{50} above 2000 mg/kg and a no-observed-adverse-effect level (NOAEL) of 1000 mg/kg/day in subchronic dermal investigations. Furthermore, acute and repeated-dose studies have generally reported no substantial adverse alterations in major organ functions, hematological parameters, or biochemical profiles at the tested doses. However, some studies have documented dose-dependent biochemical changes, including variations in liver and kidney function markers, alongside mild histopathological alterations following high-dose or prolonged administration. These findings suggest that the safety of V. negundo may vary according to the plant part, extract type, route of administration, dose, and duration of exposure. Therefore, despite its promising preclinical safety profile, comprehensive toxicological assessments, including standardized dose-ranging studies and long-term evaluations, are necessary to establish its safety for therapeutic applications in humans.

Challenges and imitations

Despite promising preclinical findings, the therapeutic potential of Vitex negundo remains limited by the scarcity of large-scale, well-designed human clinical trials. Inadequate pharmacokinetic and ADMET data also restrict understanding of its absorption, distribution, metabolism, excretion, safety, and therapeutic dosage. Furthermore, incomplete characterization of biosynthetic pathways and limited investigation of individual phytoconstituents hinder the elucidation of their specific molecular mechanisms and potential adverse effects.

Pharmaceutical challenges include the lack of standardized extraction methods, validated analytical markers, and consistent quality-control procedures, resulting in batch-to-batch variability. Adulteration or substitution with morphologically similar species may further compromise product quality and efficacy. Additionally, geographical, climatic, seasonal, and soil variations can influence the plant's phytochemical composition. Increasing demand also raises concerns regarding sustainable sourcing and the need for scalable cultivation and plant biotechnology approaches. Addressing these challenges through rigorous clinical evaluation, pharmacokinetic studies, phytochemical standardization, authentication, and sustainable cultivation is essential to establish the safety, efficacy, and pharmaceutical applicability of V. negundo.

Future Perspectives of Vitex Negundo

Vitex negundo has considerable potential for future pharmaceutical development owing to its diverse bioactive constituents and reported anti-inflammatory, antifibrotic, neuroprotective, anticancer, and antimicrobial properties. Compounds such as negundoside, agnuside, casticin, and vitexin warrant further investigation as potential therapeutic leads. Their applications in combination therapies and targeted drug delivery also merit exploration, although current evidence remains predominantly preclinical. Plant biotechnology approaches, including micropropagation, tissue culture, elicitation, and metabolic engineering, may support the sustainable production of valuable secondary metabolites and conservation of plant resources. Additionally, green synthesis of nanoparticles using V. negundo extracts and the development of plant-derived biopesticides represent promising areas for biomedical and agricultural research. However, clinical translation requires validated analytical methods, standardized formulations, reliable marker compounds, and comprehensive pharmacokinetic and toxicological evaluation. Well-designed human clinical trials are essential to establish appropriate dosages, efficacy, long-term safety, and potential drug interactions. Integrating phytochemical research, biotechnology, pharmaceutical formulation, and clinical evaluation could facilitate the development of standardized and evidence-based products from V. negundo.

Conclusion

Vitex negundo is a medicinal plant with considerable potential for anticancer drug discovery due to its diverse phytochemical constituents, including flavonoids, iridoids, lignans, and terpenoids. Pharmacognostic and phytochemical investigations provide a foundation for its identification, quality assessment, and standardization, while experimental studies suggest that its extracts and selected compounds may inhibit cancer cell proliferation through apoptosis induction, cell-cycle regulation, and modulation of cancer-associated signaling pathways. However, most available evidence remains preclinical, and the specific mechanisms, selectivity, bioavailability, and long-term safety of its bioactive constituents require further investigation. The lack of standardized formulations, comprehensive pharmacokinetic data, and well-designed clinical trials remains a major barrier to therapeutic application. Future research should focus on isolating and characterizing active compounds, validating their molecular mechanisms, developing standardized formulations, and conducting rigorous preclinical and clinical evaluations. In conclusion, V. negundo represents a promising source of potential anticancer agents, but further scientific validation is essential before its efficacy and safety can be established for clinical use.

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