

Abstract

Natural products are rich in bioactive constituents that serve as a reservoir of the resources of medicines. In recent times, the use of natural products are increasing progressively over the world and potentiating their use in the traditional healthcare system. Since the early time of civilization, natural products, especially the herbal medicines have been used to maintain and promote the health status and today, the medicinal herbs contribute to formulate the modern medicines as well. The plant derived medicines have gained popularity because of higher efficiency and level of safety, less abusiveness like side effects or adverse effects than the synthetic drugs. Most of the people are not knowledgeable enough about usual traditional medicine and ecology indicating the non-toxicity of the natural products (Rates, 2001). So, the scientific study is required to justify their traditional uses and to establish the plants as the possible drug candidates in near future.

Vitex negundo (Linn.) is commonly used in folk medicines and widely distributed in India, Pakistan, Afghanistan, Sri Lanka, Thailand, Malaysia, eastern Africa and Madagascar especially in moist places. The plant is also commercially cultivated in some regions of Asia, Europe, North America and West Indies. However, all parts of *V. negundo* are used to treat different ailments, but leaves are strongly effective for medicinal uses (Khare, 2004). Still the potency of leaf oil and comparison with leaf organic extract is unexplored. Therefore, to justify its traditional use, we proposed this research plan, which can develop a future substantial value of this plant into the scientific discipline.

In the present study, we investigated the chemical composition of the methanol extract and essential oil of *Vitex negundo* leaves and also evaluated the comparative antioxidant, antibacterial, anti-inflammatory and anticancer potentials of the bioactive molecules. We estimated the total phenolics and flavonoid contents of the leaf extracts. The methanol extract

and the essential oil of *Vitex negundo* leaves had been evaluated for β -sitosterol, polyphenol and tocopherol contents. The leaf extracts are also employed to measure its potential against the urinary tract infection pathogens and anti-inflammatory potentials by employing the RAW 264.7 cell line models. Besides, the *in-vitro* anticancer activities were assessed against the PC₃ cell line models.

The objective of this research work to explore the bioactive compounds which are presented in methanol extract and essential oil of *Vitex negundo* leaves and investigated for the comparative bioactive potential by evaluating the *in-vitro* antioxidant activity, antibacterial activity, anti-inflammatory activity and anticancer activity.

Preparation of plant extract and essential oil were performed by using microwave assisted extraction and microwave assisted hydro-diffusion method respectively. The volatile organic compounds of methanol extract and fresh leaf oil were investigated by Gas chromatography-mass spectrometry (GC-MS). The identification and quantitative estimation of β -sitosterol, polyphenols and tocopherol that were presented in methanol extract and fresh leaf oil were examined by using High Performance Liquid Chromatography (HPLC). The antioxidant properties of methanol extract and essential oil were evaluated by using DPPH free radical scavenging activity method, ABTS radical scavenging activity method and Hydrogen peroxide (H₂O₂) scavenging activity. The antibacterial properties of methanol extract and essential oil were evaluated by using Agar disk diffusion assay method. The anti-inflammatory potential of methanol extract and essential oil were evaluated by estimating of the levels of IL-1 β , IL-6, and IL-10 using ELISA kits (Abcam ELISA Kit, Cambridge, MA, USA) employing the RAW 264.7 cell lines. The anticancer properties of methanol extract and essential oil were evaluated by using the MTT assay method against the PC₃ cell lines and the IC₅₀ values were determined.

The research findings revealed the existence of major volatile components including isocaryophyllene, caryophyllene, β -myrcene, 1-methylethyl ester hexanoic acid, 3-methyl-decanoic acid, α -farnesene, thunbergol, (isomer 1) cis-trans citral, phytol, oleic acid, (o-nitrophenyl)-hydrazone valeraldehyde, 2-myristynoyl-glycinamide, squalene, maltol, lactose, 4-methyl-2-heptanol, 2-myristynoyl-glycinamide, camphor, γ -sitosterol, β -sitosterol, asarone. The methanol extract showed the stronger antioxidant potential than the essential oil. The HPLC reports also revealed presence of higher amount of β -sitosterol, polyphenols and tocopherol in methanol extract than the essential oil. The leaf extracts demonstrated significant antibacterial activities against the urinary tract infection causing pathogens including *S. aureus*, *E. faecalis* and *E. coli*, *P. aeruginosa* with inhibition zones of 17 ± 0.58 to 27 ± 0.82 mm to MIC values of 1.66 to 12.72 mg. mL⁻¹. The dose-dependent anti-inflammatory activities of the methanolic extract and essential oil were validated. It was observed that the 50 μ g/ml dose was found to be significant ($p < 0.001$) against the pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and IL-6. In case of anti-inflammatory cytokine such as IL-10, the 50 μ g/ml dose was found to produce significant effects where the statistical significance was $p < 0.001$ and $p < 0.01$ for the methanolic extract and essential oil, respectively. Results also demonstrated the stronger anticancer potentials of methanol extract (with a value of IC₅₀ 70 μ g/ml, at 24 h) than the essential oil (with a value of IC₅₀ 95 μ g/ml, at 48 h) against PC₃ cell lines. The 100 μ g/ml dose was determined as the strong significant dose with a value of $p < 0.0001$ for both methanol extract and essential oil respectively.

This research study also comes up with proofs on the worth of *Vitex negundo* leaves as an excellent source of volatile organic compounds. The results of this scientific study indicate that the methanol extract and essential oil of the plant extracts contain substantial amount of antioxidants (β -sitosterol, polyphenols and α -tocopherol). Combinedly, β -sitosterol and polyphenols can enable the free radical scavenging activities which suggests that the plant is a

natural source of antioxidants. The methanol extract and essential oil are coupled with strong antibacterial activities against UTI causing pathogens including *Staphylococcus aureus*, *Enterococcus faecalis* and *Escherichia coli*, *Pseudomonas aeruginosa*. The data also revealed that the methanol extract and essential oil of the plant extracts possess significant anti-inflammatory properties. The key role of obtained phyto-constituents is in alleviation from chronic disorders like cancer and inflammatory diseases. Our results vividly demonstrated that the methanol extract is more active against prostate carcinoma cell lines as compared to the essential oil and that suggested the potential use of these extracts as a holistic approach to manage the life-threatening diseases including prostate cancer, risk factors for developing prostate cancer, bacterial infections and inflammation which are emerging issues in global health. For all the cases, the methanol extract exhibited the higher inhibitory potentials than the leaf essential oil. Here, as the anticancer and antibacterial activities were checked on the human prostate cancer cells and the UTI pathogens respectively. So, the research findings also correlate the antibacterial, anti-inflammatory and anticancer potentials and suggest that the significant reduction of UTI pathogens could be helpful to lower the risk factors of subsequent development of prostate cancer. Therefore, the research findings justify its traditional use, which develops a future substantial value of this plant into the scientific discipline.
