ORIGINAL ARTICLE

In-vitro-Scientific evaluation of anti-inflammatory potential of leaf extracts from *Vitex negundo*: as a promising future drug candidate

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Abstract

Introduction: *Vitex negundo* (Linn.) is commonly used in folk medicines and widely distributed in India, especially in moist places. However, all parts of *V. negundo* are used to treat different pathophysiology, but leaves are strongly effective for medicinal uses. The aim of this study is to compare the anti-inflammatory potential of the methanolic extract and essential oil of *V. negundo* leaves. A comparative study of the bioactive compounds that were obtained from the methanolic extract and essential oil of *V. negundo* leaves was accomplished. **Materials and Methods:** First, the methanolic extract and essential oil were evaluated for their anti-inflammatory activities employing the RAW 264.7 cells. Subsequently, the identification and quantification of the β-sitosterol of methanolic extract and essential oil of leaves were evaluated using high-performance liquid chromatography (HPLC). The quantitative evaluations of polyphenolics were executed using HPLC. **Results:** The dose-dependent anti-inflammatory activities of the methanolic extract and essential oil were validated. Moreover, it was observed that the 50 µg/ml dose was found to be significant ($P < 0.001$) against the pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-6. In case of anti-inflammatory cytokine such as IL-10, the 50 µg/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. Adequate amounts of β-sitosterol and polyphenols were found out in the methanolic extract and essential oil of leaves of *V. negundo*. **Conclusion:** The research findings suggest the significant anti-inflammatory properties of the methanolic extract and essential oil, but the methanolic extract showed a stronger effect. Furthermore, the essential oil of *V. negundo* could be used for the development of an ideal pharmaceutical formulation for effective delivery to people. Here, results justify its traditional use, which develops a future substantial value of this plant into the scientific discipline.

Key words: Anti-inflammatory activity, polyphenols, *Vitex negundo*, β-sitosterol

INTRODUCTION

Inflammation is extensively comprehended as a multifactorial and prolonged developmental process, where the inflammatory cytokines trigger the upregulation of inflammatory reactions by activating the stimulating factors and signaling cascades. Abundant production of inflammatory mediators prostaglandin E2, chemokines (e.g., chemokine C-C motif ligand 2 and chemokine CXC motif ligand 8), and pro-inflammatory mediators such as tumor

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necrosis factor (TNF)-α, interleukin (IL)-2, and IL-6 is the key molecules which are used to recruit to build up a high-affinity binding receptor complex leading to develop the inflammation.[1-3] Evidence suggests that a delicate balance between pro-inflammatory and anti-inflammatory cytokines is believed to be essential for maintenance of cellular redox homeostatic state of health. These essential biomarkers instigate the transcription factor such as nuclear factor kappa B (NF-kB), activator of transcription 3, activator protein-1, NF-E2 related factor-2, NF of activated T cells, hypoxia-inducible factor-1α, and subsequently activate the signal transduction cascades, leads to commence the cellular stress reactions.[4]

Since the primitive age, natural products have been using for the management of various ailments and to improve the quality of life. Commonly, the medicinal plants are the principal sources of polyphenols and phytosterols. Briefly, plant polyphenols are the natural compounds which decrease the catalytic action of enzymes which are responsible for reactive oxygen species (ROS) generation, reduce the production of nitric oxide synthases (NOS), modulate other metabolizing enzymes including cyclooxygenase (COX), lipoxygenase, downregulate the transcription factor NF-κB, and expression of some inflammatory cytokines such as IL-1β, TNF-α, and IL-10 involved in inflammatory response.[5-7] Lin *et al*., 2016, reported that the polyphenols enable the antioxidant activity and enhance the defense responses including anti-aging, anti-inflammatory, antioxidant, and antiproliferative activities. Rashid *et al*., 2018, also reported the strong anticancer activity due to the presence of polyphenolic compounds such as flavonoids, phenols, triterpenoids, and tannins content in the plant *Diospyros melanoxylon* Roxb.[8,9] β-sitosterol is classified as phytosterol and extensively found in medicinal plants. A wide range of health benefits on chronic diseases such as cardiovascular diseases and diabetes with β-sitosterol has been reported remarkably.

Nowadays, the popularity and widespread use of essential oil is increasing with regard to its bioactivities. The reports of literature review revealed that the research interest and importance of essential oil are due to a range of therapeutic benefits including depression, indigestion, headache, insomnia, muscular pain, respiratory problems, skin diseases, swollen joints, and urine associated complications. In general, the oils can be useful in traditional, alternative or complementary therapies for relieving the uncomfortable feeling of inflammation.[10] Considering the above-mentioned facts, the essential oil was chosen for evaluating its potential bioactivity toward inflammation; through a planned scientific exploration.

In this study, the leaf of *Vitex negundo* was selected for investigation of its anti-inflammatory activity. *V. negundo* is one of the traditionally used medicinal plants and ascertained for the medicinal importance including anticancer, inflammations, antiseptic, antipyretic, diuretic, antihistamine, antioxidant, antibacterial, antifungal, snake venom neutralization, mosquito repellant activity, insecticidal, larvicidal efficacy, anti-nociceptive, anti-androgenic, hepatoprotective, anti-fertility, skin aging inhibitor, and antidopaminergic effects.[11]

The aim of this study is to investigate the potential anti-inflammatory activity of the methanolic extract and essential oil of *V. negundo* leaves. However, the research evidences claim the relevance of the use of *V. negundo* plant in modern medicine as adjuvant and alternative therapies. This comparative study involves a systemized endeavor to compare the efficacy of the methanolic extract and essential oil through the *in vitro* anti-inflammatory assay.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol was purchased from Spectrochem Pvt. Ltd., Mumbai, India; β-sitosterol, ibuprofen from HiMedia Laboratories Pvt. Ltd., Mumbai, India, and Sigma-Aldrich. Dulbecco's Modified Eagle's Medium (DMEM) was procured from Gibco, Grand Island, NY 14072, USA1-716-774-6700, and the ELISA kits were obtained from Abcam Elisa kit, Cambridge, MA, USA. All the chemicals and reagents used in this research were of high analytical grade.

Preparation of Extracts

Microwave assisted extraction (MAE)

Dried powdered leaves of *V. negundo* were subjected to MAE as per the method described by Rashid *et al*., 2018 using methanol as the extraction solvent.[12] Briefly, the extraction time was 10 min and 140 W microwave power was used for extraction.

Microwave assisted hydro-diffusion

Fresh leaves of *V. negundo* were placed into the microwave extractor, and microwave-assisted hydro-diffusion was executed as per the method described by Chouhan *et al*., 2019.[13]

Cell Line and Culture Conditions

Murine RAW264.7 macrophage cell line was purchased from American Type Culture Collection. The cells were cultured in DMEM with 10% heat-inactivated fetal bovine serum and 1% penicillin-streptomycin and supplemented in a humidified atmosphere at 37°C in 5% CO_2. Plate the cells onto 96-well plate at a density of 2×10^4 and incubated for 24 h. For this experiment, the cells were pretreated with lipopolysaccharide (LPS).

Cell Viability Assay of RAW Macrophages

The cell viability of RAW 264.7 cell was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium

bromide (MTT) assay which was earlier narrated by Nelson *et al*., 2016, and was used with slight modification.[14] About 70% confluent cells were treated with different concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 75 µg/ml) of plant extracts and incubated for 24 h. Thereafter, the culture medium was replaced by the fresh culture medium and MTT solution (0.5 mg/ml) was added. Subsequently, the plate was kept incubated for 3 h. In next step, the MTT solution was removed and 100 µl dimethyl sulfoxide was incorporated into each well to solubilize the formed formazan crystals. Finally, the absorbance was measured at 540 nm using a microplate reader. Cell viability of the test samples was expressed as percentage compared to the control solution.

LPS Stimulation and Assessment of Inflammatory Cytokines Production (IL-1β, IL-6, and IL-10 Level)

To investigate the inhibitory effect of the leaf extracts on cytokine levels, the RAW 264.7 cells were pretreated with LPS (1 µg/mL). LPS-induced RAW 264.7 cells (2×10^4) were seeded into 96 well-plates, and after 24 h incubation period the cells were again treated with the various concentrations (12.5 µg/ml, 25 µg/ml, and 50 µg/ml) of the methanolic extract and essential oil; and then incubated for 24 h at 37°C, 5% CO_2. Supernatants were separated and stored at −20°C. Cells were thawed and used for the estimation of the levels of IL-1β, IL-6, and IL-10 using ELISA kits (Abcam ELISA Kit, Cambridge, MA, USA). These three experiments were carried out in accordance with the clear instructions that were provided by the manufacturers. Eventually, the absorbance was checked at 450 nm using a microplate reader. Ibuprophane (1 µg/ml) was employed as standard and LPS was employed as the control.[15]

Chromatographic Analysis

Individual polyphenolic compounds (aspartic acid, gallic acid, dihydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, rutin, trans-cinnamic acid, ferulic acid, quercetin, apigenin, kaempferol, tannic acid, and ellagic acid) and phytosterol (β-sitosterol) were identified using high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) detector. The experiment was carried out as per the method proposed by Zhah *et al*., 2010, with slight modification.[16] β-sitosterol was qualitatively and quantitatively analyzed using HPLC system compatible with the Xbridge C_{18} Column (4.6 mm × 50 mm, 3.5 µm i.d.) at 25°C. The mobile phase was composed of a ratio of acetonitrile and water (95:5 v/v). The chromatographic separation was accomplished in an isocratic mode at the flow rate of 1 ml/min. Finally, the chromatogram was registered at 210 nm; against the standard compound which was purchased from Sigma-Aldrich. The qualitative and quantitative estimation of polyphenols was performed using the HPLC system. The experiment was conducted with a slight modification of method as depicted Wang by *et al*., 2019.[17] The separation of test samples was performed using column symmetry C18 (4.6 mm × 150 mm, 5 µm i.d.) at 30°C. The mobile phase consisted of 0.17% acetic acid (A) and acetonitrile (B) with a flow rate of 1 ml/min in a gradient manner. Eventually, the chromatogram was recorded at 320 nm and quantified with comparison to external standards.

Statistical Analysis

Each experiment was performed in triplicate. The results were represented as a mean ± SD ($n = 3$) and one-way analysis of variance followed by Bonferroni's multiple comparison test was performed using GraphPad Prism (version 5.03) software.

RESULTS AND DISCUSSION

Effects of Methanolic Extract and Essential Oil on Viability of RAW Macrophages

To assess the effect of cell viability on Raw 264.7 macrophage cell lines, the MTT assay was carried out at 24 h with various concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, and 75 µg/ml) of the methanolic extract and essential oil; and ibuprofen (1 µg/ml).

Figure 1a shows the percentage of cell viability of the methanolic extract on RAW 264.7 macrophages. The results exhibited a slight reduction (16.3–24.4%) in cell viability with gradual increment of the concentrations.

Figure 1b represents the no obvious cytotoxicity of essential oil on RAW 264.7 cells. The figure also displayed a little reduction (13–24.4%) in cell viability at different concentrations up to 75 µg/ml. In this experiment, Figure 1a and b demonstrates almost similar cytotoxic effects on RAW 264.7 cells.

Hence, the concentrations were used on or under 75 µg/ml, producing negative effect on cell viability and considered as the appropriate concentrations for evaluating the anti-inflammatory activity on RAW 264.7 macrophages [Figure 1a and b]. The concentration of ibuprofen (1 µg/ml) was used also demonstrating a non-toxic effect on cell viability, and thus, that particular dose warrants for assessing the anti-inflammatory activity on RAW 264.7 macrophages.

Effects of Methanolic Extract and Essential Oil on LPS Induced Inflammatory Cytokines Production

To investigate the anti-inflammation activity of *V. negundo* leaf extracts, the ELISA based assay was performed on RAW 264.7 macrophage cells.

The strong inhibitory effects of leaf extracts were found out against two types of pro-inflammatory cytokines, including IL-1β, IL-6, and reinforcing effects toward the

anti-inflammatory cytokine such as IL-10. Figure 2a-c demonstrates the ability of the methanolic extract to restrict the *in vitro* release of pro-inflammatory cytokines and enhance the release of anti-inflammatory cytokine using LPS-stimulated RAW264.7 cells against a range of three different (12.5 µg/ml, 25 µg/ml, and 50 µg/ml) effective doses. Based on the effectiveness of methanolic extract, the 50 µg/ml dose has been considered as a strong significant dose with a value of $P < 0.001$ for all the biomarkers, when the effective dose was compared with LPS-induced inflammatory groups. Interestingly, somewhere the 50 µg/ml dose exhibited the significant difference and even the stronger anti-inflammatory activity when the 50 µg/ml dose was compared with the positive drug ibuprofen (1 µg/ml).

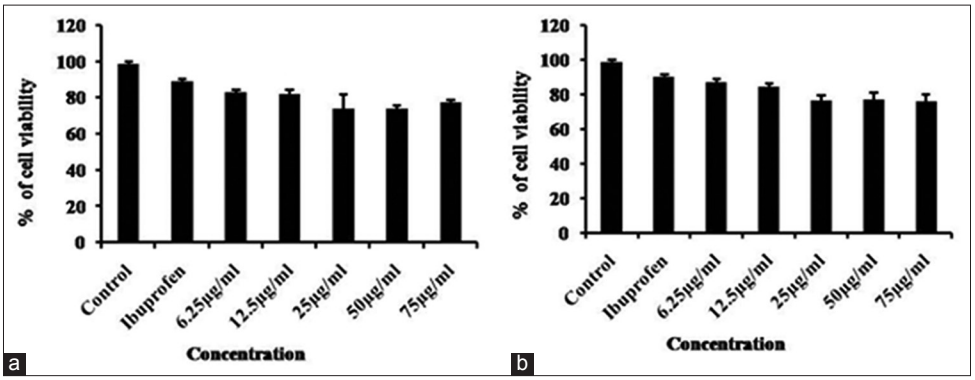

Figure 1: Concentration-dependent cell viability of (a) methanolic extract and (b) essential oil against RAW macrophage cell line at 24 h. Ibuprofen (1 µg/ml) is showing no cytotoxicity to RAW macrophage cells. The values are expressed as the percentage in comparison with the untreated (control) cell group and each bar in the graph is represented as mean ± SD ($n = 3$)

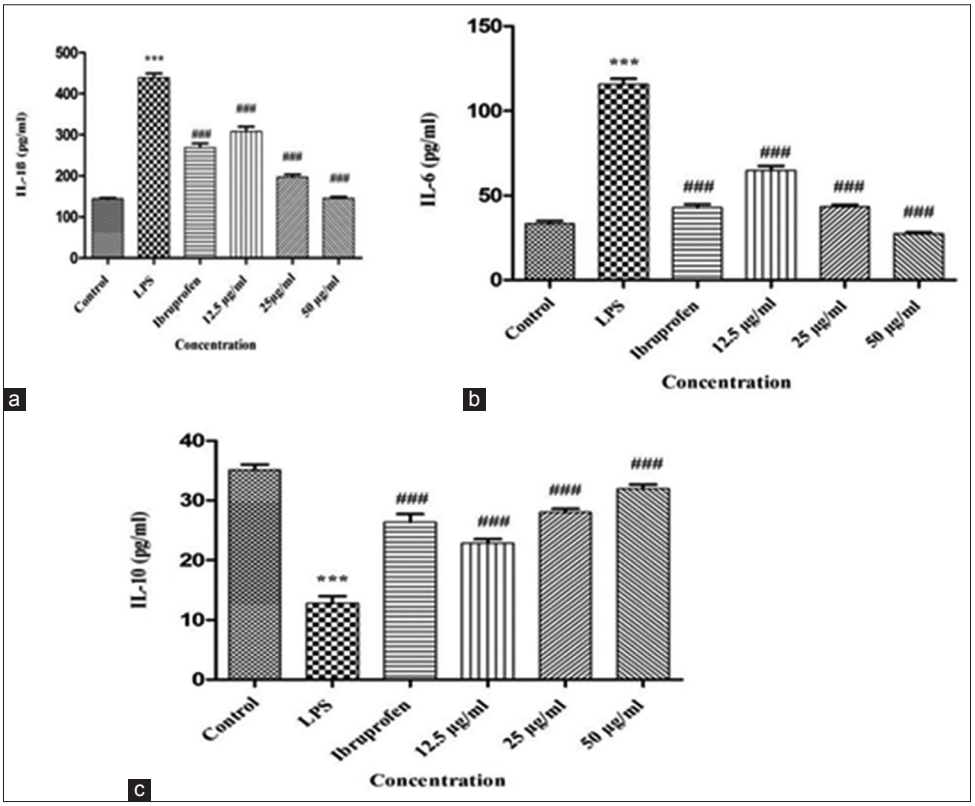

Figure 2: Concentration-dependent anti-inflammatory effects of methanolic extract of *Vitex negundo* on RAW 264.7 macrophages. The RAW 264.7 cells were treated with leaf extract (12.5, 25, and 50 µg/ml) in the presence of lipopolysaccharide (LPS) (1 µg/ml). The concentrations of (a) IL-1β, (b) IL-6, and (c) IL-10 were measured in culture media using the booklets that were provided with the commercial ELISA kits. Results are presented as the mean ± SD of at least triplicates of each experiment. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, statistically significant in comparison with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, statistically significant in comparison with LPS-induced inflammatory groups by oneway ANOVA and followed by Bonferroni's multiple comparison test

Figure 3a-c represents the potential of essential oil toward the *in vitro* anti-inflammatory activity. Afterward, the anti-inflammatory assay of essential oil revealed almost the similar effects on different cytokines, as produced by the methanolic extract. The three graphs (Figure 3a-c) displayed the dose-dependent anti-inflammatory property of essential oil, where the 50 µg/ml dose was again proved as the most effective dose (for all biomarkers; except IL-10) with a value of $P < 0.001$. Just in one exception, the leaf oil exhibited the maximum activity on IL-10 at the 50 µg/ml dose with a statistical significance $P < 0.01$. Results also showed that the bio-activities of essential oil were directly proportional with the effective doses, and the 50 µg/ml dose exhibited a significant difference in comparison with the positive drug ibuprofen (1 µg/ml).

In case of extract, IL-6 exhibited evidently maximum rate of inhibition whereas in case of essential oil IL-6 again showed the maximum degree of preventive effects. In comparison with essential oil the methanolic extract exhibited distinctly higher inhibitory activity on RAW cells. IL-6 is conventionally known as a major secretory factor and generally secrets in the feedback reaction of cell damage or infection.

IL-6 plays a contributory role to promote tumorigenesis and followed by modulating related factors are responsible to form cancer by triggering relevant cell-signaling pathways including JAK/STAT3, Ras/ERK, and PI3 K/Akt which induce cell proliferation, invasion, metastasis, and angiogenesis and most evidently the metabolism. Thus, the IL-6 dominated cell-signaling might be playing a counter-regulatory role independently or in adjuvant therapy for the treatment of chronic diseases.[18] IL-1β is widely known as a prototypic pro-inflammatory cytokine and having a crucial role to produce acute and chronic inflammation, which ultimately may responsible for autoimmune disorders. Although IL-1β plays a key role to maintain the homeostatic balance (such as in the regulation of feeding, sleep, and temperature), aberrant production of IL-1β initiates a various types of inflammatory diseases including rheumatoid arthritis, neuropathic pain, inflammatory bowel disease, osteoarthritis, vascular disease, multiple sclerosis, and Alzheimer's disease.[19] In current study, the plant extracts showed evidence of blocking effect against IL-1β which suggests the blockade of IL-1β receptor as a potential therapeutic strategy for the management of different inflammatory diseases. IL-10 is commonly appreciated as a potent anti-inflammatory cytokine and serves a key role in regulating host immune response toward pathogens such as viruses, bacteria, fungi, and protozoa. Inadequacy or aberrant expression of IL-10 may cause to develop several microbial infections and lead to progression of inflammatory bowel disease and numerous autoimmune diseases. In this experiment, the plant extracts exhibited the upregulated expression against IL-10. By coordinating the response of pro-inflammatory and anti-inflammatory cytokines, IL-10 maintains the immunomodulatory balance in living system and can be considered as a therapeutic opportunity.[20]

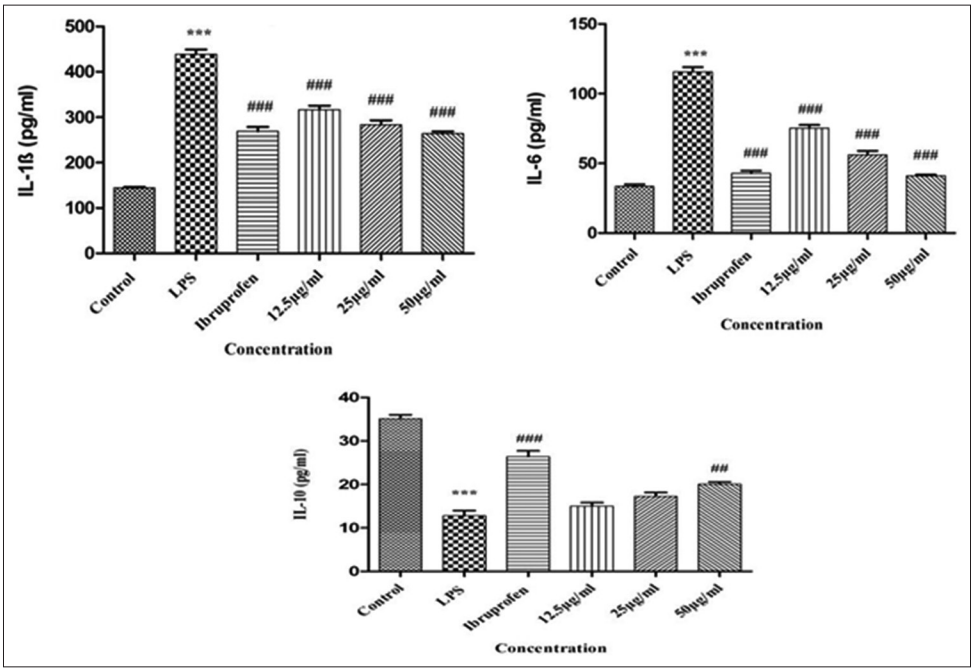

Figure 3: Concentration-dependent anti-inflammatory effects of essential oil of *Vitex negundo* on RAW 264.7 macrophages. The RAW 264.7 cells were treated with leaf oil (12.5, 25, and 50 µg/ml) in the presence of lipopolysaccharide (LPS) (1 µg/ml). The concentrations of (a) IL-1β, (b) IL-6, and (c) IL-10 were measured in culture media using the booklets that were provided with the commercial ELISA kits. Results are presented as the mean ± SD of at least triplicates of each experiment. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$, statistically significant in comparison with control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.001$, statistically significant in comparison with LPS-induced inflammatory groups by one-way ANOVA and followed by Bonferroni's multiple comparison test

Chromatographic Analysis

In the present study, β-sitosterol was identified and quantified by an optimized HPLC method. Figure 4a displays the chromatogram of β-sitosterol methanolic extract of *Vitex* leaves. The amount of β-sitosterol was obtained to be 425.45 µg/g in the methanolic extract.

Figure 4b demonstrates the chromatogram of β-sitosterol and the amount was found to be 23.42 µg/g in essential oil. This experiment revealed that the methanolic extract possessed much higher quantity of β-sitosterol than the essential oil.

Table 1 represents that the quantifications of β-sitosterol were obtained from methanolic extract and essential oil of *Vitex* leaves and Figure 4c shows the chromatogram of β-sitosterol of standard compound.

β-sitosterol is one of the important phytosterols, naturally occurring vegetable-containing diets. β-sitosterol is one of the remarkable components of functional foods and having a crucial role in the management of inflammatory diseases. Many of the scientific investigations previously proved a significant role of β-sitosterol in attenuating topical inflammation and exhibiting its anti-inflammatory property by interfering with arachidonic acid cascade. Furthermore, due to enhancement of antioxidant defense system, reduction in serum cholesterol levels, effectively blocking the expression of vascular adhesion molecules vascular cell adhesion molecule-1 and intercellular adhesion molecule-1; makes β-sitosterol a well-proven cardioprotective agent.[21]

An array of polyphenols was detected by HPLC separation associated with UV detection. The HPLC fingerprints showed the appearance of being a collage of 13 compounds such as aspartic acid, gallic acid, dihydroxy benzoic acid, catechin, chlorogenic acid, vanillic acid, rutin, trans-cinnamic acid, ferulic acid, quercetin, apigenin, kaempferol, and tannic acid with different elution times.

Figure 5a demonstrates the chromatogram for polyphenols analysis of methanolic extract of *Vitex* leaves, and Table 2 reveals that the amounts of polyphenols were presented in methanolic extract. Among the above-mentioned compounds, the rutin (480.39 µg/g) and vanillic acid (976.86 µg/g) were extensively found out in methanolic extract. Rutin is known as a glycoside of flavonoid quercetin and widely distributed in plant materials. It has been reported to reduce the inflammation by lowering the production of pro-inflammatory cytokines, modulating the antioxidant enzyme activities, and activation of the mitogen-activated protein kinase cascade.[22] The research evidence suggested that the vanillic acid exerts its anti-inflammatory property by inhibiting oxidative stress,

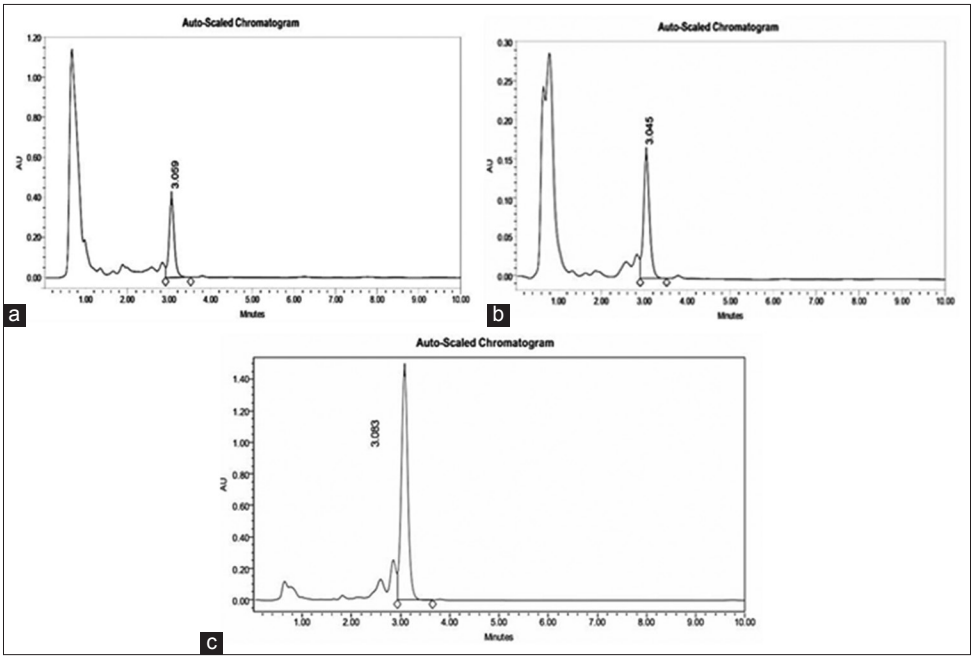

Figure 4: Reversed phase-high-performance liquid chromatography chromatogram of β-sitosterol was found in (a) methanolic extract (b) essential oil of *Vitex negundo* and (c) standard compound

Table 1: Quantification of β-sitosterol in the methanolic extract and essential oil of *Vitex negundo* leaf

S. No.	Peak name	Retention time	Area	Height	Amount (µg/g)
1.	β-Sitosterol$_{(methanolic\ extract)}$	3.059	3478289	398596	425.45
2.	β-Sitosterol$_{(E.\ oil)}$	3.045	1564544	160783	23.42

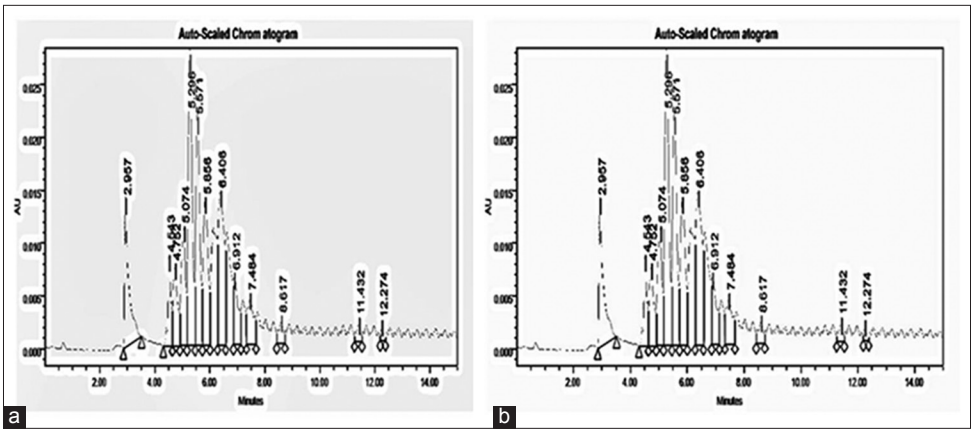

Figure 5: High-performance liquid chromatography of polyphenols were detected in (a) methanolic extract and (b) essential oil of *Vitex negundo* leaf

Table 2: Quantification of polyphenols in methanolic extract of *Vitex negundo* leaf

S. No.	Peak name	Retention time	Area	Height	Amount (µg/g)
1.	Aspartic acid	2.957	142228	13685	7.93
2.	Gallic acid	4.543	57473	7994	10.04
3.	Dihydroxy benzoic acid	4.752	78588	6999	87.43
4.	Catechin	5.074	113245	10590	82.77
5.	Chlorogenic acid	5.296	266070	26762	28.20
6.	Vanillic acid	5.571	213456	20861	976.86
7.	Rutin	5.856	158276	13253	480.39
8.	Transcinnamic acid	6.406	208049	13806	66.66
9.	Ferulic acid	6.912	70162	5965	7.02
10.	Quercetin	7.484	64826	4013	95.43
11.	Apigenin	8.617	22583	1775	6.96
12.	Kaempferol	11.432	14965	1420	0.896
13.	Tannic acid	12.274	8812	1149	1.785

pro-inflammatory cytokine generation, and NF-κB activation in rodent inflammation models.[23]

Figure 5b demonstrates the chromatogram for polyphenols analysis of essential oil and Table 3 demonstrates that the amounts of polyphenols were presented in essential oil. As compared to essential oil, the methanolic extract possessed a higher amount of individual polyphenolic compounds and which leads to exhibit the potential of the methanolic extract. Two major polyphenols such as vanillic acid (81.87 µg/g) and trans-cinnamic acid (55.35 µg/g) were detected in essential oil. Trans-cinnamic acid belongs to the group of styrene and recorded for exerting a wide range of therapeutic activities, including antioxidant, anti-inflammatory, and anticancer properties.[24]

The qualitative and quantitative estimation of polyphenols was obtained from the plant extracts would be compared against the chromatograms of each standard compound. Apigenin, catechin, and rutin are three important phenolic compounds and they are registered to represent for significant anti-inflammatory properties by modulating the activation of NLRP3 inflammasome. Anti-inflammatory effect of polyphenols has been already documented and action may be exerted notably through regulating the various transcription factors (AP-1, NF-kB, and protooncogenes), second messengers (cGMP, cAMP, protein kinases, and calcium), enzymes and compounds (iNOS, and COX-2), cytokines (IL-1β, and TNF-α), neuropeptides, and proteases which are directly involved in inflammatory reactions.[5]

Predominantly, chronic diseases linked with the excessive generation of ROS which leads to a wide spectrum of oxidative stress reactions. Many signaling molecules and peroxiredoxin 2 are released after protein oxidations, which eventually initiate the inflammatory reactions. Phenolics are most appreciated as radical scavenger and have been reported for the treatment of several diseases, including cancer, cardiovascular and neurodegenerative diseases, or for use in anti-aging or cosmetic products. The publication trends revealed that the flavonoids are pharmacologically potent bioactive compounds which are associated with antipyretic,

Table 3: Quantification of polyphenols in the essential oil of *Vitex negundo* leaf

S. No.	Peak name	Retention time	Area	Height	Amount (µg/g)
1.	Aspartic acid	2.993	121392	15916	0.829
2.	Gallic acid	4.615	59887	8584	1.22
3.	Dihydroxy benzoic acid	4.824	94935	8254	1.29
4.	Catechin	5.063	205650	18078	18.39
5.	Chlorogenic acid	5.318	171837	15009	2.23
6.	Vanillic acid	5.595	146223	11837	81.87
7.	Rutin	5.876	85217	8479	0.32
8.	Transcinnamic acid	6.597	141925	10490	55.35
9.	Ferulic acid	6.844	59645	6734	0.73
10.	Quercetin	7.698	36503	2926	6.57
11.	Apigenin	8.801	34787	2685	1.31
12.	Kaempferol	11.411	45572	2761	0.33
13.	Tannic acid	12.226	33111	2788	0.82

analgesic, anti-inflammatory, anti-arthritic, antioxidant, and immunomodulatory properties.[25] Hence, phenolics and flavonoids provide therapeutic synergism which indicates toward plant-based bioactivity by deactivating the pro-oxidative enzyme promotes health status and quality of life.[9,26]

CONCLUSION

The results of this scientific study indicate that the methanolic extract and essential oil of the plant extracts possess strong anti-inflammatory properties. The research findings claim that the essential oil can be employed to develop an ideal pharmaceutical formulation for effective delivery to people. For having an adequate amount of flavonoid contents and anti-inflammatory properties, this plant can play a contributory role to manage the age-related degenerative disorders. The data also revealed that the methanolic extract and essential oil of the plant extracts contain a substantial amount of β-sitosterol and polyphenols. Combined, β-sitosterol, and polyphenols can enable free radical scavenging activities, which suggest that the plant is a natural source of antioxidants. Hence, this plant could be effective as dietary supplements leading to improve the quality of life. Based on the research evidence, this medicinal plant can be considered as an excellent drug candidate in near future and also eligible for more developmental research work which can be highly regarded as normative comparison approach.

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REFERENCES

1. Ghosh N, Ali A, Ghosh R, Das S, Mandal SC, Pal M. Chronic inflammatory diseases: Progress and prospect with herbal medicine. Curr Pharm Des 2015;22:247-64.
2. Boominathan R, Parimaladevi B, Mandal SC, Ghoshal SK. Anti-inflammatory evaluation of *Ionidium suffruticosam* Ging in rats. J Ethnopharmacol 2004;91:367-70.
3. Nirmal SA, Pal SC, Mandal SC, Patil AN. Analgesic and anti-inflammatory activity of ß-sitosterol isolated from *Nyctanthes arbor-tristis* leaves. Inflammopharmacology 2012;20:219-24.
4. Arulselvan P, Fard MT, Tan WS, Gothai S, Fakurazi S, Norhaizan ME, *et al*. Role of antioxidants and natural products in inflammation. Oxid Med Cell Longev 2016;2016:15.
5. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative stress and inflammation: What polyphenols can do for us? Oxid Med Cell Longev 2016;2016:9.
6. Pal S, Bhattacharjee A, Ali A, Mandal NC, Mandal SC, Pal M. Chronic inflammation and cancer: Potential chemoprevention through nuclear factor kappa B and p53 mutual antagonism. J Inflamm (Lond) 2014;11:23.
7. Maiti PP, Ghosh N, Kundu A, Panda S, De B, Mandal SC. Evaluation of anti-inflammatory and antinociceptive activity of methanol extract of *Calotropis gigantea* root. Int J Green Pharm 2017;11:198-05.
8. Lin D, Xiao M, Zhao J, Li Z, Xing B, Li X, *et al*. An Overview of plant phenolic compounds and their importance in human nutrition and management of Type 2 diabetes. Molecules 2016;21:1374.
9. Rashid Md. HA, Bharadwaj PV, Majumder S, Mandal V, Pal M, Mandal SC, *et al*. Antioxidant and anticancer activity of extract and fractions obtained from *Diospyros melanoxylon* Roxb. Leaves and correlation with their polyphenolic profiles. Int J Pharm Pharm Sci

2018;10:6-16.
10. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed 2015;5:601-11.
11. Ambika S, Sundrarajan M. Antibacterial behaviour of *Vitex negundo* extract assisted ZnO nanoparticles against pathogenic bacteria. J Photochem Photobiol B 2015;146:52-7.
12. Rashid Md. HA, Bharadwaj PV, Majumder S, Mandal V, Pal M, Mandal SC, *et al*. Antioxidant and anticancer activity of extract and fractions obtained from *Diospyros melanoxylon* Roxb. leaves and correlation with their polyphenolic profiles. Int J Pharm Pharm Sci 2018;10:6-16.
13. Chouhan KB, Tandey R, Sen KK, Mehta R, Mandal V. A unique model of gravity assisted solvent free microwave based extraction of essential oil from *Mentha* leaves ensuring biorefinery of left over waste biomass for extraction of nutraceuticals: Towards cleaner and greener technology. J Clean Prod 2019;225:587-98.
14. Nelson VK, Ali A, Dutta N, Ghosh S, Jana M, Ganguli A, *et al*. Azadiradione ameliorates polyglutamine expansion disease in *Drosophila* by potentiating DNA binding activity of heat shock factor 1. Oncotarget 2016;7:78281-96.
15. Kim S, Kundu A, Chun R, Han SH, Pandey AK, Yoo S *et al*. Direct synthesis of 2-acyl acridines using aldimines and anthranils: Evaluation of cytotoxicity and anti-inflammatory activity. Asian J Org Chem 2018;7:2069-75.
16. Zhah UM, Patel SM, Patel PH, Hingorani L, Jadhav RB. Development and validation of a simple isocratic HPLC method for simultaneous estimation of phytosterols in *Cissus quadrangularis*. Indian J Pharm Sci 2010;72:753-58.
17. Wang J, Vanga SK, Raghavan V. High-intensity ultrasound processing of kiwifruit juice: Effects on the ascorbic acid, total phenolics, flavonoids and antioxidant capacity. LWT Food Sci Technol 2019;107:299-7.
18. Kumari N, Dwarakanath BS, Das A, Bhat AN. Role of interleukin-6 in cancer progression and therapeutic resistance. Tumour Biol 2016;37:11553-72.
19. Rena K, Torres R. Role of interleukin-1β during pain and inflammation. Brain Res Rev 2009;60:57-64.
20. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Crit Rev Immunol 2012;32:23-63.
21. Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. β-Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells. Mol Nutr Food Res 2010;54:551-58.
22. Enogieru AB, Haylett W, Hiss DC, Bardien S, Ekpo OE. Rutin as a potent antioxidant: Implications for neurodegenerative disorders. Oxid Med Cell Longev 2018;2018:17.
23. Calixto-Campos C, Carvalho TT, Hohmann MS, Pinho-Ribeiro FA, Fattori V, Manchope MF, *et al*. Vanillic acid inhibits inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine production, and NFκB activation in mice. J Nat Prod 2015;78:1799-808.
24. Zhu B, Shang B, Li Y, Zhen Y. Inhibition of histone deacetylases by trans-cinnamic acid and its antitumor effect against colon cancer xenografts in athymic mice. Mol Med Rep 2016;13:4159-66.
25. Gupta M, Sasmal S, Majumdar S, Mukherjee A. HPLC profiles of standard phenolic compounds present in medicinal plants. Int Pharm Phytochem Res 2012;4:162-67.
26. Kala HK, Mehta R, Tandey R, Sen KK, Mandal V. Ten years of research on phenolics (2005e2015): A status report. Pac Sci Rev A 2016;18:1-4.

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