



PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY AND ACID-NEUTRALIZING ACTIVITIES OF ETHANOLIC EXTRACT OF VITEX NEGUNDO FLOWERS

Mr. P. Sasi M. Pharm.*¹, Dr. S. Chandra M. Pharm., Ph.D., D. Litt.², A. Mainikandan³, S. Manoj⁴, P. Mohan⁵, K. Mohanapriya⁶

¹Assistant Professor of Department Pharmaceutical Chemistry Sri Lakshminarayan College of Pharmacy, Dharmapuri.

²Principal, Sri Lakshminarayan College of Pharmacy, Dharmapuri.

^{3,4,5,6}Students, Sri Lakshminarayan College of Pharmacy, Dharmapuri.



***Corresponding Author: Mr. P. Sasi M. Pharm**

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ABSTRACT

Background: Vitex negundo is a medicinal plant traditionally used in several therapeutic applications. The present study evaluated the phytochemical profile and selected in vitro pharmacological properties of an ethanolic extract of its flowers (VNFET). **Methods:** Vitex negundo flowers were extracted with ethanol by heating 10 g of material with 100 mL ethanol for 5 h under a reflux condenser in a water bath, followed by filtration and vacuum evaporation. Preliminary phytochemical screening, total flavonoid and total phenolic estimation, DPPH radical-scavenging assay, FRAP assay, egg-albumin denaturation assay and acid-neutralizing capacity were evaluated. Qualitative screening demonstrated alkaloids, flavonoids, phenols, proteins and carbohydrates, while saponins were absent. The reported maximum total flavonoid content was 75 mg at a concentration of 300, and the maximum total phenol content was 120 mg at a concentration of 200. In the DPPH assay, VNFET showed 85% inhibition at 10 mg with an IC₅₀ of 7.48 mg, compared with 3.373 mg for ascorbic acid. FRAP absorbance reached 1.73 at 15 mg. In the albumin-denaturation assay, VNFET produced 63% inhibition at 5 mg with an IC₅₀ of 10.59 mg; diclofenac sodium showed an IC₅₀ of 12.129 mg. Acid-neutralizing capacity was 19.49 mEq for VNFET compared with 7.92 mEq for sodium carbonate. Under the conditions reported in this study, VNFET demonstrated measurable antioxidant, anti-inflammatory and acid-neutralizing activities together with detectable flavonoid and phenolic constituents.

KEYWORDS: Vitex negundo; flower extract; phytochemical screening; DPPH; FRAP; albumin denaturation; acid-neutralizing capacity.

1. INTRODUCTION

Medicinal plants remain an important source of biologically active constituents and continue to be investigated for antioxidant and anti-inflammatory properties. Plant-derived phenolic compounds and flavonoids are frequently associated with free-radical-scavenging and reducing activities. The source study describes Vitex negundo, commonly known as Nirgundi or Nochi, as a medicinal shrub whose flowers have traditional relevance in digestive disorders, fever and liver-related conditions.

The flowers were selected for the present investigation because the study aimed to examine their phytochemical composition and selected pharmacological activities.

Oxidative stress is associated with an imbalance between reactive oxygen species and antioxidant defenses. In vitro assays such as DPPH and FRAP provide complementary chemical measures: DPPH estimates radical-scavenging capacity, whereas FRAP-type procedures assess reducing power. Phenolic and flavonoid constituents may contribute to these activities through electron- or hydrogen-donation mechanisms.

Inflammation is accompanied by structural and functional changes in proteins. The egg-albumin denaturation assay is an in vitro screening method used to assess the ability of a test substance to inhibit heat-associated protein denaturation. The study also evaluated acid-neutralizing capacity using a back-titration approach, providing a chemical measure of acid-neutralizing ability.

1.1 Rationale and objective

The objective was to evaluate the qualitative phytochemical profile and investigate the antioxidant, anti-inflammatory and acid-neutralizing activities of the ethanolic flower extract of *Vitex negundo*.

1.2 Botanical information

Parameter	Reported information
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Verbenaceae
Genus	Vitex
Species	<i>Vitex negundo</i>
Tamil name	Nochi
Part used	Flower



Botanical information of *Vitex negundo*.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Vitex negundo flowers used in the investigation were obtained from Yazhini Biotech Laboratory, Srivilliputhur, Tamil Nadu.

2.2 Preparation of ethanolic extract

Ten grams of *Vitex negundo* flower material were heated with 100 mL of ethanol for 5 h under a reflux condenser in a water bath. The mixture was cooled and filtered. The filtrate was evaporated under vacuum to obtain the ethanolic extract, designated VNFET.

2.3 Preliminary phytochemical screening

The extract was screened qualitatively for selected phytoconstituents. Alkaloids were assessed using Mayer's and Dragendorff's tests; flavonoids using alkaline-reagent and lead-acetate tests; saponins by the

froth test; steroids by the described chloroform/sulfuric-acid procedure; and tannins by ferric-chloride and lead-acetate tests. The source results also report tests for phenols, proteins and carbohydrates.

2.4 Total flavonoid estimation

Total flavonoids were estimated using the aluminium-chloride method. Sample and quercetin standard solutions were reacted with sodium nitrite and aluminium chloride, followed by sodium hydroxide, and absorbance was measured at 510 nm against a reagent blank. Measurements were performed in triplicate and the reported content was expressed in milligrams of quercetin equivalents.

2.5 Total phenolic estimation

Total phenolic content was estimated using the Folin-Ciocalteu procedure. Sample and gallic-acid standard solutions were reacted with Folin-Ciocalteu reagent and sodium carbonate, incubated at room temperature, and measured at 550 nm against a reagent blank. Measurements were performed in triplicate.

2.6 DPPH radical-scavenging assay

A DPPH solution of 6×10^{-5} M in methanol was prepared. Aliquots of each test concentration were mixed with DPPH solution, shaken and maintained at room temperature for 30 min. Absorbance was measured at 520 nm. Ascorbic acid was used as the positive control and distilled water as the negative control. Each concentration was tested three times.

The calculated actual absorbance by subtracting plant blank absorbance from assay absorbance. Percentage inhibition was calculated as: % inhibition = [(blank DPPH absorbance – actual absorbance) / blank DPPH absorbance] $\times$ 100.

2.7 FRAP assay

For the reducing-power assay, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide were added to the test mixture. The mixture was incubated at 50°C for approximately 20 min, followed by addition of 2.5 mL of 10% trichloroacetic acid and centrifugation at 3000 rpm for 10 min. A 2.5 mL portion of supernatant was mixed with 0.5 mL ferric chloride and absorbance was measured at 700 nm. Ascorbic acid served as positive control. The test was repeated three times.

2.8 Egg-albumin denaturation assay

The reaction mixture contained 0.5 mL sample and 0.45 mL of 5% bovine egg albumin. The pH was adjusted to 6.3 with 0.1 N HCl and the mixture was maintained at 37°C for 20 min, followed by heating at 57°C for 30 min. After cooling, absorbance was measured at 660 nm. Diclofenac sodium was used as standard and distilled water as control.

Percentage inhibition was calculated as: $[(\text{control absorbance} - \text{test-sample absorbance}) / \text{control absorbance}] \times 100$.

2.9 Acid-neutralizing capacity

Acid-neutralizing capacity was determined by a back-titration procedure. The test sample was reacted with an excess of standardized 0.1 N hydrochloric acid, after which the remaining acid was titrated using standardized 0.1 N sodium hydroxide. The difference between blank

and sample titration was used to calculate the reported acid-neutralizing capacity.

3. RESULTS

3.1 Preliminary phytochemical screening

The preliminary phytochemical investigation showed the presence of alkaloids, flavonoids, phenols, proteins and carbohydrates, whereas saponins were absent. These findings establish the qualitative phytochemical profile reported for VNFET.

Table 1. Preliminary phytochemical screening	VNFET
Alkaloids	+
Flavonoids	+
Phenols	+
Proteins	+
Saponins	—
Carbohydrates	+

The observed phytochemical profile is consistent with the study objective of identifying secondary metabolites

that may contribute to the measured in vitro activities.

3.2 Total flavonoid content

Concentration	Average OD	Reported total flavonoids
100	0.04	57
200	0.07	63
300	0.09	75

The reported flavonoid content increased across the evaluated concentrations, reaching the highest reported

value of 75 mg at a concentration of 300.

3.3 Total phenolic content

Concentration	Sample OD	Standard OD	Reported total phenol
200	0.12	0.10	120 mg
400	0.14	0.13	107 mg
600	0.10	0.10	100 mg
800	0.10	0.10	100 mg
1000	0.09	0.11	81 mg

The highest reported total phenolic value was 120 mg at a concentration of 200. The subsequent concentrations

produced reported values of 107, 100, 100 and 81 mg.

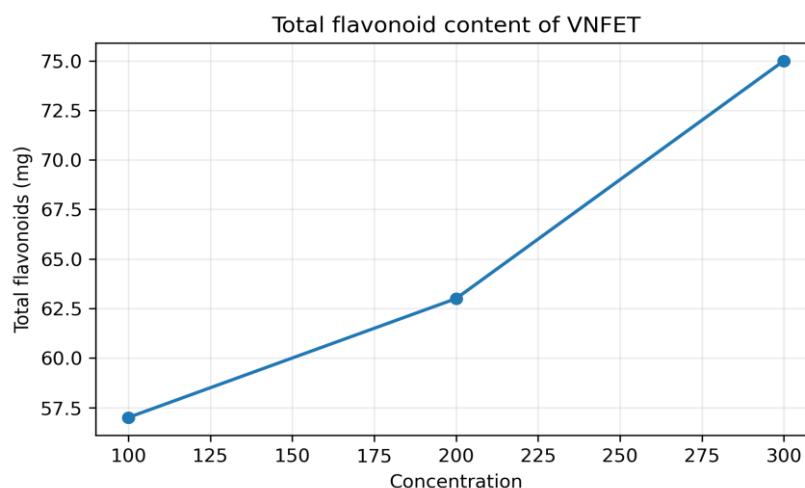


Figure 1: Total flavonoid content of VNFET.

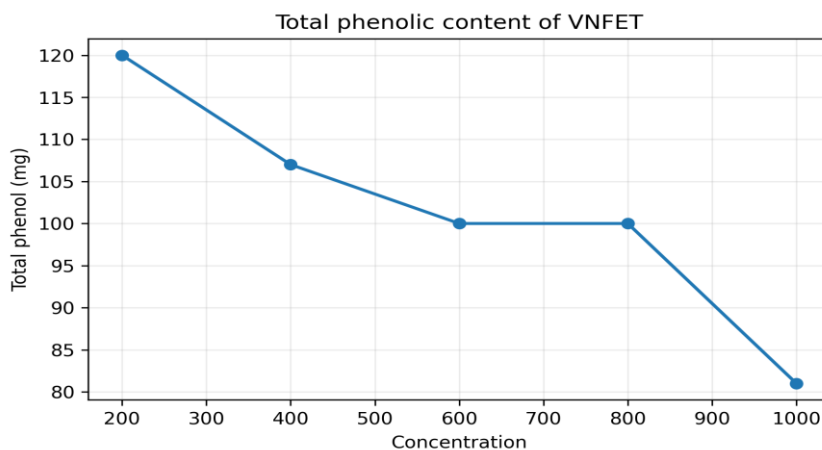


Figure 2: Total phenolic content of VNFET.

3.4 DPPH radical-scavenging activity.

Concentration	Average % inhibition	Reported IC50
5 mg	70.6%	7.48 mg
10 mg	84.6%	7.48 mg
15 mg	83.3%	7.48 mg

The source dataset reports 71% inhibition at 5 mg, 85% at 10 mg and 83% at 15 mg for VNFET, with an IC₅₀ of 7.48 mg. The 10 mg concentration was therefore

associated with the highest reported inhibition among the three tested VNFET concentrations.

Ascorbic acid concentration	Average % inhibition
5 mg	91%
10 mg	87%
15 mg	86%
20 mg	92%
25 mg	84%

Ascorbic acid showed a reported IC₅₀ of 3.373 mg. The lower IC₅₀ of the standard indicates greater radical-

scavenging potency under the reported assay conditions.

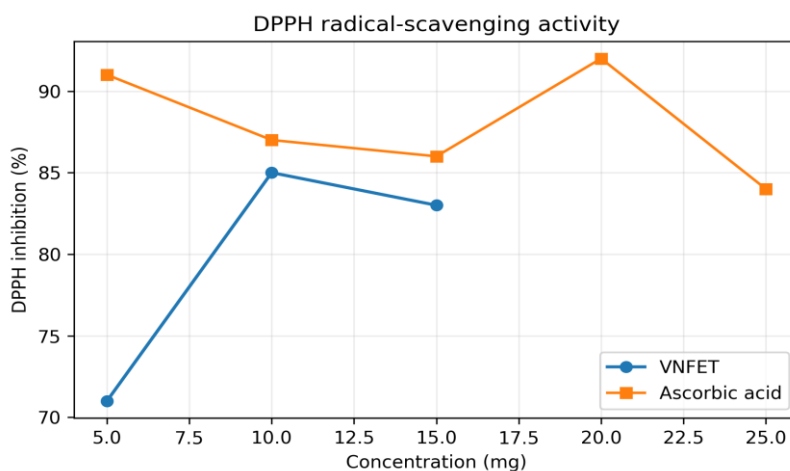


Figure 3: DPPH radical-scavenging activity of VNFET compared with ascorbic acid.

3.5 FRAP reducing activity.

Concentration	VNFET average OD	Ascorbic acid average OD
5 mg	1.56	1.52
10 mg	1.65	1.63
15 mg	1.73	1.74

VNFET showed increasing absorbance with increasing concentration, with the highest reported absorbance of

1.73 at 15 mg. The corresponding ascorbic-acid value was 1.74.

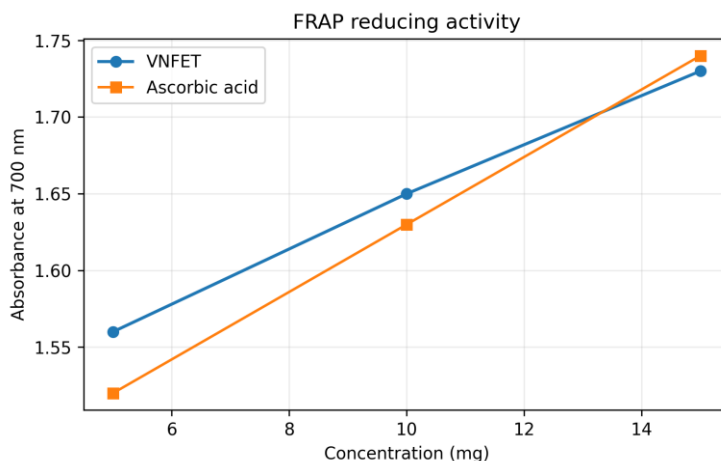


Figure 4: FRAP reducing activity of VNFET and ascorbic acid.

3.6 In vitro anti-inflammatory activity.

Concentration	Average % inhibition	Reported IC50
5 mg	63%	10.59 mg
10 mg	57%	10.59 mg
15 mg	49%	10.59 mg

The albumin-denaturation assay showed 63% inhibition at 5 mg, followed by 57% at 10 mg and 49% at 15 mg.

The reported IC50 of VNFET was 10.59 mg.

3.7 Comparison with diclofenac sodium

Diclofenac sodium concentration	Average % inhibition
5 mg	91%
10 mg	93%
15 mg	85%
20 mg	89%
25 mg	88%

Diclofenac sodium was reported to have an IC50 of 12.129 mg. Under the source study's assay conditions, the VNFET IC50 was numerically lower (10.59 mg).

This comparison is limited to the in vitro albumin-denaturation assay and should not be interpreted as evidence of clinical superiority.

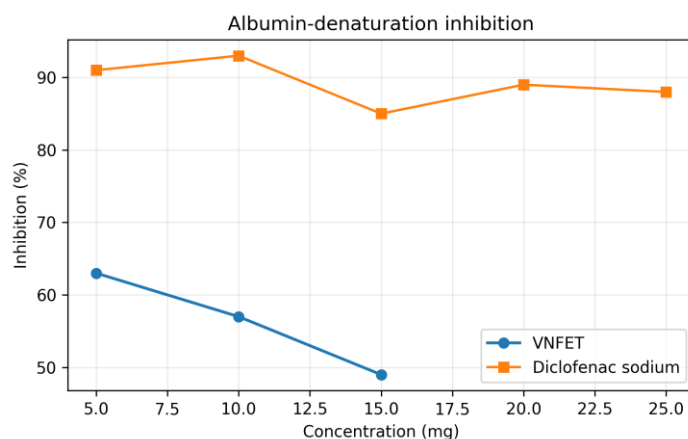


Figure 5: In vitro albumin-denaturation inhibition by VNFET and diclofenac sodium.

3.8 Acid-neutralizing capacity.

Sample	Quantity	ANC (mEq)
Standard sodium carbonate	Quantity equivalent to 1 g	7.92
VNFET sample	Quantity equivalent to 10 mL	19.49

VNFET demonstrated a reported acid-neutralizing capacity of 19.49 mEq, whereas the sodium-carbonate standard showed 7.92 mEq. Thus, the reported VNFET

value was higher than the comparator in this chemical assay.

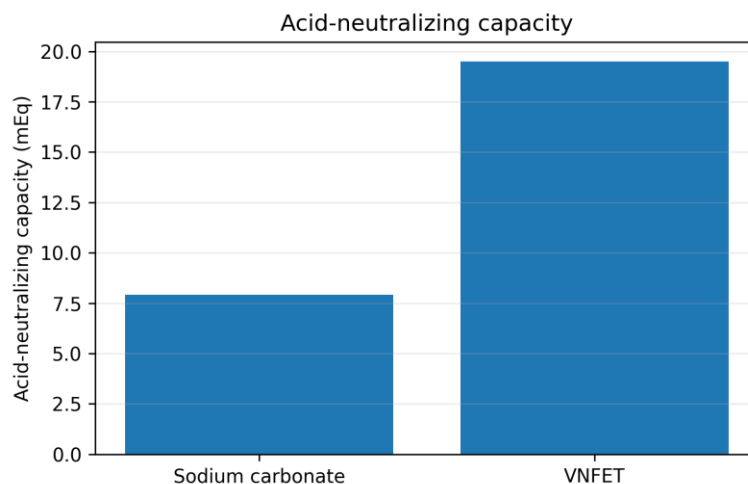


Figure 6. Reported acid-neutralizing capacity of VNFET and sodium carbonate.

4. DISCUSSION

The preliminary phytochemical screening demonstrated a positive response for alkaloids, flavonoids, phenols, proteins and carbohydrates, with a negative response for saponins. The presence of flavonoids and phenolic constituents is relevant to the antioxidant assays because these classes can participate in electron- and hydrogen-transfer reactions.

The quantitative screening showed a reported maximum flavonoid value of 75 mg at a concentration of 300 and a maximum phenolic value of 120 mg at a concentration of 200. Because the source thesis reports these values simply in milligrams and does not provide a standardized mass-of-extract denominator, the manuscript retains the original reported units rather than converting them to another expression.

The DPPH results demonstrated measurable radical-scavenging activity. VNFET reached 85% inhibition at 10 mg, while the reported IC₅₀ was 7.48 mg. Ascorbic acid showed a lower IC₅₀ of 3.373 mg, indicating stronger potency for the reference standard under the reported conditions. Nevertheless, the extract showed substantial chemical radical-scavenging activity.

FRAP measurements increased from 1.56 at 5 mg to 1.65 at 10 mg and 1.73 at 15 mg. This concentration-associated increase in absorbance indicates increasing reducing activity within the evaluated VNFET range.

4.1 Anti-inflammatory findings

The albumin-denaturation assay produced 63% inhibition at 5 mg, with progressively lower reported inhibition at 10 and 15 mg. The reported VNFET IC₅₀ was 10.59 mg, while diclofenac sodium had an IC₅₀ of 12.129 mg. These results support the presence of an in vitro protein-stabilizing/denaturation-inhibitory effect in the test system.

The source study describes this assay as an in vitro indicator of anti-inflammatory activity. The findings should therefore be interpreted as preliminary pharmacological screening results rather than proof of therapeutic anti-inflammatory efficacy in humans or animals.

4.2 Acid-neutralizing activity

The acid-neutralizing capacity of VNFET was reported as 19.49 mEq compared with 7.92 mEq for sodium carbonate. The higher value indicates that, in the reported back-titration experiment, the VNFET sample neutralized a greater quantity of acid than the comparator. This observation supports further investigation of the extract's acid-neutralizing properties.

4.3 Relationship between phytochemicals and activity

The coexistence of flavonoids and phenols with measurable DPPH and FRAP activity provides a plausible chemical association between the phytochemical profile and antioxidant measurements. However, the present dataset does not isolate individual constituents or establish a direct causal relationship

between any single phytochemical and a pharmacological endpoint.

4.4 Comparison with previous literature

The source thesis cites earlier work describing antioxidant, analgesic, anti-inflammatory and other biological activities of different parts of *Vitex negundo*. The present study adds flower-extract data focused on phytochemical screening, DPPH, FRAP, albumin denaturation and acid-neutralizing capacity.

5. Strengths and limitations

5.1 Strengths

The investigation evaluated multiple complementary endpoints using a single ethanolic flower extract. Qualitative phytochemical screening was supported by quantitative flavonoid and phenolic measurements, while antioxidant activity was examined using both radical-scavenging and reducing-power assays. The study also included an in vitro protein-denaturation model and an acid-neutralization assay.

6. CONCLUSION

The ethanolic extract of *Vitex negundo* flowers (VNFET) demonstrated a defined qualitative phytochemical profile with alkaloids, flavonoids,

phenols, proteins and carbohydrates detected, while saponins were absent. The reported maximum total flavonoid and phenolic values were 75 mg and 120 mg, respectively.

VNFET exhibited measurable antioxidant activity in both DPPH and FRAP assays, with 85% DPPH inhibition at 10 mg, an IC₅₀ of 7.48 mg and FRAP absorbance of 1.73 at 15 mg. In the albumin-denaturation assay, 63% inhibition was reported at 5 mg with an IC₅₀ of 10.59 mg. The extract also showed a reported acid-neutralizing capacity of 19.49 mEq compared with 7.92 mEq for sodium carbonate.

The findings support further phytochemical characterization and pharmacological investigation of *Vitex negundo* flower extract. The present results are limited to the in vitro experimental conditions described in the source study.

7. Data tables for publication

The following consolidated tables reproduce the numerical results reported in the source study in a publication-oriented format.

Table 2: Total flavonoid content of VNFET.

Concentration	Replicate OD values	Average OD	Reported total flavonoids
100	0.04, 0.05, 0.05	0.04	57 mg
200	0.06, 0.07, 0.08	0.07	63 mg
300	0.10, 0.09, 0.09	0.09	75 mg

Table 3: Total phenolic content of VNFET.

Concentration	Sample OD	Standard OD	Reported total phenol
200	0.12	0.10	120 mg
400	0.14	0.13	107 mg
600	0.10	0.10	100 mg
800	0.10	0.10	100 mg
1000	0.09	0.11	81 mg

Table 4: DPPH activity of VNFET.

Concentration	Average inhibition	IC ₅₀
5 mg	71%	7.48 mg
10 mg	85%	7.48 mg
15 mg	83%	7.48 mg

Table 5: FRAP activity.

Concentration	VNFET OD	Standard OD
5 mg	1.56	1.52
10 mg	1.65	1.63
15 mg	1.73	1.74

Table 6: In vitro anti-inflammatory activity of VNFET.

Concentration	Average inhibition	IC ₅₀
5 mg	63%	10.59 mg
10 mg	57%	10.59 mg
15 mg	49%	10.59 mg

Table 7: Anti-inflammatory activity of diclofenac sodium.

Concentration	Average inhibition
5 mg	91%
10 mg	93%
15 mg	85%
20 mg	89%
25 mg	88%

Table 8: Acid-neutralizing capacity.

Sample	Quantity	ANC (mEq)
Standard sodium carbonate	Quantity equivalent to 1 g	7.92
VNFET sample	Quantity equivalent to 10 mL	19.49

Statistical reporting

The source study states that measurements were repeated three times for the DPPH, FRAP and relevant spectrophotometric determinations. The reported tables present replicate readings or averages where provided. No inferential statistical comparison (such as a reported p value) is presented in the source results; therefore, no new inferential statistics have been added to this manuscript.

8. Declarations**8.1 Ethics approval and consent to participate**

Not applicable to the in vitro experimental work reported in this manuscript.

8.2 Consent for publication

Not applicable.

9: Abbreviations.

Abbreviation	Meaning
VNFET	Vitex negundo flower ethanolic extract
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric reducing antioxidant power
ANC	Acid-neutralizing capacity
OD	Optical density/absorbance
IC50	Concentration producing 50% inhibition

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