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Phytochemical investigation and antioxidant potential for *Vitex negundo* L. Leaves Methanolic extract

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Abstract

This research aimed to analyze the phytochemical constituents and antioxidant capabilities of crude methanolic extract of *Vitex negundo* L. leaves, which considered as one of medical plant. Leaves were gathered for examination followed by methanolic and essential oil extraction for biological application. Through screening processes, important compounds of therapeutic effects were investigated such flavonoids, polyphenols, and alkaloids in lower content. The quantitative assay revealed that the major content was flavonoids (513,34±23,15µg/mL), while the amount of total phenols was (350,67±27,68mg/mL). Antioxidant tests were carried out using DPPH and FRAP methods, for evaluation purposes in the current study. For DPPH, result indicated that the scavenging ability of *V. negundo* L. methanolic extract was achieved in relative to vitamin C the potential antioxidant agent at concentration (83.34±3.51µg/mL). In the FRAP experiment conducted on *V. negundo* L. plant samples showed a ferric reduction capacity of (1.92±30mg/mL) at the concentration (0.545±15mg/mL) in relative to Trolox standard. These significant findings highlight *V. negundo* L. potential as a natural source of antioxidants. These results indicate that *V. negundo* L. may be an option, for medical purposes in addressing conditions linked to oxidative stress. This supports its standing use and positions it as a remedy, in contemporary medicine.

Keywords: *Vitex negundo* L, Methanol Extraction, antioxidant activity, Phytochemical Screening.

Introduction

The Chinese chaste tree or five leaved chaste tree, with the scientific name *Vitex negundo* Linn (*V. negundo* L) is a medicinal plant belongs to the Lamiaceae family that thrives in tropical and subtropical areas like Iraq, fig 1, Southeast Asia and some parts of Africa. The plant is a fragrant shrub growing, up to 5 meters tall with compound leaves made up of five lance shaped leaflets, blue flowers and dark drupe like

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fruits. It flourishes in both habitats and manmade gardens is highly valued in ancient healing practices like Ayurvedic medicine and traditional Chinese medicine for its strong anti-inflammatory properties as well as antimicrobial and pain-relieving benefits [1].

Figure 1 Filed of *Vitex negundo* L. leaves

In Southeast Asias medicine practices *V. negundo* known as "Nirguna" in Ayurvedic medicine has played a crucial role by relieving pain and inflammation along; as recent studies indicate that *V. negundo* commonly known as the five leaved chaste tree or nirgundi in Ayurveda medicine [2], in India may have benefits in fighting cancer and has garnered interest within the field of oncology as a result of this discovery, with treating respiratory problems and female reproductive ailments. It has been utilized to address arthritis, asthma, bronchitis, menstrual disorders and enhance the healing of wounds. The diverse biological capabilities of *V. negundo* credited to a range of substances found in its leaves and bark such as flavonoids and alkaloids, among others have demonstrated effectiveness, in regulating inflammation and combating oxidative stress [3].

Studies have highlighted that extracts derived from *V. negundo* possess antioxidant properties that can regular cell growth and trigger programmed cell death in cells. Crucial components such as flavonoids and phenolic acids found in *V. negundo* are thought to be responsible for these effects which could potentially position this herb as a complementary approach, to conventional cancer treatments. In the leaves of the plant *V. negundo* various substances found; like luteolin and casticin have been found to have effects in reducing stress and inflammation—both known factors associated with the development of cancer [4]. Moreover, the essential oils from this plant contain compounds such as β caryophyllene oxide that exhibit properties like regulating the system fight against certain bacteria and reduce inflammation. These

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combined effects suggest that the plant may play a role, in alleviating cancer symptoms and potentially enhancing the efficacy of cancer treatments [5].

In addition, to its effects on cancer treatment *Vitex negundo* also exhibits anti-inflammatory and pain-relieving qualities that are beneficial for addressing long term inflammatory issues like arthritis and rheumatism. Research indicates that compounds found in the leaves of this plant have the ability to block agents such, as cytokines and enzymes that contribute to pain and inflammation. The anti-inflammatory effects of *V. negundo* plant are mostly attributed to substances such, as flavonoids and essential oils that help in decreasing inflammation by regulating pathways like COC and LOY and reducing cytokines like interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) according to Nguyen [2] study in 2024 *V. negundo* could serve as an alternative to nonsteroidal inflammatory drugs (NSAIDs) as it offers pain relief, with fewer adverse reactions as discussed by Sharma [3].

The medicinal advantages of the plant *V. negundo* also aid in maintaining health historically used in infusions or steam inhalations to alleviate respiratory problems such, as coughs and asthma symptoms while also alleviating bronchitis symptoms. The plants effects that widen the airways and reduce inflammation are attributed to substances, like α pinene and γ terpinene that assist in relaxing the muscles and decreasing mucus production. This age-old remedy shows potential as a solution, for handling term respiratory problems and improving lung wellness [6].

Aside, from its properties *V. negundo* is highly regarded for its impact on the environment as well. It is commonly used in agroforestry as a natural insect deterrent and organic fertilizer to enhance soil quality. The leaves of this plant contain substances that repel insects. When planted near crops it can naturally help control pest populations and boost crop yields. Additionally, as a shrub it plays a role, in preventing soil erosion and restoring land promoting eco agricultural methods that are sustainable [7].

Materials and Methods

Plant collection

Vitex negundo L. leaves were collected from local markets of Baghdad – Iraq and recognized by Dr. Ibrahim S. Al–Jubouri, College of Pharmacy, Al–Mustansiriyah University, during October,2023. The collected plant leaves were thoroughly washed with distilled water to remove impurities, air-dried in a shaded area to preserve phytochemicals, and then ground into a fine powder using an electric grinder. The powdered material was stored in a desiccator until further use biological experiment.

Preparation of plant methanolic extract

Approximately (50g) of the dried leaf powder was extracted with (85%) methanol (250mL) using Soxhlet apparatus at (65°C) for three hours. The obtained extract was concentrated using a rotary evaporator under reduced pressure, yielding a dry methanol extract. This extract was then stored at (-20°C) in a sealed container to prevent degradation of bioactive compounds [8].

Phytochemical screening

Preliminary phytochemical analyses were conducted to identify the presence of major bioactive compounds in *V. negundo* L., including alkaloids, flavonoids, tannins, saponins, phenolic compounds, and glycosides, at Biotechnology research center – Al-Nahrain University.

Quantification of phytochemicals

Determined total flavonoid content and total phenolic content from methanolic extraction

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Total flavonoid content (TFC)

Total flavonoid content determined by Sakanaka [9], (3mg) of *V. negundo* L. methanolic extract in (5mL) of methanol 50%, followed; added (1mL) $NaNO_3$ 5% and waited (6min.). Then, added (1mL) of $AlCl_3$ and leaved (5min) before added (10mL) of NaOH 10%. After that, mixed well with same time made up to (50mL) with D.W. Lastly, the mixture was left for 15 minutes, then the absorption was measured at 510nm for all replicates. TFC was calculated based on a rutin calibration curve [y=0.0012x+0.1109 R^2=0.9317] and expressed as mg rutin equivalents per gram of extract.

Total phenolic content (TPC)

TPC was measured by the Folin–Ciocalteu method [10]. (1mL) from *V. negundo* L. methanolic extracts were mixed with (2.5mL) Folin–Ciocalteu reagent and after (5min) added (2mL) of sodium carbonate solution, followed by incubation at (50°C) for (10min). The absorbance was recorded at (765nm), and TPC was expressed as mg gallic acid equivalents (GAE) per gram of extract using a standard curve prepared with gallic acid [y=0.8101x-0.7595 R^2=0.99958].

Antioxidant activity assays

Antioxidant activity was evaluated through 2-2-Diphenyl-1-picrylhydrazyl (DPPH) assessing radical scavenging capacity and ferric reducing antioxidant power (FRAP) determining ferric ion reducing power.

2-2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was performed to evaluate the radical scavenging activity [12] of *V. negundo* L. extracts among vitamin C. Different concentrations of the methanolic extract (0.0625, 0.125, 0.250, 0.500mg/mL) were prepared by (1mL) of DPPH to the four tubes, and then incubated for (30min.) at (37°C); The absorbance was measured at (517nm), fig. 2, and the radical scavenging activity was calculated as follows:

$$\boldsymbol{DPPH\ scarving\ activity} = \frac{\boldsymbol{absorbance\ of control - absorbance\ of\ sample}}{\boldsymbol{absorbance\ of\ control}} \times \mathbf{100}$$

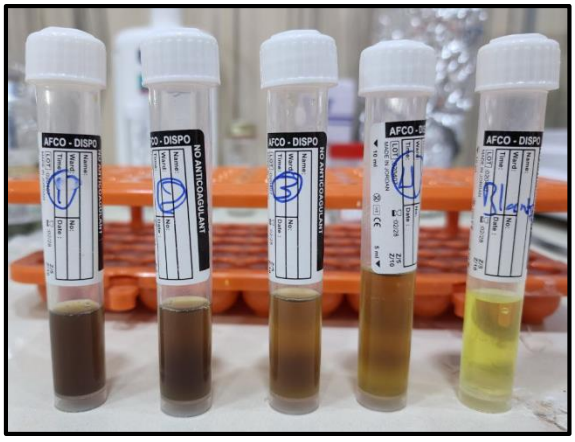

Figure 2 Preparation of DPPH for *Vitex negundo* L. methanolic extraction

Ferric reducing antioxidant power (FRAP) Assay

The FRAP assay was conducted according to Fu [12], to assess the ferric reducing capacity of the extracts. Briefly, samples of *V. negundo* L. extract was prepared in concentrations of (0.64, 0.32, 0.16, 0.08mg/mL). Each sample was combined with phosphate buffer (0.2M, pH6.6) and (1%) potassium ferricyanide and incubated at (50°C) for (20min). After adding trichloroacetic acid (10%) and centrifuging, the supernatant was mixed with distilled water and freshly prepared ferric chloride (1%) solution.

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Absorbance was measured at (700 nm). Results were compared with a Trolox standard curve in concentrations of (0.64, 0.32, 0.16, 0.08mg/mL), and data were expressed in Trolox equivalents, fig 3.

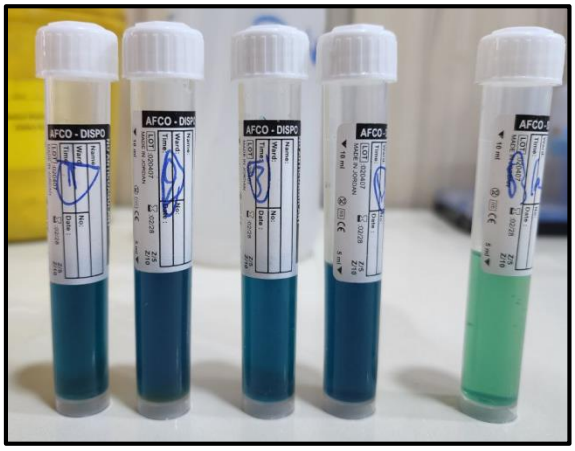
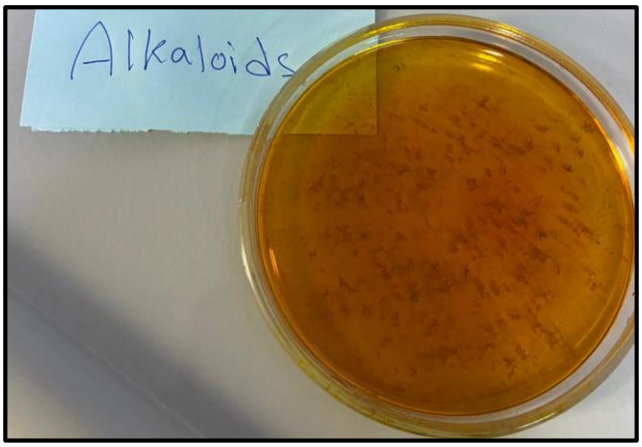
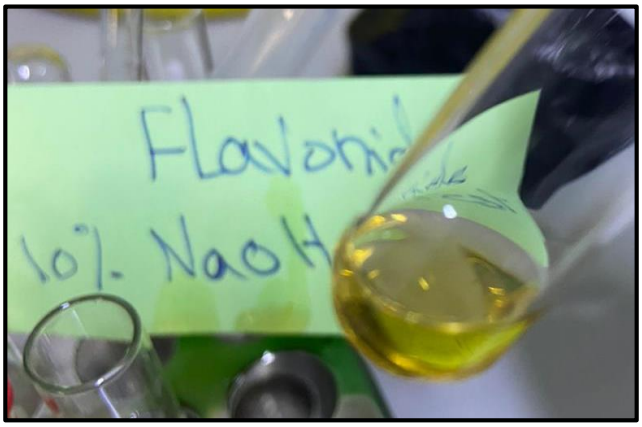


Figure 3 Preparation of FRAP for *Vitex negundo* L. methanolic extraction

Results and Discussion

Phytochemical analysis showed;

Detection test	Result	
Alkaloids by • Mayer reagent • Dragendroff reagent	+	
Flavonoids	+++	

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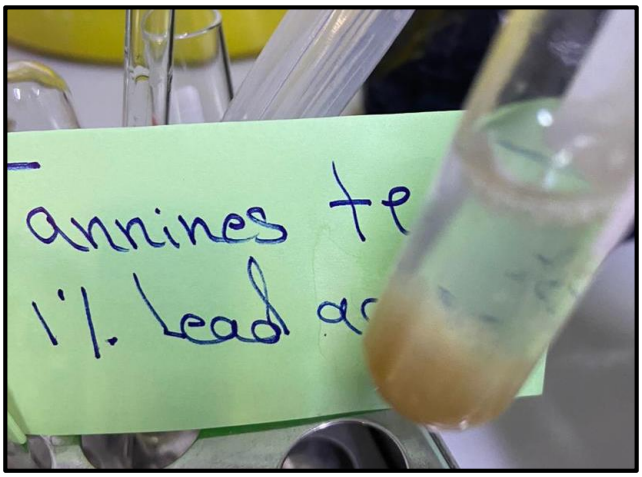
Polyphenolic	+	
Polysaccharides	++	
Tannins	++	

** + mild, ++ moderate, +++ strong

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The appearance of alkaloids, although in mild levels, is significant due to literature pharmacological effects, as ex. analgesic, antimicrobial, and anti-inflammatory activities. These combinations can react with cellular pathways, intensifying the herb's potential as a therapeutic agent [13].

The plenty of flavonoids indicates rich antioxidant capacity, as these flavonoids are known for their capacity to donate a lot of electrons with stabilizing free radicals. This activity is important in lightening oxidative stress and save cells from damage [14].

Polyphenols supply extra antioxidant activity, aid to reduction of ROS and intensifying the overall cellular structures stability. Both Polyphenols and flavonoids, generating a synergistic antioxidant outcome [15].

Polysaccharides share in immune effects, that's support immune in its responses and serve health. Due to that, *V. negundo* may aid in improving immune function, further more exceed visibility range its potential medicinal uses [16].

The moderate level of tannins assists special properties, that can help in healing of wound and reduction the inflammation. In addition, tannins possess antimicrobial properties, given more benefit to *V. negundo* therapeutic protocol [1].

Quantitative assay for Total Flavonoids and Total phenolic compounds

Total flavonoid content was spectro-photochemically determined in methanolic extract of *V. negundo* as rutin equivalent. The extract was found to contain (513.34±23.15µg/mL) flavonoids, in compared with stander curve fig (2-A) [19], while total phenolic content was (350.67±27.68mg/mL), in compared with stander curve fig (2-B) [18].

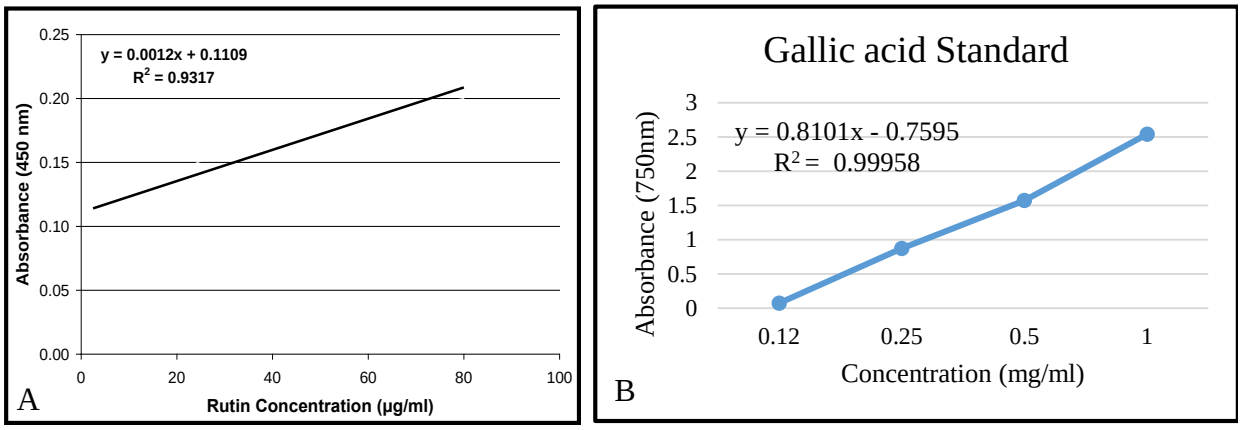

Figure 2. A – stander curve of Rutin, B – stander curve of gallic acid

The Antioxidant Activity

Result of DPPH radical scavenging activity

Methanolic extract of *V. negundo* was more effective in DPPH radical scavenging activity than vitamin C at the four concentrations tested [19]. The concentrations (0.500 and 0.250mg/mL) of plant extract shared an approximated radical scavenging activity (83.34±3.51 and 74.01±2.64%, respectively) and shown low activity at the concentrations (0.125 and 0.0620mg/mL) as (47.01±5.02 and 32.67±5.50%, respectively), as explain in fig. 3-A.

Result of FRAP

While, methanolic extract of *V. negundo* was more effective in FRAP than Trolox (vitamin E) at the four concentrations tested [20]. The concentrations (0.64 and 0.32mg/mL) of plant extract shared an

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approximated radical scavenging activity (1.92±0.30 and 1.33±0.29mg/mL, respectively) and shown low activity at the concentrations (0.16 and 0.08mg/mL, respectively) as (1.14±0.04 and 0.85±0.03mg/mL, respectively), as explain in fig. 3-B.

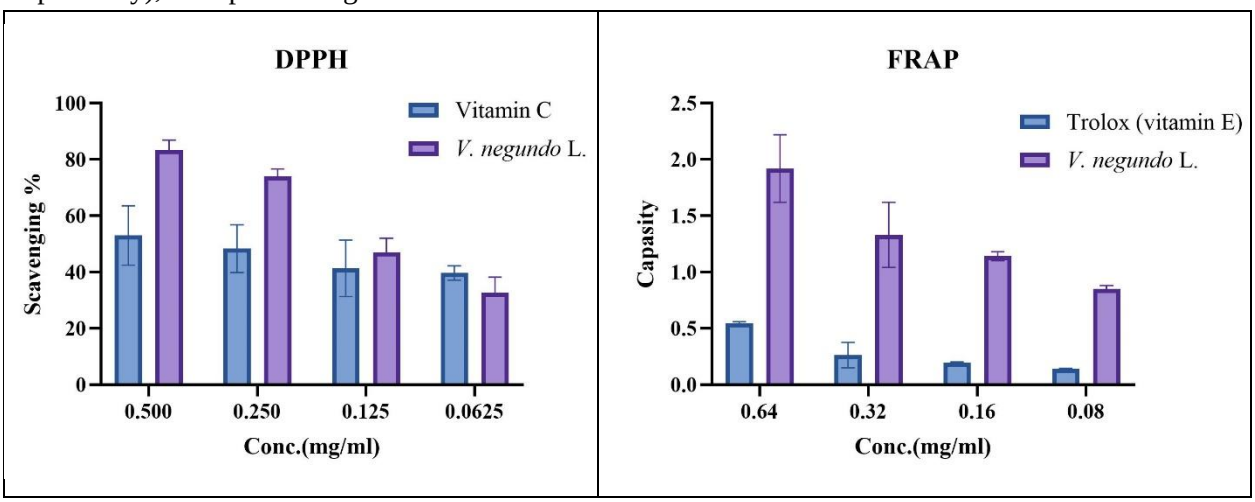

Figure 3. A – DPPH radical scavenging action of *V. negundo* L.
B – Reductive ability over *V. negundo* L. methanolic banish then trolox (vitamin E)

Conclusions

Result indicated that the scavenging ability of *V. negundo* L. methanolic extract was achieved in relative to vitamin C the potential antioxidant agent at concentration (83.34±3.51µg/mL). In the FRAP experiment conducted on *V. negundo* L. plant samples showed a ferric reduction capacity of (1.92±30mg/mL) at the concentration (0.545±15mg/mL) in relative to Trolox standard. These significant findings highlight *V. negundo* L. potential as a natural source of antioxidants. These results indicate that *V. negundo* L. may be an option, for medical purposes in addressing conditions linked to oxidative stress.

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

[First Author]: Conducted some experiments, data rearrangement and drafted the initial manuscript.

[Second Author]: Contributed to the conception and design of the study, and conducted some characteristics of the products.

[Third Author]: Facilitated processes to ensure the project ran smoothly.

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