RESEARCH ARTICLE

Utilizing *In-vitro* Techniques to Evaluate the Antioxidant Potential of *Vitex negundo* Leaves Extract

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ABSTRACT

In this study, *Vitex negundo* leaf extracts were screened for phytochemical composition and antioxidant activity. Antioxidants are essential for preventing and battling oxidative stress and, ultimately, ailments it leads to. The study that employed FRAP, nitric oxide scavenging, superoxide radical scavenging, 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) to test the antioxidant activity of *V. negundo* leaves extract revealed antioxidant activity. The result of the phytochemical examination of the extract shows that entity of total phenolic content, reducing sugar, saponin. The findings suggest that this herb can be a very beneficial component for conditions that call for antioxidant therapy and are brought on by oxidative stress. The V. negundo shows the average TPC of 235.24 GAE/g weight of ethanolic extract. The TFC in the ethanolic extract was found to be 157.53 mg QE/g of extract. Outcomes demonstrated the significant antioxidant activity of ethanolic extracts of *V. negundo* leaves.

Keywords: *Vitex negundo*, DPPH, FRAP, Ethanolic extract, Superoxide radical.

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INTRODUCTION

Many different ailments have been treated using medicinal plants since the beginning of humanity. A third of the population still trusts on plants for medical treatment. India, the birthplace of the Ayurvedic medical system, is a country rich in medicinal herbs. Herbal medications are typically regarded as safe medications with less harmful effects.[1, 2]

Free radicals, which are substances with unpaired electrons, are a source of the condition known as oxidative stress.[3] Additionally, oxidative stress is brought on by reactive oxygen species (ROS), produced both internally during aerobic cellular respiration and externally as a result of pollution and ionizing radiation.[4] Many metabolic diseases, like; cancer, stomach ulcers, diabetes and atherosclerosis, as well as abnormalities of the neurological system, are either directly or indirectly caused by both of these.[5] Living things protect themselves from such injury by either ingesting dietary antioxidants or using endogenous antioxidant defense mechanisms. Many chronic diseases and the progression of those diseases can be avoided by enhancing the body's natural antioxidant defenses or by consuming more dietary antioxidants. Antioxidants work by blocking the synthesis of free radicals or by halting and upsetting oxidative chain reactions, among other mechanisms.[6-8]

To combat the damaging consequences of oxidative stress, researchers are concentrating on naturally occurring antioxidants that bind potentially dangerous free radicals.[9] Recent advances in medical technology have made it simpler to identify specific chemical components of plants and assess their potential antioxidant action.[10] Antioxidants from natural or herbal sources can be utilized to control oxidative stress because they are healthy, safe, and effective.[11]

Since early times, medicinal plants have been a significant source of therapeutic compounds used to treat human ailments.[12] Almost all medicines used were obtained from plants. *"Vitex negundo"* is one of the well-known plant of the Verbenaceae/Lamiaceae family. It is widely used in folk medicines. It is familiar bythe name- "*Chinese chaste tree"*. Sometimes it is "five-leaved or three-leaved chaste tree", or "horseshoe Vitex" or nishida. *V. negundo* Linn is also referred to as "sarvaroganivarani" in the traditional Indian medical system, which is a treatment for all illnesses. In India, former Ayurvedic physicians used fresh juice of leaves of *V. negundo* by applying externally to treat various skin problems, like

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dermatitis, eczema and acne. Leaves of *V. negundo* are useful in toothache, inflammation, leucoderma, rheumatoid arthritis, and skin ulcers, It has anti-inflammatory, analgesic, anti: ulcerogenic, bacterial, and fungal effect and anti-microbial activity.[13,14]

In particular, if it demonstrates greater therapeutic efficacy, a decreased profile of adverse effects, or both, Investigating this herb's potential to treat serious illnesses is necessary. The antioxidant impact of this drug's extract is assessed to see if it has a stronger effect.

MATERIALS AND METHOD

An ecological survey of the area will be conducted. During the flowering and fruiting periods, *V. negundo*'s fresh leaves will be collected. With the aid of the RTMNU, the Nagpur botany department's flora, the collected plants will be recognized. Drugs were dried in the shade, crushed into coarse powder, and kept in an airtight container in a cold, dry place. The remaining of extra reagents were purchased as an AR grade.

Methods

Selection of the extraction method will depend upon the selection of solvent showing maximum extract value. The powdered leaf is extracted with 100% ethanol using soxhlet equipment at 60 to 65°C for three hours. Three additional times were used for the extraction. The leaf extracts were concentrated and vacuum-dried after being filtered through polypropylene cloth. To create a solid mass from the semisolid extract, it was then dried, packaged, and labeled. The extract obtained will be further used for phytochemical analysis.[15]

Preliminary phytochemical screening

To find diverse phytochemicals, a preliminary phytochemical screening of the extract was conducted.

Estimation of total phenolic content

It was calculated for extracts using the Folin-Ciocalteu technique. UV visible spectrophotometer was used to measure absorbance at 765 nm following the addition of sample extract to 1.2 mL of 7.5% w/v Na_2CO_3, 1.5 mL of 10% v/v Folin-reagent, Ciocalteu's, and 30 minutes of dark incubation. Therefore, mg per 100 g of extract was used to determine gallic acid equivalents.[16]

Estimation of total flavonoid content

Quercetin as reference material, TFC was evaluated in an aluminum chloride complex assay. Quercetin solutions were produced as follows: 500 uL of distilled H_2O_2, 100 µL of 5% sodium nitrate, and 100 µL each of following quercetin concentrations: 0.1, 0.5, 1.0, 2.5, and 5 mg/mL in methanol. 150 µL of 10% aluminum chloride solution were added after about 6 minutes, left in situ for 5 minutes. Add 200 µL of a 1M sodium hydroxide solution to each dilution after five minutes, and then use UV-visible spectrophotometer to measure absorbance at 510 nm. TFC was calculated mgQE/g of quercetin after the samples were analysed using the same procedure.[17]

Evaluation of *In-vitro* Antioxidant Activity

DPPH scavenging assay

In order to gauge antioxidant activity, ascorbic acid was used reference material in DPPH free radical scavenging assay. Freshly made sample was collected and 0.1 mL were put into test tubes. Tubes were filled with 6 mL of DPPH solution (0.1 mM), and they were then kept in the dark for an hr. At 517 nm, color was reciting. Calculating the difference between DPPH solution's O.D. and sample that included the solution allowed for the evaluation of scavenging activity.[18]

Hydroxyl radical scavenging method

In 20 mM phosphate buffer was mixed with 100 µL of H_2O_2 de (1 mM), 100 µL of deoxyribose (2.8 mM), 200 µL of EDTA (0.1 mM), 200 µL of ferric chloride (0.1 mM), and 100 µL of ascorbic acid (0.1 mM). The reaction mixture was individually supplemented with different extract concentrations (500 µL), and it was then incubated at 37°C for an hour. The reaction mixture received 1-mL of trichloroacetic acid (2.8%) and 1.0 mL of thiobarbituric acid (1%), followed by 20 minutes of heating in a water bath. The preparation of control and blank was the same. UV set at 532 nm was used to compare each sample's absorbance to a blank. To calculate the percentage of inhibition, the sample's and the control's absorbance values were used.[19]

Nitric oxide scavenging technique

A range of extract concentrations (0.2–0.8 mg/mL) were collected with 0.5 mL of saline phosphate buffered (pH 7.4), 0.5 mL of sodium nitroprusside (10 mM), 1.0 mL of Gries reagent. Combination was then incubated at 30° C for 2.5 hours. A UV spectrophotometer was used to evaluate absorbance at 548 nm in contrast to blank surface. The formation of nitric oxide was firmly inhibited by comparing an extract's absorbance to the control.[20]

Ferric reducing antioxidant power (FRAP assay)

A new working FRAP solution was made by mixing acetate buffer (pH 3.6), 2, 4, and 6-tripyridyl-s-triazine (10 mM), ferric chloride solution (20 mM). This solution was then kept at 37°C. After adding 2.8 mL of the FRAP solution, it was left in dark for 30 minutes to see how it responded. The absorbance at 593 nm was estimated using UV light. Outcomes were expressed g Fe (II)/g dry bulk.[21]

Superoxide radical scavenging method

Superoxide anions were created using a non-enzymatic procedure termed phenazine methoxy sulfate-nicotinamide adenine dinucleotide synthesis, and the efficiency of these anions was evaluated by reducing nitro blue tetrazolium (NBT). Phosphate buffer, pH 7.4, 1-mL of NBT (50 M), and 1-mL of NADH (78 M) solution were all combined to produce solutions with extract concentration. To start reaction, 1-mL of PMS solution (60 M) was added to the mixtures. Reaction was then let to stand at 25°C for 5 minutes. UV was used to detect absorbance at 560 nm and compared to a blank.[22]

RESULTS

The *V. negundo* drug powder was treated with 100% ethanol as a solvent foe the extraction process. To extract the material, Soxhlet equipment was employed. After being concentrated in a vacuum, the extract was dried in vacuum desiccators. The yield obtained was 36%w/w and the then used for further procedure, Table 1.

Phytochemical Analysis

The findings of the analysis of several phytochemicals in the ethanolic extract of *V. negundo* are tabulated below. It displays TPC of *V. negundo* extract. Gallic acid standard curve was used to compute results mg GAE/g of extract. Average TPC for *V. negundo* was 38.82 ± 0.3 mg GAE/g of extract. The antioxidant effects of phenolic compounds are assumed to be caused by their redox characteristics. The redox potential neutralises and blocks free radicals, neutralises singlet and triplet oxygen, breaksdown peroxides. TFC of extract of *V. negundo* individually is presented in Table 2. Results were calculated using the quercetin standard curve as mg QE/g of extract. The *V. negundo* showed average TFC of 152.04 ± 0.5 mg QE/g of extract. The significant antioxidant action of the flavonoid molecules may be due to their scavenging or chelating capacity, which allows them to deactivate highly active oxidative moieties.

DPPH free Radical Scavenging

Antioxidant potential of *V. negundo* extracts (5.0 to 30.0 mg/mL) was assessed utilizing *in-vitro* DPPH free radical scavenging technique. Extract from *V. negundo* leaves demonstrated 68.03% scavenging. A similar quantity of ascorbic acid showed scavenging at 90.49%. The activity increased linearly with concentration and was concentration-dependent. The high TPC and TFC concentrations and potent proton-donor properties of the investigated extracts may be the root of their antioxidant activity.

Hydroxyl Radical Scavenging

To evaluate extracts' capacity to scavenge hydroxyl radicals, ascorbic acid was active as reference standard varying from 5.0 to 30.0 mg/m. The *V. negundo* extract showed 48.76% scavenging. The same quantity of ascorbic acid showed 98.89% scavenging. Activity rises with higher concentration, according to the positive association between concentration and activity.

Nitric Oxide Radical Scavenging

Stable molecules nitrate and nitrite, which are both extremely reactive, unstable species, are produced when nitric oxide combines with oxygen. By detecting reduction in nitrous acid in presence of scavenging agent using Griess reagent at 546 nm, level of antioxidant activity may be ascertained. Extract's capacity to scavenge nitric oxide radicals was assessed at various dose levels, ranging from 5.0 to 30.0 mg/mL, using ascorbic acid as reference. As concentration levels increased, all test samples became more effective at scavenging nitric oxide radicals. In contrast to ascorbic acid, which displayed 97.88% scavenging at an identical concentration, *V. negundo* extract demonstrated 58.22% scavenging.

Table 1: Phytochemical analysis of extracts *V. negundo*

Constituents	*V. negudo*
Steroids	+
Carbohydrates	-
Alkaloids	+++
Phenolic compounds	+++
Saponins	+
Tannins	+++
Flavonoids	++
Glycosides	+

(+) indicated presence and
(–) indicated absence

Table 2: Total phenols, flavonoids and antioxidant activity in leaves of *V. negundo*

Parameters examined (mg/100 g)	*Values achieved*
Phenols	38.82 ± 0.3
Flavonoids	152.04 ± 0.5
Antioxidant activity	29.41 ± 0.5

Superoxide Radical Scavenging Activity

With ascorbic acid serving as the reference, ability of *V. negundo* extract to scavenge superoxide radicals was examined at absorptions ranging from 5.0 to 30.0 mg/mL. The outcome at the greatest concentration level was 98.23. A maximal inhibition of 68.56% was reported in a *V. negundo* leaf extract at 30 mg/mL.

Ferric Reducing Antioxidant Power

Basic idea behind a ferric-reducing antioxidant assay is that antioxidants have ability to convert Fe3+ to Fe2+ in the presence of 2,4,6-tri (2-pyridyl)-s-triazine (TPTZ). Optimal pH for reaction, which is pH-dependent, is 3.6. The endpoint is an intensely blue Fe^{2+}-TPTZ combination with a maximum absorbance of 593 nm. With increase in antioxidant concentration, absorbance falls. Antioxidant power activity of a *V. negundo* extract was assessed at various doses. 5.0 to 30.0 mg/mL using ferrous sulphate reference point. *V. negundo* leaves extract showed maximum FRAP value of 88.16% at 30 mg/mL.

IC_{50} Determination

The half maximum Inhibitory Concentration value, or IC_{50}, is the sample concentration needed to use free radical scavenging procedures to remove 50% of free radicals. Low IC_{50} values indicate high antioxidant capacity because it is inversely connected with sample's antioxidant capacity. IC_{50} value was considered by means of interpolation from the linear regression analysis. Results demonstrated that antioxidant activity of extract was very high and comparable to ascorbic acid.

DISCUSSION

Antioxidants interact with free radicals and destroy them. Since they stop cellular harm that free radicals produce, they are frequently referred to as scavengers of free radicals.[23]

Endogenous antioxidants are specific antioxidants produced by body to combat free radicals. They must, however, be supplemented with extra external (exogenous) oxidants, usually through diet or medicine. *In-vitro* antioxidant capacity of *V. negundo* extract was measured using nitric oxide, superoxide, DPPH, H2O2, and FRAP photometric tests.[24] According to *in-vitro* antioxidant testing, this drug has substantial antioxidant activity comparable to vitamin C served as indication standard. Antioxidant chemicals produce colored moiety that may be quantified calorimetrically using a spectrophotometer in order to battle free radical nature of. Degree of color or discoloration indicates how effective an antioxidant or extract is in scavenging free radicals through hydrogen-donating. Extracts from *V. negundo* leaves exhibit strong antioxidant activity.[25,26]

CONCLUSION

Due to the actions of free radicals, many diseases are either triggered or made worse. Strong and effective antioxidant therapy can be highly beneficial in the management of such disorders. Since ancient times, *V. negundo* has been utilized to cure a wide range of ailments due to its potent antioxidant benefits. Results from the study of this medication using different approaches showed good antioxidant activity.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHOR CONTRIBUTIONS

The study's design and concepts were worked on by all three writers. Ms. M. B. Wawre conducted experiments and wrote manuscripts. Dr. Deepak Khobragade provided supervision, review, and editing. The authors have given their approval for the final manuscript to be published.

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