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In vitro Antioxidant Properties and Bioavailability of Biological Application of *Vitex negundo*

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A B S T R A C T

The aim of this study was to screen solvent extracts of whole plant of *V. negundo* to display potent antioxidant activity in vitro antioxidant contents in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations. Our results SOD with the percentage of inhibition was 8.23% and 58.23.% for the leaf extract and vitamin C, Hydroxyl with the percentage of inhibition was 8.23% and 58.23.% for the leaf extract and vitamin C respectively at 150 µg/mL and total antioxidant assay maximum inhibition assay. The findings suggest that perhaps a significant number of ADME/toxicity compounds were considered to be a positive cancer drug molecule, and that the analysis chosen compounds fulfil the ADME and Toxicity parameters. A significant relationship between antioxidant capacity and total phenolic content was found, indicating that phenolic compounds are the major contributors to the antioxidant properties of these plants. It is expected that the important phytochemical properties recognized by our study in the indigenous of *V. negundo* plant will be very useful in the curing of various diseases.

***Key words*:** *Vitex negundo*, ADMET, SOD, Total antioxidant, Hydroxyl

Chemical compounds derived from medicinal plants are utilized as raw materials in the manufacture of pharmaceutical medications, which are employed by humans to alleviate specific health problems [1]. Medicinal plants have been significant to humans since prehistoric times due to their antioxidant and natural therapeutic capabilities [2], since plants contain bioactive phytochemical substances that act on a broad variety of ailments, including neurological disorders. There is presently a great deal of research being conducted on the isolation of these bioactive compounds [1], which has resulted in the identification of novel medications of natural origin using contemporary procedures. Antioxidants are recognized to be critical for health because they neutralize free radicals created by stress, hence reducing oxidative damage [3]. Plant extracts are used as an alternative to synthetic antioxidants in the treatment of a variety of diseases [4], due to their pharmacological activity, economic viability, and low toxicity, as opposed to synthetic antioxidants, which may have adverse effects on human health [5]. These extracts include natural polyphenolic components that help prevent some illnesses and are connected with oxidative stress, since polyphenols are associated with antioxidant activity [6], but have a pro-oxidant effect in cancer cells [7]. The purpose of this research was to determine the antimicrobial activity of plant extracts and phytochemicals against both conventional microbial strains and multidrug-resistant bacteria. The current effort included the extraction and screening of leaves of *V. negundo* for antioxidant activity.

All reactive oxygen species, including free radicals such as superoxide (O_2), hydroxyl (OH), and peroxyl (ROO), as well as non-radical species such as hydrogen peroxide, are reactive oxygen species (H_2O_2). In vivo, these compounds are closely coupled at the source or are detoxified by endogenous antioxidant defences in order to sustain cellular function [8]. However, in sick states, detoxification procedures often fail owing to high ROS generation. If the pro-oxidant-antioxidant balance is not restored, the resulting pro-oxidant shift may result in destruction of cellular components such as DNA, polysaccharides, polyunsaturated lipids, and proteins, as well as irreversible cell dysfunction and death. Additionally, ROS are involved in the patho-physiology of a variety of diseases. Antioxidant phytochemicals are sought after because they have the potential to inhibit free radical reactions and protect the body against disease [9]. The Vitex (Lamiaceae) genus has 270 species, each with its own unique combination of medicinal active components and properties. Most of these plants are trees and shrubs that thrive in tropical and subtropical regions. Other species' fruits, seeds, and roots are also employed in traditional medicine. Among the Vitex species found in India are *V. negundo*, *V. glabrata*, *V. leucoxylon*, *V. penduncularis*, *V. pinnata*, and *V. trifolia* [10]. These species are used in traditional medicine to treat a number of ailments, including depression, venereal diseases, asthma, allergies, skin disorders, snakebite, and muscular pains [11-12]. Vitex plants are utilized

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biologically for a range of purposes, including cough treatment, wound healing, larvicidal, anti-HIV, anticancer, and trypanocidal activity [13] *V. negundo* leaf extract is often used to repel insects from grains [14]. Additionally, it contains polyphenols, terpenoids, glycosidic iridoids, and alkaloids. It also includes flavonoids such as luteolin, lutein-7-O-glucoside, corymbosin (a flavonoid), 5-Odesmethylnobiletin (a flavonoid), and 3′,4′,5,5,6,7,8, heptamethoxyflavone (a flavonoid). Additionally, it is used to treat eye infection, toothache, inflammation, leukoderma, spleen enlargement, skin ulcers, catarrhal fever, rheumatoid arthritis, gonorrhoea, and bronchitis. Additionally, it functions as an antihistaminic and antibacterial. *V. negundo* oil is effective for sinus and scrofulous lesions. The extract's oncogenic potential was determined in Swiss albino mice using Dalton ascites tumour cells [15]. Lagundi tablets (Ascof®, Rose Pharmacy, Mandaue, Philippines) are used for the treatment of moderate to severe bronchial asthma and cough [16].

MATERIALS AND METHODS

Collection of plant material and extraction

Leaves of *V. negundo* Roxb (Verbeaceae) were obtained from Boduvarayan malai in the Kalvarayan hills, Tamil Nadu, India. N 11 47' and 12 33' latitudes and E 77 02' longitude. The voucher number for this plant is PU/SRC/1/18/2020/PP/124. The powder extract (10 g) was macerated with 100 ml of water solution and filtered through Whatman No.1 filter paper. The powder extract and plant samples were kept at -20°C and temperature regulated for further analysis.

Assays for antioxidants

Assay for scavenging superoxide anion

The riboflavin-light-NBT system was used to support the test for superoxide anion radical scavenging activity [17]. 0.5 ml phosphate buffer (50 mM, pH 7.6), 0.3 ml riboflavin (50 mM), 0.25 ml PMS (20 mM), and 0.1 ml NBT were added to 1 ml of sample at various doses (25 to 150 g/ml) (0.5 mM). The reaction was initiated by utilizing a fluorescent light to illuminate the reaction mixture. After 20 minutes of incubation, the absorbance at 560 nm was determined. Ascorbic acid was employed as a reference substance. The following equation was used to evaluate the scavenging ability of the plant extract:

Assay for phosphomolybdate (Total antioxidant capacity)

The total antioxidant capacity of the fractions was evaluated using the phosphomolybdate technique as a standard [18]. A 0.1 ml aliquot of sample solution was combined with 1 ml reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were sealed and incubated for 90 minutes at 95°C in a water bath. After the samples had cooled to room temperature, the mixture's absorbance at 765 nm was determined in comparison to a blank. A typical blank was prepared using 1 ml of the reagent solution and the appropriate amount of solvent and incubated under the same conditions as the sample. Ascorbic acid was employed as a reference substance.

Hydroxyl radical scavenging assay

The capacity of various fractions of *V. negundo* extract to scavenge hydroxyl radicals produced by the $Fe3^{+}$-ascorbate-EDTA-H_2O_2 system (Fenton reaction) was determined [19]. The reaction mixture consisted 500 l of 2-deoxyribose (2.8 mM) in phosphate buffer (50 mM, pH 7.4), 200 l of a 1:1 solution of ferric chloride (100 mM) and EDTA (100 mM), and 100 l of H_2O_2 (200 mM) with or without the extract solution (100 l). 25 l of 150 mM ascorbate was added to initiate the reaction, which was then incubated for 1 hour at 37°C. 0.5 ml of the reaction mixture was added to 1 ml of TCA (2.8 percent; w/v; aqueous solution), followed by 1 ml of 1% aqueous TBA. The mixture was cooked over a boiling water bath for 15 minutes. After cooling the combination, the absorbance at 532 nm was measured in comparison to a blank (the same solution but without the reagent) for hydroxyl radical scavenging activity.

Lipinski's rule and in silico ADME prediction

The drug-like properties of newly synthesized compounds with anticipated biological and/or pharmacological activity were evaluated using Lipinski's rule of five ADME, which was used to determine whether these compounds possess the properties necessary to be a potentially orally active drug for humans. Our compounds' drug-like activity has been anticipated using the Swiss ademt module. The 1,2,4-triazole compounds were synthesised using the LigPrep module v3.1; Schrodinger 2015-1 and utilized as previously described [20].

RESULTS AND DISCUSSION

Numerous approaches have been employed to evaluate the antioxidant activity of compounds in vitro in order to facilitate quick screening, since drugs with low antioxidant activity in vitro are likely to have poor activity in vivo. It is well established that free radicals play a significant role in a broad range of clinical symptoms. Antioxidants combat free radicals and shield us from a variety of ailments. They work by scavenging reactive oxygen species or by safeguarding the antioxidant defence systems.

Table 1 ADMET studies bioactive compounds in *Vitex negundo*

Molecule	Formula	MW	H-bond acceptors	H-donors	TPSA	GI absorption	BBB	Pgp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	log Kp (cm/s)	Lipinski violations	Bioavailability score	Synthetic accessibility
Molecule 1	C5H10O2	102.13	2	0	26.3	High	Yes	No	No	No	No	No	No	-6.06	0	0.55	1
Molecule 2	C16H32O2	256.42	2	1	37.3	High	Yes	No	Yes	No	Yes	No	No	-2.77	1	0.85	2.31
Molecule 3	C16H22O4	278.34	4	0	52.6	High	Yes	No	Yes	Yes	No	No	No	-5.08	0	0.55	2.36
Molecule 4	C24H38O4	390.56	4	0	52.6	High	No	Yes	No	No	Yes	No	Yes	-3.39	1	0.55	4.12

The percentage yield of V. negundo extracts

The (Table 1) shows the % yields of n-hexane, diethyl ether, ethanol, and aqueous extracts of *V. negundo*. The maximum yield was obtained from the aqueous extract. The yield of 13.25 ethanol extract was 8.20 percent, followed by diethyl ether extract (6.00 percent). This indicated that the yield of crude *V. negundo* leaf extracts rose with increasing polarity of the extraction solvent utilized. According to Nagarsekar [21], flavonoids were the major constituents of the ethanol extract of *V. negendo*, along with a few terpenoids. Finally, the supercritical fluid extract and steam distilled oil were mostly composed of essential oils (terpenoids).

V. negundo antioxidant assay

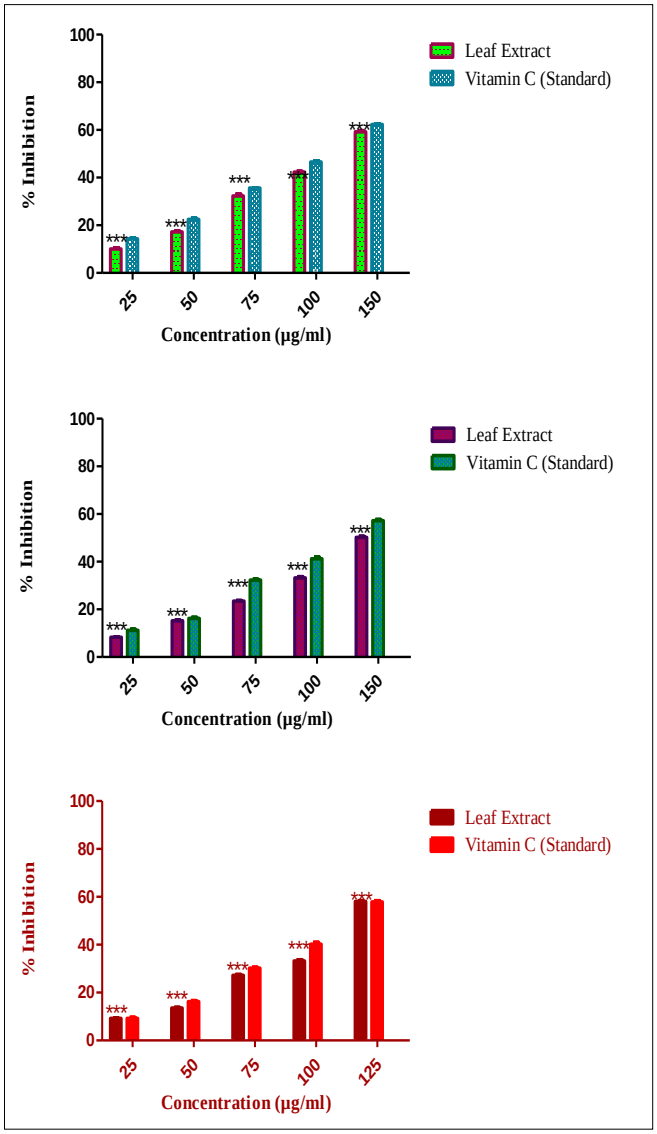

Fig 1-3 *In-vitro* antioxidant activity of using aqueous *Vitex negundo* leaf a) SOD radical scavenging activity, b) Hydroxyl radical scavenging activity and total antioxidant assay. The value are mean ± SD, One way ANOVA was used to analyze the significant difference among groups. The symbol (***) indicate the significant difference (P < 0.001)

The superoxide radical is a key generator of reactive oxygen species in biological systems [22]. Although superoxide anion is a weak oxidant, it generates highly reactive hydroxyl radicals and singlet oxygen, both of which contribute to oxidative stress [23]. At 150 g/mL, the leaf extract inhibited SOD by 8.23 percent and vitamin C by 58.23 percent, respectively. The antioxidant capacity of the fractions was determined spectrophotometrically using the phosphomolybdenum technique, which is based on the test sample reducing Mo (VI) to Mo (V) and forming green phosphate/Mo (V) compounds with a maximum absorbance at 765 nm. The hydroxyl radical is a highly reactive oxygen species found in biological systems. It interacts with the polyunsaturated fatty acid moieties in phospholipids found in cell membranes, causing cell damage [24]. The hydroxyl radical is considered a harmful species in pathophysiological processes because it is capable of causing damage to almost every molecule in a biological system and so contributes to carcinogenesis, mutagenesis, and cytotoxicity [25]. Hydroxyl radicals were formed as a result of the interaction between H_2O_2 and ferrous, which would react with 2-deoxyribose. The reaction was terminated by adding TBA reagent, which would have a red colour if malonaldehyde had been generated as a consequence of the radical reacting with 2-deoxyribose. The ability of an extract to scavenge hydroxyl radicals is directly related to its antioxidant activity, as shown by the extract's low intensity of red colour [26]. At 150 g/Ml, hydroxyl had an inhibition percentage of 8.23 percent and 58.23 percent for the leaf extract and vitamin C, respectively. Similar suppression was observed for the DPPH stable radical by *V. negundo* extract at 79.43 percent (IC_{50} 13.31 0.18), toluene at 82.53 percent (IC_{50}13.8 0.14), and gallic acid at 89.51 percent (IC_{50}3.1 0.08). In comparison to two commercially available antioxidants, BHT and ascorbic acid, V. negundo extract has the highest capacity for scavenging free radicals [27]. It was revealed that *V. negundo* has a greater antioxidant capacity than *V. trifolia*. The antioxidant potential of polyphenols is influenced by their chemical structure [28].

Prediction of drug likelihood and ADME in silico

In silico prediction of molecular physicochemical characteristics, bioavailability, and pharmacokinetics becomes more relevant for the exploration of efficient prospective medicinal compounds from a drug development perspective. Theoretical investigations are critical in giving trustworthy data in a timely and convenient way. Numerous free online platforms have been established recently to facilitate screening and to minimize the time and expense associated with drug candidate research (no animal testing). The ADME qualities governing the proposed drug's entry to the target or its removal by the organism are required throughout the early stages of the drug development process. These characteristics may be validated using in silico calculations of physicochemical standards. The latter place a higher premium on lipophilicity, water solubility, molecular size, polarity, saturation, and flexibility. Similar entities have been shown to have a lipophilicity of 0.21 XLOGP3. The insolubility logarithm of -2.84 indicates that vitexin is soluble, although the Csp3 unsaturation value is 0.29 [29].

CONCLUSION

The substitution of natural antioxidants with synthetic antioxidants (due to the consequences for human health) may be favourable. The current research examines the scavenging activity of free radicals. However, more comprehensive research are required to identify the secondary metabolites responsible for the reported biological activities and to deduce the underlying mechanism behind these therapeutic effects.

Conflict of interests

The authors have no conflict of interests to declare.

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Author's contributions

GU: Credit contribution not specified; methodology; formal analysis; visualization; writing-original draft preparation. PP: validation; investigation. KS: CRediT contribution not specified.

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