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Research Article

Phytochemical Characterization and *In-vitro* Nitric Oxide Scavenging Activity of Hydroalcoholic Seed Extract of *Ocimum gratissimum*

Syeda Qadar Unnisa[1*], Afshan Shadab[2], Tuba Aziz[1], Ameena Begum[1], Mariyam Sultana[1], Siddiqa Tabassum[1], Fatima Begum[1]

[1]*Department of Pharmacology, Shadan Women's College of Pharmacy, JNTU Hyderabad, Telangana, India.*
[2]*Department of Pharmacy Practice, Shadan Women's College of Pharmacy, JNTU Hyderabad, Telangana, India*

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ABSTRACT

Ocimum gratissimum (Lamiaceae) is a medicinal plant traditionally used to manage several disorders. Although its therapeutic properties have been extensively explored, limited studies have investigated the phytochemical profile and *in-vitro* nitric oxide scavenging activity of its hydroalcoholic seed extract. The present study aimed to evaluate the phytochemical profile and *in-vitro* NO scavenging activity of the hydroalcoholic seed extract of O. *gratissimum*. Nitric oxide (NO) scavenging activity was assessed using a nitric oxide scavenging assay. The absorbance of treated samples was measured and compared with the control using UV-visible spectrophotometry, and the percentage inhibition of nitric oxide radicals was assessed. The hydroalcoholic extract exhibited concentration-dependent nitric oxide (NO) scavenging activity with an IC50 value of 87.5 µg/mL. The standard drug, curcumin, showed greater activity with an IC50 value of 48.3 µg/mL. Despite lower potency compared to curcumin, the extract demonstrated notable activity, indicating the presence of antioxidant phytoconstituents that may contribute to the observed nitric oxide scavenging activity. The hydroalcoholic seed extract of O. *gratissimum* demonstrated concentration-dependent NO scavenging activity with an IC50 of 87.5 µg/mL. These findings indicate antioxidant potential under *in-vitro* conditions. However, additional mechanistic and animal studies are required to establish antihypertensive efficacy.

INTRODUCTION

Hypertension remains one of the leading health concerns, contributing markedly to cardiovascular morbidity & mortality. Although several classes of antihypertensive medications are available, limitations such as adverse side effects, high cost, and limited accessibility particularly in low-resource settings have sustained scientific interest in identifying safer, plant-based alternatives. Medicinal plants continue to receive attention due to their bioactive compounds capable of tempering vascular tone, oxidative stress, & endothelial function.

Ocimum gratissimum L. is an aromatic medicinal herb classified under the Lamiaceae family and is widely recognized by the common names clove basil and African basil. Traditionally, it is used to manage inflammatory conditions, respiratory ailments, infections, and cardiovascular disorders. The phytochemical composition of *O. gratissimum* comprises essential oil constituents, including eugenol, thymol, and linalool, together with secondary metabolites such as flavonoids, phenolic acids, tannins, alkaloids, and saponins. While the leaves have been studied extensively, the seeds remain understudied despite indications that they contain unique polyphenols and fixed oils with potential therapeutic relevance. Hydroalcoholic extraction is particularly appropriate for recovering a broad range of phytoconstituents.[1,2]

***Corresponding Author:** Ms. Syeda Qadar Unnisa

Address: Department of Pharmacology, Shadan Women's College of Pharmacy, JNTU Hyderabad, India.

Email ✉**:** sqadarunnisa23@gmail.com

Tel.: 91-6304200871

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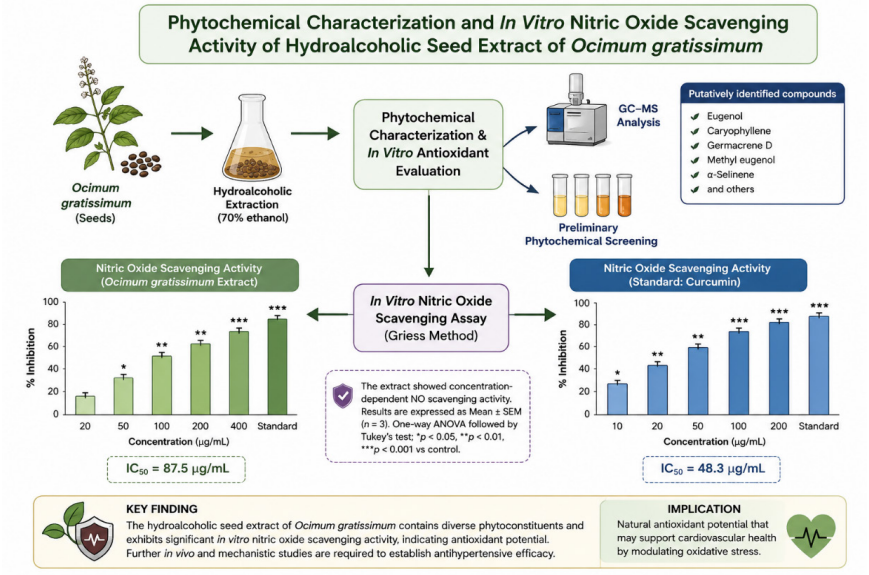

Fig 1: Graphical Abstract

Nitric oxide (NO) plays a foremost role in vascular homeostasis, and diminished NO availability is closely associated with endothelial dysfunction in hypertension. Oxidative stress further reduces bioactive NO by promoting its interaction with reactive oxygen species. Therefore, in-vitro nitric oxide scavenging assays provide a valuable preliminary measure of the antioxidant and vasomodulatory potential of plant extracts.[3]

Extracts of *Uncaria rhynchophylla* exert calcium channel–blocking effects that contribute to lower blood pressure.[4] *Nigella sativa* seeds demonstrate antihypertensive activity via diuretic, antioxidant, and NO-enhancing mechanisms.[5] *Camellia sinensis* (green tea) improves endothelial NO levels through catechin-mediated antioxidant activity[6], and *Crataegus monogyna* (hawthorn) exhibits vasodilatory and ACE-inhibitory properties due to its flavonoid and proanthocyanidin content[7], while *Terminalia arjuna* enhances endothelial function and reduces oxidative stress.[8] *Allium sativum* (garlic) improves NO bioavailability due to its sulfur-containing compounds.[9]

Beyond its cardiovascular potential, *O. gratissimum* possesses a wide array of pharmacological activities. Leaf extracts show antimutagenic activity, including inhibition of *Salmonella typhimurium*, supporting their role in preventing oxidative and genetic damage. Its essential oil constituents have been associated with anti-inflammatory, analgesic, antistress, anthelmintic, antidiarrheal, antipyretic, anti-ulcer, hepatoprotective, sedative, and fungicidal actions. The plant's antiseptic properties support its inclusion in toothpaste, mouthwash, and topical formulations. Traditionally, it is used for managing sore throat, tonsillitis, cough, and gastrointestinal helminths, and it has been reported to offer benefits for individuals living with HIV/AIDS. In West Africa, it is used as a febrifuge and included in herbal antimalarial remedies, while crushed leaves are applied for conjunctivitis, and the essential oil is used in wound care and postpartum hygiene. Fresh aerial parts are often consumed as vegetables, and dried leaves are incorporated into various traditional dishes.[10,11]

Although antioxidant properties of *O. gratissimum* have been extensively investigated, information regarding the nitric oxide scavenging activity and phytochemical profile of its hydroalcoholic seed extract remains limited. We hypothesized that the seed extract contains phytochemicals capable of scavenging nitric oxide radicals *in-vitro*. Therefore, this study aimed to describe the phytochemical configuration of the extract and evaluate its nitric oxide scavenging activity.

MATERIALS AND METHODS

Chemicals Required

O. gratissimum seeds, ethanol, distilled water, Griess reagent, sodium nitroprusside, phosphate buffer.

Apparatus

Measuring cylinder, glass jar, filter paper, rotary evaporator or water bath.

Extraction Procedure

Collection of Plant

The Medicinal plant *O. gratissimum* (Linn) with the common name clove basil, which belongs to the family Lamiaceae, was collected, which was taxonomically identified and authenticated by Dr. L. Rasingam.

Preparation of Seed Extract by Maceration Process

Take the seeds of *O. gratissimum* exposed to shade drying. On complete drying, the pieces were finely powdered.

Prepare hydro alcoholic solvent {ethanol + distilled water (7:3)}. Weigh 500 gm of *O. gratissimum* seed powder and placed in a glass jar. Add 1 litre of hydro alcoholic solvent (7:3 v/v ratio). The powdered seed material was immersed in the extraction solvent for 48 to 72 hours, with intermittent agitation to improve extraction efficiency. Filter the extract through muslin cloth. Evaporate the solvent using a rotatory evaporation or gentle heat on a water bath. Collect the concentrated extract and store in an air tight amber coloured bottle as shown in Fig. 2. The concentrated extract was preserved for subsequent phytochemical investigation and evaluation of nitric oxide radical scavenging activity.[12]

Extract Preparation

Seeds of *O. gratissimum* were collected, authenticated, and shade-dried. The dried seeds were powdered and subjected to hydroalcoholic extraction (ethanol & water, 70 & 30% v/v, respectively) using maceration. The extract was filtered, concentrated under reduced pressure, and stored at a cool temperature until further use. Prior to phytochemical screening, 2 to 5 mL of the hydroalcoholic extract was slightly acidified with 1 percent HCl and gently warmed. Insoluble residues were removed by filtration.

Preliminary Phytochemical Screening

Qualitative tests were performed on the hydroalcoholic seed extract following standard protocols to distinguish the incidence of major secondary metabolites like alkaloids, saponins, flavonoids, terpenoids, tannins, & glycosides.[13]

GC-MS Analysis of Hydro alcoholic Extract

The hydro alcoholic extract of *Og* were imperiled to GC–MS analysis using QP 2010 system by Shimadzu following standard procedure.[14]

In-vitro Nitric Oxide Scavenging Assay

Aim

To assess the hydroalcoholic seed extract of *O. gratissimum* through nitric oxide scavenging activity.

Apparatus

Test tubes, pipettes, beakers, incubator, UV-visible spectrophotometer, volumetric flasks, water bath, and analytical balance.

Reagents and Chemicals

Sodium nitroprusside (10 mM), phosphate buffer, Griess reagent containing sulphanilamide, orthophosphoric acid and N-(1-naphthyl) ethylenediamine dihydrochloride (NED), methanol, hydroalcoholic extract of *O. gratissimum*, and a standard antioxidant such as ascorbic acid.

Principle

Under physiological conditions, sodium nitroprusside continuously liberates nitric oxide in phosphate buffer. The released nitric oxide undergoes oxidation to form nitrite ions, which subsequently react with Griess reagent to produce a pink-colored azo compound. The intensity of the developed color is directly related to the amount of nitrite formed. Antioxidants capable of scavenging nitric oxide reduce nitrite formation, resulting in lower absorbance values that can be quantified spectrophotometrically at 550 nm.

Fig. 2: Extraction of seeds by maceration

Procedure

A stock solution of sodium nitroprusside was prepared by dissolving 10 mg of the compound in 5 mL of distilled water. Phosphate-buffered saline (PBS, pH 7.4) was used as the reaction medium. For each assay, 3 mL of sodium nitroprusside solution was mixed with different concentrations of the hydroalcoholic seed extract of *O. gratissimum* (50, 100, 150, and 200 µg/mL). A control containing sodium nitroprusside without the extract was prepared in parallel.

The reaction mixtures were maintained at 30°C for 2 hours to facilitate nitric oxide generation. After incubation, 5 mL of freshly prepared Griess reagent was added, and the solutions were allowed to develop color. Absorbance was then recorded at 550 nm using a UV-visible spectrophotometer. Nitric oxide scavenging activity was expressed as percentage inhibition relative to the control, and the IC_{50} value was estimated from the concentration-response curve.[15]

Statistical Analysis Of Data

All experimental observations were obtained from three independent determinations (n = 3) and are presented as mean ± standard error of the mean (SEM). The IC_{50} values were estimated from concentration–response curves generated using GraphPad Prism version 8.0. Differences among the experimental groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's multiple

Table 1: Nitric oxide scavenging activity by *O. gratissimum*

S. No	*Concentration (µg/ml)*	*Mean absorbance extract (nm)*	*% Inhibition*	*P. Value*	*IC_{50}*
1	Control	0.75	-	-	-
2.	20	0.70 ± 0.010	6.91 ± 1.33	0.0009	-
3.	40	0.63 ± 0.01	15.78 ± 0.74	0.0001	-
4.	60	0.55 ± 0.01	27.22 ± 1.02	0.0001	87.5 µg/ml
5.	80	0.43 ± 0.01	42.29 ± 0.28	0.0010	-
6.	100	0.23 ± 0.01	68.79 ± 1.55*	0.0100	-

Investigational groups were correlated with the control. *p < 0.001 indicates highly significant activity of *O. gratissimum* hydroalcoholic extract. Mean Absorbance of Hydroalcoholic O.G Extract

comparison test for pairwise comparisons. Statistical significance was accepted at a *p-value* < 0.05.

RESULTS

Preliminary Phytochemical Screening Result Interpretation

Qualitative phytochemical analysis of the hydroalcoholic seed extract of *O. gratissimum* demonstrated the presence of several biologically important secondary metabolites. Positive reactions were observed for alkaloids using Mayer's, Wagner's, and Hager's tests, confirming their occurrence in the extract. Flavonoids, terpenoids, glycosides, carbohydrates, steroids, and saponins were also detected through their respective qualitative tests. In contrast, tannins did not produce a positive response under the experimental conditions employed. Detection of these metabolites demonstrates that the extract possesses a chemically diverse composition that may contribute to its biological activity.

GC-MS Identified Compounds of Hydroalcoholic Extract

Interpretation

Gas chromatography–mass spectrometry (GC–MS) analysis revealed a complex phytochemical composition of the hydroalcoholic seed extract of *O. gratissimum*. Compound identification was performed by matching the recorded mass spectra with entries available in the NIST spectral database. The principal compounds detected included eugenol, methyl eugenol, eucalyptol, β-caryophyllene, germacrene D, α-selinene, acetylated methyl eugenol, and myristicin found in chromatogram as shown in Fig. 3. Earlier investigations have associated many of these constituents with antioxidant and other pharmacological activities. Although compound identification was based on spectral matching and requires confirmation using advanced analytical techniques such as LC–MS/MS or authentic reference standards, the obtained chromatographic profile provides valuable information regarding the phytochemical composition of the extract.

In-vitro Test Result

Nitric Oxide Scavenging Activity by O. gratissimum

Increasing concentrations of the extract produced progressively greater nitric oxide radical inhibition throughout the study. As the extract concentration increased from 20 to 100 µg/mL, a progressive reduction in absorbance was observed, indicating enhanced scavenging of nitric oxide radicals. The corresponding percentage inhibition increased from 6.91 ± 1.33% at 20 µg/mL to 68.79 ± 1.55% at 100 µg/mL. The calculated IC_{50} value of 87.5 µg/mL indicates moderate nitric oxide scavenging capacity under the experimental conditions as shown in Table 1 and Figs 4 and 5. The observed antioxidant activity may be attributed to the presence of phenolic

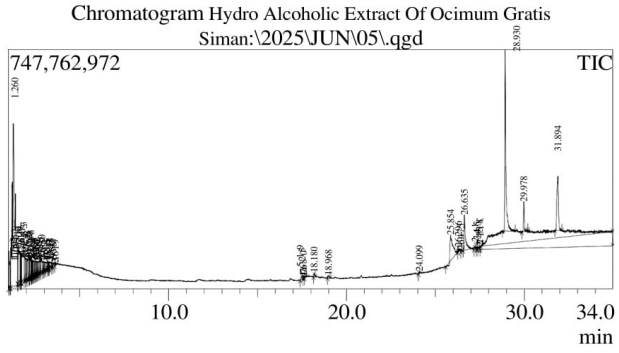

Fig. 3: Chromatogram of extract

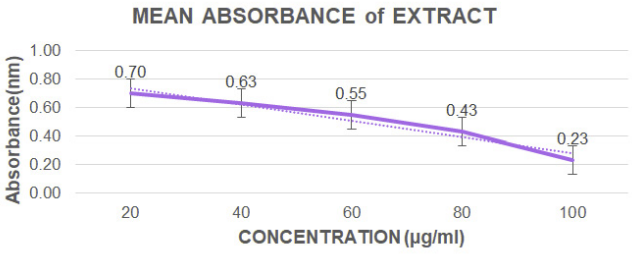

Fig 4: Mean absorbance of *O. gratissimum* hydroalcoholic extract at different concentrations (20–100 µg/mL) during the NO scavenging assay. A concentration-dependent decrease in absorbance was observed.

compounds, flavonoids, terpenoids, and other bioactive phytoconstituents identified during phytochemical screening and GC–MS analysis. The observed response indicates that the extract possesses appreciable in-vitro antioxidant activity.

Nitric Oxide Scavenging Activity by Curcumin

Curcumin, used as the reference antioxidant, demonstrated significantly greater nitric oxide scavenging activity than the plant extract at all tested concentrations. The percentage inhibition increased from 27.13 ± 1.41% at 20 µg/mL to 80.90 ± 2.50% at 100 µg/mL, accompanied by a concentration-dependent decline in absorbance values as depicted in Table 2 and Figs 6 and 7.

The IC_{50} value of curcumin was calculated as 48.34 µg/ mL, confirming its superior free radical scavenging efficiency compared with the hydroalcoholic extract of *O. gratissimum.* The response obtained with curcumin served as a reference for comparing the antioxidant performance of the plant extract.

DISCUSSION

Medicinal plants are valuable sources of bioactive phytochemicals with diverse pharmacological properties.

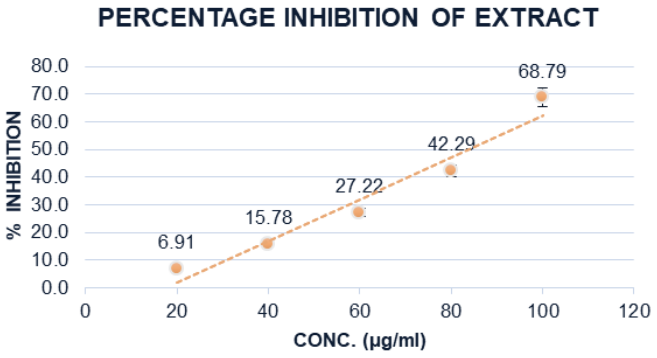

Fig 5: Concentration-dependent NO scavenging activity of *O. gratissimum* hydroalcoholic extract showing percentage inhibition at different concentrations (20–100 µg/mL).

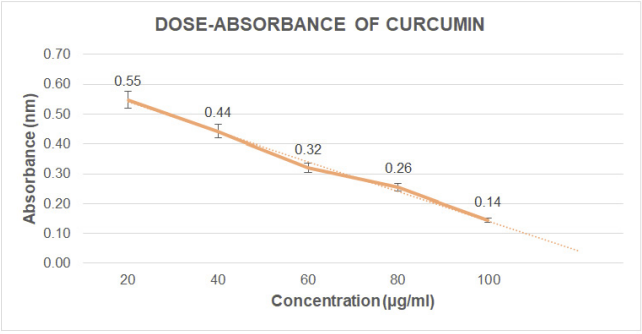

Fig 6: Mean absorbance of curcumin at different concentrations (20 to 100 µg/mL) during the NO scavenging assay. A concentration-dependent decrease in absorbance was observed, indicating increased nitric oxide scavenging assay with increasing extract concentration.

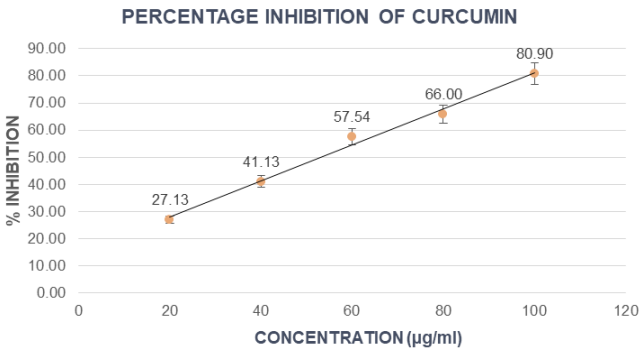

Fig. 7: Concentration-dependent NO scavenging activity of *curcumin* showing percentage inhibition at different concentrations (20–100 µg/mL).

Among these, flavonoids, phenolic compounds, alkaloids, and terpenoids are recognized for their antioxidant potential and their ability to reduce oxidative stress associated with several chronic diseases.[16 -21]

In the present study, the hydroalcoholic seed extract of *O. gratissimum* demonstrated the presence of alkaloids, tannins, glycosides, steroids, flavonoids, triterpenoids, and carbohydrates during preliminary phytochemical screening, indicating a chemically diverse extract.[16] GC–MS analysis further identified several bioactive constituents, including eugenol, methyl eugenol, eucalyptol, β-caryophyllene, germacrene D, α-selinene, acetylated methyl eugenol, and myristicin. These compounds have previously been reported to possess antioxidant and free radical scavenging properties, which may contribute to the biological activity observed in the present study.[20, 21]

The hydroalcoholic extract exhibited concentration-dependent nitric oxide scavenging activity with an IC_{50} value of 87.5 µg/mL, indicating moderate antioxidant potential. In comparison, curcumin showed a lower IC_{50} value (48.34 µg/mL), reflecting its higher antioxidant potency. Comparable antioxidant responses have been described for different *Ocimum* species, although the magnitude of activity varies according to extraction conditions, plant material, and experimental design.[20, 21]

Nitric oxide scavenging assays are widely employed to evaluate the ability of plant extracts to neutralize reactive nitrogen species under *in-vitro* conditions. Excessive production of reactive oxygen and reactive nitrogen species contributes to oxidative stress, endothelial dysfunction, and tissue damage involved in the progression of cardiovascular and other chronic diseases.[18, 19] Therefore, the antioxidant activity observed in the present study may be attributed to the combined action of phenolic compounds, flavonoids, and other phytoconstituents capable of scavenging free radicals.[17, 19]

Although the extract exhibited significant nitric oxide scavenging activity, these findings should not be interpreted as direct evidence of antihypertensive efficacy. Blood pressure regulation involves multiple

Table 2: Nitric oxide scavenging activity by curcumin

S. No	*Concentration (µg/ml)*	*Triplicate Mean Absorbance Extract (nm)*	*% Inhibition*	IC_{50}
1	Control	0.75	-	-
2.	20	0.55 ± 0.010	27.13 ± 1.41	-
3.	40	0.44 ± 0.01	41.13 ± 1.89	48.34 µg / ml
4.	60	0.32 ± 0.01	57.54 ± 1.92	-
5.	80	0.26 ± 0.01	66.00 ± 1.82	-
6.	100	0.14 ± 0.01	80.90 ± 2.50	-

Values are represented as mean ± SEM (n = 3). Investigational groups were related with the control. *p < 0.001 indicates highly significant.

physiological pathways, including endothelial nitric oxide production, vascular reactivity, the renin–angiotensin–aldosterone system, autonomic regulation, and renal function.[18,19] Therefore, further *in-vivo* studies and mechanistic investigations are required to establish the antihypertensive potential of *O. gratissimum.*

LIMITATIONS

The present study has certain limitations. The biological activity was evaluated using only three independent replicates (n = 3), and curcumin was the only reference antioxidant included for comparison. Furthermore, compound identification by GC–MS was tentative, as no LC–MS/MS analysis or authentic reference standards were used for confirmation. Finally, the biological activity was assessed only by an *in-vitro* nitric oxide scavenging assay, and no mechanistic, vascular, or *in-vivo* studies were performed. Therefore, further investigations employing LC–MS/MS characterization, additional antioxidant standards, larger biological sample sizes, and suitable animal models are necessary to validate these findings and identify the phytoconstituents responsible for the observed activity.

Overall, the results demonstrate that the hydroalcoholic seed extract of *O. gratissimum* possesses appreciable *in-vitro* nitric oxide scavenging activity, which is likely attributable to the synergistic action of its phytochemical constituents. These findings support the antioxidant potential of the extract and provide a basis for future phytochemical and pharmacological investigations.

The hydroalcoholic seed extract of *O. gratissimum* demonstrated a concentration-dependent capacity to scavenge nitric oxide radicals under *in-vitro* conditions, indicating the presence of antioxidant phytochemicals. Qualitative phytochemical screening and GC–MS profiling revealed a diverse range of bioactive constituents that may collectively contribute to the observed free radical scavenging activity. Although the extract exhibited lower antioxidant potency than curcumin, its measurable activity suggests that *O. gratissimum* seeds could serve as a promising natural source of antioxidant compounds. By reducing oxidative and nitrosative stress, these phytochemicals may help preserve endothelial function, a process closely associated with cardiovascular health. Nevertheless, the current findings are limited to an *in-vitro* antioxidant model and cannot be considered direct evidence of antihypertensive efficacy. Further *in-vivo* investigations and mechanistic studies are required to establish whether these antioxidant effects translate into clinically relevant blood pressure-lowering activity.

Future Scope

Further studies using appropriate *in-vivo* experimental models are required to evaluate the pharmacological potential of *O. gratissimum* and elucidate its underlying mechanisms of action. Isolation, purification, and structural characterization of the active phytoconstituents, supported by LC–MS/MS and other advanced analytical techniques, will help identify the compounds responsible for antioxidant activity. Additional mechanistic studies, including antioxidant and endothelial function assays, are warranted to better understand its biological effects. Furthermore, comprehensive toxicological evaluations and well-designed clinical studies are necessary to establish the safety, efficacy, and therapeutic potential of the extract.

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