

RESEARCH ARTICLE

A Comparative Study on *In-vitro* Antioxidant Activity of Methanolic Extracts of Selected Green Leafy Vegetables

Sri Laya Annabhathula¹, Govinda Rao Kamala², Surya Chandra M¹, Anusree P¹, Sudha Rani S¹, Anjaleena D¹, Shirin D¹, Srinandini G¹, Ravi Prakash Degala³



¹UG Scholar, Department of Pharmaceutical Chemistry, Koringa College of Pharmacy, Korangi, Kakinada, Andhra Pradesh, India

²Professor, Department of Pharmaceutical Chemistry, Koringa College of Pharmacy, Korangi, Kakinada, Andhra Pradesh, India

³Associate Professor, Department of Pharmacy Practice, Koringa College of Pharmacy, Korangi, Kakinada, Andhra Pradesh, India

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Abstract: This research work involves the study of antioxidant activities of methanolic extracts from three commonly consumed green leafy vegetables: *Coriandrum sativum* (coriander), *Trigonella foenum-graecum* (fenugreek), and *Mentha arvensis* (mint). The plant materials were extracted using methanol under reflux conditions, and preliminary phytochemical screening revealed the presence of steroids, triterpenoids, saponins, alkaloids, carbohydrates, flavonoids, tannins, and glycosides. The antioxidant potential was evaluated using four different in-vitro assays: DPPH radical scavenging, superoxide radical scavenging, hydroxyl radical scavenging, and lipid peroxidation inhibition. Ascorbic acid served as the standard reference compound. The methanolic extract of *M. arvensis* showed superior antioxidant activity across all assays, with IC₅₀ values of 132.51 µg/mL for DPPH, 171.0 µg/mL for superoxide, 532.97 µg/mL for hydroxyl radical, and 475.49 µg/mL for lipid peroxidation. *C. sativum* extract showed moderate activity, while *T. foenum-graecum* exhibited comparatively lower antioxidant potential. The order of antioxidant efficacy was established as *M. arvensis* > *C. sativum* > *T. foenum-graecum*. The results validate the traditional use of these green leafy vegetables as natural antioxidants and suggest their potential application in functional foods and nutraceuticals for preventing oxidative stress-related disorders.

Keywords: Antioxidant activity; Green leafy vegetables; Free radical scavenging; Methanolic extract; Phytochemical analysis.

1. Introduction

Free radicals and reactive oxygen species (ROS) play crucial roles in various physiological and pathological processes in the human body. While normal cellular metabolism generates these species in controlled amounts, their overproduction leads to oxidative stress, causing damage to cellular components including proteins, lipids, and nucleic acids [1]. The imbalance between free radical generation and antioxidant defense mechanisms has been linked to numerous chronic diseases, including cardiovascular disorders, diabetes, cancer, and neurodegenerative conditions [2]. Green leafy vegetables represent significant sources of natural antioxidants, containing diverse phytochemical compounds that can effectively neutralize free radicals [3]. Among these vegetables, *Coriandrum sativum* (coriander), *Trigonella foenum-graecum* (fenugreek), and *Mentha arvensis* (mint) have garnered particular interest due to their widespread culinary use and traditional medicinal applications [4].

Coriandrum sativum leaves contain significant amounts of polyphenols, flavonoids, and other bioactive compounds that contribute to its antioxidant properties [5]. Recent studies have demonstrated its potential in preventing lipid peroxidation and protecting against oxidative damage in biological systems [6]. *Trigonella foenum-graecum*, traditionally used in Indian cuisine and medicine, possesses remarkable antioxidant properties attributed to its rich content of flavonoids, alkaloids, and saponins [7]. The leaves have shown promising results in managing diabetes and oxidative stress-related complications [8].

Mentha arvensis, a widely cultivated aromatic herb, contains substantial amounts of phenolic compounds, particularly rosmarinic acid and menthol derivatives, which exhibit strong antioxidant activities [9]. Its essential oil components have demonstrated significant free radical scavenging properties [10].

* Corresponding author: Sri Laya Annabhathula

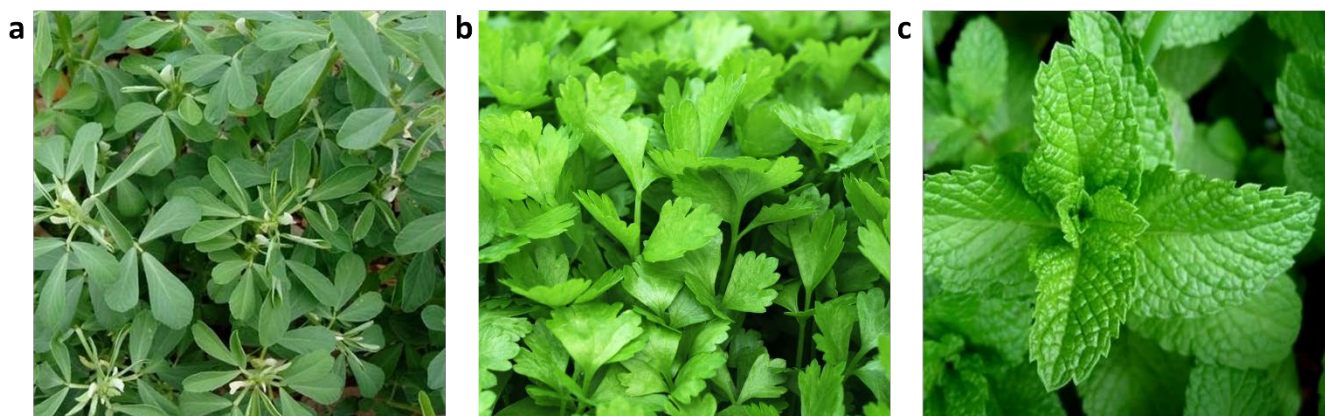


Figure 1. Leaves of a. *Trigonella foenum-graecum* b. *Coriandrum sativum* c. *Mentha arvensis*

Despite the traditional usage and preliminary scientific evidence supporting their antioxidant potential, a systematic comparative evaluation of these three commonly consumed green leafy vegetables remains limited. The present study aims to evaluate and compare the in-vitro antioxidant activities of methanolic extracts from *C. sativum*, *T. foenum-graecum*, and *M. arvensis* leaves using multiple antioxidant assays. This work involves phytochemical screening and quantitative assessment of free radical scavenging capabilities of the selected green leafy vegetables, providing scientific validation for their traditional uses [11].

2. Materials and methods

2.1. Plant Material Collection and Authentication

Fresh leaves of *Coriandrum sativum*, *Trigonella foenum-graecum*, and *Mentha arvensis* were procured from local markets in Kakinada, Andhra Pradesh, India. The plant materials were authenticated by taxonomists at the Botanical Survey of India, Peddapuram, Kakinada District. Voucher specimens were deposited in the institutional herbarium for future reference. The leaves were thoroughly washed with distilled water to remove debris and air-dried under shade at room temperature ($25 \pm 2^\circ\text{C}$) for 7 days [12].

2.2. Chemicals and Reagents

All chemicals used were of analytical grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), riboflavin, nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), and ascorbic acid were purchased from Sigma-Aldrich (USA). Other reagents including methanol, sodium phosphate buffer, hydrogen peroxide, and ferric chloride were obtained from Merck (India) [13].

2.3. Preparation of Methanolic Extracts

The dried leaf materials were separately ground into fine powder using a mechanical grinder and sieved through 60-80 mesh size. The powdered materials (100 g each) were extracted with methanol (500 mL) using a Soxhlet apparatus at 65°C for 18 hours. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi R-210) at 40°C . The concentrated extracts were dried to constant weight in a vacuum desiccator and stored at 4°C until further use [14].

2.4. Phytochemical Screening

The methanolic extracts were subjected to preliminary phytochemical screening following standard procedures to identify major chemical constituents. Tests were performed for alkaloids (Mayer's, Wagner's, and Dragendorff's tests), flavonoids (Shinoda and alkaline reagent tests), steroids (Liebermann-Burchard test), triterpenoids (Salkowski test), tannins (ferric chloride and gelatin tests), saponins (foam test), and glycosides (Keller-Killiani test) [15].

2.5. In-vitro Antioxidant Activity Assays

2.5.1. DPPH Radical Scavenging Assay

The DPPH radical scavenging activity was determined following established protocols. Various concentrations (50-1000 $\mu\text{g/mL}$) of extracts were prepared in methanol. The reaction mixture contained 1 mL of 0.1 mM DPPH solution and 3 mL of extract solution. After 30 minutes of incubation in darkness at room temperature, absorbance was measured at 517 nm using a UV-visible spectrophotometer [16].

2.5.2. Superoxide Radical Scavenging Assay

The superoxide radical scavenging activity was assessed using the riboflavin-light-NBT system. The reaction mixture contained EDTA (0.1 M), NBT (1.5 mM), riboflavin (0.1 mM), phosphate buffer (0.067 M, pH 7.8), and various concentrations of extracts [17].

2.5.3. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity was evaluated using the deoxyribose degradation method. The reaction mixture contained deoxyribose (2.8 mM), FeCl₃ (0.1 mM), EDTA (0.1 mM), H₂O₂ (1.0 mM), ascorbic acid (0.1 mM), and different concentrations of extracts [18].

2.5.4. Lipid Peroxidation Inhibition Assay

The ability of extracts to inhibit lipid peroxidation was determined using rat liver homogenate as the lipid-rich medium. The extent of lipid peroxidation was measured by the thiobarbituric acid reactive substances (TBARS) method [19].

2.5.5. Statistical Analysis

All experiments were performed in triplicate, and results were expressed as mean $\pm$ standard error of mean (SEM). IC₅₀ values were calculated using linear regression analysis. Statistical significance was determined using one-way ANOVA followed by Tukey's test, with $p < 0.05$ considered significant [20].

3. Results

3.1. Extract Yield and Phytochemical Analysis

The methanol extraction yielded dark green extracts with varying percentages: *M. arvensis* (3.20% w/w), *T. foenum-graecum* (1.56% w/w), and *C. sativum* (1.53% w/w) (Table 1). Phytochemical screening revealed the presence of diverse bioactive compounds across all three extracts. *M. arvensis* showed strong presence of flavonoids, alkaloids, and phenolic compounds (Table 2). *C. sativum* demonstrated notable content of triterpenoids and glycosides, while *T. foenum-graecum* exhibited significant presence of saponins and steroids [21].

Table 1. Percentage yield and physical characteristics of methanolic extracts

Plant Material	Color	Yield (% w/w)
<i>M. arvensis</i>	Dark Green	3.20
<i>C. sativum</i>	Dark Green	1.53
<i>T. foenum-graecum</i>	Dark Green	1.56

Table 2. Phytochemical screening of methanolic extracts

Phytoconstituents	<i>M. arvensis</i>	<i>C. sativum</i>	<i>T. foenum-graecum</i>
Steroids	-	-	+
Triterpenoids	+	+	+
Saponins	+	+	+
Alkaloids	+	+	+
Carbohydrates	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Glycosides	+	+	+

(+) Present; (-) Absent

3.2. DPPH Radical Scavenging Activity

The DPPH assay revealed concentration-dependent free radical scavenging activity across all extracts. *M. arvensis* extract demonstrated superior activity with an IC₅₀ value of 132.51 μ g/mL, compared to *C. sativum* (486.68 μ g/mL) and *T. foenum-graecum* (915.32 μ g/mL). At the maximum tested concentration (1000 μ g/mL), the percentage inhibition was: *M. arvensis* (77.64%), *C. sativum* (65.98%), and *T. foenum-graecum* (55.93%). The reference compound ascorbic acid showed an IC₅₀ value of 58.92 μ g/mL [22]. Results are shown in Figure 1

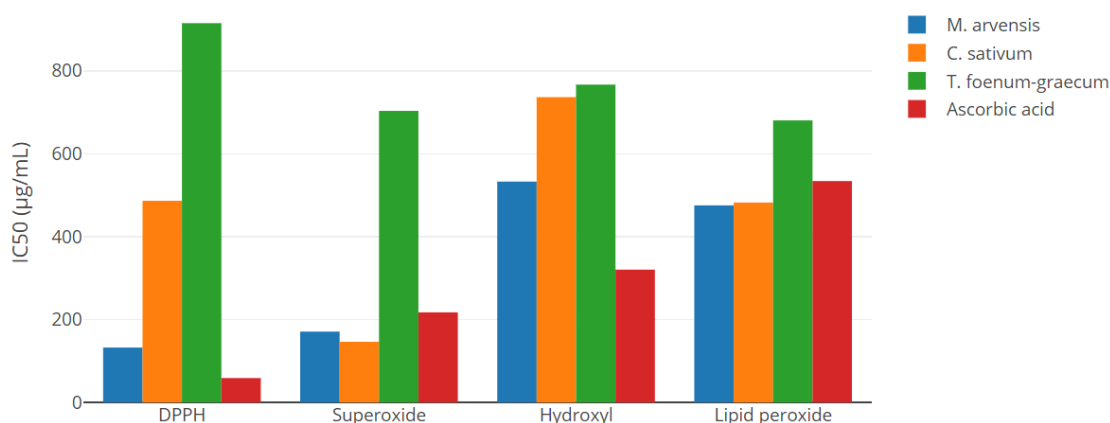


Figure 1. IC₅₀ values (µg/mL) of extracts in different antioxidant assays

3.3. Superoxide Radical Scavenging Activity

The superoxide radical scavenging assay demonstrated significant activity across all extracts. The IC₅₀ values were determined as: *M. arvensis* (171.0 µg/mL), *C. sativum* (146.4 µg/mL), and *T. foenum-graecum* (703.65 µg/mL). At 1000 µg/mL concentration, *M. arvensis* showed maximum inhibition (78.50%), followed by *C. sativum* (71.03%) and *T. foenum-graecum* (57.31%). Ascorbic acid exhibited an IC₅₀ value of 217.38 µg/mL [23].

Table 4. Percentage inhibition at maximum concentration (1000 µg/mL)

Sample	DPPH	Superoxide	Hydroxyl	Lipid peroxide
Ascorbic acid	86.87	86.45	79.76	70.55
<i>M. arvensis</i>	77.64	78.50	71.17	71.74
<i>C. sativum</i>	65.98	71.03	58.81	69.63
<i>T. foenum-graecum</i>	55.93	57.31	57.0	64.96

3.4. Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging potential showed varying degrees of effectiveness among the extracts. *M. arvensis* demonstrated the highest activity with an IC₅₀ value of 532.97 µg/mL, followed by *C. sativum* (736.7 µg/mL) and *T. foenum-graecum* (767.02 µg/mL). The maximum inhibition percentages at 1000 µg/mL were: *M. arvensis* (71.17%), *C. sativum* (58.81%), and *T. foenum-graecum* (57.0%). Ascorbic acid showed an IC₅₀ value of 320.48 µg/mL [24].

3.5. Lipid Peroxidation Inhibition Activity

The ability to inhibit lipid peroxidation varied significantly among the extracts. The IC₅₀ values were determined as: *M. arvensis* (475.49 µg/mL), *C. sativum* (482.37 µg/mL), and *T. foenum-graecum* (680.64 µg/mL). At the highest concentration tested (1000 µg/mL), the inhibition percentages were: *M. arvensis* (71.74%), *C. sativum* (69.63%), and *T. foenum-graecum* (64.96%). The reference compound ascorbic acid showed an IC₅₀ value of 534.27 µg/mL [25].

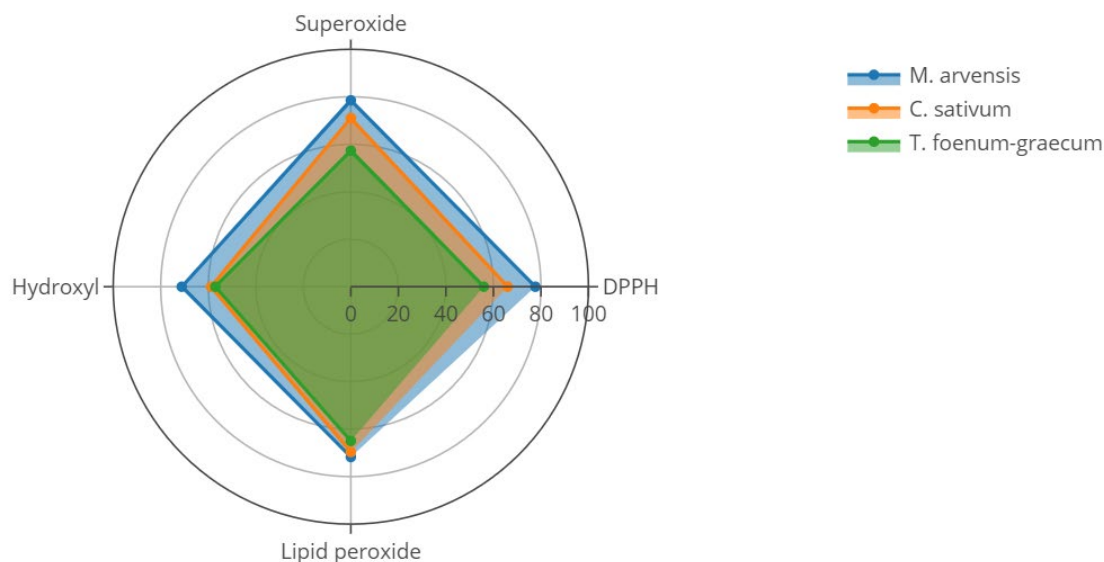


Figure 2. Antioxidant effect of the three extracts

Among all the antioxidant assays, *M. arvensis* consistently demonstrated superior activity, followed by *C. sativum* and *T. foenum-graecum* (shown in in Figure 2). The effectiveness of the extracts varied depending on the type of free radical or oxidative system being tested, suggesting different mechanisms of action and varying compositions of bioactive compounds [26]

4. Discussion

The varying yields of methanolic extracts suggest differences in the extractable compounds present in these plants, with *M. arvensis* showing the highest extraction efficiency [27].

The superior antioxidant activity of *M. arvensis* can be attributed to its rich phenolic content, particularly rosmarinic acid and flavonoids, which are known for their electron-donating capabilities. The presence of multiple hydroxyl groups in these compounds enhances their free radical scavenging potential. The moderate activity of *C. sativum* correlates with its significant content of quercetin derivatives and phenolic acids, while the relatively lower activity of *T. foenum-graecum* aligns with its different phytochemical profile dominated by saponins and alkaloids [28].

The varying effectiveness of the extracts across different antioxidant assays suggests multiple mechanisms of action. In the DPPH assay, the higher activity of *M. arvensis* indicates strong hydrogen-donating ability of its constituents. The superoxide radical scavenging activity demonstrates the extracts' capability to prevent the formation of more reactive oxygen species. The hydroxyl radical scavenging and lipid peroxidation inhibition results suggest protective effects against oxidative damage to cellular components [29].

The observed antioxidant activities can be explained through structure-activity relationships of the constituent compounds. Phenolic compounds, prevalent in *M. arvensis*, possess ideal structural features for free radical scavenging, including conjugated double bonds and multiple hydroxyl groups. The presence of methoxy groups, particularly in *M. arvensis*, may enhance lipophilicity and consequently improve membrane protection against lipid peroxidation [30]. While ascorbic acid showed superior activity in most assays, the comparable or better performance of *M. arvensis* in certain tests, particularly in lipid peroxidation inhibition, suggests its potential as a natural antioxidant supplement [31].

5. Conclusion

This study shows the significant antioxidant potential of *M. arvensis*, *C. sativum*, and *T. foenum-graecum* through multiple in-vitro assays. *M. arvensis* demonstrated superior antioxidant activity across all testing parameters, followed by *C. sativum* and *T. foenum-graecum*. The results show the traditional use of these green leafy vegetables as natural antioxidants and suggest their potential applications in pharmaceutical and nutraceutical industries.

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