Trop J Nat Prod Res, July 2025; 9(7): 3214 - 3224

**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

# Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

*Original Research Article*

# Phytochemical Profiling, GC-MS Analysis, and Antioxidant Activity of Polar Solvent Extracts of *Conoclinium coelestinum* Leaves

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ARTICLE INFO

*Article history:*
Received 21 February 2025
Revised 05 June 2025
Accepted 08 June 2025
Published online 01 August 2025



ABSTRACT

The importance of medicinal plants as source of potent drugs for therapeutic purposes is currently of global interest to researchers. Phytochemicals are compounds from plants used for prevention and treatment of health disorders. Antioxidants are molecules that inhibit free radicals. This research aimed to investigate the phytochemical profile, and antioxidant activity of polar solvent extracts of *Conoclinium coelestinum* leaves. The pulverized leaves was extracted with methanol, ethanol, and water using Soxhlet apparatus. Phytochemical screening of the extracts was done using chemical methods and by gas chromatography-mass spectrometry (GC-MS). Antioxidant activity was evaluated using DPPH, FRAP, ABTS, lipid peroxidation, $H_2O_2$, nitric oxide, and superoxide radical scavenging assays. Phytochemical screening revealed the presence of alkaloids, glycosides, saponins, flavonoids, terpenoids, and steroids in all three solvent extracts. GC-MS analysis identified twenty-seven (27) bioactive compounds, with hexadecanoic acid ethyl ester (8.89%), 2(3H)-furanone, dihydro-5-pentyl (6.52%), and butanal oxime (5.18%) as the major compounds. Of all the three extracts, the methanol extract significantly ($p < 0.05$) demonstrated exceptional antioxidant activity, achieving 94.15% DPPH inhibition at 400 µg/mL ($IC_{50}$ = 95.65 µg/mL) and FRAP activity of 81.12% ($IC_{50}$ = 100.90 µg/mL). The methanol extract also demonstrated significant ($P < 0.05$) lipid peroxidation inhibitory activity, $H_2O_2$, superoxide, and nitric oxide scavenging activities, with percentage inhibition of 64.07%, 76.12%, 90.12%, and 60.07%, respectively at 400 µg/mL, highlighting its capacity to neutralize reactive oxygen species and protect against oxidative damage. These findings affirm *C. coelestinum* as a potential source of natural antioxidants, supported by its rich bioactive compounds profile.

***Keywords:*** *Conoclinium coelestinum*, Gas Chromatography-Mass Spectrometry, Phytochemicals, Antioxidant.

## Introduction

Phytochemicals are chemical substances produced by plants, and they have the ability to combat fungal, bacterial, and viral infections in plants. Some foods like beans, fruits and vegetables possess phytochemicals that can help prevent the onset of diseases.[1,2] They possess antioxidant properties, and aid in eliminating harmful free radicals.[3,4] Antioxidants are substances that can remove free radicals from body cells and prevents radical-induced oxidative damage. They are present in numerous plant-derived foods such as spinach, arugula, kale, etc. A variety of medicinal plants have been thoroughly assessed for their antioxidant properties lately.[5] Antioxidants derived from medicinal and aromatic plants are used to create natural antioxidant formulations for food, health, and cosmetic purposes.[6] Phytochemical screening is a crucial technique used experimentally to assess the antioxidant compounds present in plants.[7] *Conoclinium coelestinum*, belonging to the Asteraceae family, is popularly referred to as "Blue Mistflower" and is a herbaceous flowering plant found in North America. *Conoclinium coelestinum* grows in damp soils and along the edges of woods. It acts as a beacon for butterflies, transforming gardens into centers of activity. In conventional medicine, its blossoms and roots are used to treat and manage conditions like fever and abdominal pain.[8] The plant can be found in the eastern, central, and Midwest regions of the United States, where it has been

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Citation: Odeghe B O, Onobrudu A D, Orororo C O, Ikoya S , Apitikori-Owumi E J, Egbune O E, Nwogueze C B, Ojugbeli T E, Agboola E O, Awhin P E, Ekakitie I L, Oviri O M, Ofoke H I, Cyril C. Dunkwu , Enyi C K, Derhie A V, Isaac O P, Mordi C J ,Adebayo O. Adegoke O A. Phytochemical Profiling, GC-MS Analysis, and Antioxidant Activity of Polar Solvent Extracts of *Conoclinium coelestinum* Leaves. Trop J Nat Prod Res. 2025; 9(7): 3214 – 3224 https://doi.org/10.26538/tjnpr/v9i7.48

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

traditionally utilized for the management of asthma, treatment of fever, cough, sore throats, skin infections, and arrest bleeding from injuries.[9,10] The volatile substances found in the aerial parts of *C. coelestinum* consist of germacrene D, (2E)-hexenal, *β*-caryophyllene, and (Z, Z)-α-farnesene. On the other hand, the non-volatile substances include intermedine, gardenin A, nobiletin, amorphane sesquiterpenoid 5, 8-epoxy-4, 6-dihydroxy-3-cadinanone, 7-hydroxytoxol 7-*O*-glucoside, luteolin, and its derivatives.[10] The increase in awareness of the relevance of medicinal plants use and the growing request for detailed information regarding the potency and side effects of plant extract employed traditionally in the treatment of diseases necessitate this study. Currently, there is no documented information regarding the phytochemical profile, GC-MS analysis and *in vitro* antioxidant potentials of *C. coelestinum* polar leaves, hence this study was designed to determine and provide the basic scientific knowledge relating to this gap.

## Materials and Methods

*Collection, identification and preparation of plant material*

Fresh leaves of *C. coelestinum* was collected from Abraka Community at GPS coordinates 5°47' 24.828" N and 6° 6' 17.208 E in Ethiope East Local Government Area, Delta State, Nigeria in June, 2024. Taxonomic identification and authentication of the plant was done by Mr. Wilson O. Egboduku of the Department of Botany, Faculty of Science, Delta State University, Abraka, Nigeria. Herbarium specimen with voucher number DELSUN 269 was deposited at the University Herbarium in the Department of Botany, Faculty of Science. The leaves were washed and shade dried at room temperature in a clean and hygienic conditions. The dried leaves (500 g) were ground into powder and extracted separately with methanol (MeOH), ethanol (EtOH) and water ($H_2O$) using Soxhlet apparatus based on the modified method described by Hussen and Endalew (2023).[11] The resulting plant extracts were kept at 4°C until further experiment.

*Phytochemical screening*

*Qualitative assay*

The Qualitative phytochemical screening of the leaf extracts was done based on previously described methods.[1,11,12]

*Quantitative Assay by GC-MS*

The GC-MS Analysis was done according to the method previously described by Njoku *et al*. (2021).[13]

*In vitro antioxidant assays*

*DPPH radical scavenging assay*

The antioxidant potential of *C. coelestinum* leaf extracts was determined using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging assay.[14] Different dilutions of the extracts (25, 50, 100, 200 and 400 µg/mL) were prepared, and 2 mL each of the different concentrations of the extract was added to 3 mL of 0.1 mM DPPH solution in methanol. The reaction mixture was incubated at room temperature in the dark for 30 minutes. The absorbance of the mixture was measured at 517 nm using a UV-Visible spectrophotometer.

Ascorbic acid at similar concentrations as the extracts was used as the reference standard. Methanol, ethanol, and distilled water were used as the blank for the respective extracts. All determinations were performed in triplicate. The percentage DPPH radical scavenging activity was calculated using the formula below:

$$\% \text{ Inhibition of DPPH} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Where, $A_{control}$ is the absorbance of the mixture of methanol/ethanol/water and DPPH solution, and $A_{sample}$ is the absorbance of the mixture of sample extract and DPPH solution.

The percentage inhibition was plotted against concentration from which $IC_{50}$ values were calculated.

*Ferric reducing antioxidant power (FRAP) assay*

The FRAP of the different extracts of *C. coelestinum* leaves was evaluated using the modified method of Shahinuzzaman *et al*. (2020).[15] The extracts solution (100 µL aliquots) was added to 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After 20 minutes of incubation at 50°C, 2.5 mL of trichloroacetic acid (10%) was added. From the mixture, 2.5 mL was taken and mixed with 2.5 mL of water and 0.5 mL of 1% $FeCl_3$. The resulting solution was allowed to stand for 30 minutes, then the absorbance was measured at 593 nm. Trolox was used as the reference standard. The results were expressed as Trolox equivalent antioxidant capacity (TEAC) in mM/L. TEAC was calculated by preparing a Trolox standard curve (Equation: $y = 0.0007x + 0.0645$, $R^2 = 0.9998$) using 31.25 µg/mL to 1.0 mg/mL of standard Trolox solution. Each experiment was carried out in triplicate.

*ABTS radical scavenging assay*

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of the different extracts of *C. coelestinum* leaves was assessed using a modified method of González-Palma *et al*. (2016).[16] The ABTS radical cation ($ABTS^{\bullet+}$) was produced after dissolving ABTS in water to a final concentration of 7 mM by reacting ABTS stock solution with 2.45 mM potassium persulfate. The $ABTS^{\bullet+}$ solution was diluted with water until an absorbance of 0.70 (±0.02) at 734 nm was achieved. The reaction mixture comprised 0.07 mL of extract and 3 mL of the ABTS radical solution. The reaction mixture was incubated for 6 minutes, and immediately the absorbance was measured at 734 nm. The ABTS radical scavenging activity was calculated using the formula below:

$$\% \text{ ABTS Inhibition} = \frac{A \text{ control} - A \text{ sample}}{A \text{ control}} \times 100$$

Where; $A_{control}$ is the absorbance of a mixtures of 10 mL of 7 mM ABTS, 2.45 mM $K_2S_2O_8$ with blank solvents, and Asample is the absorbance of the mixture of sample extract/standard and ABTS. The scavenging activity of *C. coelestinum* leaf extract against ABTS was expressed as $IC_{50}$.

*$H_2O_2$ radical scavenging assay*

The $H_2O_2$ radical scavenging activity was determined by the method of Agrawal *et al*. (2021).[1] A potassium phosphate buffer was prepared by mixing 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions. Equal volume of hydrogen peroxide and potassium phosphate buffer were mixed to generate free radicals, and the solution was kept at room temperature for 5 minutes for the reaction process to be completed. The hydrogen peroxide radical solution (0.6 mL) was added to the 1 mL of the extract, and the absorbance was measured using a spectrophotometer at 230 nm against phosphate buffer solution blank. The percentage inhibition of $H_2O_2$ was calculated using the formula:

$$\text{Percentage } H_2O_2 \text{ Inhibition} = \frac{A1 - A2}{A1} \times 100$$

Where A1 is the control absorbance and A2 is the absorbance in the presence of the extract and standard

*Lipid peroxidation inhibition assay*

The ability of *C. coelestinum* leaf extracts to inhibit lipid peroxidation *was evaluated in vitro* following a the method described by Moriasi *et al*. (2020).[17] Briefly, the reaction mixtures contained 2.0 mL of the trichloroacetic acid-thiobarbituric acid-hydrochloric acid (TCA-TBA-HCl) reagent (15% [w/v] TCA, 0.375% [w/v] TBA, and 0.25 N HCl) and 1 mL of the extracts or standard (L-ascorbic acid) at concentrations of 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL. The resulting mixtures were incubated in a water bath set at 90°C for 10 minutes, cooled, and centrifuged at 10 000 rpm for 15 minutes. The absorbance of the supernatant was measured at 532 nm. The percentage inhibition of lipid peroxidation (LP) was calculated using the formula below:

$$\% \text{ LP Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample2}}{\text{Absorbance of control}} \times 100$$

*Nitric oxide radical scavenging assay*

The ability of the extracts to scavenge nitric oxide (NO) generated from sodium nitroprusside (SNP) was evaluated using the modified method of Odeghe *et al*. (2016).[12] The reaction mixture (SNP, with or without extract) was incubated close to visible polychromatic light source for 180 minutes at 25ºC. The produced NO radical was

interacted with oxygen and the resulting nitrite ion ($NO^{2-}$) was investigated at 30 min intervals by mixing equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride). The mixture was incubated, the absorbance of the purple azo dye produced during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was determined at 546 nm. The nitrite formed with or without the leaf extract was calculated based on sodium nitrite solutions of known concentrations using a standard curve.

*Superoxide radical scavenging assay*

The scavenging of superoxide ($O_2^{\bullet-}$) anion radical was determined by the modified method described by Lalhminghlui and Jagetia (2018).[18] The reaction mixture consisted of 1 mL of alkaline DMSO, 0.3 mL of the leaf extract and 0.1 mL of *Nitro blue tetrazolium* (NBT). The absorbance of the reaction mixture was measured at 560 nm using a UV–VIS spectrophotometer. Pure DMSO was used as the blank, while ascorbic acid was used as the standard.

*Statistical analysis*

Data were analyzed using statistical package for social sciences (SPSS) for windows version 23.0. The data were expressed as mean ± standard error of mean (Mean ± SEM, n = 3). Significant differences between means were determined by one-way analysis of variance (ANOVA) followed by post hoc analysis. P-value < 0.05 was considered statistically significant.

## Results and Discussion

*Percentage yield of the extracts*

The crude extract yields from 500 g of *C. coelestinum* leaves were 69 g for methanol extract, 58 g for ethanol extract, and 35 g for aqueous extract. These results indicate that methanol produced the highest yield compared to the other solvents (Table 1).

**Table 1:** Extraction yields of *Conoclinium coelestinum* leaves

| Sample | Weight (g) | Percentage yield (%) |
|---|---|---|
| Methanol Extract | 69 | 42.59 |
| Ethanol Extract | 58 | 35.80 |
| Aqueous Extract | 35 | 21.60 |
| Total Yield | 162 | 100 |

*Phytochemical constituents*

This study examined the phytochemical composition, chemical profile, and antioxidant activity of the aqueous, methanol and ethanol extracts of *C. coelestinum* leaves. The purpose of this analysis was to provide scientific validation for the plant's traditional medicinal applications, particularly its antioxidant potentials. A preliminary qualitative phytochemical screening of the leaf extracts revealed the presence of alkaloids, glycosides, saponins, proteins, flavonoids, triterpenoids, steroids, and tannins (Table 2). These compounds are extensively documented for their ability to counteract oxidative stress, a condition associated with neurodegenerative disorders and inflammation.[19,20] The GC-MS analysis offered a more detailed identification of these phytochemicals, emphasizing their structural and functional diversity, which plays a crucial role in the observed antioxidant properties.[21]

*Gas chromatography-mass spectrometric profile of C. coelestinum*

The GC-MS analysis identified 27 bioactive constituents from the methanol extract of *C. coelestinum* leaves. The chromatogram is presented in Figure 1, while the bioactive constituents with their structures, molecular formula, molecular weight (MW), peak area (%), and retention time (RT) are presented in Table 3. The chemical compounds identified are Hexadecanoic acid ethyl ester, Pyrrole, 2-methyl-5-phenyl, Butanal, Oxime, Lidocaine, 1,2,4-Trithiolane,3,5-diethyl, Carpaine, Cedrene, 1,2,4-Thiadiazole-3,5-diamine, gamma-Muurolene, 2(3H)-Furanone,dihydro-5-pentyl, Alpha-Terpineol, IsoCaryophyllene, trans-Sesquisabinene hydrate, Methacrolein, n-Caprylic acid, 2(5H)-Furanone,methyl, D-Limonene, 2-Furancarboxaldehyde, 2-Propanone,1-hydroxy, Methyl,8-Methyl-nonanoate, 8-Octadecenoic acid,methyl ester, 1-Butanol,3-methyl, Phenol,3-pentadecyl, Linolenic acid, Pterin-6-carboxylic acid, Tetraacetyl-d-xylonic nitrile, and Propanoic acid.

Among these, hexadecanoic acid ethyl ester exhibited the highest peak area, indicating its prominence as a major compound. Previous studies have reported this compound to possess antioxidant, anti-inflammatory, and hypocholesterolemic activities.[15] Similarly, linolenic acid, a well-known polyunsaturated fatty acid, has demonstrated roles in mitigating oxidative stress and promoting cardiovascular health.[13]

Linolenic acid, with a peak area of 3.47%, is a polyunsaturated fatty acid critical for reducing inflammation and protecting against cardiovascular diseases. Its role in mitigating oxidative damage is well-documented, making it a valuable compound in preventing chronic illnesses.[16] Also, gamma-muurolene (3.36%), a sesquiterpene, has demonstrated antimicrobial and anti-inflammatory activities, supporting its potential application in managing infections and inflammatory disorders.[21]

Terpenoids, such as gamma-muurolene and alpha-terpineol, identified in moderate quantities, are recognized for their anti-inflammatory and antimicrobial activities. These findings are consistent with the plant's traditional use in managing inflammation-related conditions. Flavonoids like D-limonene were also detected, widely acknowledged for their strong antioxidant and anticancer properties.[22]

Other notable compounds include alpha-terpineol (3.63%), a monoterpene alcohol with significant antioxidant and anti-inflammatory properties, and phenol, 3-pentadecyl (1.59%), recognized for its robust free radical scavenging capacity. The presence of D-limonene (1.78%), a monoterpene, adds to the extract's anticancer and antioxidant potential, as reported in various studies.[16]

Compounds like 2-furancarboxaldehyde (0.62%) and 2-propanone, 1-hydroxy (3.94%) are known for their antioxidant activity and contribute to the extract's ability to neutralize reactive oxygen species. Additionally, terpenoids such as gamma-muurolene and trans-sesquisabinene hydrate (1.74%) enhance the plant's pharmacological properties, particularly in antimicrobial and anti-inflammatory applications.[9]

Unique compounds like carpaine (2.64%), a plant alkaloid, have demonstrated cardioprotective and antimicrobial effects, while methyl linoleate (1.79%) is a known anti-inflammatory agent. The identification of beta-caryophyllene, a sesquiterpene, further corroborates the extract's potential for managing oxidative stress and inflammatory conditions.

*In vitro antioxidant activity of C. coelestinum leave extracts*

The *in vitro* antioxidant activity of *C. coelestinum* leaf extracts are shown in Tables 4 – 10. Antioxidants are chemicals capable of inhibiting or preventing the oxidation of lipids or other molecules by halting the initiation of oxidative chain reactions. Consequently, they can prevent or reverse damage caused by oxidative stress to the body cells.[23] Natural antioxidants are increasingly favored for their health-promoting properties and their role in disease prevention.[23]

The significance of bioactive compounds in traditional medicinal plants cannot be overemphasized owing to their multifaceted therapeutic applications. Studies suggest that plants containing a diverse array of secondary metabolites like those in *C. coelestinum* may exhibit enhanced pharmacological effects due to their synergistic and multifunctional activities. This aligns with the growing interest in utilizing plant-based extracts as natural remedies for managing oxidative stress and inflammation-driven disorders.

The antioxidant potential of *C. coelestinum* extracts is corroborated by compounds such as phenol, 3-pentadecyl, and D-limonene. Phenolic compounds are well-recognized for their strong radical scavenging abilities, as demonstrated in assays like ABTS (70.15% inhibition at 400 µg/mL) and FRAP (61.67% inhibition at 400 µg/mL). D-limonene, a monoterpene, has been extensively researched for its antioxidant and anti-carcinogenic properties.[11]

The methanol extract exhibited significant antioxidant activities across various free radical scavenging assays, including DPPH, ABTS, FRAP, lipid peroxidation, hydrogen peroxide ($H_2O_2$), nitric oxide (NO), and superoxide radical scavenging assays. These findings

emphasize the extract's capability to remove reactive oxygen and nitrogen species, which are critical contributors to oxidative stress.

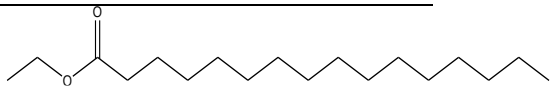
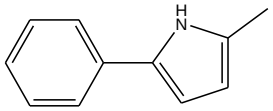
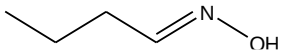
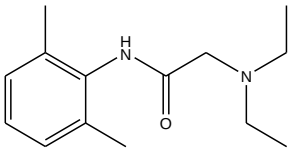
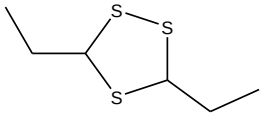
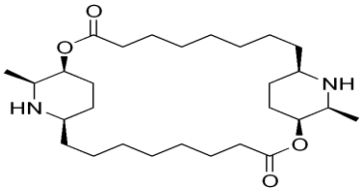
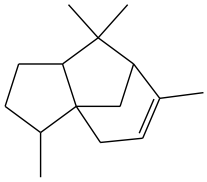
In the DPPH assay, the methanol extract significantly ($p < 0.05$) displayed 94.15% inhibition at 400 µg/mL, with an $IC_{50}$ value of 95.65 (Table 4). DPPH radical has a deep violet colour in solution, turning colourless or pale yellow upon neutralization and conversion to DPPH-H.[11,18] Numerous plant extracts have been reported to scavenge DPPH radicals *in vitro*.[24] Similarly, extracts of *C. coelestinum* scavenged

**Table 2:** Phytochemical constituents of *Conoclinium coelestinum* leaves

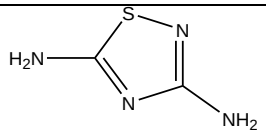
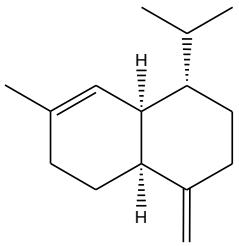
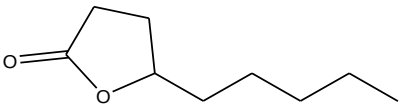
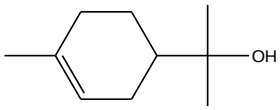
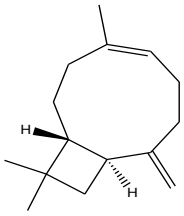
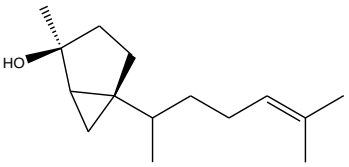
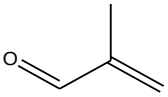
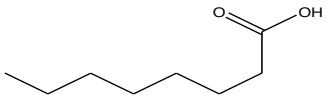
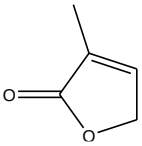
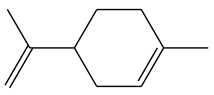
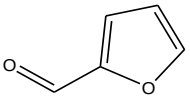
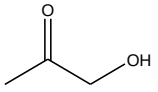
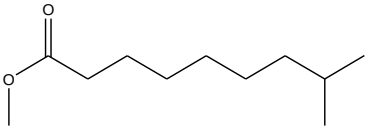
| Bioactive Compound | Observation | Inference | | |
|---|---|---|---|---|
| | | MeOH Extract | EtOH Extract | $H_2O$ Extract |
| Alkaloids | Creamy precipitate | + | + | + |
| Glycosides | Reddish brown precipitate | + | + | + |
| Saponins | Persistent foaming | + | + | + |
| Proteins | White precipitate | - | + | + |
| Flavonoids | Yellow colouration | + | + | + |
| Terpenoids | Reddish brown | + | + | + |
| Steroids | Deep yellow | + | + | + |
| Tannins | Dark green colouration | + | - | + |

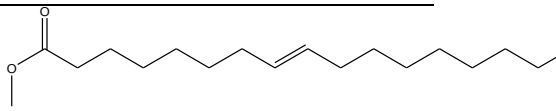
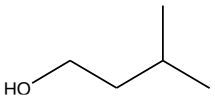
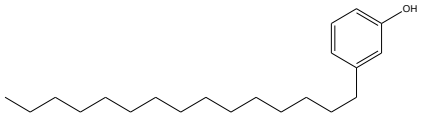
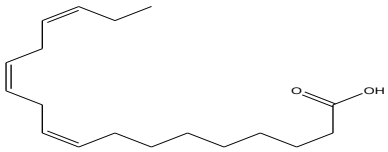
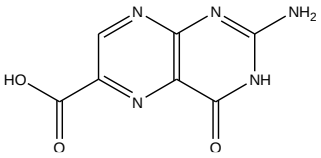
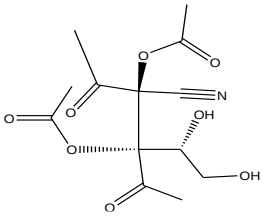
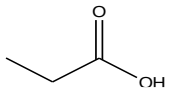
Key: - Absent, + Positive.

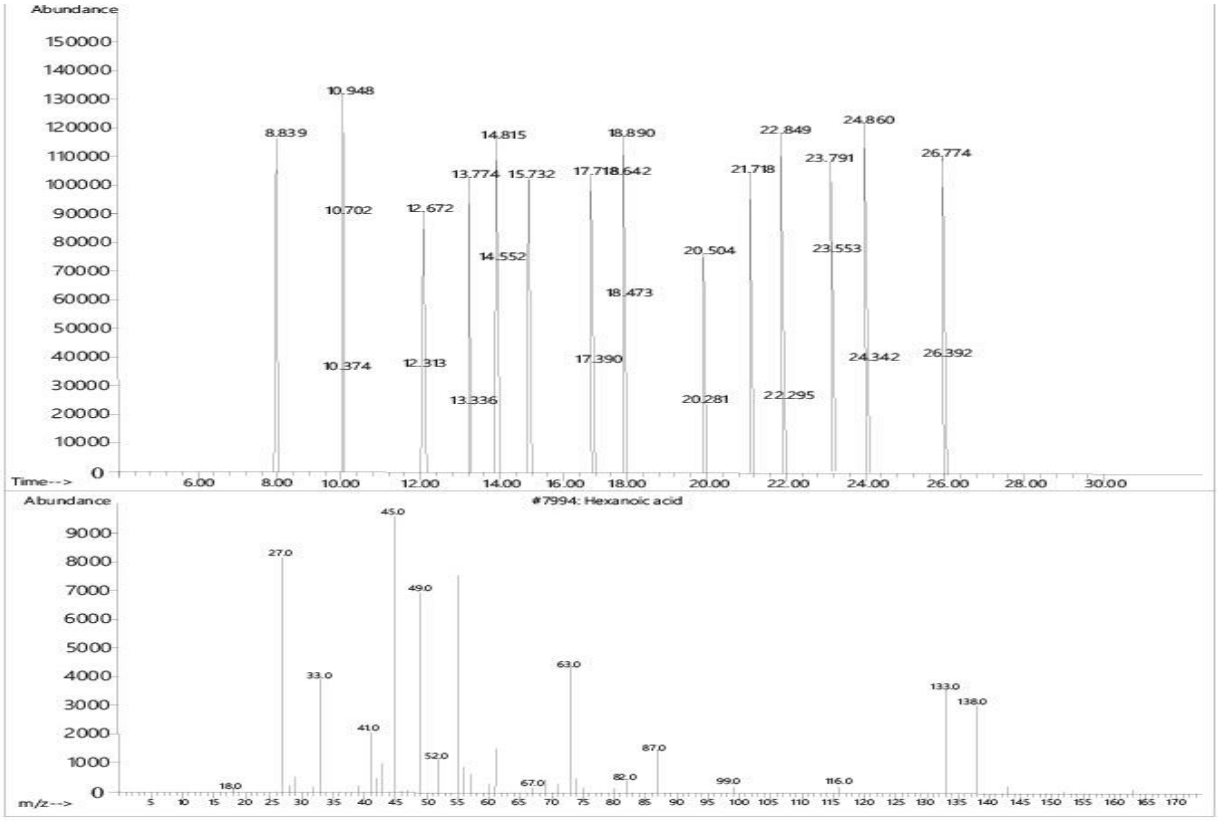
**Table 3: C**ompounds identified from the GC-MS analysis of *Conoclinium coelestinum* leaves

| S/No. | Name of Compound | Molecular Formula | Molecular Weight (g/Mol) | Peak Area (%) | RT (Min) | Structure of Compound |
|---|---|---|---|---|---|---|
| 1. | Hexadecanoic acid ethyl ester | $C_{18}H_{36}O_2$ | 284.4 | 8.89 | 8.839 |  |
| 2. | Pyrrole,2-methyl-5-phenyl | $C_{11}H_{11}N$ | 157.21 | 4.74 | 10.702 |  |
| 3. | Butanal oxime | $C_4H_9NO$ | 87.12 | 5.18 | 10.948 |  |
| 4. | Lidocaine | $C_{14}H_{22}N_2O$ | 234.34 | 3.26 | 12.313 |  |
| 5.. | 1,2,4-Trithiolane,3,5-diethyl | $C_6H_{12}S_3$ | 180.4 | 2.31 | 12.672 |  |
| 6. | Carpaine | **$C_{28}H_{50}N_2O_4$** | 478.7 | 2.64 | 13.336 |  |
| 7. | Cedrene | $C_{15}H_{24}$ | 204.35 | 3.94 | 13.774 |  |

**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

| | | | | | | |
|---|---|---|---|---|---|---|
| 8. | 1,2,4-Thiadiazole-3,5-diamine | $C_2H_4N_4S$ | 116.15 | 4.21 | 14.552 |  |
| 9. | Gamma-Muurolene | $C_{15}H_{24}$ | 204.35 | 3.36 | 14.815 |  |
| 10. | 2(3*H*)-Furanone,dihydro-5-pentyl | $C_9H_{16}O_2$ | 156.22 | 6.52 | 15.732 |  |
| 11. | Alpha-Terpineol | $C_{10}H_{18}O$ | 154.25 | 3.63 | 17.718 |  |
| 12. | Isocaryophyllene | $C_{15}H_{24}$ | 204.35 | 4.92 | 18.473 |  |
| 13. | trans-Sesquisabinene hydrate | $C_{15}H_{26}O$ | 222.37 | 1.74 | 18.890 |  |
| 14. | Methacrolein | $C_4H_6O$ | 70.09 | 0.85 | 20.281 |  |
| 15. | n-Caprylic acid | $C_8H_{16}O_2$ | 144.21 | 3.01 | 20.504 |  |
| 16. | 2(5*H*)-Furanone,methyl | $C_5H_6O_2$ | 98.1 | 2.63 | 21.718 |  |
| 17. | D-Limonene | $C_{10}H_{16}$ | 136.23 | 1.78 | 22.295 |  |
| 18. | 2-Furancarboxaldehyde | $C_5H_4O_2$ | 96.08 | 0.62 | 22.849 |  |
| 19. | 2-Propanone,1-hydroxy | $C_3H_6O_2$ | 74.07 | 3.94 | 23.553 |  |
| 20. | Methyl,8-Methyl-nonanoate | $C_{11}H_{22}O_2$ | 186.29 | 2.51 | 23.791 |  |

| | | | | | | |
|---|---|---|---|---|---|---|
| 21. | 8-Octadecenoic acid methyl ester | $C_{19}H_{36}O_2$ | 296.51 | 1.79 | 24.342 |  |
| 22. | 1-Butanol,3-methyl | $C_5H_{12}O$ | 88.15 | 1.63 | 24.860 |  |
| 23. | Phenol,3-pentadecyl | $C_{21}H_{36}O$ | 304.5 | 1.59 | 26.392 |  |
| 24. | Linolenic acid | $C_{18}H_{30}O_2$ | 278.43 | 3.47 | 26.774 |  |
| 25. | Pterin-6-carboxylic acid | $C_7H_5N_5O_3$ | 207.15 | 2.22 | 26.912 |  |
| 26. | Tetraacetyl-D-xylonic nitrile | $C_{14}H_{17}NO_9$ | 343.286 | 2.69 | 28.374 |  |
| 27. | Propanoic acid | $C_3H_6O_2$ | 74.08 | 1.24 | 28.726 |  |



**ISSN 2616-0684 (Print)**
**ISSN 2616-0692 (Electronic)**

**Figure 1:** GC-MS Chromatogram of methanol extract of *Conoclinium coelestinum* leaves

**Table 4:** DPPH radical scavenging activity of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 31 ± 0.09@ | 70.15 | 20.87 | 0.22 ± 0.03@ | 92.15 | 95.65 | 0.24 ± 0.04@ | 91.85 | 95.97 | 0.12 ± 0.06 | 93.15 | 92.90 |
| 50 | 0.08@ | 77.18 | | 0.20 ± 0.03@ | 92.42 | | 0.22 ± 0.03@ | 92.16 | | 0.10 ± 0.05 | 93.56 | |
| 100 | 76 ± 0.07@ | 84.13 | | 0.19 ± 0.02* | 93.15 | | 0.20 ± 0.02@ | 92.47 | | 0.09 ± 0.04 | 93.99 | |
| 200 | 72 ± 0.07@ | 89.19 | | 0.16 ± 0.01@ | 93.86 | | 0.19 ± 0.02@ | 92.96 | | 0.08 ± 0.03 | 94.52 | |
| 400 | 58 ± 0.05* | 94.45 | | 0.11 ± 0.04* | 94.15 | | 0.15 ± 0.01* | 93.45 | | 0.08 ± 0.03 | 95.01 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference (P < 0.05), while values with superscript (@) indicate no significant difference (P > 0.05). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

**Table 5:** Ferric reducing antioxidant power (FRAP) of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 59 ± 0.07* | 60.15 | 192.77 | 0.49 ± 0.04@ | 80.01 | 100.9 | 0.52 ± 0.03@ | 83.73 | 110.1 | 0.39 ± 0.04 | 85.12 | 99.97 |
| 50 | 0.07@ | 60.45 | | 0.47 ± 0.02@ | 80.23 | | 0.50 ± 0.02@ | 84.10 | | 0.37 ± 0.03 | 85.59 | |
| 100 | 55 ± 0.06* | 60.93. | | 0.45 ± 0.03@ | 80.45 | | 0.48 ± 0.03@ | 84.45 | | 0.35 ± 0.01 | 85.96 | |
| 200 | 52 ± 0.05@ | 61.24 | | 0.44 ± 0.00* | 80.75 | | 0.45 ± 0.00* | 84.94 | | 0.32 ± 0.02 | 86.32 | |
| 400 | 59 ± 0.03* | 61.67 | | 0.39 ± 0.03* | 81.12 | | 0.43 ± 0.01* | 85.03 | | 0.29 ± 0.00 | 87.01 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference (P < 0.05), while values with superscript (@) indicate no significant difference (P > 0.05). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

**Table 6:** ABTS scavenging activity of of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 56 ± 0.04@ | 14.45 | 353.97 | 0.43 ± 0.04* | 28.45 | 190.97 | 0.49 ± 0.02@ | 21.15 | 287.97 | 0.32 ± 0.05 | 5.15 | 130.97 |
| 50 | 0.03@ | 18.98 | | 0.40 ± 0.03@ | 43.12 | | 0.46 ± 0.02* | 32.06 | | 0.29 ± 0.04 | 5.02 | |
| 100 | 49 ± 0.02@ | 29.43 | | 0.39 ± 0.03* | 58.15 | | 0.43 ± 0.02 | 41.01 | | 0.26 ± 0.02 | 7.11 | |
| 200 | 45 ± 0.01* | 35.89 | | 0.36 ± 0.02* | 63.10 | | 0.39 ± 0.01* | 48.14 | | 0.24 ± 0.03 | 9.19 | |
| 400 | 39 ± 0.00@ | 48.15 | | 0.31 ± 0.01@ | 70.15 | | 0.35 ± 0.01@ | 55.18 | | 0.20 ± 0.01 | 0.15 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference (P < 0.05), while values with superscript (@) indicate no significant difference (P > 0.05). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

**Table 7:** $H_2O_2$ scavenging activity of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 65 ± 0.03@ | 61.56 | 285.77 | 0.46 ± 0.02@ | 74.01 | 196.19 | 0.49 ± 0.02* | 69.73 | 228.10 | 0.33 ± 0.04 | 75.12 | 184.14 |
| 50 | 0.02* | 61.91 | | 0.43 ± 0.03* | 75.23 | | 0.46 ± 0.02@ | 70.10 | | 0.31 ± 0.03 | 75.59 | |
| 100 | 50 ± 0.01@ | 62.13 | | 0.41 ± 0.02@ | 75.45 | | 0.44 ± 0.02* | 70.45 | | 0.29 ± 0.03 | 75.96 | |
| 200 | 48 ± 0.03* | 62.67 | | 0.38 ± 0.01* | 75.75 | | 0.42 ± 0.01@ | 70.94 | | 0.27 ± 0.01 | 76.32 | |
| 400 | 46 ± 0.01* | 63.07 | | 0.36 ± 0.00* | 76.12 | | 0.39 ± 0.01* | 71.03 | | 0.23 ± 0.02 | 7.01 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference ($P < 0.05$), while values with superscript (@) indicate no significant difference ($P > 0.05$). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

**Table 8:** Lipid peroxidation inhibitory activity of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 63 ± 0.02* | 62.26 | 276.28 | 0.49 ± 0.00@ | 68.01 | 186.12 | 0.55 ± 0.03@ | 66.73 | 195.10 | 0.45 ± 0.03 | 72.12 | 175.23 |
| 50 | 0.03* | 62.91 | | 0.48 ± 0.03@ | 68.13 | | 0.52 ± 0.01@ | 67.10 | | 0.42 ± 0.04 | 72.48 | |
| 100 | 58 ± 0.02@ | 63.13 | | 0.46 ± 0.02 | 68.44 | | 0.50 ± 0.01@ | 67.45 | | 0.40 ± 0.00 | 72.85 | |
| 200 | 57 ± 0.02@ | 63.67 | | 0.45 ± 0.00* | 68.76 | | 0.48 ± 0.02@ | 67.74 | | 0.37 ± 0.03 | 73.32 | |
| 400 | 55 ± 0.01* | 64.07 | | 0.42 ± 0.00* | 69.11 | | 0.47 ± 0.03* | 68.00 | | 0.35 ± 0.01 | 74.00 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference ($P < 0.05$), while values with superscript (@) indicate no significant difference ($P > 0.05$). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

**Table 9:** Nitric oxide scavenging activity of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $C_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | 75 ± 0.05* | 58.36 | 302.13 | 0.59 ± 0.02* | 62.24 | 201.12 | 0.66 ± 0.02* | 61.73 | 235.76 | 0.52 ± 0.05 | 67.12 | 182.95 |
| 50 | 0.04@ | 58.91 | | 0.57 ± 0.03* | 62.23 | | 0.63 ± 0.00 | 62.33 | | 0.50 ± 0.03 | 67.45 | |
| 100 | 72 ± 0.03@ | 59.22 | | 0.54 ± 0.01* | 62.45 | | 0.62 ± 0.01* | 62.45 | | 0.48 ± 0.02 | 67.86 | |
| 200 | 69 ± 0.01* | 59.65 | | 0.53 ± 0.04@ | 63.75 | | 0.59 ± 0.01@ | 62.94 | | 0.45 ± 0.00 | 68.15 | |
| 400 | 67 ± 0.02* | 60.07 | | 0.49 ± 0.00* | 64.12 | | 0.57 ± 0.02* | 63.03 | | 0.42 ± 0.02 | 69.01 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference ($P < 0.05$), while values with superscript (@) indicate no significant difference ($P > 0.05$). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

**Table 10:** Superoxide radical scavenging activity of *Conoclinium coelestinum* leaf extracts

| Conc. (µg/mL) | $H_2O$ Extract | | | MeOH Extract | | | EtOH Extract | | | AA | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) | A | % Inhibition | $IC_{50}$ (µg/mL) |
| 25 | '2 ± 0.06$^{@}$ | 80.36 | 139.00 | 0.49 ± 0.04$^{*}$ | 39.01 | 120.12 | 0.57 ± 0.03 | 90.73 | 105.64 | 0.40 ± 0.06 | 92.12 | 94.87 |
| 50 | 0.05$^{@}$ | 80.91 | | 0.46 ± 0.03$^{@}$ | 89.23 | | 0.53 ± 0.06* | 91.13 | | 0.39 ± 0.04 | 92.59 | |
| 100 | 39 ± 0.01$^{@}$ | 81.13 | | 0.42 ± 0.01* | 89.45 | | 0.51 ± 0.01$^{*}$ | 91.46 | | 0.37 ± 0.01 | 92.96 | |
| 200 | 37 ± 0.00$^{*}$ | 81.67 | | 0.40 ± 0.04$^{*}$ | 89.75 | | 0.49 ± 0.03$^{*}$ | 91.92 | | 0.33 ± 0.03 | 92.32 | |
| 400 | 42 ± 0.02* | 82.07 | | 0.38 ± 0.01$^{*}$ | 90.12 | | 0.43 ± 0.00$^{*}$ | 92.03 | | 0.29 ± 0.04 | 4.01 | |

Data represent mean ± S.E.M (n = 3). Values with superscript (*) denote significant difference ($P < 0.05$), while values with superscript (@) indicate no significant difference ($P > 0.05$). A = Absorbance, AA = Ascorbic Acid, $IC_{50}$ = Half-maximal inhibitory concentration.

DPPH radicals in a concentration-dependent manner. Comparable effects have been observed in various tea extracts rich in polyphenols, which also exhibit potent DPPH radical scavenging activity.[18] Other phytochemicals such as mangiferin and naringin have been noted to scavenge DPPH radicals effectively in a concentration-dependent manner.[18,25] The DPPH scavenging ability of *C. coelestinum* can thus be attributed to its abundant flavonoids and polyphenols, as highlighted in this study.

The reducing power of the extracts, as measured by the FRAP assay, further demonstrated the superiority of MeOH and EtOH extracts, with significant ($p < 0.05$) percentage inhibitions of 81.12% and 85.03%, respectively, compared to the aqueous extract (61.67%). Their $IC_{50}$ values followed a similar pattern, with MeOH (100.90 µg/mL) slightly outperforming EtOH (110.10 µg/mL) (Table 5). This result underscores the extract's capability to reduce ferric ($Fe^{3+}$) ions to ferrous ($Fe^{2+}$) ions, indicative of its reducing power. Such activity is strongly linked to its phenolic and flavonoid contents, which stabilizes reactive oxygen species by donating electrons. Compounds like hexadecanoic acid and beta-tocopherol, identified in the GC-MS analysis, contribute significantly to the extract's reducing power and antioxidant properties. The FRAP assay has been widely used due to its simplicity and reliability in determining antioxidant activity.[26] Extracts of *C. coelestinum* showed a concentration-dependent increase in FRAP values, consistent with other plant extracts reported to exhibit high antioxidant activity *in vitro*.[26,27]

In the ABTS assay, the extracts demonstrated lower scavenging activity compared to DPPH and FRAP assays. The MeOH extract exhibited the highest inhibition (70.15%), followed by EtOH (55.18%) and $H_2O$ extract (48.15%), while ascorbic acid showed superior activity (90.15%). The higher $IC_{50}$ values of MeOH (190.97 µg/mL), EtOH (256.89 µg/mL), and $H_2O$ extract (334.93 µg/mL) indicate reduced potency compared to DPPH and FRAP assays (Table 6). This comparatively lower activity may be attributed to the reduced reactivity of certain compounds toward ABTS radicals. These assays reflect the extract's ability to reduce oxidized radicals and ferric ions, demonstrating its robust reducing potential. Compounds like beta-tocopherol and linolenic acid further support these findings. The ABTS radical cation is generated through the reaction between ABTS and potassium persulfate, resulting in a blue cation that absorbs light at 734 nm.[28] ABTS is highly reactive toward antioxidants, including phenols, thiols, and vitamin C.[27] Extracts of *C. coelestinum* displayed concentration-dependent inhibition of ABTS radicals. A similar effect has been observed in extracts of *Syzygium cumini*, naringin, and mangiferin.[18] Additionally, kaempferol, a flavonoid reported to scavenge ABTS radicals, may also contribute to the antioxidant activity of *C. coelestinum* leaves observed in this study.[29]

Similarly, the $H_2O_2$ scavenging activity was significant, with 76.12% inhibition at 400 µg/mL (Table 7). These outcomes align with the roles of terpenoids and phenolics, which are well-known for their protective effects against oxidative stress. $H_2O_2$ is an essential substance because of it potential to pass through biological membranes. While $H_2O_2$ itself is not highly reactive, it can become toxic by generating hydroxyl radicals within cells.[30] The scavenging of $H_2O_2$ by the extracts may be attributed to the presence of phenolics, which donate electrons to neutralize $H_2O_2$ into water.[31] The data suggests that all extracts exhibit robust $H_2O_2$ scavenging activity, likely owing to their antioxidant compounds. As effective electron donors, these components facilitate the conversion of $H_2O_2$ to $H_2O$.

The lipid peroxidation assay revealed a 64.07% inhibition at 400 µg/mL, demonstrating the extract's capacity to prevent oxidative damage to lipids, which is essential for maintaining cellular integrity (Table 8). The nitric oxide scavenging assay showed 60.07% inhibition at 400 µg/mL, indicating the extract's potential in mitigating inflammation mediated by NO radicals (Table 9). Furthermore, the superoxide radical scavenging activity was notably high, with 90.12% inhibition at the same concentration, highlighting the extract's effectiveness in countering mitochondrial oxidative stress (Table 10). The nitric oxide radical (NO•) is known for its cytotoxicity, particularly when reacting with oxygen or superoxide anion radicals. The *C. coelestinum* extracts demonstrated a concentration-dependent reduction in NO• generation. Also, several plant extracts and formulations have shown the ability to scavenge NO• in a concentration-dependent manner.[32] Compounds such as betanin, phyllocactin, and betanidin have been previously documented to scavenge NO radicals effectively.[33] Additionally, flavonoids like kaempferol, myricetin, epigallocatechin gallate, catechin, epicatechin, and resveratrol, along with delphinidin, pelargonidin, malvin, mangiferin, and naringin, have been reported to neutralize NO radicals in earlier studies.[18,28,31,34]

Differences in observations could be attributed to variations in the cultivars used or the influence of the local environment where the plants were grown. For instance, contrary to these findings, it has been reported that polyphenol extracts from mango leaves failed to scavenge hydroxyl radicals across all tested concentrations.[35]

However, DPPH scavenging of mango leaf alcoholic extracts, as reported by Ndel Famen *et al*. (2020)[36] aligns with the present observations. These authors emphasized that the type of extraction solvent and plant variety play pivotal roles in determining phenolic content and antioxidant activity.[18] Four primary phenolic compounds identified in mango leaves contributed significantly to antioxidant properties.[26]

The diverse phytochemicals identified through GC-MS analysis, coupled with the robust antioxidant activity, highlight the pharmacological potential of *C. coelestinum*. These compounds possess anti-inflammatory and cardioprotective activities. For instance, flavonoids are associated with antitumor, anti-inflammatory, and antidiarrheal properties, while terpenoids exhibit activities such as anti-HIV, antitumor, and antimycobacterial effects.[37] These findings reinforce the therapeutic relevance of *C. coelestinum* for managing oxidative stress and associated disorders.

## Conclusion

The GC-MS analysis of *C. coelestinum* leaf extracts highlight the richness and diversity of its bioactive compounds. The methanol and ethanol extracts of *C. coelestinum* leaves exhibit potent antioxidant activity, with methanol extract showing slightly superior efficacy. Among all the antioxidant assays, the DPPH and FRAP assays demonstrated the highest scavenging activities, highlighting the extracts' potential to neutralize free radicals and reduce oxidative stress. These findings, in conjunction with the strong radical scavenging activity observed in the assays, validate the plant's traditional use in medicine and underscore its potential as a source of natural antioxidants and anti-inflammatory agents. Future studies should focus on isolating and characterizing the bioactive compounds to better understand their individual and synergistic roles in therapeutic applications.

## Conflict of Interest

The authors declare no conflict of interest.

## Author's Deceleration

The authors hereby declare that the work presented in this article is original and that any liability for claim relating to the content of this article will be borne by them.

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