Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 9s (2025)
https://www.jneonatalsurg.com

Extraction, Antioxidant Activity and HPLC Study of Bioactive Compound Present in Leaves Extract of Onosma Bracteatum

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Cite this paper as: Subodh Vishnukant Kamble, Dr. Prashant Soni, (2025) Extraction, Antioxidant Activity and HPLC Study of Bioactive Compound Present in Leaves Extract of Onosma Bracteatum. *Journal of Neonatal Surgery*, 14 (9s), 458-465.

ABSTRACT

The leaves of *Onosma bracteatum*, a member of the Boraginaceae family, are known for their medicinal properties, which are attributed to the presence of bioactive compounds such as flavonoids and phenolic acids. This study aims to investigate the antioxidant activity and bioactive compound profile of the ethanolic extract of *Onosma bracteatum* leaves, using DPPH and nitric oxide (NO) inhibition assays, along with High-Performance Liquid Chromatography (HPLC) for the quantification of key compounds. The DPPH and NO assays revealed that the ethanolic extract exhibited significant antioxidant activity, although not as potent as ascorbic acid. The IC50 values for the DPPH and NO assays were 75.62 µg/ml and 83.39 µg/ml, respectively. HPLC analysis identified quercetin and gallic acid as the major bioactive compounds in the extract, with 0.831% quercetin and 0.940% gallic acid. The results suggest that *Onosma bracteatum* leaves are a valuable source of natural antioxidants, which could have potential therapeutic applications in the treatment of oxidative stress-related diseases. Further studies are needed to explore the full range of pharmacological properties and possible clinical uses of this plant.

Keywords: *Onosma bracteatum*, *Antioxidant activity*, *DPPH assay*, *Nitric oxide assay*, *Quercetin*, *Gallic acid*, *High-Performance Liquid Chromatography (HPLC).*

1. INTRODUCTION

Onosma bracteatum, a member of the Boraginaceae family, has gained considerable attention in traditional medicine due to its wide range of therapeutic properties. Commonly referred to as "Indian Lithospermum," this plant is predominantly found in temperate and subtropical regions. The medicinal potential of *Onosma bracteatum* is largely attributed to its rich phytochemical profile, which includes alkaloids, flavonoids, phenolic compounds, terpenoids, and saponins, among others. These bioactive compounds contribute to its anti-inflammatory, antimicrobial, antioxidant, and anticancer activities (Patel et al., 2020). The extraction of bioactive compounds from plant materials is a critical process in herbal medicine, as it directly influences the concentration and biological activity of the compounds present. Various extraction methods, including maceration, Soxhlet extraction, and successive solvent extraction, have been employed to obtain plant extracts with high bioactive content (Mathew & Abraham, 2006). Solvents such as ethanol, methanol, acetone, and water are commonly used, with the extraction yield and efficiency varying based on the solvent's polarity and the specific compounds targeted. Among the most significant bioactive compounds present in *Onosma bracteatum* are phenolic compounds and flavonoids. These compounds have attracted considerable interest due to their potent antioxidant properties, which are crucial in combating oxidative stress and related diseases, such as cancer, cardiovascular disorders, and neurodegenerative diseases (Sánchez-Rangel et al., 2013). Antioxidants act by scavenging free radicals and reactive oxygen species (ROS), preventing cellular damage and mitigating the risk of chronic diseases (Gómez-Caravaca et al., 2006). High-Performance Liquid Chromatography (HPLC) has emerged as one of the most powerful techniques for the separation and identification of individual bioactive compounds in plant extracts. HPLC provides a precise, reproducible, and sensitive method for the quantification of phenolic acids, flavonoids, and other polyphenolic compounds (Nawaz et al., 2014). It has been widely used to profile the phytochemical composition of various medicinal plants and is particularly useful in determining the presence of compounds that contribute to antioxidant activity. This study aims to evaluate the extraction efficiency, antioxidant activity, and HPLC profile of bioactive compounds in the leaf extracts of *Onosma bracteatum*. The primary objectives are to determine the total phenolic content (TPC) and total flavonoid content (TFC) of the extracts and to identify the individual bioactive compounds using HPLC analysis. This research will provide further insight into the medicinal potential of *Onosma bracteatum* leaves, particularly in relation to their antioxidant properties.

2. MATERIALS AND METHODS

Chemicals and equipment

Diphenyl-1-picrylhydrazyl (DPPH), **Nitric Oxide (NO),** ascorbic acid, methanol, sulphanilamide, 2% phosphoric acid (H_3PO_4), and 0.1% naphthylethylenediamine dihydrochloride used in this study. All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai). A thermospectronic model of Labindia 3000 + UV/VIS Spectrophotometer with 1cm. matched quartz cells was used for determination of λ_{max}. The HPLC system (Waters) consisted of a pump, a U.V. Visible detector, a Thermo C_{18} (250 X 4.6 mm, 5µm) column, a N2000 Chromatography Data System.

Methods

Extraction by maceration process

Dried defatted leaves of *Onosma bracteatum* were exhaustively extracted with ethanol and using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts (Mukherjee, 2007).

In-vitro antioxidant activity using different methods

DPPH method

Total free radical scavenging capacity of the ethanolic extract from *Onosma bracteatum* were estimated according to the previously reported method with slight modification (Parkhe and Jain, 2018). Solution of DPPH (6 mg in 100ml methanol) was prepared and stored in dark place. Different concentration of standard and test (10- 100 µg/ml) was prepared. 1.5 ml of DPPH and 1.5 ml of each standard and test was taken in separate test tube; absorbance of this solution was taken immediately at 517nm. 1.5 ml of DPPH and 1.5 ml of the methanol was taken as control absorbance at 517nm.

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%.

Nitric oxide method

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing (Marcocci *et al.*, 1994). Sodium nitroprusside (10 mmol / L) was mixed with various extract concentrations in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a positive control. The inhibition proportion was evaluated using the following formula:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times100$

Where Acontrol is the absorption (without extract) of the control and where Atest is the absorption in the presence of the extract / standard.

Identification of marker compound (Quercetin) of ethanolic extract of *Onosma bracteatum* leaves by HPLC

Table 1: Selection of Separation Variable

Variable	Condition
Column	
Dimension.	250mm x 4.60mm
Particle Size	5 µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Acetonitrile	50

Methanol	50
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	256 nm
Retention time	2.315± 0.005 min

Preparation of standard solution

10mg of Quercetin was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the methanol to give a stock solution of 1000ppm (Acharya *et al.*, 2019). From stock solutions of Quercetin 1 ml was taken and diluted up to 10 ml. from this solution 0.2, 0.4, 0.6, 0.8, 1.0ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 2, 4, 6, 8, 10µg/ ml concentration.

Analysis of extract

10 mg of ethanolic extract of *Onosma bracteatum* were taken in 10 ml volumetric flask and dilute upto the mark with Methanol; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000µg/ml. Inject 20µl of this solution in injector and record the chromatogram.

Identification of marker compound (Gallic acid) of ethanolic extract of *Onosma bracteatum* leaves by HPLC

Table 2: Selection of Separation Variable

Preparation of standard solution

Variable	**Condition**
Column	
Dimension	250mm x 4.60mm
Particle Size	5µm
Bonded Phase	Octadecylsilane (C_{18})
Mobile Phase	
Water	80
Acetonitrile	20
pH with OPA	3.0
Flow rate	1ml/min
Temperature	Room temp.
Sample Size	20 µl
Detection wavelength	272 nm
Retention time	5.346± 0.004 min

10mg of Gallic acid was weighed accurately and transferred to a 10ml volumetric flask, and the volume was adjusted to the mark with the mobile phase to give a stock solution of 1000ppm. From stock solutions of Gallic acid 1 ml was taken and diluted up to 10 ml. from this solution 0.1, 0.2, 0.3, 0.4, 0.5ml solutions were transferred to 10ml volumetric flasks and make up the volume up to 10 ml with mobile phase, gives standard drug solution of 1, 2, 3, 4, 5µg/ ml concentration.

Analysis of extract

10 mg of ethanolic extract of *Onosma bracteatum* were taken in 10 ml volumetric flask and dilute upto the mark with mobile phase; resultant solution was filtered through Whatmann filter paper and finally volume made up to mark with same solvent to obtain concentration of 1000 µg/ml. Inject 20µl of this solution in injector and record the chromatogram.

3. RESULTS AND DISCUSSION

The results presented in this study provide a detailed evaluation of the antioxidant potential and bioactive compound profile of *Onosma bracteatum* leaves through various assays, including DPPH and nitric oxide (NO) inhibition methods, and through HPLC analysis for the quantification of quercetin and gallic acid. These findings demonstrate the plant's promising antioxidant activity and provide valuable insight into the compounds that may contribute to its therapeutic potential. The antioxidant activity of *Onosma bracteatum* leaves was assessed using two common methods: the DPPH (2,2-diphenyl-1-picrylhydrazyl) and nitric oxide (NO) inhibition assays. In the DPPH method, the ethanolic extract of *Onosma bracteatum* exhibited a dose-dependent increase in % inhibition, although the inhibition was lower compared to ascorbic acid, a standard antioxidant (Table 3). The IC50 value for ascorbic acid was found to be 18.16 µg/ml, indicating strong antioxidant activity, while the ethanolic extract showed an IC50 value of 75.62 µg/ml. This suggests that while the ethanolic extract exhibits notable antioxidant potential, it is less potent than ascorbic acid, which is widely recognized for its strong antioxidant properties. The increased inhibition with higher concentrations of the extract indicates that the leaves contain compounds with antioxidant activity, likely due to the presence of phenolic compounds and flavonoids. The nitric oxide inhibition assay further confirmed the antioxidant activity of the ethanolic extract (Table 4). Similar to the DPPH assay, the % inhibition increased with the concentration of the extract. At higher concentrations (100 µg/ml), the ethanolic extract showed 59.6% inhibition, compared to 78.69% for ascorbic acid. The IC50 value for ascorbic acid was 22.60 µg/ml, while the ethanolic extract's IC50 value was 83.39 µg/ml, indicating a moderate antioxidant capacity. The NO scavenging activity of the ethanolic extract reinforces the hypothesis that *Onosma bracteatum* possesses antioxidant properties, likely due to the phenolic and flavonoid compounds present in the plant. These results suggest that the antioxidant potential of *Onosma bracteatum* leaves, while not as strong as ascorbic acid, is still considerable and may be attributed to the plant's polyphenolic content. The extract may serve as a useful source of natural antioxidants, particularly for combating oxidative stress-related conditions. High-Performance Liquid Chromatography (HPLC) analysis was conducted to identify and quantify key bioactive compounds in the ethanolic extract of *Onosma bracteatum*. The chromatograms for standard quercetin and gallic acid were compared with those of the ethanolic extract to identify and estimate the concentrations of these compounds. Quercetin is a well-known flavonoid with potent antioxidant and anti-inflammatory properties. In the HPLC chromatogram (Figure 3), the standard quercetin exhibited a retention time (RT) of 2.515 minutes. The ethanolic extract of *Onosma bracteatum* also showed a peak at the same retention time (Figure 4), confirming the presence of quercetin in the extract. The quantitative estimation (Table 5) revealed that the ethanolic extract contained 0.831% quercetin, indicating that this compound is present in significant amounts and could contribute to the observed antioxidant activity of the extract. Gallic acid is a phenolic compound with strong antioxidant properties. In the chromatogram of the ethanolic extract (Figure 6), the RT for gallic acid was found to be 5.342 minutes, matching the RT of the standard gallic acid (Figure 5). The quantitative analysis (Table 6) showed that the ethanolic extract contained 0.940% gallic acid. This further supports the hypothesis that the antioxidant activity of the plant extract may be due to the combined action of both quercetin and gallic acid, among other phenolic compounds. The antioxidant activity of *Onosma bracteatum* leaves observed in this study aligns with findings from other plants in the *Boraginaceae* family. Studies have demonstrated that members of this family are rich in phenolic compounds, flavonoids, and other secondary metabolites that exhibit antioxidant properties. While the antioxidant activity of *Onosma bracteatum* is moderate compared to ascorbic acid, it is still significant enough to warrant further investigation for potential therapeutic uses in managing oxidative stress-related disorders. The concentration of quercetin (0.831%) in the ethanolic extract of *Onosma bracteatum* is consistent with reports of flavonoid content in other medicinal plants known for their antioxidant properties. The presence of gallic acid (0.940%) in the ethanolic extract adds further support to the antioxidant potential of the plant, as gallic acid is widely recognized for its ability to scavenge free radicals and reduce oxidative damage (Gómez-Caravaca et al., 2006). The significant antioxidant activity and the presence of bioactive compounds such as quercetin and gallic acid suggest that *Onosma bracteatum* leaves could be a valuable source of natural antioxidants. These compounds have well-documented health benefits, including anti-inflammatory, anticancer, and neuroprotective effects. The findings from this study suggest that *Onosma bracteatum* could be further explored for its potential in treating conditions associated with oxidative stress, such as cancer, cardiovascular diseases, and neurodegenerative disorders.

Table 3: % Inhibition of ascorbic acid and extract of *Onosma bracteatum* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	45.25	20.78
2	20	50.98	31.44
3	40	61.51	36.02
4	60	72.64	42.67
5	80	80.57	51.93
6	100	82.92	63.51
IC $_{50}$ value		**18.16**	**75.62**

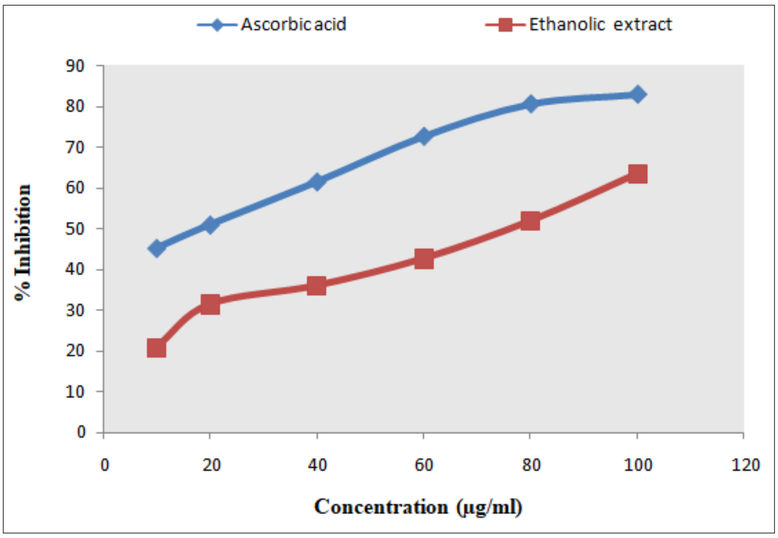

Figure 1: % Inhibition of ascorbic acid and extract of *Onosma bracteatum* using DPPH method

Table 4: % Inhibition of ascorbic acid and extract of *Onosma bracteatum* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	50.71	19.54
2	40	53.45	28.73
3	60	64.98	39.47
4	80	70.37	45.81
5	100	78.69	59.6
IC $_{50}$ value		**22.60**	**83.39**

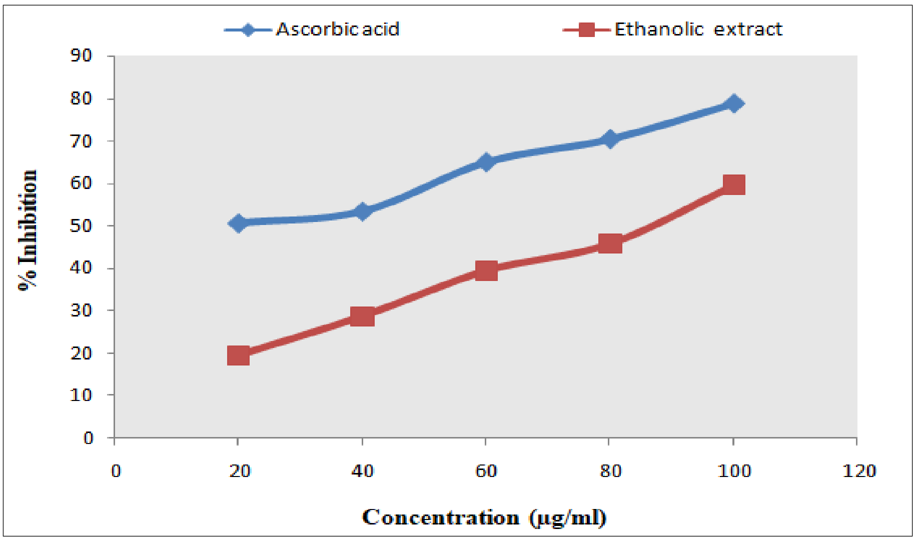

Figure 2: % Inhibition of ascorbic acid and extract of *Onosma bracteatum* using NO method

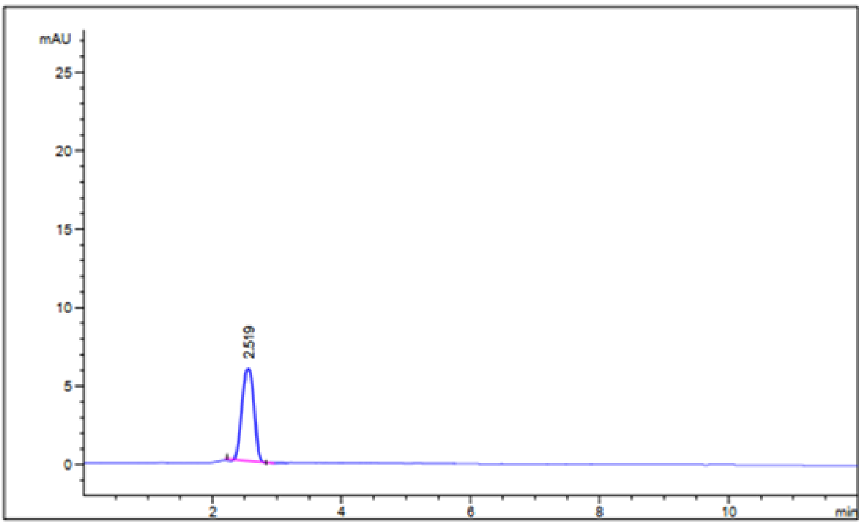

Figure 3: Chromatogram of standard Quercetin (4µg/ml)

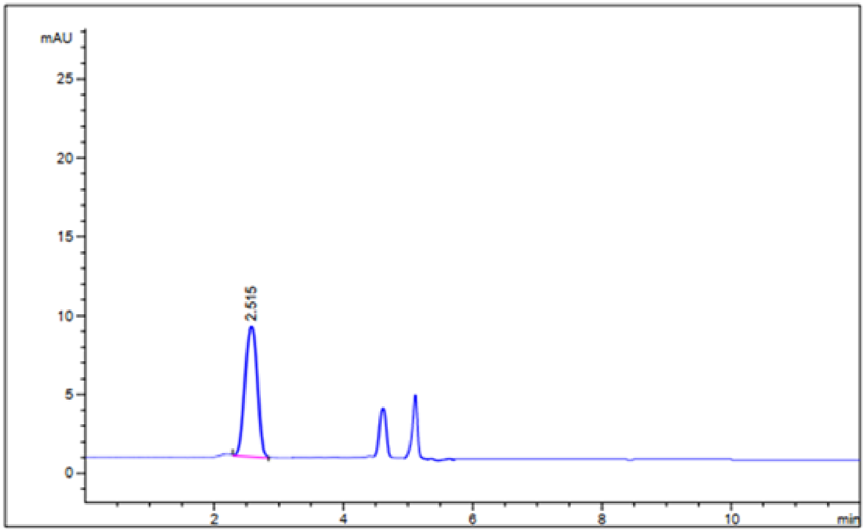

Figure 4: Chromatogram of ethanolic extract of *Onosma bracteatum*

Table 5: Quantitative estimation of Quercetin in *Onosma bracteatum* extract

S. No.	Standard/ Extracts	RT	Area	% Assay
1.	**Quercetin**	2.515		
2.	Ethanolic Extract	2.515	10568.232	0.831

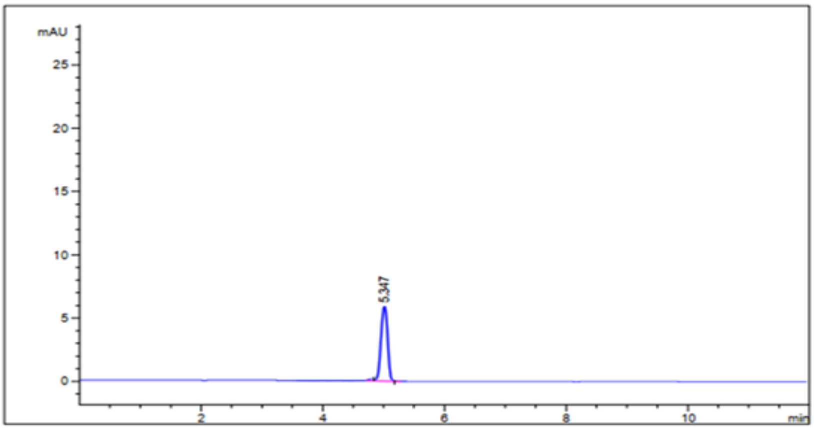

Figure 5: Chromatogram of standard Gallic acid (8µg/ml)

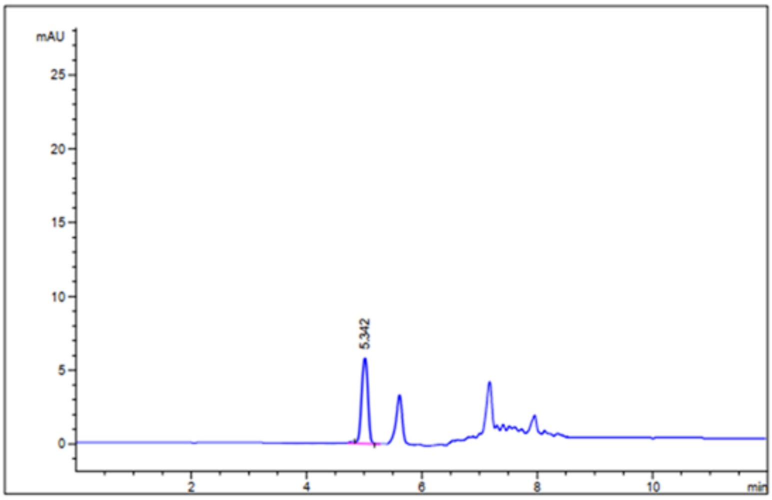

Figure 6: Chromatogram of ethanolic extract of *Onosma bracteatum*

Table 6: Quantitative estimation of Gallic acid in *Onosma bracteatum* extract

S. No.	Standard/ Extracts	RT	Area	% Assay
1.	**Quercetin**	5.342		
2.	Ethanolic Extract	5.342	1165.254	0.940

4. CONCLUSION

The ethanolic extract of *Onosma bracteatum* demonstrates promising antioxidant activity as evidenced by both the DPPH and NO inhibition assays. The presence of quercetin and gallic acid, as determined through HPLC analysis, may play a key role in this activity. The moderate antioxidant capacity of the extract suggests its potential for use in pharmaceutical and nutraceutical applications aimed at combating oxidative stress-related diseases. Further research, including in vivo studies, is necessary to fully explore the therapeutic potential of this plant.

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