

Antioxidant activity and phytochemical analysis of *Clerodendrum infortunatum* from Morang, Nepal

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Abstract

The objective of the study was to carry out the phytochemical screening of all parts (stem, root, leaves, and flower) of *Clerodendrum infortunatum*, while the antioxidant assay was conducted only on flower extracts in ethanol and methanol solvents. Standard Chemical test method was used for qualitative screening of plant extracts. For the antioxidant assay, the ascorbic-iron-EDTA model was used to prepare free hydroxyl radicals (OH•). Various concentrations of floral extract (250, 500, 750, and 1000 µg/ml) were taken and treated with formulated hydroxyl radicals. The absorbance of the treated extract and control was measured at 412 nm using a UV-Vis Spectrophotometer. Antioxidant activities were determined by the calculation of % inhibition of free radicals from the absorbance value. The phytochemical screening revealed the presence of carbohydrate, alkaloid, terpenoid, flavonoid, coumarin, steroid, except glycoside and anthraquinone in all parts of the plant extract. The study also exhibited the greatest antioxidant activity with 84.58 ± 2.04 % of inhibition at 1000 µg/ml and IC₅₀ at 630 µg/ml against OH• by the methanolic flower extract of *Clerodendrum infortunatum*, whereas the ethanolic flower extract revealed 55.12 ± 1.14 % of inhibition at 1000 µg/ml and IC₅₀ at 940 µg/ml. The antioxidant activity experiment was carried out in triplicate and showed a significant ($p < 0.05$), concentration-dependent increase in OH• scavenging activity. The findings suggest that the flower of *Clerodendrum infortunatum* is a rich source of bioactive compounds. However, isolation and characterization of active phytochemicals following in-vivo validation will be an important step to verify their pharmacological potential.

Keywords: *Clerodendrum infortunatum*, Phytochemical analysis, Antioxidant activity, Hydroxyl radical scavenging, IC₅₀

1.0 Introduction

Plants have been employed for medicinal purposes since the early days of human civilization, long before the beginning of recorded history (Israyilova et al., 2025). Phytochemicals are either primary or secondary metabolites that are present in plants naturally. Nowadays, these phytochemicals become more popular due to their countless medicinal uses (Rabizadeh et al., 2022). The release of numerous secondary metabolites is to improve health by producing enzyme inhibitors, antibiotics, immune modulators, growth promoters, and antitumor agents of animals and plants (Thirumurugan et al., 2018). Flavonoids, a group of plant-derived secondary metabolites, are rich in plant-based foods and beverages and are valued for their antioxidant and physicochemical properties and role in preventing diseases such as Alzheimer's, atherosclerosis, and cancer (Valko et al., 2007).

Free radicals are very unstable and reactive substances that are either created by the body naturally during regular metabolism or are brought in by environmental factors such as pollution, carbon monoxide, sulfur dioxide, etc. (Phaniendra et al., 2015). It leads to modification in the structure and causes damage to the DNA, cell, or tissue, which results in carcinogenesis, cardiovascular disease, Parkinson's disease, Alzheimer's disease, neurodegenerative diseases, etc. (Hajam et al., 2022; Kumar et al., 2012). Identification of the medicinal plants, which is necessary for medicinal activity and drug manufacture, depends critically on the qualitative analysis (Chitravadivu et al., 2009). Antioxidants are the free radical scavengers, which are capable of inhibiting the oxidation of the free radicals and other molecules (Frankel, 2012).

The *Clerodendrum infortunatum* (*C. infortunatum*) is found across the world's tropical and subtropical climates, especially in Nepal and India. This plant species belongs to the family Lamiaceae and is known for its multiple ethno-medicinal uses (Nandi & Mawkhlieng, 2016). Kumari and Singh reported that the ethanolic leaf and flower extracts of *C. infortunatum* collected from Bhagalpur, Bihar, India, contained various phytochemicals, including phenolics, terpenoids, flavonoids, and glycosides, and multiple assays further confirmed that flower extracts exhibited stronger antioxidant activity than both leaf extracts and the standard (BHT) reference (Kumari & Singh, 2025). Methanolic extracts of *C. infortunatum*, especially from the leaves, demonstrated substantial antioxidant activity, suggesting its potential as a treatment for neurodegenerative illnesses by reducing oxidative stress and cognitive dysfunction (Verma et al., 2025). Gallic acid was identified by phytochemical analysis as the primary source of the antioxidant action of the methanolic leaf extract of *C. infortunatum* (Kaveri et al., 2024). Due to its high content of secondary metabolites and strong antioxidant activity, the methanolic leaf extract of *C. infortunatum* helps cells reduce oxidative stress (Sharma & Thakur, 2022).



Figure 1: *Clerodendrum infortunatum* plant captured through mobile camera in its natural habitat at Biratnagar, Morang, Nepal

Free radicals such as hydroxyl (HO^\bullet), superoxide ($\text{O}_2^{\bullet-}$), trichloromethyl (CCl_3^\bullet), nitric oxide (NO^\bullet) and peroxy (ROO^\bullet) are produced by the metabolism of living things, but non-radical derivatives like hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2) can also be produced by foods and

biological systems (Halliwell et al., 1995). The hydroxyl radical is an extremely reactive free radical with a rate constant of 10^9 - 10^{10} $M^{-1}s^{-1}$. It strongly combines with inorganic and organic molecules involving proteins, DNA, carbohydrates, and lipids, and inflicts greater cellular damage than any other ROS (Halliwell, 1990). DPPH is a synthetic radical that is rather stable in comparison to the extremely reactive hydroxyl and superoxide species (Heim et al., 2002). The plant is chosen, as local healers are using it for medicinal purposes, and no prior research has been done on its flower extracts from the Morang area, although the biochemical features of *C. infortunatum* have been examined in earlier research. The main aim of this study was to investigate the phytochemical constituents in different parts (root, stem, leaves, and flower) of *C. infortunatum* and to assess the antioxidant activity of its flower extracts from the Morang district of Nepal.

2.0 Materials and Methods

2.1 Chemicals

Every chemical that had been used was of laboratory standards. For phytochemical screening, the chemical reagents used were Molisch's reagent, conc. HCl, conc. H_2SO_4 , ferric chloride, NaOH, Wagner's reagent, chloroform, glacial acetic acid, NaOH, ammonia solution. For antioxidant activities analysis, DMSO, ascorbic acid, EDTA, Ferrous ammonium sulphate, TCA, ammonium acetate, and acetone were procured.

2.2 Preparation of plant extract

Plant extracts were prepared through the maceration method (Bitwell et al., 2023). The different parts (stem, leaves, root, and flower) of the plant were collected from the remote area of Biratnagar, Morang, Nepal, in May 2024. The image of the plant species is depicted in Fig.1. Collected plant parts were carefully separated, washed, dried in the shade, and powdered in a mechanical grinder. Each sample of 20 gm powder was taken and soaked for 48 hours in 100 ml of ethanol and methanol separately. Whatman filter paper 1 was used to filter the extracts. A water bath set at $55^\circ C$ was used to concentrate the filtrates until all the solvent evaporated. The semi-solid extracts obtained called crude drug were kept at $4^\circ C$ for later usage.

2.3 Phytochemical screening

Plant extracts were dissolved in 70% ethanol and methanol separately to prepare 1 mg/ml concentration of extract solution for the qualitative test of secondary metabolites. Each test was conducted twice. The tests for phytochemical screening of plant extracts were done following different literature, as tabulated in the third column of Table 1. The results are presented in Table 2 of Section 3.1.

Table 1: Tests for phytochemical screening of plant extracts.

S. No.	Phytochemicals	Tests	Observation	References
1	Carbohydrate	2 ml of plant extract + 2 drops of Molisch's reagent + 2 drops of conc. H_2SO_4	Purple hue indicating the presence of carbohydrate.	(Auwal et al., 2014)
2	Tannin	1 ml plant extract + 1ml Ferric chloride (10%)	Dark blue or greenish black color indicating the presence of tannin.	(Auwal et al., 2014)

3	Saponin	2 ml of plant extract + 2 ml of distilled water + shaken vigorously	Foam production indicating the presence of saponin.	(Auwal et al., 2014)
4	Flavonoid	2 ml of plant extract + 1 ml of 2N NaOH	Yellow hue indicating the presence of flavonoid.	(Ali et al., 2018)
5	Alkaloid	2 ml of plant extract + 2 ml of Wagner's reagent	Dark or reddish brown indicating the presence of alkaloid.	(Alemu et al., 2024)
6	Quinone	1 ml plant extract + 1 ml of conc. H ₂ SO ₄	Red hue indicating the presence of quinone.	(Ali et al., 2018)
7	Glycoside	2 ml of plant extract + 3 ml of CHCl ₃ + 3 drops of NH ₃ solution (10%)	Pink hue indicating the presence of glycoside.	(Ali et al., 2018)
8	Cardiac glycoside	0.5 ml of plant extract + 2 ml of glacial CH ₃ COOH + few drops of 5% FeCl ₃ + 2 drops of conc. H ₂ SO ₄	A blue layer formed at the interface indicating the presence of cardiac glycoside.	(Alemu et al., 2024)
9	Terpenoid	0.5 ml of plant extract + 2 ml of CHCl ₃ + few drops of conc. H ₂ SO ₄	Reddish-brown color formed at the interface, indicating the presence of terpenoid.	(Alemu et al., 2024)
10	Phenol	1 ml of plant extract + 2 ml of distilled water + few drops of 10% FeCl ₃	Blue green or black hue indicating the presence of phenol.	(Alemu et al., 2024)
11	Coumarin	1 ml of plant extract + 1 ml of 10% NaOH solution	Yellow hue indicating the presence of coumarin.	(Ali et al., 2018)
12	Steroid	1 ml of plant extract + 1 ml of CHCl ₃ + few drops of conc. H ₂ SO ₄	Brown ring indicating the presence of steroid.	(Auwal et al., 2014)
13	Phlobatannin	1 ml of plant extract + few drops of 2% HCl	Red precipitate indicating the presence of phlobatannin.	(Auwal et al., 2014)
14	Anthroquinone	1 ml of plant extract + few drops of 10% NH ₃ solution	Pink precipitate indicating the presence of anthraquinone.	(Batool et al., 2019)

2.4 Antioxidant activity

2.4.1 Preparation of different concentrations of flower extract

Following the dilution process, a stock solution of floral extract was prepared. 10mg of crude drug was dissolved in 1ml of ethanol and methanol separately to prepare the stock solution of 10,000µg/ml. Using the dilution formula, $C_1V_1=C_2V_2$, the different concentrations (1000, 750, 500, 250µg/ml) of floral extract were obtained, where C_1 = stock concentration (µg/ml), V_1 = volume to be taken from stock solution (ml), C_2 = desired concentration (µg/ml) and V_2 = final volume upon dilution (ml).

2.4.2 Preparation of stock solution to generate Free Hydroxyl Radical (OH•)

For the generation of free hydroxyl radicals, different stock solutions had to be prepared (Klein et al., 1981). Iron EDTA solution was prepared by mixing 0.26 gm of EDTA, 0.13gm $FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$ in 100 ml of distilled water. 1.17gm of EDTA was mixed in 100 ml of distilled water to prepare the EDTA solution. The TCA solution was prepared by mixing 110.132gm of TCA into 100 ml of distilled water. Nash reagent was prepared by mixing 7.5gm of $NH_4CH_3CO_2$, 0.5ml of glacial acetic acid and 0.2ml of C_3H_6O in 100 ml of distilled water.

2.4.3 Scavenging of hydroxyl radical (OH•)

The antioxidant activity of *C. infortunatum* flowers was assessed using the hydroxyl free radical assay, with a few minor modifications (Mulla et al., 2010). To start the reaction, the different concentrations, i.e., 250, 500, 750, and 1000µg/ml of the flower extract called samples were taken in different Eppendorf tubes. Each Eppendorf tube containing a sample was treated with EDTA solution (0.5 ml), iron EDTA solution (1 ml), DMSO (1 ml), and ascorbic acid (0.5 ml). In a water bath, these tubes were securely sealed and placed in them at 80-90°C for 15 minutes. To terminate the reaction, 1ml of ice-cold TCA was added to each sample tube, and the tubes were then left for five minutes. The reaction resulted in formaldehyde. The amount of formaldehyde formed is directly proportional to the number of hydroxyl free radicals. As formaldehyde is colorless, its intensity cannot be measured directly by UV-Vis spectrophotometer. To this, Nash reagent (3ml) was added, and this reaction mixture was set aside for 15 minutes for color development. For the control, solvent (ethanol and methanol separately) and OH• radical (prepared solution to generate hydroxyl radical) were taken without plant extract. Using UV-Vis spectrophotometer, the absorbance value was measured for both the sample and control at 412 nm in triplicate. The percentage of inhibition of free radicals was calculated using the absorbance data of the sample and control; $\% \text{ Inhibition} = \frac{(A_C - A_S) \times 100}{A_C}$ where A_C = Absorbance of control (OH• radical in solvent) and A_S = Absorbance of sample (plant extracts).

The IC₅₀ value was determined by plotting the graph between the percentage of inhibition of hydroxyl free radical and concentrations.

2.5 Statistical analysis

Experiments were conducted in triplicate, and results (inhibition) are presented as mean \pm standard deviation. Statistical analysis was performed using a one-tailed t-test, with $p < 0.05$ considered statistically significant.

3.0 Results

3.1 Phytochemical screening

The different bioactive compounds in root, stem, leaves and flowers in ethanol and methanol extract have been detected using various tests described in Table 1 of the section 2.3. The findings of the phytochemical screening of floral metabolites are shown in Table 2.

Table 2: Phytochemical screening of *C. infortunatum* flower

Plant metabolites	Solvent		Plant metabolites	Solvent	
	Ethanol	Methanol		Ethanol	Methanol
Carbohydrate	+	+	Cardiac glycoside	+	+
Tannin	+	+	Terpenoid	+	+
Saponin	+	+	Phenol	+	+
Flavonoid	+	+	Coumarin	+	+
Alkaloid	+	+	Steroid	+	+
Quinone	+	+	Phlobatannin	-	-
Glycoside	-	-	Anthraquinone	-	-

The qualitative tests indicate the presence of carbohydrates, flavonoids, alkaloids, terpenoids, coumarin, and steroids in all parts of the extracts of *C. infortunatum*. Tannins and saponins were absent in the methanolic root extract but present in other extracts. Quinone was detected only in the flower extract, while glycosides and anthraquinones were absent in all parts. Phlobatannins were detected only in the methanol leaf extract. Cardiac glycosides were absent in the ethanol leaf extract but present in other parts. Phenols were absent from the ethanol root extract but were found in the stem, leaf, and flower.

3.2 Antioxidant Scavenging Activity

In antioxidant scavenging activity analysis, hydroxyl free radical inhibition was analyzed in the methanol and ethanol extracts of flower of *C. infortunatum*. The antioxidant activity was expressed in terms of % inhibition. It was found that the % inhibition of hydroxyl free radical in the methanol extract is higher than in the ethanol extract. The observations for hydroxyl free radical inhibition are depicted in Table 3 and Fig. 2 for ethanol extract, and Table 4 and Fig. 3 for methanol extract.

Table 3: Result of OH• radical detoxification by the flower of *C. infortunatum* in ethanol solvent. The % inhibition values contain mean \pm standard deviation.

Sample	Concentration ($\mu\text{g/ml}$)	Abs 1	Abs 2	Abs 3	Abs Mean	% Inhibition \pm sd
Control	--	0.3185	0.3191	0.3197	0.3191	--
1	250	0.3080	0.3084	0.3082	0.3082	3.41 \pm 1.21
2	500	0.2400	0.2409	0.2406	0.2405	24.63 \pm 3.46
3	750	0.2095	0.2106	0.2114	0.2105	34.05 \pm 3.55
4	1000	0.1430	0.1429	0.1437	0.1432	55.12 \pm 1.14

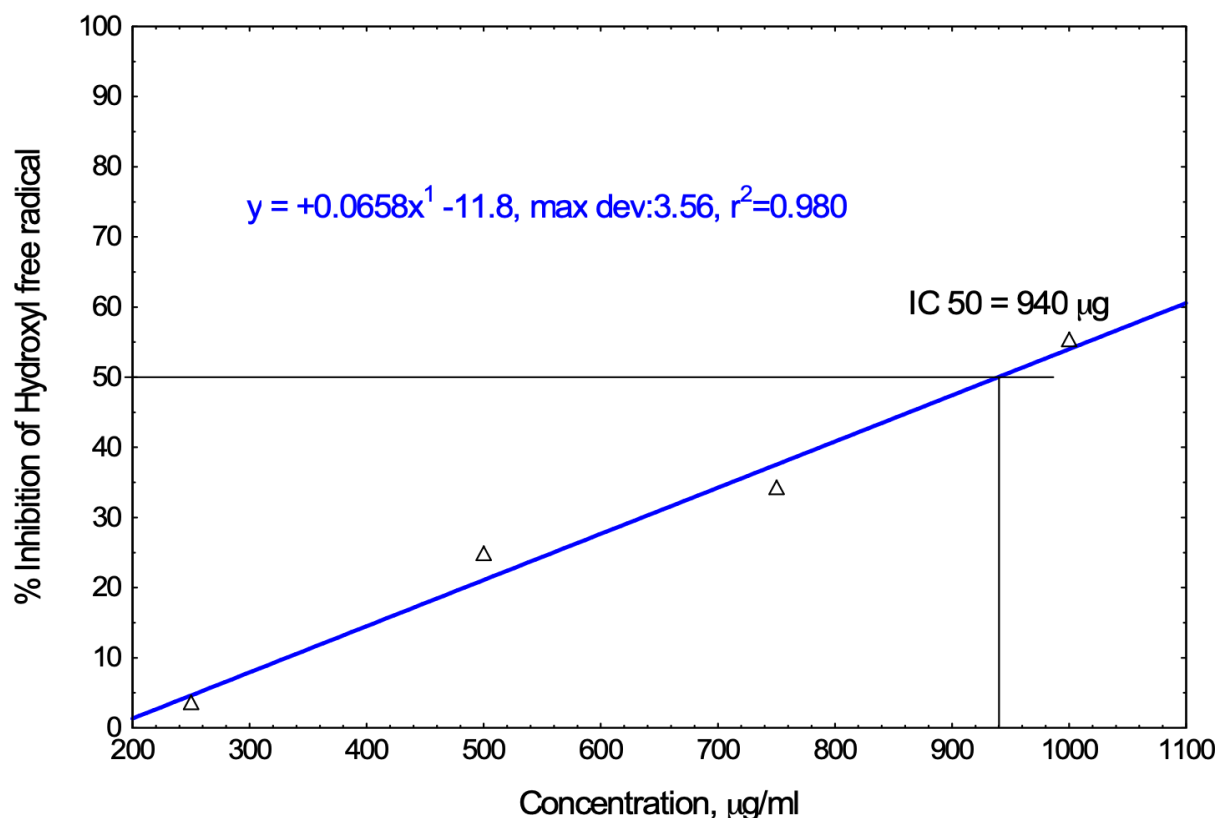


Figure 2: OH• radical detoxification by flower of *C. infortunatum* in ethanol solvent.

Table 4: Result of OH• radical detoxification by flower of *C. infortunatum* in methanol solvent.

Sample	Concentration (µg/ml)	Abs 1	Abs 2	Abs 3	Abs Mean	% Inhibition ± sd
Control	--	0.9963	0.9969	0.9969	0.9967	--
1	250	0.856	0.848	0.843	0.849	14.81± 1.78
2	500	0.555	0.559	0.554	0.556	44.21± 5.61
3	750	0.4525	0.4531	0.4528	0.4528	54.57± 5.93
4	1000	0.1533	0.1538	0.1537	0.1536	84.58± 2.04

The extracts' capacity to scavenge hydroxyl radicals differs with the extraction solvent and its concentration. In this study, the hydroxyl scavenging activities were enhanced with higher concentrations of the plant extract. The OH• scavenging activities of the floral extract in ethanol were found to be 3.41±1.21%, 24.63±3.46%, 34.05±3.55% and 55.12±1.14% at concentrations of 250, 500, 750, and 1000µg/ml, respectively. Methanol extract showed 14.81±1.78%, 44.21±5.61%, 54.57±5.93% and 84.58±2.04% of OH• scavenging activity at 250, 500, 750, and 1000µg/ml, respectively. Ethanolic control (ethanol +OH•) and methanolic control (methanol +

OH•) showed absorbance values of 0.3191 and 0.9967, respectively, with no inhibition towards OH• by either, as they do not contain any antioxidants or plant extract.

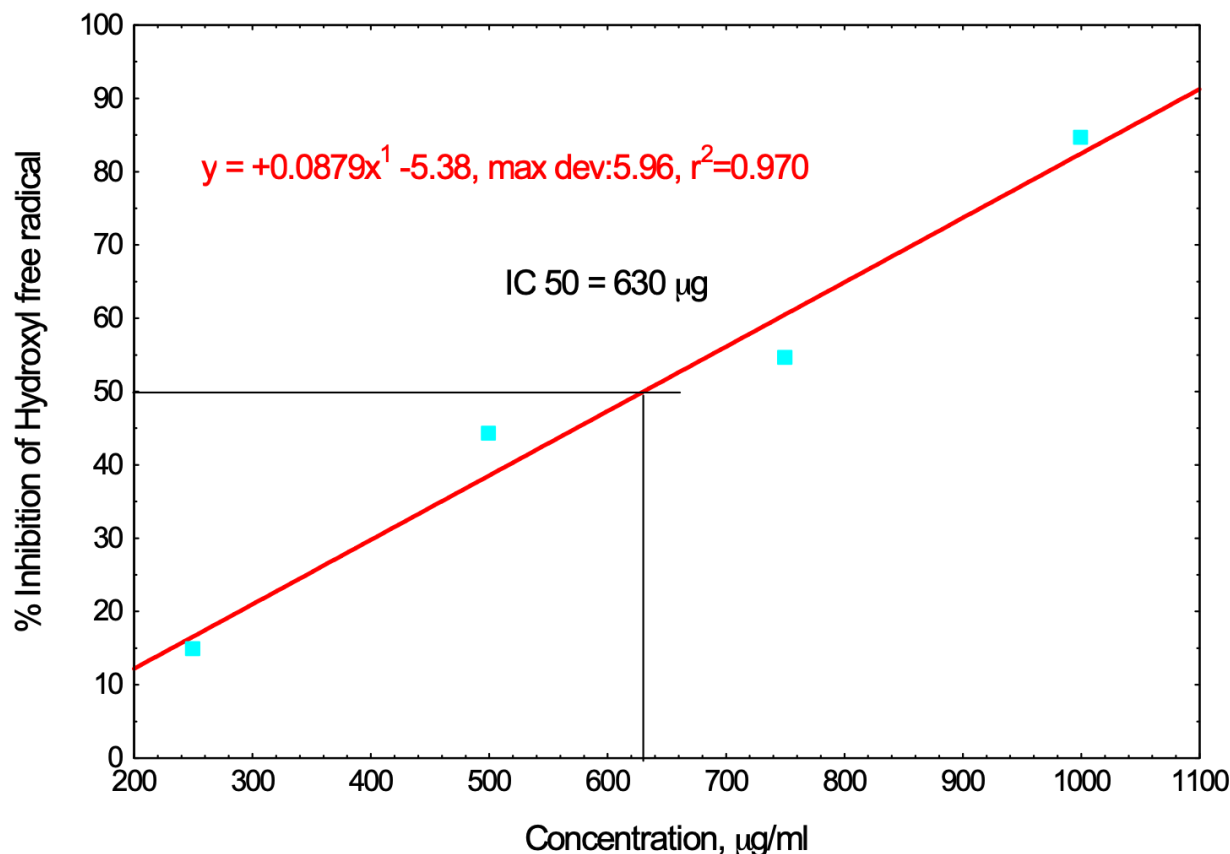


Figure 3: OH• radical detoxification by the flower of *C. infortunatum* in methanol solvent.

The linear regression equation $y = +0.0658x^1 - 11.8$ from the concentration-inhibition curve for ethanolic extract (Fig. 2) and $y = +0.0879x^1 - 5.38$ for methanolic extract (Fig. 3) indicated the percentage inhibition increased with an increase in concentration. The high value of the coefficient of determination for ethanolic extract ($r^2 = 0.980$) and methanolic extract ($r^2 = 0.970$) indicated the strong linear relationship between concentration and inhibition. IC₅₀ value of the ethanolic and methanolic floral extract was 940 µg/ml and 630 µg/ml respectively, which were determined from the curves shown in Figs. 2 and 3.

4.0 Discussion

4.1 Phytochemical screening

The phytochemical screening of *C. infortunatum*'s stem, root, leaves, and flower revealed positive results for alkaloids, carbohydrates, saponins, terpenoids, coumarins, and steroids in all plant sections examined. Similar work carried out by Kumari and Singh showed similar results by flower extract, except for glycoside and anthraquinone (Kumari & Singh, 2025). Leaf extract showed the presence of flavonoid, coumarin, glycoside, saponin, quinone, meanwhile negative for alkaloid (Sharma & Thakur, 2022). Minor differences in the results may be due to different factors like chemical quality, laboratory setup, extraction techniques, environmental factors, collecting seasons, solvent, temperature, time, etc. (Changade et al., 2022; Helen & Latha, 2014; Yan et al., 2022). Reddy & Reddy explained the well-known medicinal use of alkaloids as anesthetics, cardiac protectants, anti-diabetics, anti-cancer, immune modulatory, anti-oxidant, anti-microbial, and anti-toxic medications (Heinrich et al., 2021; Reddy & Reddy, 2015). Ullah et al. reported the potential

uses of flavonoids in medical chemistry as anticancer, anti-inflammatory, antioxidant, antimicrobial, antimalarial, antiangiogenic, antitumor, neuroprotective and anti-proliferative agents (Ullah, et al., 2020). Plant steroids have the perfect structural chemistry to reduce inflammation (Patel & Savjani, 2015). Cardiac glycosides are one of the most often utilized medication types for treating arrhythmias and heart failure (Smith et al., 2019). Quinone exposure in industries like chemical, tanning, textiles, dye, and cosmetics can cause skin dermatitis and eye irritation with acute exposure. Prolonged inhalation may lead to eye abnormalities, while extended skin contact can result in skin ulcers (Devi & Mehendale, 2014).

4.2 Antioxidant Scavenging Activity

Antioxidant activities of the flower of *C. infortunatum* have been carried out by hydroxyl free radical assay. Free radical has been prepared by following the ascorbic-iron-EDTA model (Mulla et al., 2009). Using UV-Vis spectrophotometer, the degree of yellowness that developed was determined at 412 nm, which confirms the presence of hydroxyl radical, similar to the literature (Dawidowicz & Olszowy, 2013).

Table 5: % inhibition of hydroxyl radical using ascorbic acid (Utami et al., 2021).

Ascorbic Acid ($\mu\text{g/ml}$)	125	250	500	1000
% Inhibition	44.44	50	61.11	81.97

The data of % inhibition vs concentrations of ascorbic acid tabulated in Table 5 is plotted with EasyPlot software (Fig. 4), and the % inhibition of 750 $\mu\text{g/ml}$ was estimated to be 71.3% from the straight-line where the coefficient of determination (r^2) fits to be 1. These results of the inhibition of ascorbic acid were compared with our experimental data.

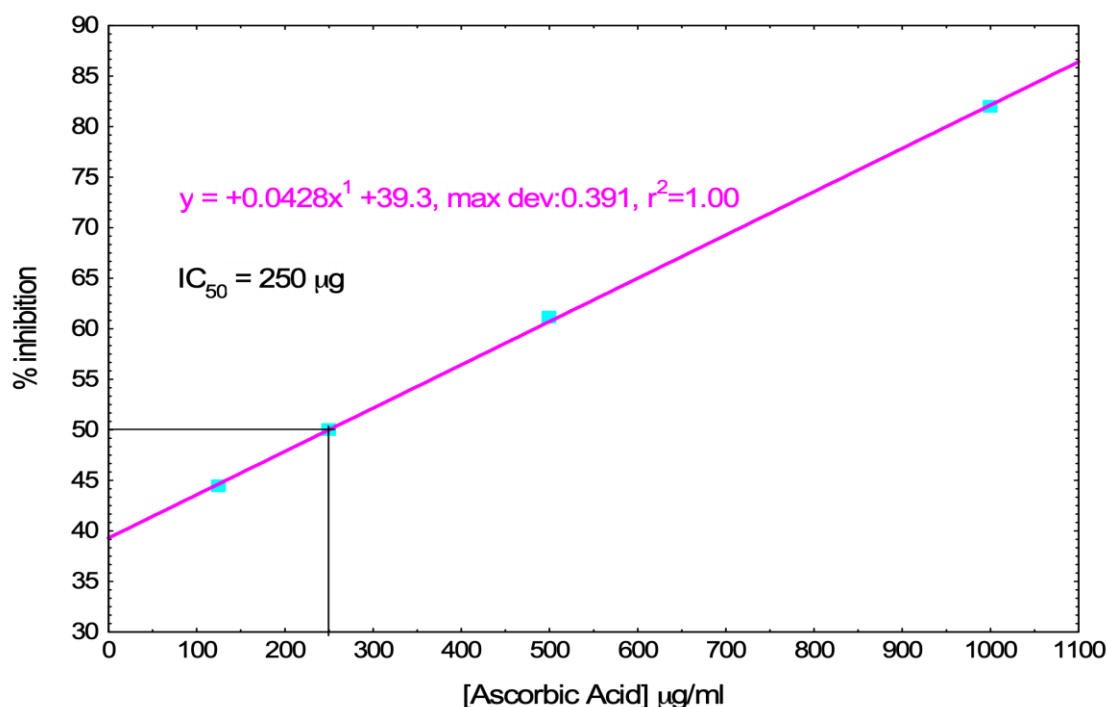


Figure 4: Variation of % inhibition of hydroxyl free radical through ascorbic acid of various concentrations

From Fig. 5, it is seen that the % inhibition of methanol extract at 1000 $\mu\text{g/ml}$ shows better inhibition characteristics than ascorbic acid. The level of significance (p-value) between the mean

standard reference (ascorbic acid) and the mean sample was estimated with one tailed t-test and was found to be less than 0.05, as tabulated in Table 6. It means that the % inhibition of the sample (ethanol extract) is less than that of the standard reference (ascorbic acid). In the methanol extract, the p-value was less than 0.05 except at 1000 µg/ml. It suggested that the % inhibition of methanol extract at 1000 µg/ml is higher than that of ascorbic acid.

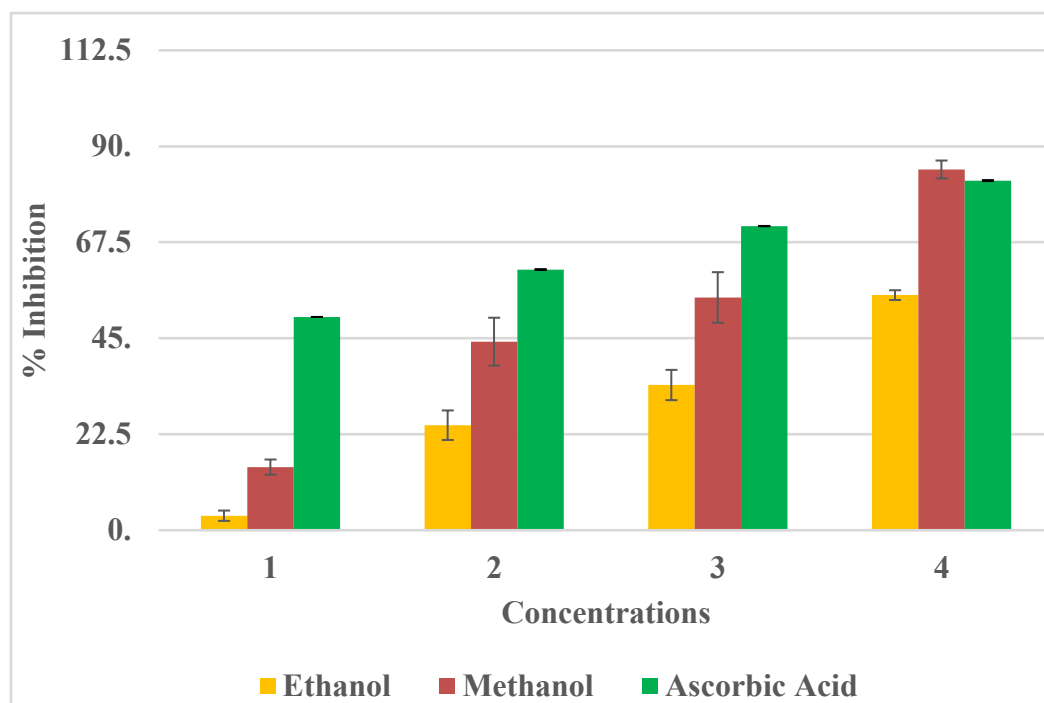


Figure 5: Comparative bar graph of % inhibition of ethanol and methanol extract with ascorbic acid.

According to Kumari and Singh, *Clerodendrum* flower (CF) showed the highest free radical scavenging activity in the DPPH assay, followed by *Clerodendrum* leaf (CL) and BHT (standard). Its IC₅₀ value was the lowest at 482.014 µg/ml, compared to CL and BHT, with BHT having the highest IC₅₀ at 581.927 µg/ml, confirming CF as the most effective antioxidant (Kumari & Singh, 2025). Akhil et al. reported the notable anticancer effects against HPV-positive cervical

Table 6: The % inhibition values (mean ± standard deviation) of ethanol extract, methanol extract, and ascorbic acid (Utami et al., 2021) with the level of significance (p value).

Concentration µg/ml	Ascorbic Acid	Methanol Extract		Ethanol Extract	
	% inhibition	% inhibition	p value	% inhibition	p value
250	50 ± 0.001	14.81 ± 1.78	0.000425	3.41 ± 1.21	0.000112
500	61.11 ± 0.012	44.21 ± 5.61	0.017341	24.63 ± 3.46	0.001482
750	71.3 ± 0.033	54.57 ± 5.93	0.019504	34.05 ± 3.55	0.001479
1000	81.97 ± 0.11	84.58 ± 2.04	0.074937	55.12 ± 1.14	0.000245

cancer by the root extract of *C. infortunatum*, providing scientific support for its traditional application by healers in treating this disease (Akhil et al., 2023). The alcoholic flower extract of

C. paniculatum demonstrated an IC₅₀ value of 2362.71 µg/ml and 62.28 µg/ml in the ABTS and DPPH assay, respectively, whereas the standard ascorbic acid demonstrated 941.09 µg/ml and 48.74 µg/ml in the ABTS and DPPH assay, respectively. In the DPPH assay, the alcoholic extract showed significant antioxidant activity, with an IC₅₀ value of 62.28 µg/mL, in contrast to the standard ascorbic acid, which had an IC₅₀ value of 48.74 µg/ml (Kopilakkal et al., 2021). Zhou et al. performed antioxidant activity on leaf extract of *C. cyrtophyllum* against hydroxyl radicals and reported the scavenging activity in increasing order: Remaining fraction RF (3.41 mg/ml) < ethanolic crude extract ECE (1.99 mg/ml) < ethyl acetate fraction EAF (1.07 mg/ml) < petroleum ether fraction PEF (0.90 mg/ml) < dichloromethane fraction DMF (0.84 mg/ml) n-butyl alcohol fraction BAF (0.65 mg/ml) < Vitamin-C VC (0.17 mg/ml) (Zhou et al., 2020). The IC₅₀ values of *Clerodendrum inerme* stem extracts exhibited the antioxidant activity of the tested samples in the following decreasing order: BHT (20.5) > methanol (24.1) > petroleum ether (34.1) > ethyl acetate (46) > petroleum ether: chloroform (46.4) > ethyl acetate: chloroform (54.9) > ethanol (67.7) > chloroform (81.2) mg/ml (Khan et al., 2013). The present study found the IC₅₀ value of methanolic flower extract as 630 µg/ml, carrying more antioxidant property than ethanolic extract with 940 µg/ml IC₅₀ value, which is supported by polarity dependent that results in high extraction yield, free radical scavenging, and antioxidant property (Nawaz et al., 2020). Antioxidants prevent the harmful impact of free radicals, which contribute to the onset of numerous diseases and the aging process (Soni & Sosa, 2013). It is important to scavenge the OH[•] free radical as it has high reactivity properties, which allow it to interact with a variety of compounds present in cellular systems, including lipids, amino acids, sugars, and nucleotides (Wang et al., 2008).

5. Conclusion

It is concluded from the study that the extracts of *C. infortunatum* showed notable biochemical characteristics like alkaloids, phenols, flavonoids, terpenoids, etc., including antioxidant capabilities. The high value of the coefficient of determination for ethanolic extract ($r^2=0.980$) and methanolic extract ($r^2=0.970$) indicated the strong linear relationship between concentration and inhibition. Methanolic flower extract possesses high free radical scavenging property, which is better than the reference taken as ascorbic acid at 1000 µg/ml and ethanolic flower extract, highlighting its novelty. Flower of *C. infortunatum* can be suggested as a valuable source of bioactive compounds. However, further studies involving the isolation and characterization of the active phytochemicals through bioassay-guided fractionation, followed by in-vivo validation, are required to fully substantiate their therapeutic potential.

References

- Akhil, B. S., Ravi, R. P., Lekshmi, A., Abeesh, P., Guruvayoorappan, C., Radhakrishnan, K. V., & Sujathan, K. (2023). *Exploring the Phytochemical Profile and Biological Activities of Clerodendrum infortunatum*. <https://doi.org/10.1021/acsomega.2c08080>
- Alemu, M., Lulekal, E., Asfaw, Z., Warkineh, B., Debella, A., Abebe, A., Degu, S., & Debebe, E. (2024). Antibacterial activity and phytochemical screening of traditional medicinal plants most preferred for treating infectious diseases in Habru District, North Wollo Zone, Amhara Region, Ethiopia. *PLOS ONE*, 19(3), e0300060. <https://doi.org/10.1371/journal.pone.0300060>
- Ali, S., Khan, M. R., Irfanullah, Sajid, M., & Zahra, Z. (2018). Phytochemical investigation and antimicrobial appraisal of *Parrotiopsis jacquemontiana* (Decne) Rehder. *BMC Complementary and Alternative Medicine*, 18, 43. <https://doi.org/10.1186/s12906-018-2114-z>
- Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts

- of *Acacia nilotica* (Thorn mimosa). *Veterinary Research Forum: An International Quarterly Journal*, 5(2), 95–100.
- Batool, R., Khan, M. R., Sajid, M., Ali, S., & Zahra, Z. (2019). Estimation of phytochemical constituents and in vitro antioxidant potencies of *Brachychiton populneus* (Schott & Endl.) R.Br. *BMC Chemistry*, 13(1), 32. <https://doi.org/10.1186/s13065-019-0549-z>
- Bitwell, C., Indra, S. S., Luke, C., & Kakoma, M. K. (2023). A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. *Scientific African*, 19, e01585. <https://doi.org/10.1016/j.sciaf.2023.e01585>
- Changade, J. V., Thomas, A., Tanvi, H. A., Ghangale, N., Shirkande, A., & Sable, P. D. (2022). Phytochemical analysis of *Clerodendron infortunatum* Linn. Leaves in various seasons by different solvent and extraction techniques. *International Journal of Ayurvedic Medicine*, 13(3), 744–748. <https://doi.org/10.47552/ijam.v13i3.2683>
- Chitravadivu, C., Manian, S., & Kalaichelvi, K. (2009). *Qualitative Analysis of Selected Medicinal Plants, Tamilnadu, India*.
- Dawidowicz, A. L., & Olszowy, M. (2013). The importance of solvent type in estimating antioxidant properties of phenolic compounds by ABTS assay. *European Food Research and Technology*, 236(6), 1099–1105. <https://doi.org/10.1007/s00217-013-1982-1>
- Devi, S. S., & Mehendale, H. M. (2014). Quinone. In *Encyclopedia of Toxicology* (pp. 26–28). Elsevier. <https://doi.org/10.1016/B978-0-12-386454-3.00350-X>
- Frankel, E. N. (2012). Chapter 9—Antioxidants. In E. N. Frankel (Ed.), *Lipid Oxidation (Second Edition)* (pp. 209–258). Woodhead Publishing. <https://doi.org/10.1533/9780857097927.209>
- Hajam, Y. A., Rani, R., Ganie, S. Y., Sheikh, T. A., Javaid, D., Qadri, S. S., Pramodh, S., Alsulimani, A., Alkhanani, M. F., Harakeh, S., Hussain, A., Haque, S., & Reshi, M. S. (2022). Oxidative Stress in Human Pathology and Aging: Molecular Mechanisms and Perspectives. *Cells*, 11(3), 552. <https://doi.org/10.3390/cells11030552>
- Halliwell, B. (1990). How to Characterize a Biological Antioxidant. *Free Radical Research Communications*, 9(1), 1–32. <https://doi.org/10.3109/10715769009148569>
- Halliwell, B., Murcia, M. A., Chirico, S., & Aruoma, O. I. (1995). Free radicals and antioxidants in food and in vivo: What they do and how they work. *Critical Reviews in Food Science and Nutrition*, 35(1–2), 7–20. <https://doi.org/10.1080/10408399509527682>
- Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *The Journal of Nutritional Biochemistry*, 13(10), 572–584. [https://doi.org/10.1016/S0955-2863\(02\)00208-5](https://doi.org/10.1016/S0955-2863(02)00208-5)
- Heinrich, M., Mah, J., & Amirkia, V. (2021). Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity—An Update and Forward Look. *Molecules*, 26(7), 1836. <https://doi.org/10.3390/molecules26071836>
- Helen, L. R., & Latha, M. J. and M. S. (2014). Phytochemical and Antioxidant Screening of Various Extracts of The Root Bark and Leaf of *Clerodendrum Infortunatum* Linn. *Pharmacophore*, 5(2–2014), 343–351. <https://doi.org/10.51847/gPRhFE1>
- Israyilova, A., Peykova, T. Z., Kittleson, B., Sprowl, P. C., Mohammed, T. O., & Quave, C. L. (2025). From Plant to Patient: A Historical Perspective and Review of Selected Medicinal Plants in Dermatology. *JID Innovations*, 5(1), 100321. <https://doi.org/10.1016/j.xjidi.2024.100321>

- Kaveri, S. J., Dhiya, P., Aiswarya, M. S., Anjali, S., Sneha, S., & Rejithamol, R. (2024). Extraction, identification, and phytochemical analysis of the leaves of the methanolic extract of *Clerodendrum Infortunatum* Linn. *AIP Conference Proceedings*, 3122(1), 020007. <https://doi.org/10.1063/5.0216256>
- Khan, S. A., Rasool, N., Riaz, M., Nadeem, R., Rashid, U., Rizwan, K., Zubair, M., Bukhari, I. H., & Gulzar, T. (2013). Evaluation of Antioxidant and Cytotoxicity Studies of *Clerodendrum inerme*. *Asian Journal of Chemistry*, 25(13), 7457–7462. <https://doi.org/10.14233/ajchem.2013.14831>
- Klein, S. M., Cohen, G., & Cederbaum, A. I. (1981). Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. *Biochemistry*, 20(21), 6006–6012. <https://doi.org/10.1021/bi00524a013>
- Kopilakkal, R., Chanda, K., & Balamurali, M. M. (2021). Hepatoprotective and Antioxidant Capacity of *Clerodendrum paniculatum* Flower Extracts against Carbon Tetrachloride-Induced Hepatotoxicity in Rats. *ACS Omega*, 6(40), 26489–26498. <https://doi.org/10.1021/acsomega.1c03722>
- Kumar, H., Lim, H.-W., More, S. V., Kim, B.-W., Koppula, S., Kim, I. S., & Choi, D.-K. (2012). The Role of Free Radicals in the Aging Brain and Parkinson's Disease: Convergence and Parallelism. *International Journal of Molecular Sciences*, 13(8), 10478–10504. <https://doi.org/10.3390/ijms130810478>
- Kumari, P., & Singh, M. (2025). Phytochemical Screening, Antifungal and Antioxidant Activities of Ethanolic Leaf and Flower Extracts of *Clerodendrum Infortunatum* from Bhagalpur Region. *Journal of Scientific Research*, 17, 341–354. <https://doi.org/10.3329/jsr.v17i1.74922>
- Mulla, W. A., Kuchekar, S. B., Thorat, V. S., Chopade, A. R., & Kuchekar, B. S. (2010). Antioxidant, Antinociceptive Anti-inflammatory Activities of Ethanolic Extract of Leaves of *Alocasia indica* (Schott.). *Journal of Young Pharmacists*, 2(2), 137–143. <https://doi.org/10.4103/0975-1483.63152>
- Mulla, W. A., Salunkhe, V. R., Kuchekar, S. B., & Qureshi, M. N. (2009). Free Radical Scavenging Activity of Leaves of *Alocasia indica* (Linn). *Indian Journal of Pharmaceutical Sciences*, 71(3), 303–307. <https://doi.org/10.4103/0250-474X.56036>
- Nandi, S., & Mawkhlieng, L. (2016). *Clerodendrum viscosum*: Traditional uses, pharmacological activities and phytochemical constituents. *Natural Product Research*, 30(5), 497–506. <https://doi.org/10.1080/14786419.2015.1025229>
- Nawaz, H., Shad, M., Rehman, N., Andaleeb, H., & Ullah, N. (2020). Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (*Phaseolus vulgaris*) seeds. *Brazilian Journal of Pharmaceutical Sciences*, 56. <https://doi.org/10.1590/s2175-97902019000417129>
- Patel, S. S., & Savjani, J. K. (2015). Systematic review of plant steroids as potential antiinflammatory agents: Current status and future perspectives. *The Journal of Phytopharmacology*, 4(2), 121–125. <https://doi.org/10.31254/phyto.2015.4212>
- Phaniendra, A., Jestadi, D. B., & Periyasamy, L. (2015). Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian Journal of Clinical Biochemistry*, 30(1), 11–26. <https://doi.org/10.1007/s12291-014-0446-0>
- Rabizadeh, F., Mirian, M. S., Doosti, R., Kiani-Anbouhi, R., & Eftekhari, E. (2022). Phytochemical Classification of Medicinal Plants Used in the Treatment of Kidney Disease

- Based on Traditional Persian Medicine. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2022, 8022599. <https://doi.org/10.1155/2022/8022599>
- Reddy, N. M., & Reddy, R. (2015). *Tinospora cordifolia* Chemical Constituents and Medicinal Properties: A Review. <https://www.semanticscholar.org/paper/Tinospora-cordifolia-Chemical-Constituents-and-%3A-A-Reddy-Reddy/ae1f772faf1da791902657dab1eb7413978bbb54>
- Sharma, K. R., & Thakur, K. (2022). Determination of Phenolic and Flavonoid Content, Antioxidant and α -Amylase Inhibitory Activity of Leaf and Flower Extracts of *Clerodendrum infortunatum* and *Hibiscus rosa sinensis* Growing in Bara Nepal. *Journal of Balkumari College*, 11(1), 20–26. <https://doi.org/10.3126/jbk.v11i1.53019>
- Smith, G. F., Figueiredo, E., & van Wyk, A. E. (2019). Chapter 9—Biocultural Significance and Toxicity. In G. F. Smith, E. Figueiredo, & A. E. van Wyk (Eds.), *Kalanchoe (Crassulaceae) in Southern Africa* (pp. 105–110). Academic Press. <https://doi.org/10.1016/B978-0-12-814007-9.00009-8>
- Soni, A., & Sosa, S. (2013). *Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts*.
- Thirumurugan, D., Cholarajan, A., Vijayakumar, S. S. S. R. and R., Thirumurugan, D., Cholarajan, A., & Vijayakumar, S. S. S. R. and R. (2018). An Introductory Chapter: Secondary Metabolites. In *Secondary Metabolites—Sources and Applications*. IntechOpen. <https://doi.org/10.5772/intechopen.79766>
- Ullah, A., Munir, S., Badshah, S. L., Khan, N., Ghani, L., Poulson, B. G., Emwas, A.-H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules*, 25(22), 5243. <https://doi.org/10.3390/molecules25225243>
- Utami, J., Wasiaturrahmah, Y., & Kania, D. (2021). Hydroxyl Radical Scavenging Activity of *Stachytarpheta jamaecensis* Root Extract using In Vitro Deoxyribose Degradation Assay. *Majalah Obat Tradisional*, 26, 103. <https://doi.org/10.22146/mot.61746>
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, 39(1), 44–84. <https://doi.org/10.1016/j.biocel.2006.07.001>
- Verma, N., Singh, S., Gupta, A., & Srivastava, A. K. (2025). Antioxidant, anti-inflammatory, and nootropic activities in flavonoid-rich fractions derived from *Clerodendrum infortunatum* leaf and root extracts: A comparative analysis. *Fitoterapia*, 186, 106842. <https://doi.org/10.1016/j.fitote.2025.106842>
- Wang, H., Gao, X. D., Zhou, G. C., Cai, L., & Yao, W. B. (2008). In vitro and in vivo antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. *Food Chemistry*, 106(3), 888–895. <https://doi.org/10.1016/j.foodchem.2007.05.068>
- Yan, K., Cheng, X.-J., Bian, G.-L., Gao, Y.-X., & Li, D.-Q. (2022). The Influence of Different Extraction Techniques on the Chemical Profile and Biological Properties of *Oroxylum indicum*: Multifunctional Aspects for Potential Pharmaceutical Applications. *Evidence-Based Complementary and Alternative Medicine: eCAM*, 2022, 8975320. <https://doi.org/10.1155/2022/8975320>
- Zhou, J., Yang, Q., Zhu, X., Lin, T., Hao, D., & Xu, J. (2020). Antioxidant activities of *Clerodendrum cyrtophyllum* Turcz leaf extracts and their major components. *PLOS ONE*, 15(6), e0234435. <https://doi.org/10.1371/journal.pone.0234435>