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Phytochemical Screening and Antioxidant Activity of Different Parts of Low Rattlebox (*Crotalaria pumila*)

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Abstract

This study investigated the phytochemical composition and antioxidant activity of the leaves, stems, seeds, and roots of *Crotalaria pumila* (Low Rattlebox). Methanolic extracts of the plant parts were subjected to standard qualitative phytochemical screening and antioxidant evaluation using the DPPH radical scavenging assay. The qualitative analysis revealed the presence of key bioactive compounds, including alkaloids, flavonoids, saponins, phenols, and quinones, with varying concentrations across plant parts. Alkaloids and saponins were abundant in the stem and seed, while phenols were prominent in the leaves. Antioxidant assessment showed that all extracts exhibited dose-dependent radical scavenging activity. The leaves recorded the highest antioxidant potential (IC_{50} = 25.81 µg/mL), closely comparable to vitamin C (IC_{50} = 21.95 µg/mL), followed by the root (44.67 µg/mL), stem (95.69 µg/mL), and seed (225.13 µg/mL). Statistical analysis using one-way ANOVA revealed significant differences ($p < 0.05$) among the plant parts. The strong antioxidant activity, particularly in the leaves, is attributed to the high content of phenolic and flavonoid compounds. The results suggest that *C. pumila* is a promising natural source of natural antioxidants and bioactive compounds with potential applications in pharmaceutical and nutraceutical formulations. Further research should focus on the isolation and structural characterization of the active phytochemicals in *Crotalaria pumila*. In vivo and clinical investigations are also recommended to validate its efficacy and safety for pharmaceutical and nutraceutical applications.

Keywords: *Crotalaria pumila*, Phytochemical Screening, Antioxidant Activity, DPPH Assay, IC_{50} value, Bioactive Compounds.

Introduction

Traditional herbal medicine is the most ancient form of medicine known to man, and plant-based substances account for more than half of all modern clinical drugs (Najmi *et al.,* 2022). In the pharmaceutical industry, natural products serve significant functions in drug formulation. Plants have historically served as a great source of drugs, and nearly all of the medications available today originated from plant-based substances either directly or indirectly (Rajput *et al.,* 2025). The World Health Organization (WHO), reported that about 80 % of the world's population relies on traditional or plant-based medications for primary healthcare. These may serve as the foundation of proficient traditional medicine systems and are said to provide great prospects for the development of novel drugs (Li *et al.,* 2020).

Most of the African communities rely on traditional medicines for the treatment of various ailments (Birhan *et al.,* 2011). This is necessary because of the medicinal properties that have been exhibited in herbal mixtures and decoctions, reflecting their effectiveness, affordability, and availability.

A medicinal plant is defined as any plant that contains substances known as phytochemicals in one or more of its organs that may be used to treat ailments or act as precursors for the synthesis of drugs (Najmi *et al.,* 2022). Phytochemicals are nonnutritive chemical components in plants. They occur during metabolic processes and have been shown possess various therapeutic properties which are helpful in the treatment or alleviation of symptoms of a variety of disease conditions (Opoku and Akoto, 2014). They are secondary metabolites in the leaves, stems, roots, or any part of the plants. Importantly, various researches have shown that they play an important role in human disease prevention and cure (Said & Abba*,* 2021). Studies have shown that most plant species possess phytochemical such as alkaloids, flavonoids, saponins, cardiac glycosides, steroids, tannins, terpenoids, phenolics, triterpenoids, anthraquinones, and so on (Adjouzem *et al.,* 2020). These phytochemicals are said to confer pharmacological properties like antimicrobial, antioxidant, anthelmintic, anti-inflammatory, anticancer, anti-diabetic, hypoglycemic, etc, on the plants (Alara *et al.,* 2019).

Crotalaria pumila, commonly known as Low Rattlebox, is a species of flowering plant belonging to the Fabaceae family. This plant is widely distributed in tropical and subtropical regions and has been traditionally used for various medicinal purposes. The leaves and stems of *Crotalaria pumila* are known to contain a variety of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which contribute to its medicinal properties (Martínez-Zaragoza *et al.,* 2023).

Phytochemicals, the naturally occurring compounds in plants, play a significant role in the prevention and treatment of various diseases. These compounds are responsible for the antioxidant properties of plants, which protect the body from oxidative stress and free radical damage. Oxidative stress is implicated in the pathogenesis of numerous chronic diseases, including cancer,

cardiovascular diseases, and neurodegenerative disorders (Ghasemzadeh *et al.,* 2018). The screening of phytochemicals and the extraction of antioxidants from plant materials are essential steps in identifying and utilizing plants for medicinal purposes. The leaves and stems of *Crotalaria pumila* have been reported to possess significant antioxidant activity, which may be attributed to the presence of phytochemicals such as flavonoids and phenolic acids. These compounds have been shown to scavenge free radicals, inhibit lipid peroxidation, and reduce oxidative damage to cells (Kumar and Pandey, 2015).

Given the increasing interest in natural antioxidants for health promotion and disease prevention, it is crucial to explore the phytochemical composition and antioxidant potential of *Crotalaria pumila*. This study aims to provide a comprehensive analysis of the phytochemicals present in the leaves and stems of *Crotalaria pumila* and to evaluate their antioxidant activity. Despite the growing interest in the medicinal properties of *Crotalaria pumila*, there is a limited understanding of the specific phytochemicals responsible for its antioxidant activity. Furthermore, there is a lack of standardized methods for the extraction and quantification of these compounds. The absence of detailed phytochemical and antioxidant profiles hinders the full utilization of *Crotalaria pumila* in pharmaceutical and nutraceutical applications.

The problem, therefore, is the insufficient knowledge of the phytochemical constituents and antioxidant potential of *Crotalaria pumila* leaves and stems. Addressing this gap is crucial for the development of effective natural antioxidant products and for advancing the use of this plant in traditional and modern medicine. Therefore, the aim of this Study is determining the relative phytochemical screening and antioxidants effect of leaves, stem, seed and root of *Crotalaria pumila* (low rattlebox).

MATERIALS AND METHODS

Sample Collection and Authentication

The research took place in Damaturu, the administrative centre of Yobe State in northeastern Nigeria. Geographically, the city lies between latitudes 11°39′30″ and 11°47′00″ North, and longitudes 11°54′00″ to 12°02′00″ East (Figure 1). Spanning an area of 2,366 square kilometres, Damaturu had a recorded population of 88,014 as of the 2006 census (NPC, 2006). It shares boundaries with Tarmuwa LGA to the north, Gujba LGA to the south, Fune LGA to the west, and Kaga LGA in neighbouring Borno State to the east. The climate is characterized by semi-arid savannah conditions, with average peak temperatures ranging from 38 to 42 °C. The area experiences extended dry seasons and significant desertification, leading to sandy landscapes during dry periods and muddy terrain during the rains (Gana & Sa'id, 2022).

The entire specimen of *Crotalaria pumila* (commonly known as low rattlebox) was gathered from Mal Matari in Damaturu, Yobe State. A certified taxonomist at the Herbarium Unit of the Department of Biological Sciences, Yobe State University, Damaturu, carried out its identification and authentication. For long-term reference, voucher specimens were archived in the university's herbarium under the accession number YSUH 122.

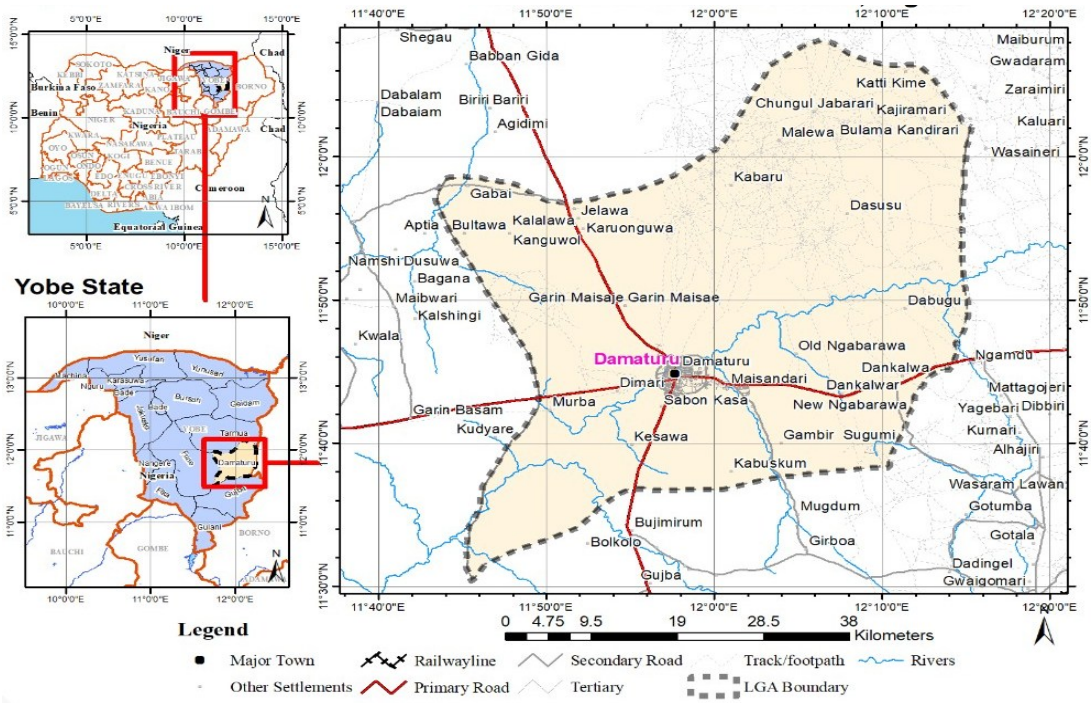


Figure 1: Map Showing Study Site of Plant Collection
Source: (Gana and Sa'id, 2022)

Preparation of Plant Extract

The collected samples were washed under running tap water to eliminate dust and other foreign particles and to dried at room temperature in the department laboratory and reduce to fine powder using kitchen blender (pulverized) to powder and store at room temperature until required for analysis.

Extraction

Methanolic extraction was performed by separately soaking 250 g of the powdered leaves, stems, seeds, and roots in 700 mL of methanol. The mixtures were agitated on a mechanical shaker for 24 hours at room temperature, after which the supernatants were filtered and the filtrates concentrated to dryness using a hot-air oven.

Preparation of Reagents

Sodium hydroxide solution (20 %)

Twenty grams (20 g) of sodium hydroxide pellets was dissolved in little amount of distilled water and then the volume was made up to 100 ml mark of a volumetric flask.

Ferric Chloride Solution (5 and 10 %)

The ferric chloride 1.95 and 2.5 g were each dissolved in a 250 ml of water distilled in a volumetric flask.

Hydrochloric acid (1 and 2%)

These solutions were diluted in 0.25 and 0.5 ml of concentrated HCI with water to a final volume of 100 ml to give that I and 2 % HCI.

Phytochemical Screening of the Extract

The methanol extract of *Crotalaria pumila* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituent present in the samples as outlined below:
The following standard procedures were employed:

1. **Alkaloids:** The presence of alkaloids was tested using Dragendorff's reagent. A few drops of the reagent were added to 1 mL of the extract, and the formation of a reddish-brown precipitate indicated the presence of alkaloids (Harborne, 1998).
2. **Saponins:** Saponins were identified using the froth test. The extract was vigorously shaken with distilled water, and the formation of a stable froth indicated the presence of saponins (Edeoga *et al.,* 2005).
3. **Phenolic Acids:** Phenolic acids were detected using the ferric chloride test, similar to tannins, with a green or blue-black coloration indicating their presence (Evans, 2002).
4. **Tannins**: Tested by adding 5% ferric chloride solution to the extract, indicated by blue-black or greenish precipitate. Standard Method: Ferric Chloride Test (Evans, 2002).
5. **Quinones:** Detected by adding concentrated sulfuric acid to the extract with a resultant red coloration. Standard Method: Sulfuric Acid Test (Sofowora, 1993).
6. **Flavonoids:** Identified by adding 10% sodium hydroxide to the extract, producing yellow color changing to colorless on acidification. Standard Method: Sodium Hydroxide Test (Harborne, 1973).
7. **Cardiac Glycosides:** Screened by adding glacial acetic acid and ferric chloride followed by sulfuric acid; brown ring formation indicates presence. Standard Method: Keller-Kiliani Test (Evans, 2002).

Antioxidant Activity Assays

The antioxidant activity of the leaf, stem, seed and root extracts was evaluated using the following in vitro assays:

DPPH Radical Scavenging Assay: The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was performed to measure the free radical scavenging activity of the extracts. A solution of DPPH in methanol was mixed with different concentrations of the extract, and the absorbance was measured at 517 nm after 30 minutes of incubation in the dark. Ascorbic acid was used as the standard, and the antioxidant activity was expressed as IC_{50}, which is the concentration required to inhibit 50 % of the DPPH radicals (Baliyan *et al.,* 2022).

Data Analysis

Qualitative phytochemical data were analyzed descriptively using symbols (+++, ++, +, –) to indicate the relative presence of each compound across plant parts. Antioxidant assay results were expressed as mean ± standard deviation (SD) of triplicate determinations. EC_{50} values were obtained from dose–response curves using nonlinear regression analysis. Differences in antioxidant activity among plant parts were evaluated using one-way ANOVA followed by Tukey's post hoc test at $p < 0.05$.

RESULTS

Phytochemical screening

Qualitative phytochemical test of *Crotalaria pumila* (Low Rattlebox) Leaves, Stem, Seed and Root.

Table 1: Qualitative Analysis of Phytochemical Screening of *Crotalaria pumila* (Low Rattlebox) Leaves, Stem, Seed and Root

Phytochemicals	Leaves	Stem	Seed	Root
Alkaloid	+	++	++	+
Saponins	+	++	++	+
Phenol	++	-	+	+
Tannins	-	-	-	-
Quinone	+	++	+	+
Flavonoid	+	+	+	+
Cardiac Glycoside	-	-	-	+

KEY: +++ = high concentration, ++ = moderate concentration, + = low concentration and - = absent

The qualitative phytochemical analysis revealed the presence of various bioactive compounds across the leaves, stem, seed, and root of *Crotalaria pumila*. Alkaloids and saponins were detected in all parts of the plant, showing moderate concentrations in the stem and seed and lower levels in the leaves and root. Phenols were abundant in the leaves but occurred in lower concentrations in the seed and root, while being absent in the stem. Quinones and flavonoids were uniformly present in all parts, though quinones were relatively higher in the stem. Tannins were absent in all samples, whereas cardiac glycosides were detected only in the root at low concentration. Overall, the stem and seed exhibited a richer phytochemical profile compared to the leaves and root, indicating their potential as more potent sources of bioactive compounds (Table 1).

Antioxidant Activity

Concentration-dependent activity of leaves, stem, and seed, root of *Crotalaria pumila* (Low Rattlebox).

Antioxidant Activity of Leaves and Stem:

Both stem and leaves show an increase in inhibition percentage as the concentration increases from 1 to 500 µg/mL, indicating that the antioxidant activity is dose-dependent for both aboveground parts.

Stem performance:

The stem (green line) shows a gradual increase in antioxidant activity across all concentrations. It reaches about 72% inhibition at 500 µg/mL.

Leaves performance:

The leaves (red line) demonstrate a rapid increase in antioxidant activity, especially between 10 and 50 µg/mL. They reach a high inhibition percentage (94%) at 500 µg/mL, very close to that of Vitamin C.

Comparative potency:

The leaves consistently show higher antioxidant activity than the stem across all concentrations. The difference is particularly pronounced at mid-range concentrations (10-100 µg/mL).

Comparison to Vitamin C:

The leaves demonstrate antioxidant activity remarkably close to Vitamin C, especially at higher concentrations. The stem shows lower activity compared to both leaves and Vitamin C.

EC_{50} Values:

- Vitamin C: 21.95 µg/mL
- Stem: 95.69 µg/mL
- Leaves: 25.81 µg/mL

The leaves demonstrate significantly higher antioxidant potency compared to the stem, as evidenced by their much lower EC_{50} value. In fact, the EC_{50} of the leaves is very close to that of Vitamin C, indicating potent antioxidant activity.

The leaves show exceptionally high antioxidant activity, nearly matching Vitamin C in potency. This suggests that the leaves contain high concentrations of powerful antioxidant compounds. The stem, while less potent than the leaves, still demonstrates significant antioxidant activity, particularly at higher concentrations.

These results indicate that the aboveground parts, especially the leaves, could be valuable sources of natural antioxidants. The high antioxidant activity in leaves might be attributed to their exposure to environmental stressors like UV radiation and pathogens, which could stimulate the production of protective antioxidant compounds.

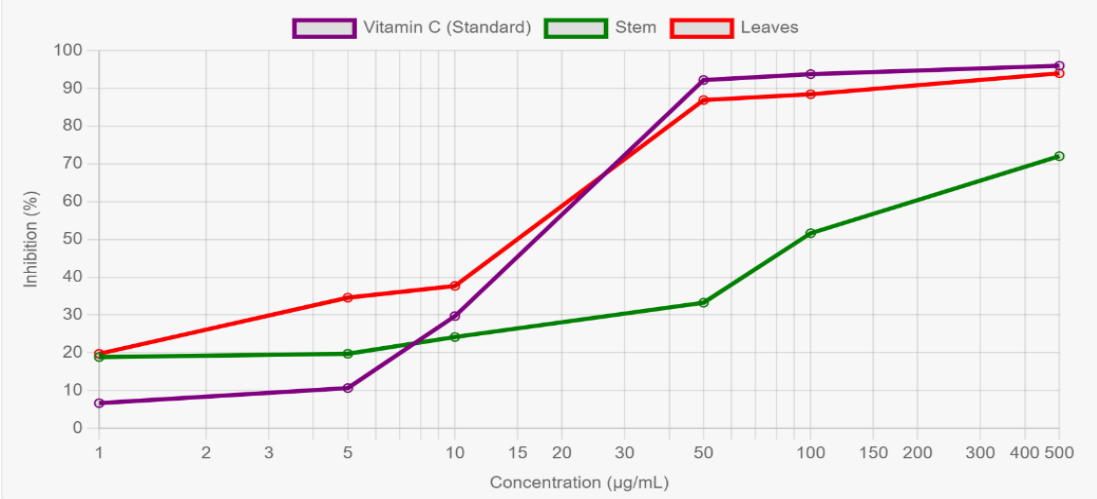


Figure 2: Concentration-dependent activity of Stem, leave and Vitamin C

The EC_{50} values of the stem and leaves to Vitamin C, which serves as a reference standard

The graph compares the EC_{50} values of the stem and leaves to Vitamin C, which serves as a reference standard.

Stem EC_{50}: 95.69 µg/mL

The stem shows a moderate EC_{50} value among the plant parts tested. This indicates that it has notable antioxidant potency, but less than the leaves and Vitamin C. The stem's EC_{50} suggests it contains antioxidant compounds, but possibly in lower concentrations or with less potency compared to the leaves. This could be due to the stem's primary functions of structural support and nutrient transport, which may not require as high levels of antioxidants as other plant parts.

Leaves EC_{50}: 25.81 µg/mL

The leaves demonstrate an impressively low EC_{50} value, very close to that of Vitamin C (21.95 µg/mL). This indicates that the leaves have the highest antioxidant potency among all the plant parts tested, nearly matching the potency of Vitamin C. The low EC_{50} value suggests that the leaves contain high concentrations of potent antioxidant compounds. This could be due to the leaves' exposure to environmental stressors such as UV radiation and atmospheric pollutants, which may stimulate the production of protective antioxidant compounds.

The significant difference between stem and leaves EC_{50} values (95.69 vs 25.81 µg/mL) underscores the importance of considering specific plant parts when seeking high antioxidant activity. The leaves, with their EC_{50} value close to Vitamin C, appear to be the most promising part of the plant for applications requiring strong antioxidant properties.

These comparisons highlight the varying antioxidant potencies across different parts of the plant. The leaves show exceptionally high activity, followed by the root, then the stem, and finally the seed. This information can be valuable for determining which plant parts might be

most beneficial for specific antioxidant-related applications, whether in the development of natural supplements, functional foods, or other products where antioxidant properties are desired.

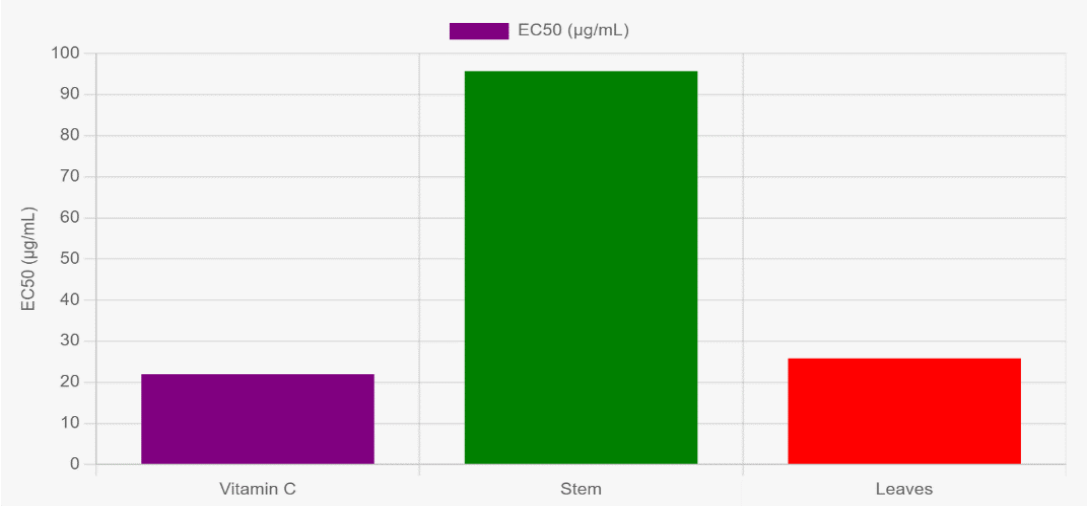


Figure 3: The Graph of the EC_{50} values of the stem and leaves to Vitamin C, which serves as a reference standard

Antioxidant Activity of Seed and Root:

Both seed and root show an increase in inhibition percentage as the concentration increases from 1 to 500 µg/mL, indicating that the antioxidant activity is dose-dependent for both underground parts.

Seed performance:

The seed (orange line) shows a gradual increase in antioxidant activity up to 50 µg/mL, followed by a steeper increase at higher concentrations. It reaches about 85% inhibition at 500 µg/mL.

Root performance:

The root (blue line) demonstrates a more rapid increase in antioxidant activity compared to the seed, especially between 5 and 100 µg/mL. It reaches its maximum inhibition of about 83% at 500 µg/mL.

Comparative potency:

The root generally shows higher antioxidant activity than the seed across most concentrations. However, at the highest concentration (500 µg/mL), their activities are very similar.

Comparison to Vitamin C:

Both underground parts show lower antioxidant activity compared to Vitamin C, especially at mid-range concentrations (10-100 µg/mL). However, at 500 µg/mL, their activity approaches that of Vitamin C.

EC_{50} Values:

- Vitamin C: 21.95 µg/mL
- Seed: 225.13 µg/mL
- Root: 44.67 µg/mL

The root demonstrates significantly higher antioxidant potency compared to the seed, as evidenced by its lower EC_{50} value. This suggests that the root contains higher concentrations of antioxidant compounds or more potent antioxidants compared to the seed.

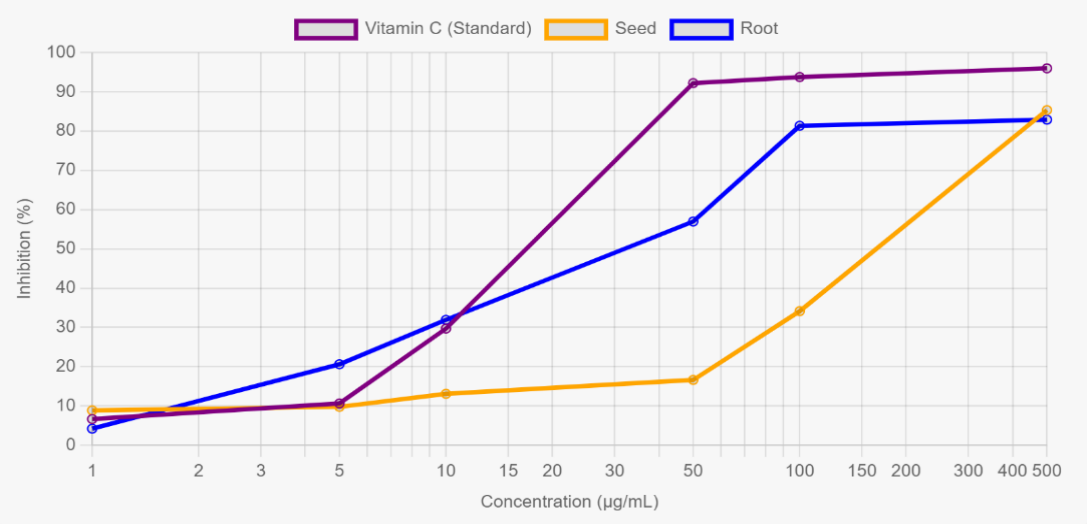


Figure 4: Concentration-dependent activity of seed, root and Vitamin C

The EC_{50} values of the seed and root to Vitamin C which serves as a reference standard

The graph compares the EC_{50} values of the seed and root to Vitamin C, which serves as a reference standard.

Seed EC_{50}: 225.13 µg/mL

The seed shows the highest EC_{50} value among all plant parts tested. This indicates that it has the lowest antioxidant potency. A higher concentration of seed extract is required to achieve 50% of its maximum antioxidant effect compared to other parts. This could be due to lower concentrations of antioxidant compounds in the seed or the presence of less potent antioxidants. Despite its lower potency, the seed still demonstrates some antioxidant activity, which might be valuable in certain applications where a milder effect is desired.

Root EC_{50}: 44.67 µg/mL

The root demonstrates a significantly lower EC_{50} value compared to the seed, indicating higher antioxidant potency. While still higher than Vitamin C (21.95 µg/mL), the root's EC_{50} suggests it contains considerable amounts of antioxidant compounds or compounds with strong antioxidant activity. This could be related to the root's role in absorbing nutrients and defending against soil-borne pathogens, which might require significant antioxidant capacity. The stark difference between seed and root EC_{50} values (225.13 vs 44.67 µg/mL) highlights the variability in antioxidant potency across different parts of the same plant. This information can be crucial for selecting the most appropriate plant part for specific antioxidant-related applications.

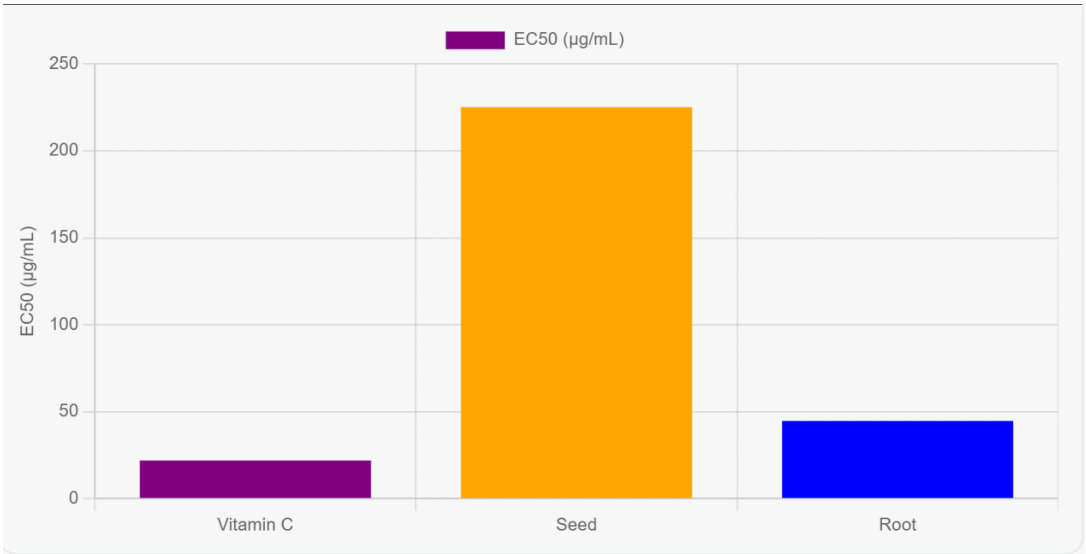


Figure 5: The graph of the EC_{50} values of the seed and root to Vitamin C which serves as a reference standard.

DISCUSSION

Phytochemical Composition

The phytochemical screening of the leaves, stems, seeds, and roots of *Crotalaria pumila* (Low Rattlebox) revealed the presence of diverse bioactive compounds that contribute significantly to its antioxidant and therapeutic potential. The study demonstrated that all parts of the plant contained varying concentrations of alkaloids, flavonoids, tannins, phenolics, and saponins, indicating that *C. pumila* is a rich source of secondary metabolites with medicinal value. These findings corroborate earlier reports by (Kumari & Kumar, 2022), who also identified similar classes of phytochemicals in *C. pumila* extracts.

The leaves and stems of *C. pumila* showed particularly high levels of phenolic acids, flavonoids, and saponins, compounds widely recognized for their antioxidant and pharmacological activities. Flavonoids, known for their capacity to scavenge free radicals and mitigate oxidative stress, were found in especially high concentrations in the leaves. This

correlates with their strong antioxidant potential as reported by Mahasawat *et al*. (2023). The observed phytochemical profile aligns with those of other members of the *Crotalaria* genus such as *C. pallida* and *C. juncea* which have been documented for their therapeutic efficacy and antioxidant properties (Sangram *et al.,* 2015). Furthermore, studies by Islam *et al*. (2018) and Mahasawat *et al*. (2023) have emphasized the vital role of these secondary metabolites in reducing oxidative stress and enhancing cellular protection, supporting the current findings.

Similarly, the seeds and roots of *C. pumila* exhibited a rich composition of flavonoids, alkaloids, tannins, and saponins, compounds associated with diverse biological functions, including antioxidant, anti-inflammatory, and antimicrobial activities. The presence of flavonoids and alkaloids in these parts reinforces their medicinal potential, as these compounds play crucial roles in neutralizing reactive oxygen species and protecting against oxidative damage (Martínez-Zaragoza *et al.,* 2023). Alkaloids isolated from *Crotalaria* species have also demonstrated cytotoxic and anticancer properties (Rahman *et al*., 2022), suggesting that *C. pumila* could possess similar therapeutic potential worthy of further pharmacological exploration.

Overall, the consistent presence of these phytochemicals across all plant parts underscores the holistic medicinal value of *C. pumila*. The antioxidant capabilities observed are largely attributable to the synergistic action of these secondary metabolites, which collectively contribute to the plant's ability to prevent oxidative stress-related diseases. These findings provide a scientific basis for the traditional use of *C. pumila* in herbal medicine and highlight its potential as a natural source of antioxidants and pharmacologically active compounds. Further studies involving compound isolation, structural elucidation, and in vivo assays are recommended to validate and enhance the understanding of its therapeutic applications.

Comparative Analysis of Plant Parts

The distribution and concentration of bioactive compounds varied across different parts of the plant. The leaves and stems exhibited higher levels of flavonoids and phenolic acids, accounting for their strong antioxidant potential (Mutha *et al*., 2021). These compounds are known to donate hydrogen atoms or electrons, thereby neutralizing free radicals and preventing oxidative cellular damage. In contrast, the seeds and roots contained appreciable amounts of alkaloids, tannins, and saponins, compounds that contribute to additional pharmacological properties such as antimicrobial, anti-inflammatory, and cytotoxic activities (Baliyan *et al*., 2022).

Flavonoids found in both the leaves and seeds act as effective scavengers of reactive oxygen species (ROS), reducing lipid peroxidation and maintaining cellular integrity (Martínez-Zaragoza *et al.,* 2023). Similarly, alkaloids identified in the seeds and roots have demonstrated cytotoxic and anticancer properties in related *Crotalaria* species (Rahman *et al*., 2022), suggesting potential for therapeutic exploitation. Saponins and tannins, present in considerable amounts across all plant parts, further enhance the medicinal value of *C. pumila* through their antimicrobial and anti-inflammatory effects.

Antioxidant Properties

Leave and Stem:

Antioxidant assays, such as DPPH and FRAP, conducted on *C. pumila* extracts suggest that leaves have slightly higher antioxidant potential compared to stems, likely due to their higher phenolic content which correspond to the study by (Seleshe *et al.,* 2022). This activity is attributed to the high concentrations of phenolic compounds in the leaves, which have been widely documented to neutralize reactive oxygen species effectively. Studies reveal a robust correlation between the plant's phenolic content and its radical scavenging capacity, supporting its use as a natural antioxidant source which is in line with the study by (Sinan *et al.*, 2020).

Seed and Root:

The antioxidant activities of *Crotalaria pumila* seed and root extracts are often attributed to their phenolic content, which is crucial for neutralizing free radicals. According to recent findings, the total phenolic and flavonoid content of *C. pumila* exhibits a positive correlation with its antioxidant efficacy, as demonstrated in DPPH and ABTS assays which is confirmatory with the study of (Sinan *et al.*, 2020). The seeds and roots have shown comparable antioxidant capacities, indicating that both parts of the plant could be viable sources of natural antioxidants.

Antioxidant Mechanisms and Medicinal Implications

The antioxidant capacity observed in *C. pumila* extracts can be attributed to the synergistic action of its phytoconstituents. Phenolic compounds act as primary antioxidants by donating hydrogen atoms to free radicals, while flavonoids and alkaloids enhance enzymatic and non-enzymatic defense systems. These mechanisms collectively protect biomolecules such as lipids, proteins, and DNA from oxidative stress, thereby mitigating cellular damage and aging processes.

The antioxidant mechanisms of *C. pumila* are particularly important considering the global interest in natural sources of antioxidants as safer alternatives to synthetic agents. The strong antioxidant response observed in the leaves and stems aligns with the findings of Garcia *et al*. (2023), who linked high flavonoid concentrations with superior radical-scavenging ability. Likewise, the alkaloid-rich seeds and roots offer additional health benefits, including potential anticancer and antimicrobial applications (Mwangi *et al.,* 2021).

Implications for Pharmaceutical and Nutraceutical Development

The combined phytochemical richness of the leaves, stems, seeds, and roots of *Crotalaria pumila* underscores the plant's potential for use in drug formulation and functional food development. The abundance of flavonoids and phenolics suggests suitability for antioxidant supplements, while the presence of alkaloids and saponins provides a foundation for future pharmacological exploration. The therapeutic profile of *C. pumila* is therefore consistent with

other *Crotalaria* species that have been utilized in traditional medicine for treating inflammatory and infectious diseases.

However, while the findings of this study affirm the medicinal potential of *C. pumila*, further investigations are recommended to isolate, characterize, and quantify individual bioactive compounds. Advanced studies involving *in vitro* and *in vivo* assays will help elucidate their specific biological mechanisms, toxicological safety, and pharmacodynamic properties.

CONCLUSION

The findings of this study demonstrated that *Crotalaria pumila* (Low Rattlebox) is a rich source of diverse phytochemicals, including alkaloids, flavonoids, saponins, phenols, and quinones, which collectively contribute to its significant antioxidant and therapeutic properties. The qualitative and quantitative analyses revealed that all plant parts possess bioactive compounds, though their concentrations vary. The leaves exhibited the strongest antioxidant activity (IC_{50} = 25.81 µg/mL), closely matching that of vitamin C, followed by the root, stem, and seed. This high antioxidant potential, particularly in the leaves, can be attributed to the abundance of flavonoids and phenolic compounds known for their free radical scavenging capabilities. The results therefore validate the traditional use of *C. pumila* in herbal medicine and underscore its potential as a natural source of compounds beneficial for the prevention and management of oxidative stress-related disorders.

Future studies should focus on the isolation, purification, and structural elucidation of individual phytochemicals responsible for the observed antioxidant effects. In vivo and clinical evaluations are also recommended to confirm their pharmacological safety, dosage, and therapeutic efficacy for potential pharmaceutical and nutraceutical development.

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