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PHYTOCHEMICAL SCREENING, MINERAL CONTENT ANALYSIS, AND ANTIOXIDANT ACTIVITY OF LEAVES, FRUITS, STEMS, AND ROOTS OF *WITHANIA COAGULANS*: IMPLICATIONS FOR MEDICINAL APPLICATIONS

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Abstract

Withania coagulans has garnered attention in therapeutic medicine due to its rich phytochemical profile and mineral content. This study aimed to evaluate the mineral composition, phytochemical profile, and antioxidant activity of various plant parts, including leaves, fruits, stems and roots. Mineral analysis was conducted using atomic spectroscopy, while standard chemical assays were employed to assess phytochemical and antioxidant properties. The results revealed that among macro minerals, calcium (Ca) concentration was significantly higher ($P < 0.05$) in fruits, roots, and leaves compared to other minerals, whereas chromium (Cr) was the most abundant micromineral ($P < 0.05$). Nutritional analysis showed that roots had the highest protein and fat content ($P < 0.05$), while leaves exhibited the highest carbohydrate, fiber, and energy content ($P < 0.05$), while leaves exhibited the highest carbohydrate, fiber, and energy content ($P < 0.05$). Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, saponins, alkaloids, terpenoids, and phenols in all plant parts with tannins detected exclusively in leaves. Notably, fruits demonstrated the highest antioxidant capacity (88.7 ± 2.003 µM TE/g) among the tested parts. These findings underscore the therapeutic potential of Withania coagulans, highlighting its antioxidant properties, nutrient richness and diverse bioactive compounds. This study paves the way for its application in developing novel medicinal therapies and nutraceutical formulations.

Key words: *Antioxidant activity, Minerals contents, Phytochemical, Traditional medicine, Withania coagulans*

INTRODUCTION

Modern world is facing many issues related to medical health sciences, especially regarding the treatment and control of diseases. The synthetic medicine prepared in laboratory or industry are very expensive, have many side effects and produce antibiotic resistance. Therefore, alternative is quite necessary for the protection and control of various diseases. Herbal medicine from the therapeutic herbs and tress, their leaves, roots, seeds, fruits and stems contain very important phytochemical that are very essential in therapeutic medicine (1). Scientist, researchers and physician are willing to discover of new therapeutic medicinal plants for treatment of various chronic and sever disease. According to Word health organization (WHO) till now 80% population of world still depending on the traditional herbal medicine to cure of various disease as they are easily available, safe and cheap (2). Therefore, *W. coagulans* is one of the best option due to presence of phytochemical constituents like flavonoids, saponins, alkaloids, terpenoids, and phenols served as anti-bacterial, antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, anticancer, antidiabetic, antihyperlipidemic, neuroprotective, cardioprotective, nephroprotective, hepatoprotective, reproductive system regulatory, and anthelmintic activities (3).

Withania coagulans Dunal is a member of Solanaceae family that is known as vegetable rennet or Indian cheese maker (4). It is distributed mostly in Asia like Pakistan, Iran, Afghanistan and northern India including Simla, Punjab, Kumaun and Garhwal. *Withania coagulans* fruits are red smooth or brownish, and closed with a leathery calyx and globose; their pulp has a brown color and having nauseous fruity odour that is used as coagulant. The husk and pulp of fruits have a withanin enzyme that is responsible for coagulation of milk due to its coagulating properties. One quarter of boiling water is mixed with fruits of *Withania coagulans* having weight of one ounce, and suspension with one spoonful of this is enough to coagulate milk of one gallon within sixty minutes(5).

The elemental composition of *Withania coagulans* will be greatly helpful in exploring the medicinal properties of this plant, as limited work has been reported on this plant. *Withania coagulans* are economically and medicinally significant being used and cultivated in several regions of Pakistan (6). *Withania coagulans* is being used as blood purifier in several regions of subcontinents. Antimicrobial, anti-hyperglycemic, cardiovascular, free radical scavenging, anti-inflammatory and central nervous system depressant activities of the *Withania coagulans* have been reported (3) . Considering all the medicinal properties, the species of genus "Withania" 'are also of great medicinal value.

Despite the extensive use of herbal medicine in therapeutic applications, there is lack of comprehensive studies exploring the phytochemical profile, minerals composition, and antioxidant potential of *W. coagulans* across its different structural parts. Existing research primarily focuses on isolated components, leaving a gap in understanding the holistic nutritional and medicinal value of the plant. This study aims to address this gap by systematically analyzing the leaves, fruits, stems, and roots of *W. coagulans* to evaluate their bioactive compounds, mineral content, and antioxidant capacity. By identifying the most nutrient-rich and bioactive plant parts, the findings are expected to provide a scientific basis the plant's therapeutic application and its potential for developing novel medicinal and nutraceutical products. In our research, we analyzed and reported the results of different parts of *W. coagulans* in detail. Therefore, this research provides a significant contribution to the scientific community regarding this plant.

MATERIALS AND METHODS

COLLECTION, PROCESSING OF PLANT MATERIALS

During the study leaves, fruits, stems and roots of *W. coagulans* were collected from different districts of Balochistan. Different air tight bags were used for separation of different parts of plant. To remove the soil dirt and foreign particles we first wash all parts properly and then sun dried them. The stem and roots were cut into small pieces, and then processed in the oven for 48 hours at 40°C. The plants parts after the drying were properly powdered by using the grinder. After that powder were mixed with different chemicals to make extract and find the nutritional profiles.

CHEMICALS

All the chemicals like Concentrated sulphuric Acid, Hydrogen per oxide and Standard solution of Zn, Cu, Na, K, Mn, Mg, Ca, Fe, Cr were purchased form Sigma Aldrich. All the chemical were analytical grade.

ACID DIGESTION

Two grams of dry powder of plant sample were soaked overnight in 100 volumetric flask containing concentrated nitric acid. The flask was then heated at 70 ℃ for forty minutes and then again heated up to 160°C. This solution was diluted with distilled water and then again boiled for 15 minutes. The samples were transferred to a volumetric flask of 250 ml volume of a proper measurement.

DETERMINATION OF MINERAL COMPOSITION

Standard protocol reported in previous literature for atomic absorption spectroscopy for determination of mineral contents was carried out (7). The details are provided in the supplementary section.

ESTIMATION OF PROXIMATE COMPOSITION

Association of Official Analytical Chemists was carried out to determine Protein, fat, carbohydrate, fiber, moisture and energy. All the measurements were performed according to well established methodologies reported in previous literature (8). However, the details are provided in the supplementary section.

ANTIOXIDANT ACTIVITY

DPPH FREE RADICAL SCAVENGING ACTIVITY

The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical is changed by antioxidants into 1, 1-diphenyl-2-picryl hydrazine. The degree of colour change from purple to yellow was used to gauge an antioxidant extract's ability to scavenge free radicals. We'll take aliquots of extract solutions (1 mg/ml) and use methanol to dilute them to a level of 3 ml. The freshly made 0.15 ml of DPPH solution was added, mixed, and allowed to stand for 30 minutes in the dark at room temperature. In place of the sample, the control solely comprises DPPH solution in methanol, with ascorbic acid serving as the standard and methanol serving as the blank. The spectrophotometer was used to record the absorbance at 517 nm. Scavenging activity was used to calculate the capacity of scavenging free radicals.

$$\%\text{DPPH radical scavenging activity} = [(A_{blank} - A_{sample})/ A_{blank}] \times 100$$

where $Abs_{control}$ is the absorbance of DPPH radical + methanol

Abs_{sample} is the absorbance of DPPH radical + sample extract/standard

MEASUREMENT OF VITAMIN C

The grinded plants parts were utilized to calculated the dehydroascorbic acid and ascorbate via spectrophotometer (Hitachi, Tokyo, Japan). During the measurement the ferric iron (Fe3+) reduce in to ferrous iron (Fe2+) that make complexes with 2,2- dipyridyl were made via spectrophotometer. At the end the sample data was recorded on the basis of absorbance.

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

All crude extracts were screened for different constituents such as flavonoids, alkaloids, saponins, carbohydrates, glycosides, phenols and tannins. For these phytochemical screening standard protocols was used (9). Whereas terpenoids, carotenoids, phlobatanins, coumarins, quinines, and terpenes was identified using accepted techniques (10). The phytochemical composition of *Withania coagulans* was analyzed using standard qualitative methods. For flavonoids, 1 mL of plant extract was treated with a few drops of ammonium hydroxide (NH_3), and the development of a yellow fluorescence indicated their presence. Terpenoids were identified by mixing 1 mL of plant extract with 2 mL of chloroform and 3 mL of concentrated sulfuric acid (H_2SO_4), where a reddish-brown interface confirmed their presence. Saponins were detected by vigorously shaking 2 mL of plant extract with 3 mL of distilled water, resulting in a stable froth. Phenols were assessed by adding a few drops of ferric chloride to 10 mL of plant extract, with the appearance of a dark green or blue color indicating their presence. Alkaloids were tested by heating 2 mL of plant extract with 5 mL of 2% hydrochloric acid (HCl), filtering, and adding 0.5 mL of Wagner's reagent, where a reddish-brown precipitate confirmed their presence. These tests provided a comprehensive profile of the phytochemical constituents in the plant.

STATISTICAL ANALYSIS

The data obtained for each experiment were recorded and statistically analyzed. The mean and standard deviation of replication of all groups were statically analyzed by one way ANOVA using GraphPAD Prism 8 software. The mean obtained were compared with the post hock Tukey's test with significance thresholds at $P < 0.05$.

RESULTS AND DISCUSSION

MINERAL COMPOSITION *WITHANIA COAGULANS*

The mineral composition of *W. coagulans* varies significantly across different parts of the plant. The fruits, roots and leaves have significantly (P<0.05) highest concentrations of calcium (Ca) as compared with other minerals. The results were observed by Fuji apple fruit having higher concentration of calcium (11), similarly, in the leaves and fruits of loquat contain higher amount of Ca (12). However, stem contain significant (P<0.05) highest concentration of Magnesium as compared with other minerals (Table I). The Stem of *Amaranthus lividus* contained higher amount of magnesium 29.77 mg/kg DW as compare with other minerals potassium and calcium (13).

Table I. Macro mineral concentration in different part of plant

	Na	K	Ca	Mg
Fruit	129.41 ± 12.12 *	176.66 ± 6.72 ***	253.10 ± 22.75 **	152.25 ± 8.16 **
Leaves	121.80 ± 3.18 **	161.41 ± 1.45 **	211.56 ± 3.63 **	125.12 ± 3.71**
Roots	130.62 ± 2.18 **	150.41 ± 4.78 ***	211.93 ± 3.55 **	134.43 ± 4.31***
Stem	98.42 ± 2.74 ***	132.77 ± 3.13 *	171.69 ± 7.93 **	122.15 ± 3.49 ***

The micro mineral concentration in different parts of *W. coagulans* also significantly differ in different parts of plant like the level of chromium (Cr) in the fruit, leaves and roots contains the significantly (p<0.05) highest as compare to other minerals. Our findings are quite similar with the results of Karahan *et al.,* (14). In contrast the roots contain iron (Fe) in higher amount as compare with the other micro minerals (Table II). The higher amount of iron (60-180 mg) in the roots of paddy rice (15) as compared with other parts of plant.

Table II. Micro mineral concentration in different part of plant

	Cr	Zn	Cu	Mn	Fe
Fruit	2.97 ± 0.12 ***	1.66 ± 0.31 **	0.66 ± 0.18 *	1.24 ± 0.11 **	0.26 ± 0.15 *
Leaves	1.80 ± 0.25 *	1.61 ± 0.45 **	1.51 ± 0.36 *	1.21 ± 0.17**	1.37 ± 0.29*
Roots	1.26 ± 0.18 **	1.14 ± 0.07 **	1.39 ± 0.05 **	1.13 ± 0.13**	1.48 ± 0.18***
Stem	1.42 ± 0.14 ***	1.27 ± 0.13 *	1.09 ± 0.15 **	1.15 ± 0.19 **	1.29 ± 0.21**

NUTRITIONAL COMPOSITION

The nutritional composition of *Withania coagulans* varies among parts of the plant (Fruit, leaves, roots and stem). The protein percentage was significantly higher (P<0.05) in roots and stem compare to the fruit and leaves, similarly fat % age significantly higher (P<0.05) among roots and leaves. On other hand percentage of carbohydrate fiber significantly higher (P<0.05) in leaves and stem while the moisture percentage and energy kcal/100g is significantly higher (P<0.05) among leaves roots and stem (Fig. 1). Previous research reported higher amount of ash, fat, crude protein and total solids were observed in the roots, leaves and fruits of *Withania coagulans* (16). The plants parts including seeds, leaves, fruits, stem and roots of collected *Withania coagulans* from Balochistan and reported that seeds and leaves due to higher nutritional composition are mostly utilized in medicinal purpose (17).

PHYTOCHEMICAL CHARACTERS OF LEAVES, FRUITS, STEM AND ROOTS OF *W. COAGULANS*

The phytochemical analysis of *Withania coagulans* reveals the presence of various bioactive compounds in different parts of the plant. Flavonoids, saponins, alkaloids, terpenoids, and phenols are consistently detected across the fruits, leaves, roots, and stem. Tannins is also present in the leaves, but absent in the fruits, roots, and stem (Table III). The different parts like fruits roots and leaves due to presence of phytochemical constituents like Flavonoids, saponins, alkaloids, terpenoids, and phenols served as anti-bacterial, antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, anticancer, antidiabetic, antihyperlipidemic, neuroprotective, cardioprotective, nephroprotective, hepatoprotective, reproductive system regulatory, and anthelmintic activities (3). Similarly, *W. coagulans* contains many phytochemicals

such as flavonoids, tannins, and β-sterols also been reported by Khan et al. (16). However the fruits contain higher amount of phytochemicals as compare to the other parts of plants (18).

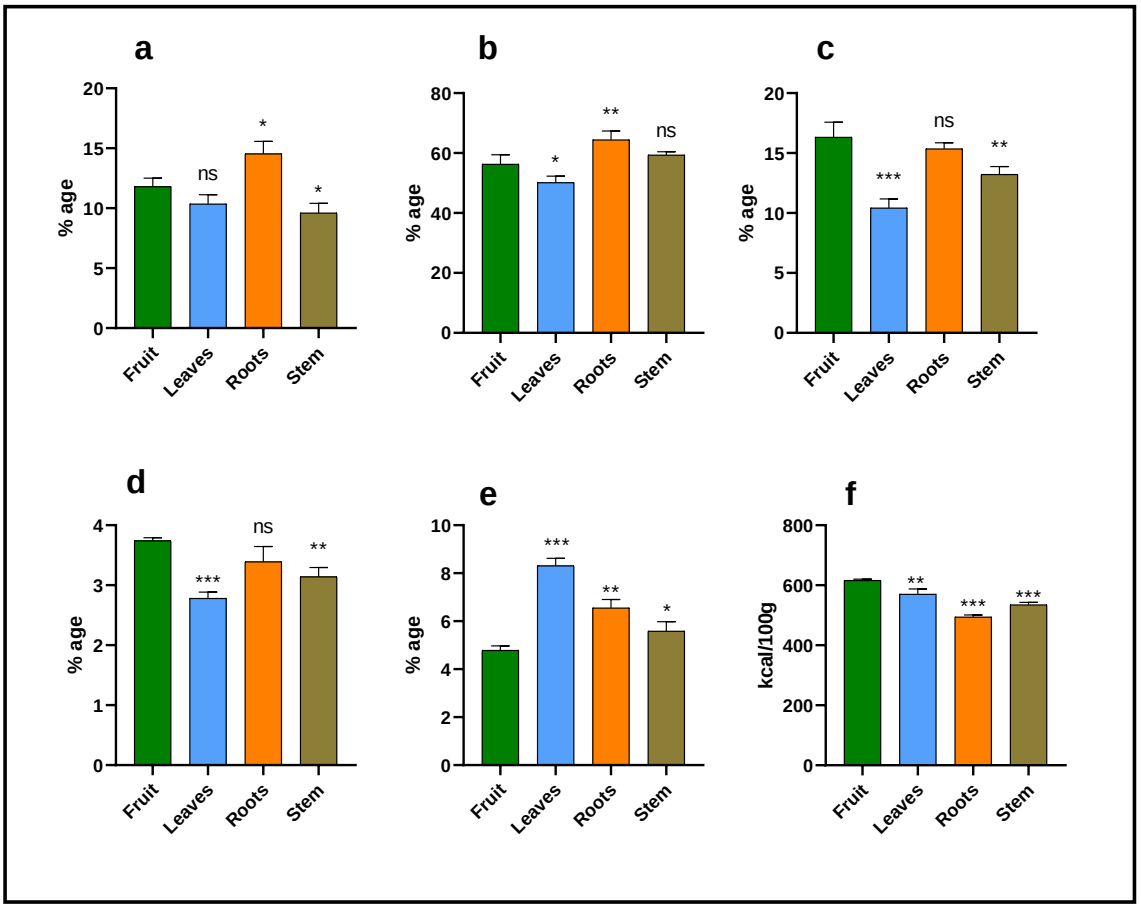

Fig. 1. Nutritional composition of different parts of plants. (a) Represents % age of protein (b) Represents % age of fat (c) Represents % age of carbohydrate (d) Represents % age of fiber (e) Represents % age of moisture (f) Represents Kcal/100 g of energy. The data was analyzed by one way ANOVA followed by post hock Tukey's test. *p<0.05 value of significantly different among the groups

VITAMIN C, TOTAL PHENOLS AND ANTIOXIDANT ACTIVITY OF *WITHANIA COAGULANS*

The vitamin C content, total phenol content, and antioxidant activity of *Withania coagulans* vary among its different parts. The leaves exhibit the highest antioxidant activity (%DPPH inhibition) at 66.68 ± 4.74%, followed by the fruit at 56.78 ± 4.85%, roots at 48.59 ± 2.14%, and stem at 45.66 ± 1.71%. The leaves also have the highest vitamin C content (32.98 ± 1.73 mg/100g), while the fruit, roots, and stem contain 29.78 ± 1.35 mg/100g, 26.32 ± 0.96 mg/100g, and 29.3 ± 1.03 mg/100g, respectively. In terms of total phenol content, the fruit is the richest with 5301.66 ± 76.17 mg GAE/100g, while the leaves, roots, and stem have slightly lower but comparable values of 5104.66 ± 61.27 mg GAE/100g, 5151.67 ± 48.64 mg GAE/100g, and 5126 ± 51.45 mg GAE/100g, respectively. The fruit also shows the highest Trolox equivalent antioxidant capacity (88.7 ± 2.003 µM TE/g), with the stem, roots, and leaves having lower values at 72.44 ± 3.21 µM TE/g, 63.22 ± 3.69 µM TE/g, and 45.62 ± 3.99 µM TE/g, respectively (Table IV).

Table III. Phytochemical constituents present in different parts of *Withania coagulans*

Phytochemical	Fruit	Leaves	Roots	Stem
Flavonoids	+	+	+	+
Saponin	+	+	+	+
Alkaloids	+	+	+	+
Tannins	-	+	-	-
Terpenoids	+	+	+	+
Phenols	+	+	+	+

*(+) = detected and (-) = not detected

The value of flavonoids and total phenolic were higher in *W. coagulans* leaves as 58.21 mg GEA/g and 47 mg RE/g. However, the value of total flavonoids and total phenolic leaves, stem and roots extracts (19). Similarly, another study found that ethanol extract of seeds of *W. coagulans* showed higher total flavonoids and total phenolic contents. Whereas, DPPH free radical scavenging assay of *W. coagulans* showed maximum % age inhibition then methanol extract. In addition to that ethanolic extract of *W. coagulans* showed strong antioxidant activities (20).

Table IV. Vitamin C content, total phenols, and antioxidant activity in different parts of *Withania coagulans*

Composition	Fruit	Leaves	Roots	Stem
Antioxidant Activity (%DPPH inhibition)	56.78 ± 4.85	66.68 ± 4.74 *	48.59 ± 2.14 ns	45.66 ± 1.71 *`
Vitamin C (mg/100g)	29.78 ± 1.35	32.98 ± 1.73 *	26.32 ± 0.96 *	29.3 ± 1.03 ns
Total Phenol (mg GAE/100 g)	5301.66 ± 76.17	5104.66 ± 61.27*	5151.67 ± 48.64*	5126 ± 51.45*
(% DPPH inhibition)	88.7 ± 2.003	45.62 ± 3.99 ***	63.22 ± 3.69 ***	72.44 ± 3.21**
Trolox equivalent (μM TE/g)				

*p<0.05 value of significantly different among the row

The findings of this study highlight the remarkable phytochemical diversity, mineral richness, and antioxidant capacity of various parts of *W. coagulans,* underlining its significant potential in medicinal applications. The high calcium (Ca) and chromium (Cr) content in fruits, roots, and leaves emphasizes their nutritional value and relevance for bone health and metabolic functions. The superior flavonoids and phenolic content in roots and carbohydrate and fiber richness in leaves suggest that different parts of plant can be selectively utilized for specific dietary and therapeutic purposes. The antioxidant capacity particularly pronounced in fruits, positions *W. coagulans,* as potent source of natural antioxidants, which are crucial for combating oxidative stress and related diseases.

Comparing to existing studies, which often focus on isolated components, our comprehensive analysis provides a broader understanding of plant's bioactive potential. For example, the presence of flavonoids, saponins, alkaloids, terpenoids, and phenols across different parts supports the plant's diverse pharmacological properties, including anti-inflammatory, antimicrobial and anticancer effects. These compounds, coupled with plant's rich mineral profile, make it an excellent candidate for the development of nutraceuticals and herbal formulations.

Further research should explore the bioavailability and synergistic effects of these compounds *in vivo* to better understand their therapeutic efficacy. Additionally, the identification of specific compounds responsible for observed antioxidant activity could facilitate the development of targeted medicinal products. *W. coagulans* is an efficient supplements, functional foods, and herbal remedies, offering a natural alternative to synthetic drug. This study provides a foundational framework for further investigation into the medicinal potential of this valuable plant.

CONCLUSION

In conclusion, this study highlights the rich phytochemical composition, mineral content, and antioxidant potential of *W. coagulans,* underscoring its significant value in herbal medicine and potential applications in pharmaceutical development. The presence of diverse bioactive compounds, such as flavonoids, alkaloids, terpenoids, and phenols, along with high antioxidant activity, particularly in the fruits, positions *W. coagulans,* as a promising candidate for developing natural remedies and nutraceuticals. These findings contribute to the growing body of evidence supporting the therapeutic potential of medicinal plants, offering new opportunities for combating oxidative stress and related diseases. However, further research should focus on in vivo studies to assess the bioavailability and efficacy of these compounds, along aside clinical trails to validate the plant's medicinal properties and safety. Mechanistic studies exploring the

interaction of bioactive compounds at the molecular level could further facilitate the development of targeted pharmaceutical applications.

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Conflict of interest:

Authors have no conflict of interest.

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