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Phytochemical evaluation of leaves and roots of *Withania somnifera* collecetd from different locations

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Abstract
The current study explores the biochemical composition of leaf and root extracts of the plant *Withania somnifera*, growing in different regions, including, Ajmer, Nagaur and Tonk. The study shows huge variability in level of primary metabolites (carbohydrates, proteins and lipids) in leaf and root extract of *Withania somnifera*, growing in different geographical regions, with leaf extract and roots of the plant from Tonk, Ajmer and Nagaur (leaves) and Ajmer, Ajmer and Nagaur (root extract) showing highest amount of carbohydrates, proteins and lipids. Furthermore, the study showed presence of highest amount of flavonoids, alkaloids, phytosterols, phenols and tannins in leaf and root extracts from Ajmer, Tonk, Ajmer, Ajmer and Nagaur respectively. As per the results of the study, leaf extracts contain higher amount of flavonoids and phenols while higher amount of phytosterols, alkaloids and tannins was found in plant root extract. Furthermore, the results show that higher antioxidant activity in both leaf and root extracts was observed when ethanol and water were used as polar solvents for extraction purpose (ET, WA, WN, EN and WT for leaf extract and ET, WA, WN and EN for root extract).

Keywords: *Withania somnifera*, carbohydrates, proteins, lipids, flavonoids, alkaloids, phytosterols, phenols, tannins

Introduction

Ever since the evolutionary period, plants have been companions of humans and aided in human survival and sustenance in a number of ways, ranging from, provision of oxygen for respiration and facilitating availability of nutrients and food products to both animals and plants. Furthermore, much beyond the conventional role of plants, lack of proper healthcare facilities led our ancestors to explore the medicinal attributes of plants, in response to which, they discovered the huge plethora of bioactive secondary metabolites, that contribute to plant defence. Presence of these bioactive metabolites not only aids in fortification of plant immune syroot, protecting it from attack of deadly phytopathogens, but also exhibit potent microbicidal, anti-inflammatory, antioxidant and immunomodulatory activity to tackle chronic human ailments (Bunge,1973; Stinca, 2022; Lu, 2017) [2, 11, 19]. One such plant is *Withania somnifera*, commonly referred to as, Ashwagandha, which holds paramount medicinal importance and has been used as a popular medicinal plant in Ayurveda for centuries. The plant helps the body to adapt to a number of stressful conditions, and is therefore regarded to possess "adaptogenic" properties. Ashwagandha possess a number of potential benefits and medicinal values, that include

- Role of Ashwagandha in stress reduction owing to its ability to balance cortisol levels in the body.
- Anti-inflammatory activity as well as ability of the plant to act as immunomodulator, and accordingly stimulate or supress immune syroot in different ailments
- Ability of the plant to augment cognitive function and memory and aid in alleviation of neurodegenerative disorders
- Anticancer ability of the plant owing to its ability to trigger apoptotic death of uncontrollably proliferating cells.
- Ability of the plant to improve cardiovascular health by mitigating triglycerides and cholesterol and improve physical health.
- Ability of the plant to effectively balance hormone levels, leading to amelioration of thyroid disorders.
- Ability to act as potent antioxidant molecule and abort progression of oxidative damage in ROS mediated disorders and act as aphrodisiac to boost overall sexual health (Bharti, 2016; Mishra *et al*, 2000; Verma *et al*, 2022; Zahirudiin *et al*, 2020) [1, 14]

Considering the medicinal attributes of the plant *Withania somnifera*, the current study has been drafted to focus on determining biochemical composition of leaf and root extracts of the *Withania somnifera*, growing in different regions, including, Ajmer, Nagaur and Tonk. Presence of both primary and secondary metabolites plays an instrumental role in contributing to the pharmacological and medicinal attributes of the plant. The current study shows huge variability in amount of primary metabolites (carbohydrates, proteins and lipids) as well as secondary metabolites (flavonoids, alkaloids, phytosterols, phenols and tannins) in leaf and root extract of *Withania somnifera* L. growing in different geographical reasons. Furthermore, both leaf and root extract have been shown to exhibit potent antioxidant activity, contributing to ROS scavenging activity of the plant.

Results

Quantitative analysis to determine amount of primary metabolites (carbohydrates, proteins and lipids) in leaf extract of *Withania somnifera* L.

The results in the Table 1 and its corresponding Graph 1 show amount of carbohydrates, proteins and lipids in leaf extract of the plant *Withania somnifera* L. collected from different geographical locations of Ajmer region, including, Ajmer, Nagaur and Tonk. The results show highest amount of carbohydrates, proteins and lipids in leaves collected

from Tonk, Ajmer and Nagaur respectively. Furthermore, leaves of the plant from different regions contained variable amount of carbohydrates, proteins and lipids with the trend in carbohydrate, proteins and lipids content as follows:
Carbohydrate: Leaf Tonk (39.50±0.14)> Leaf Nagaur (30.78±0.12)> Leaf Ajmer (28.23±0.17).
Proteins: Leaf Ajmer (18.44±0.25)> Leaf Nagaur (14.61±0.88)> Leaf Tonk (12.91±1.58).
Lipids: Leaf Nagaur (24.91±2.47)> Leaf Tonk (21.26±0.87)> Leaf Ajmer (19.21±1.11).

Quantitative analysis to determine amount of primary metabolites (carbohydrates, proteins and lipids) in root extract of *Withania somnifera* L.
The results in the Table 1 and its corresponding Graph 1 show amount of carbohydrates, proteins and lipids in root extract of the plant *Withania somnifera* L. collected from different geographical locations of Ajmer region, including, Ajmer, Nagaur and Tonk. The results show highest amount of carbohydrates, proteins and lipids in roots collected from Ajmer, Ajmer and Nagaur respectively. Furthermore, roots of the plant from different regions contained variable amount of carbohydrates, proteins and lipids with the trend in carbohydrate, proteins and lipids content as follows:
Carbohydrate: root Ajmer (31.11±0.18)> root Nagaur (31.58±0.31)>root Tonk (31.28±0.25)
Proteins: root Ajmer (17.00±0.38)> root Tonk (15.06±1.27)> root Nagaur (11.41±2.23)
Lipids: root Nagaur (22.73±3.18)> root Ajmer (20.21±1.15)> root Tonk (17.76±0.93).

Quantitative analysis to determine amount of secondary metabolites (flavonoids, alkaloids, phytosterols, phenols and tannins) in leaf extract of *Withania somnifera* L.
The results in the Table 1 and its corresponding graph 2 show amount of flavonoids, alkaloids, phytosterols, phenols and tannins in leaf extract of the plant *Withania somnifera* L. collected from different geographical locations of Ajmer region, including, Ajmer, Nagaur and Tonk. The results show highest amount of flavonoids, alkaloids, phytosterols, phenols and tannins in leaves collected from Ajmer, Tonk, Ajmer, Ajmer and Nagaur respectively. Furthermore, the results show that leaves of the plant from different regions contained variable amount of secondary metabolites with the trend in flavonoids, alkaloids, phytosterols, phenols and tannins content as follows:
Flavonoids: Leaf Ajmer (10.22±1.28)> Leaf Nagaur (9.81±0.36)> Leaf Tonk (8.77±0.47).
Alkaloids: Leaf Tonk (3.92±0.82)> Leaf Nagaur (3.14±0.66)> Leaf Ajmer (1.16±0.35).
Phytosterols: Leaf Ajmer (3.17±0.27)> Leaf Nagaur (2.95±0.14)> Leaf Tonk (2.36±0.02)
Phenols: Leaf Ajmer (20.76±1.23)> Leaf Tonk (18.28±0.11)> Leaf Nagaur (12.65±0.81).
Tannins: Leaf Nagaur (8.22±1.16)> Leaf Tonk (6.65±0.12)> Leaf Ajmer (5.27±0.48).

Quantitative analysis to determine amount of secondary metabolites (flavonoids, alkaloids, phytosterols, phenols and tannins) in root extract of *Withania somnifera* L.
The results in the Table 1 and its corresponding graph 2 show amount of flavonoids, alkaloids, phytosterols, phenols and tannins in root extract of the plant *Withania somnifera* L. collected from different geographical locations of Ajmer region, including, Ajmer, Nagaur and Tonk. Similar to what was observed in case of leaf extract, the results show highest amount of flavonoids, alkaloids, phytosterols, phenols and tannins in roots collected from Ajmer, Tonk, Ajmer, Ajmer and Nagaur respectively. Furthermore, roots of the plant from different regions contained variable amount of secondary metabolites with the trend in flavonoids, alkaloids, phytosterols, phenols and tannins content as follows:
Flavonoids: root Ajmer (8.77±1.14)> root Nagaur (7.66±0.82)> root Tonk (5.34±0.28).
Alkaloids: root Tonk (4.11±0.07)> root Nagaur (4.11±0.11)> root Ajmer (2.16±0.05).
Phytosterols: root Ajmer (4.61±0.12)> root Tonk (3.16±0.03)> root Nagaur (3.11±0.05).
Phenols: root Ajmer (16.77±1.11)> root Tonk (15.92±2.13)> root Nagaur (9.34±1.15).
Tannins: root Nagaur (10.45±2.13)> root Tonk (9.11±1.17)> root Ajmer (7.55±0.56).

Antioxidant activity of leaf extracts of *Withania somnifera* collected from different districts
The results in the table 2 show antioxidant activity of different concentrations (20 ug/ml, 40 ug/ml, 60 ug/ml, 80 ug/ml and 100 ug/ml) of leaf extracts of *Withania somnifera* L. collected from Ajmer, Nagaur and Tonk districts of Ajmer regions regions using different solvents (ethanol, water, chloroform and petroleum ether) by DPPH assay. The highest antioxidant activity was observed in leaf extracts collected using ethanol as solvent followed by water, pet ether and chloroform.
The trend in antioxidant activity according to IC 50 values is as follows:
ET (55.22±0.23)>WA (55.22±0.78)>WN (83.61±0.34)>EN (94.08±0.23) >WT (103.20±0.89)> CA (110.87±0.28) >CT (202.53±0.78)>PN (224.01±0.34)>EA (236.48±0.89)>PT (324.79±0.29) >PA (459.20±0.45)> CN (609.57±0.96)

Antioxidant activity of root extracts of *Withania somnifera* collected from different districts
The results in the table 3 show antioxidant activity of different concentrations (20 ug/ml, 40 ug/ml, 60 ug/ml, 80 ug/ml and 100 ug/ml) of root extracts of *Withania somnifera* L. collected from Ajmer, Nagaur and Tonk districts of Ajmer regions using different solvents (ethanol, water, chloroform and petroleum ether) by DPPH assay. The highest antioxidant activity was observed in root extracts collected using ethanol as solvent followed by water, chloroform and pet ether.
The trend in antioxidant activity according to IC 50 values is as follows:
ET (15.73±0.45)>WA (111.58±0.34)>WN (122.40±0.56)>EN (176.52±1.12) >WT (270.31±0.51)> CA (303.28±0.56) >CT (337.72±1.17)>PN (374.45±0.27)>EA (391.63±0.08)>PT (409.45±0.32) >PA (432.49±0.23)> CN (698.34±0.70)

Discussion
The current study compares leaf and root extract of *Withania somnifera* L. collected from different regions of Ajmer district, namely Ajmer area, Nagaur and Tonk in terms of various plant attributes, that include

- Primary metabolites (carbohydrates, proteins and lipids) in leaf and root extracts

- Secondary metabolites (flavonoids, alkaloids, phytosterols, phenols and tannins) in leaf and root extracts
- Antioxidant ability of leaf and root extracts

The results of the study show variability in amount of primary metabolites with the highest amount of carbohydrates, proteins and lipids in leaf extracts from the plant in comparison to root extract. This maybe attributed to the fact that leaves contain higher amount of soluble carbohydrates such as glucose, fructose and sucrose while roots contain higher amount of structural carbohydrates such as cellulose and hemicellulose. In addition to carbohydrates, leaves also contained higher amount of proteins and lipids; out of which, the highest protein content was found in leaves of plant from Ajmer region while highest lipid content was found in leaves of plants from Nagaur region. Similar trend was observed in root extract, where too, highest amount of carbohydrates, proteins and lipids was found in root extract from Tonk, Ajmer and Nagaur region respectively. Primary metabolites contribute to the overall growth and development of plants by fulfilling various important roles, that include

- Apart from serving as important sources of energy and fuelling the various metabolic processes occurring in plants, carbohydrates act as pillars of support and provide structural integrity to the cells by acting as components of cell wall and cell membrane.
- Proteins act as structural components of cellular membranes, organelles and cytoskeleton and catalyze the important biochemical reactions, control gene expression and aid in various signalling pathways.
- Lipids act as reservoir of energy during nutrient starvation and contribute to structural integrity by acting as component of cellular membranes.
- Amino acids act as precursors of structural proteins, hormones and enzymes and contribute to maintenance of signal transduction machinery.
- Organic acids such as citric acid maintain osmotic pressure and aid the plants in adapting to various environmental conditions.
- Chlorophyll traps light energy and aids in biosynthesis of carbohydrates using photosynthesis.
- Water facilitates nutrient uptake, waste removal, maintenance of turgor pressure and provides structural integrity and rigidity to the cell (Pott *et al*, 2019; Caretto *et al*, 2015; Salam *et al*, 2023) [3, 16, 17]

Thereafter, the researchers assessed the amount of secondary metabolites (flavonoids, alkaloids, phytosterols, phenols and tannins) in leaf and root extracts of *Withania somnifera* L. collected from different regions of Ajmer district, namely Ajmer area, Nagaur and Tonk. The results show highest amount of show highest amount of flavonoids, alkaloids, phytosterols, phenols and tannins in leaves and roots collected from Ajmer, Tonk, Ajmer, Ajmer and Nagaur respectively, wherein, comparatively higher amount of flavonoids and phenols was observed in leaves while amount of alkaloids, phytosterols and tannins was higher in root extract. Leaves generally contain higher amount of flavonoids and phenols; since both these secondary metabolites play a crucial role in plant defense against pathogens, UV rays and herbivores. Leaves are directly exposed to sunlight and UV rays, which stimulate higher production of phenols, which consequently prevent plant leaves from deleterious effects of UV radiations. Furthermore, leaves are at higher risk of herbivore attack, and presence of unpleasant taste of phenols makes leaves unpalatable to herbivores, preventing herbivore attack. Presence of phenols and flavonoids prevents microbial growth on leaves. In addition to these, the ability to execute photosynthesis makes leaves metabolically active, augmenting the need for presence of bioactive components such as as flavonoids and phenols in order to maintain cellular integrity (Kumar *et al*, 2020; Kolb *et al*, 2001; Singh *et al*, 2021) [6, 7, 18].

Furthermore, the results showed higher amount of phytosterols, alkaloids and tannins was in plant root extract. Phytosterols from components of cell membranes and provide structural rigidity and integrity to the cells. roots need to possess study cell membranes since they are involved in providing structural integrity to the plants and transport water and nutrients. Therefore, roots act as storage site for phytosterols, because of the high metabolic activity they have to undergo during plant growth and development (Mangiarotti *et al*, 2019; Leshem *et al*, 2013) [9, 13].

Secondary metabolites perform a number of crucial functions in the plant, *Withania somnifera* L., that include

- Withanolides contribute to antioxidant and free radical scavenging activity of the plant *Withania somnifera* L. and also impart adaptogenic nature to the plant.
- Alkaloids such as Withanine possess antimicrobial, anti-inflammatory and sedative effect and aid in providing neuromodulatory effect to the plant extract.
- Steroids present in the plant exhibit potent antimicrobial activity and aid in various processes involved in plant growth and development and various signalling pathways.
- Flavonoids present in the plant act as anti-inflammatory and antioxidant and boost cardiovascular health.
- Phenolics provide protection from UV induced damage and act as antimicrobial and herbivore deterrent.
- Tannins provide wound healing and tissue repair ability to the plant while also contributing to herbivore deterrence and digestive property of the plant.
- Phytosterols contribute to membrane fluidity and stability and aid in regulation of cholesterol levels (De-la-Cruz *et al*, 2013; Li *et al*, 2020; Pang *et al*, 2021; Kumar *et al*, 2018) [4, 8, 10, 15].

Presence of secondary metabolites contributes to antioxidant activity of the plant extracts, which aids in usage of plant extracts in amelioration of a number of chronic ailments characterized by sudden oxidative outbursts (Swallah *et al*, 2020; Jain *et al*, 2019; Maliar *et al*, 2023) [5, 12, 20]. Considering this, in the next part of the study, the researchers determined antioxidant potential of the leaf and root extracts obtained from *Withania somnifera* L. collected from different regions of Ajmer district, namely Ajmer area, Nagaur and Tonk using different solvents (ethanol, water, chloroform and petroleum ether).

Table 1: primary and secondary metabolites in leaf and root extracts

Primary Metabolites			
		Leaves	**root**
Carbohydrate (mg/g.dw)	Tonk	39.50±0.14	31.28±0.25
	Ajmer	28.23±0.17	31.11±0.18
	Nagaur	30.78±0.12	31.58±0.31
Proteins (mg/g.dw)	Tonk	12.91±1.58	15.06±1.27
	Ajmer	18.44±0.25	17.00±0.38
	Nagaur	14.61±0.88	11.41±2.23
Lipids (mg/g.dw)	Tonk	21.26±0.87	17.76±0.93
	Ajmer	19.21±1.11	20.21±1.15
	Nagaur	24.91±2.47	22.73±3.18
Secondary Metabolites			
		Leaves	root
Flavonoids (mg/g.dwQE)	Tonk	8.77±0.47	5.34±0.28
	Ajmer	10.22±1.28	8.77±1.14
	Nagaur	9.81±0.36	7.66±0.82
Alkaloids (mg/g.dwAE)	Tonk	3.92±0.82	4.11±0.07
	Ajmer	1.16±0.35	2.16±0.05
	Nagaur	3.14±0.66	4.11±0.11
Phytosterols (mg/g.dw)	Tonk	2.36±0.02	3.16±0.03
	Ajmer	3.17±0.27	4.61±0.12
	Nagaur	2.95±0.14	3.11±0.05
Phenols (mg/g.dwCE)	Tonk	18.28±0.11	15.92±2.13
	Ajmer	20.76±1.23	16.77±1.11
	Nagaur	12.65±0.81	9.34±1.15
Tannins (mg/g.dwGAE)	Tonk	6.65±0.12	9.11±1.17
	Ajmer	5.27±0.48	7.55±0.56
	Nagaur	8.22±1.16	10.45±2.13

Table 2: Antioxidant activity of leaf extracts

Solvent	**District**	**% Free radical scavenging activity at the given concentrations (µg/ml)**					**IC50 (µg/ml)**
		20	**40**	**60**	**80**	**100**	
Ethanol	Tonk	46.70±0.011	48.67±1.13	51.50±0.15	52.10±0.90	52.91±0.37	55.22±0.23
	Ajmer	42.50±0.07	43.40 ±0.90	45.50 ±0.23	47.77 ±0.89	48.93 ±0.23	110.87 ±0.28
	Nagaur	40.49 ±0.18	44.90±0.87	47.39±1.34	48.33±0.15	49.91±0.08	94.08±0.23
Chloroform	Tonk	14.57±0.24	19.11 ±0.09	22.92 ±1.89	24.59 ±0.27	27.81 ±0.09	236.48 ±0.89
	Ajmer	17.01 ±0.07	18.29±0.03	19.28±0.90	20.14±0.20	21.68±0.23	609.57±0.96
	Nagaur	24.76±0.30	27.85 ±0.045	31.36 ±0.07	32.39 ±0.87	34.58 ±0.89	224.01 ±0.34
Pet ether	Tonk	14.44 ±0.43	18.94±0.65	20.39±0.04	21.98±0.90	24.25±0.56	324.79±0.29
	Ajmer	34.36±1.67	35.26 ±0.23	37.15 ±0.52	38.95 ±0.14	41.35 ±0.34	202.53 ±0.78
	Nagaur	9.08±0.03	13.84±0.45	14.57±0.67	15.72±0.17	17.14±0.78	459.20±0.45
Water	Tonk	37.75 ±1.89	44.47 ±0.07	46.66±0.34	48.33 ±0.28	47.51±0.56	103.20 ±0.89
	Ajmer	40.75±1.14	45.37±0.56	47.90 ±0.98	49.40±0.24	51.50±0.29	83.61±0.34
	Nagaur	44.64±1.12	44.86 ±0.70	47.39±0.51	49.36 ±0.23	51.62±0.45	55.22 ±0.78

Table 3: Antioxidant activity of root extracts

Solvent	**District**	**% Free radical scavanging activity at the given concentrations (µg/ml)**					**IC50 (µg/ml)**
		20	**40**	**60**	**80**	**100**	
Ethanol	Tonk	49.27±0.10	52.06±0.07	52.74±0.65	52.96±1.1	53.64±±0.15	15.73±0.45
	Ajmer	19.75±0.15	22.28±0.43	22.75±0.34	23.82±0.08	27.08±0.16	391.63±0.08
	Nagaur	27.46±0.07	32.18±0.03	35.39±0.27	36.20±0.04	39.12±0.07	176.52±1.12
Chloroform	Tonk	14.35±0.22	17.05±0.76	19.32±0.03	21.72±0.03	23.14 ±0.19	337.72±1.17
	Ajmer	20.48±0.31	22.84±0.04	23.95±0.09	27.08±0.07	28.79±0.15	303.28±0.56
	Nagaur	9.55±0.07	10.54±0.07	11.70±0.32	13.07±0.09	14.27 ±0.07	698.34±0.70
Pet ether	Tonk	15.90±0.11	17.91±0.08	20.09±0.21	21.81±0.08	22.62±0.22	409.45±0.32
	Ajmer	17.44±0.32	18.68±0.89	19.84±0.03	22.49±0.11	23.48 ±0.39	432.49±0.23
	Nagaur	9.38±0.07	13.92±0.54	15.34±0.07	17.44±0.15	18.77±0.69	374.45±0.27
Water	Tonk	42.50±0.09	43.53±0.04	43.92±1.1	44.56±0.17	44.90 ±0.58	270.31±0.51
	Ajmer	45.24±0.65	46.79±0.07	47.51±1.1	48.20±0.23	49.49 ±0.42	111.58±0.34
	Nagaur	38.17±0.32	40.53±0.09	43.40±0.9	44.64±0.27	47.56±0.34	122.40±0.56

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