

Accessing the Qualitative and Bioactive Compounds in the leaves of *Withania somnifera* from Rajasthan India

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Received Date: 15 August 2025 | **Accepted Date:** 25 August 2025 | **Published Date:** 29 August 2025

Citation: Alagbe J. Olujimi (2025), Accessing the Qualitative and Bioactive Compounds in the leaves of *Withania somnifera* from Rajasthan India, *Journal of Clinical and Laboratory Research*, 8(4); DOI:10.31579/2768-0487/189

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Abstract

Medicinal plants have a long tradition as therapeutic agents because they contain numerous bioactive compounds that are considered safe, eco-friendly and have no withdrawal period. These compounds can be influenced by several factors including, age of plant, geographical location, processing method, amongst others. Accessing the bioactive compounds in herbs will help to prevent toxicity and curb the increasing cases of antimicrobial resistance. Results obtained from qualitative assessment showed that *Withania somnifera* leaf extract contained, phenolic compounds, alkaloids, saponins, glycosides, tannins, flavonoids, terpenoids and steroids which possess some pharmacological properties such as – antimicrobial, antioxidant, antifungal, anti-tumor, anti-helminthic, immuno-modulatory, antiviral, hypolipidemic, hepato-protective amongst others. Outcome on Gas Chromatography- mass spectrometry *Withania somnifera* oil revealed a total of 28 constituents representing 98.12 % composition. The major components in the oil were – β -Caryophyllene (25.06 %), δ -2-Carene (16.40 %), Longifolene (10.87 %), 1,8-Cineole (9.90 %), γ -Cadinene (7.63 %), Nonanoic acid (7.58 %) and Acetophenone (5.07 %). It was concluded that *Withania somnifera* oil has a lot of medicinal properties and can be used as natural alternative to antibiotics.

Key words: antimicrobials; phytochemicals; *withania somnifera*; oil; therapeutic; gas chromatography; mass spectrometry

Introduction

Withania somnifera is a highly medicinal evergreen shrub belonging to the family Solanaceae (Chebbac et al., 2023). The plant grows in some part of Africa, Europe and most parts of Asia including India (Rabhi et al., 2019). Phytochemical analysis of methanol and aqueous extracts of *Withania somnifera* aerial parts proves the presence of phytocomponents as flavonoids, tannins, alkaloids, saponins, steroids and terpenoids hence possess medicinal properties like anti-inflammatory (Jayaprakasam and Nair, 2003), hypoglycaemic, anti-tumor, cytotoxic (El-Moussaoui et al., 2020), anti-fertility, abortifacient, hepato-protective (Chebbac et al., 2022), antioxidant, immuno-modulatory, gastro-protective (Bakhtawar et al., 2010), cardio-protective recently its antihypertensive and anti-helminthic activities (Prakash et al., 2004). *Withania somnifera* is used in folklore in the treatment of yellow fever, malaria, diarrhea, cough, diabetes, arthritis, convulsion, respiratory disorders and dermatological infections (Chebbac et al., 2021; Chebbac et al., 2023). The bark is used for the treatment of sexually transmitted infections and immune disorders (Bhattacharya et al., 2001; Elmoussaoui et al., 2021), while the leaf is used as an emollient and for the treatment of skin eruption, stomach ache and diarrhea (Rabhi et al., 2019).

According to Bouchia et al. (2023), essential oil composition from the leaves of *Withania somnifera* revealed β -caryophyllene (20.26 %), Cadinene (18.08 %), Longifolene (11.29 %), Acetophenone (8.58 %), Santolina alcohol (8.25 %), Nonanoic acid (7.73 %), ethyl octanoate (6.35 %) and methyl linoleate (5.17 %) as their most abundant compounds. *Withania somnifera* oil have been suggested to possess antibacterial properties and are capable of inhibiting the activities of *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Candida albicans*, *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium oxysporum*. They have also been reported to exhibit a wide spectrum of pharmacological activities such as infection control, wound healing, pain relief, anti-nausea, anti-inflammation and anti-anxiety (Abdelfattah et al., 2022). However, factors such as specie, age of plant, geographical location, processing method and period/stage of harvesting have been reported to influence the chemical composition of essential oil (Alagbe, 2023). Accessing the qualitative and bioactive compounds in *Withania somnifera* oil will further help to ensure that product from the plant is safe and can also be as natural alternative to antibiotics to curb the incidence of multidrug resistance.

Materials and methods

Research area

The research was carried out at the department of Microbiology at Gandhi College of Agriculture, Rajasthan India. All laboratory procedures were done were done according to the official method of analysis of Association of Analytical Chemist (AOAC, 1997).

Equipment's/reagent required for analysis

Ferric chloride, glacial acetic acid, lead acetate, hydrochloric acid, ammonia solution, Dragendorff's reagent.

Round bottom flask, heating mantle, condenser, clavenger apparatus, seperatory funnel and beaker.

Collection of *Withania somnifera* leaves

Fresh *Withania somnifera* leaves were collected within the premises of Gandhi College of Agriculture in Rajasthan, India and taken to the department of Crop Science, Sumitra Research Institute, Gujarat for proper identification and authentication before it was assigned a voucher number (AG/2024RTC/OA). Collected leaves were sorted, washed with running tap water to remove dirt's and shade dried for 13 days and pulverized using an electric blender before extraction.

Withania somnifera oil Extraction Technique

S/N	Phytochemicals	Method of determination	Result	Reference
1	Alkaloids	5 mL of <i>Withania somnifera</i> oil + 1 % dilute hydrochloric acid + few drops of Dragendorff's reagent.	Orange precipitate was formed	Usman and Usuji, 2007T
2	Flavonoids	5 mL of <i>Withania somnifera</i> oil + concentrated Tetraoxosulphate (VI) acid + 0.5 mL of ammonia solution	Yellow colour was formed	Simlai and Roy, 2012
3	Tannins	5 mL of <i>Withania somnifera</i> oil + few drops of 1% lead acetate.	Yellowish precipitate is observed	Edeoga et al. (2005)
4	Glycosides	5.0 mL of <i>Withania somnifera</i> oil + 1 mL of glacial acetic acid + few drops of ferric chloride solution + drops of Tetraoxosulphate (VI) acid.	Reddish brown colouration at the junction of the two layers and bluish green colour in the upper layer was observed	Edeoga et al. (2005)
5	Saponins	5.0 mL of <i>Withania somnifera</i> oil + 1 mL of distilled water +agitated for 10 minutes	Formation of foam was observed	Nurdiani et al. (2012)
6	Terpenoids	5.0 mL of <i>Withania somnifera</i> oil + 0.3 ml of chloroform + 0.3 ml of concentrated Tetraoxosulphate (VI) acid.	Formation of a reddish brown colour was seen.	Sharma, 2012
7	Phenols	5.0 mL of <i>Withania somnifera</i> oil +1.0 mL of 10% ferric chloride	Formation a greenish brown or black precipitate was observed	Harbone, 1998
8	Steroids	5.0 mL of <i>Withania somnifera</i> oil + 1.0 ml Tetraoxosulphate (VI) acid	Violet to blue or green colour was observed.	Klavina et al. (2015)

Table 1: Qualitative analysis *Withania somnifera* oil.

Extraction of oil was done using stream-distillation method earlier outlined by (Alagbe, 2024). Briefly, 500 g *Withania somnifera* powder was measured into a round bottom flask, placed on a heating mantle and heated at 70°C for 20 minutes the steam produced is collected in a glass condenser then into a round bottom flask. Mixture of oil and water was separated using a laboratory separator to obtain *Withania somnifera* oil. The oil obtained was dried over anhydrous sodium sulphate and stored in vials in a refrigerator prior to analysis. Qualitative analysis of non-bioactive compounds was carried out using standard laboratory procedures as presented in Table 1.

Gas Chromatography – Mass Spectrometry Analysis of Volatile oil

Analysis of bioactive compounds in *Withania somnifera* oil was done using Truce Portable GC-MS (Model 8095A, China) helium was its carrier gas and it was maintained at constant flow of 2.003 mL/min, pressure (1.50 psi), velocity (43.00 cm/sec), temperature (300 °C), total flow (18.09 mL/min) after 5mL of *Withania somnifera* oil was injected into the machine inlet unit of the GC. The mass spectrometer was maintained at an ionization mode (70eV), temperature (230 °C) and scan mode (scanning from m/z 45 to 550 amu at 2.0s/scan rate). Identification confirmation was by comparison of their mass spectra with published spectra (Adams, 2007) and those of reference compounds from the Library of National Institute of Standard and Technology (NIST, 2011) database.

Compounds	Result
Alkaloids	+++
Glycosides	++
Flavonoids	+++
Terpenoids	+++
Steroids	++
Tannins	++
Phenols	+++
Saponins	++

Table 2: Result on the Qualitative analysis of Withania somnifera oil.

Key: ++: Moderately present; +++: Highly present

Peak	Compounds	Reaction time (min)	% volume
1	α -Terpinolene	8.08	0.99
2	β -Pinene	8.16	1.81
3	α -Cedrene	9.79	3.62
4	β -Caryophyllene	10.15	25.06
5	1,8-Cineole	10.32	0.25
6	Cis-9-Hexadecanal	10.90	9.90
7	Trans-2,4-Decadienal	11.81	1.52
8	δ -2-Carene	11.96	16.40
9	13-Tetradec-11-yn-1-ol	12.02	0.03
10	Acetophenone	12.55	5.07
11	2,6,10-Trimethyltetradecane	13.06	1.61
12	2-methyldecahydronaphthalene	13.51	0.27
13	17-Octadecynoic acid	13.84	1.90
14	Nonanoic acid	14.00	7.58
15	1,3,5,8-Undecatetraene	14.33	0.41
16	Longifolene	15.00	10.87
17	Ethylhexadecanoate	15.28	0.08
18	Hexadecanoic acid	15.33	0.12
19	17-Octadecynoic acid	15.92	0.97
20	Isopropyltetradecanoate	16.81	0.02
21	β -Elemene	17.15	0.51
22	Cubenol	17.40	0.05
23	α -Selinene	18.26	0.01
24	Trimethylbenzene	19.82	0.83
25	Isoaromadendrene epoxide	20.06	0.11
26	α -Decene	20.71	0.03
27	Cis-Carvotanacetol	21.07	0.47
28	γ -Cadinene	22.91	7.63
	Total		98.12 %

Table 3: Gas chromatography-mass spectrometry result of Withania somnifera oil.

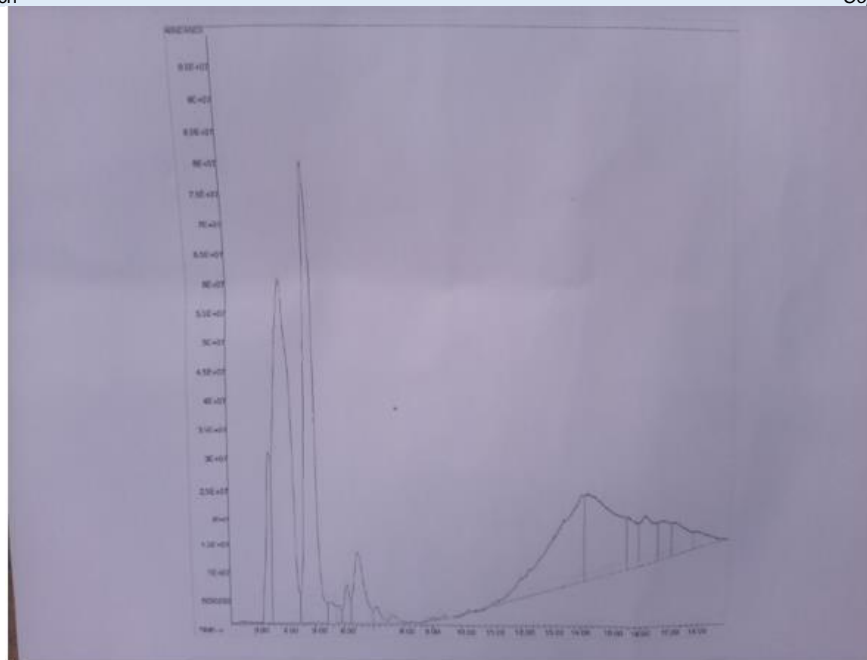


Figure 1: Gas Chromatogram of *Withania somnifera* oil.

Results and discussion

The qualitative analysis of *Withania somnifera* oil is presented in Table 2. Result obtained revealed the presence of eight phytochemicals which includes, alkaloids, terpenoids, saponins, tannins, glycosides, steroids, phenols and flavonoids. This suggests that the extract has several medicinal properties like anti-inflammatory, hypoglycaemic (Alagbe, 2023), anti-fertility, abortifacient, hepato-protective (John, 2024d; Jayaprakasam et al., 2003), cytotoxic, antimicrobial (John, 2024a; Ojediran et al., 2024b), immuno-modulatory (John, 2024b), gastro-protective, antihypertensive (John, 2024c), antitumor amongst others (Davis and Kuttan, 2000; Iuvone et al., 2003). The result obtained in this study was in agreement with the reports of Prakash et al. (2004); Sitansu and Ajay (2011). Alkaloids found in *Withania somnifera* leaf extract has also been examined for its analgesics, anti-nociceptive, anti-inflammatory and antioxidant activities (Ojediran et al., 2024a; Musa et al., 2020). The extract also shows potent antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, *Streptococcus* spp, *Salmonella* spp, *Bacillus cereus*, *Micrococcus luteus*, *Pseudomonas aeruginosa* and *Proteus mirabilis* due to the presence of tannins (Adewale et al., 2021; Omokore and Alagbe, 2019). The high concentration of phenolic compounds in *Withania somnifera* leaf extract justifies why it contains natural antioxidants which have been established to promote health by acting against oxidative stress (Alagbe, 2024; Daniel et al., 2023). Terpenoids and flavonoids have wide range of pharmacological activities including anti-tumor, anticancer, gastro-protective and hypolipidemic (Muritala et al., 2022; Singh et al., 2022). Pharmacologically, steroids have been shown to exhibit hormonal, anti-inflammatory and anti-diarrhea activities (Mamza et al., 2012). Saponins also exhibit antifungal, gastro-protective and hypolipidemic activities (Awa et al., 2012; Manilal et al., 2011). Result of Gas Chromatography- mass spectrometry *Withania somnifera* oil revealed a total of 28 constituents representing 98.12 % composition. The major components in the oil were – β -Caryophyllene (25.06 %), δ -2-Carene (16.40 %), Longifolene (10.87 %), 1,8-Cineole (9.90 %), γ -Cadinene (7.63 %), Nonanoic acid (7.58 %) and Acetophenone (5.07 %). However, Caryophyllene composition observed in this study was lower than 20.26 % reported by Mohammed et al. (2023). Abdelfattah et al. (2022)

also recorded a higher value of 9.04 %, 15.40 % and 12.05 % for Nonanoic acid, Cadinene and Longifolene respectively. El Moussaoui et al. (2020) also recorded a higher concentration of 24.11 % for Caryophyllene concentration in *Withania somnifera* oil. The differences in result could be attributed to processing technique, specie, geographical location as well as age of plant (Singh et al., 2022). In another study, the oil of *Withania somnifera* revealed that (Z)-nerolidol, 2,4- decadienal, α -farnesene, Geranyl acetone, α -Bergamotene, α -Himachalene, Acoradiene, β -Sesquiphellandrene and α -copaene (Bakhtawar et al., 2010). Caryophyllene and γ -Cadinene have also been reported to be isolated from *Mentha piperita* and *Mentha communis* oil and are commonly used for the treatment of skin inflammation and as antibacterial and antifungal agents (Yadegarinia et al., 2006). 1,8-Cineole, α -Terpinolene, Acetophenone, Nonanoic acid and 17-Octadecynoic acid are important sesquiterpene with highly potent antimalarial and antibacterial activity (Alagbe et al., 2025). β -Pinene, β -Elemene, Cubenol, α -Selinene and α -Decene which have been confirmed to be also isolated from *Carica papaya* oil exhibits significant antimicrobial and anti-helminthic activity (Sumathi, 2014). Rabhi et al. (2019) reported Cis-9-Hexadecanal, Trans-2,4-Decadienal, 13-Tetradec-11-yn-1-ol, 6,10- Trimethyltetradecane and 2-methyldecahydronaphthalene to also have antidiarrheal, anti-inflammatory and antioxidant activity (Abdelfattah et al., 2022). Hexadecanoic acid, Ethylhexadecanoate, 1,3,5,8-Undecatetraene, 17-Octadecynoic acid and Isopropyltetradecanoate have reported to have antimicrobial activity, immuno-modulatory, gastro-protective and anticancer properties (Edeoga et al., 2005). Trimethylbenzene, Isoaromadendrene epoxide and Cis-Carvotanacetol have bactericidal to pathogenic organisms including, *Staphylococcus* spp, *Salmonella* spp, *Klebsiella pneumonia*, *Aspergillus* spp, *Escherichia coli* and *Candida albicans* (Bourhia et al., 2019; Chebbac et al., 2022).

Conclusion

It was concluded that *Withania somnifera* oil is rich in bioactive compounds which have several health and medicinal benefit including: antimicrobial, anti-inflammatory, antioxidant, cytotoxic, immuno-stimulatory, hepato-protective, cardio-protective, gastro-protective, dermato-protective, anti-

helminthic, antiviral, antimalarial, analgesics, anticancer, anti-tumor among others. These compounds are generally regarded as safe, eco-friendly and can be utilized as natural alternative to antibiotics.

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