AUCTORES
Globalize your Research

Journal of Clinical and Laboratory Research
Alagbe John Olujimi*

Open Access

Research Article

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

**Alagbe John Olujimi***

Adjunct Lecturer, Department of Biochemistry, Gandhi College of Agriculture, Rajasthan, India.

***Corresponding Author:** Alagbe John Olujimi., Adjunct Lecturer, Department of Biochemistry, Gandhi College of Agriculture, Rajasthan, India.

**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(5); **DOI:10.31579/2768-0487/189**



## 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 Bourchia et al. (2023), essential oil composition from the leaves of Withania somnifera revealed β-caryophyllene (20.26 %), Cadinene (18.08 %), Longifolene (11.29 %), Aceptophenone (8.58 %), Santolina alcohol (8.25 %), Nonanoic acid (7.73 %), ethyloctanoate (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

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.

| S/N | Phytochemicals | Method of determination | Result | Reference |
|---|---|---|---|---|
| 1 | Alkaloids | 5 mL of *Withania somnifera* oil + 1 % dilute hydrochloric acid + few drops of Dragendorff's reagent. | Orange precipitate was formed | Usman and Usuji, 2007T |
| | | | | |
| 2 | Flavonoids | 5 mL of *Withania somnifera* oil + concentrated Tetraoxosulphate (VI) acid + 0.5 mL of ammonia solution | Yellow colour was formed | Simlai and Roy, 2012 |
| | | | | |
| 3 | Tannins | 5 mL of *Withania somnifera* oil + few drops of 1% lead acetate. | Yellowish precipitate is observed | Edeoga et al. (2005) |
| | | | | |
| 4 | Glycosides | 5.0 mL of *Withania somnifera* 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 *Withania somnifera* 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 *Withania somnifera* 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 *Withania somnifera* 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 *Withania somnifera* 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.

| 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-Tetradece-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.

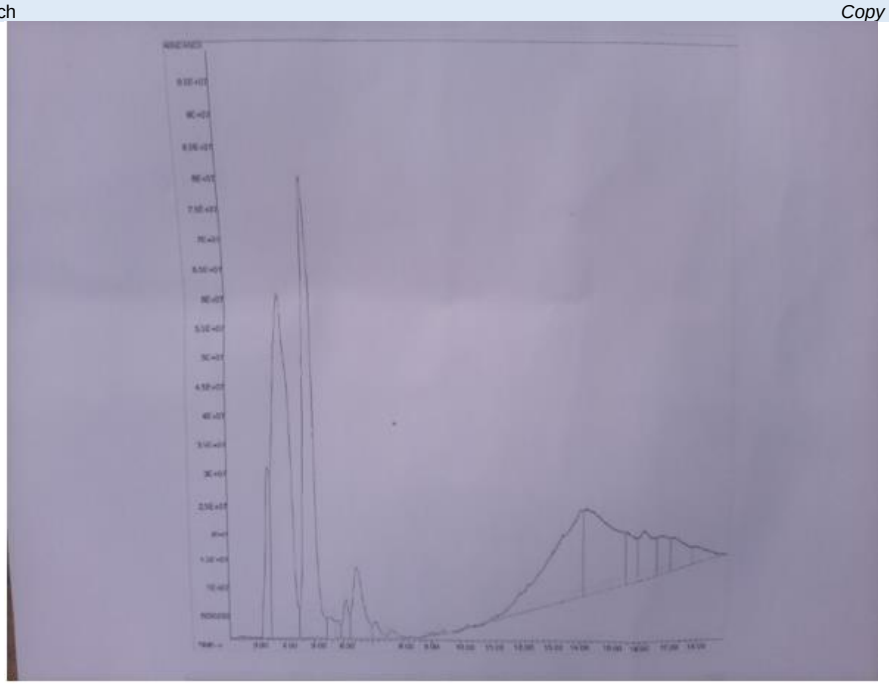

**Figure 1:** Gas Chromatogram of Withania somnifera oil.

## 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 hypo-lipidemic (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-Tetradece-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, Staphylococccus 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.

## References


1. John, A.O. (2024e). Growth performance, haemato-biochemical indices of broiler chicken fed Aristochia indica as a phytogenic feed additive. Cerrado: *Agricultural and Biological Research*, 1(1): 42-53.
2. Alagbe, J.O. (2023). Bioactive compounds in ethanolic extract of Strychnos innocua root using gas chromatography and mass spectrometry (GC-MS). *Drug Discovery*,; 17:e4dd1005.
3. Muritala, Daniel Shittu., Alagbe, J.O., Adebiyi, O.O., Ojediran, T.K. et al. (2022). Growth performance and haematological and serum biochemical parameters of broiler chickens given varied concentrations of Polyalthia longifolia leaf extract in place of conventional antibiotics. *Animal Science and Genetics* 18(2): 57-71.
4. Ojediran, T.K., Emiola, I.A., Durojaye, V and Alagbe, J.O. (2024). Analysis of Kigellia africana fruit's powder antioxidant and phytochemical properties. *Brazilian Journal of Science*, 3(7): 38-49.
5. Ojediran, T.K., Emiola, I.A., Durojaye, V and John Alagbe (2024). Proximate, vitamin and GC-MS profiling of Kigella africana powder. Cerrado: *Agricultural and Biological Research*, 1(1): 13-20.
6. John, A.O. (2024b). Clerodendron splendens leaf extract supplementation in weaner rabbits: impact on growth performance, haematology and intestinal microbial population. Cerrado: *Agricultural and Biological Research*, 1(1): 21-31.
7. John, A.O. (2024c). Effect of coconut shell extract on the growth performance and some haemato-biochemical parameters of broiler chicken. *Brazilian Journal of Science*, 3(6): 82-95.
8. John, A.O. (2024a). Impact of dietary supplementation of Rhamnus prinoides leaf extract on the growth performance, nutrient retention and intestinal microbial count of Japanese quails. *Brazilian Journal of Science*, 3(5): 40-50.
9. John, A.O. (2024d). Effect of performance, serum biochemistry and heamatological components of feeding Japanese quails phytogenic feed additions comprising Megaphrynium macrostachyum leaves. *Brazilian Journal of Science*, 3(5): 51-64.
10. Daniel Nnadozie Anorue, Friday Ubong and Alagbe Olujimi John (2023). Investigating the effects of pawpaw (Carica papaya) essential oil dietary supplementation on the growth performance and carcass characteristics of broilers. Research in: *Agricultural and Veterinary Sciences*, 7(3): 164 - 174.
11. Alagbe, J.O., Shittu, M.D and Tanimomo, Babatunde K. (2022). Influence of Anogeissusleio carpus stem bark on the fatty acid composition in meat of broiler chickens. *European Journal of Life Safety and Stability* 14(22): 13-22.
12. Alagbe, J.O (2022). Use of medicinal plants as a panacea to poultry production and food security: A review. *Gospodarka I Innowacj*e 22: 1-12.
13. Omokore, E.O and Alagbe, J.O. (2019). Efficacy of dried Phyllantus amarus leaf meal as an herbal feed additive on the growth performance, haematology and serum biochemistry of growing rabbits. *International Journal of Academic Research and Development*. 4(3): 97-104.
14. Shittu, M.D., Alagbe, J.O., Alaba, O., Okanlawon, E.O., Adelakun, F.A., et al. (2024). Effect of ginger, garlic and Negro pepper on the gut microbes, gut histomorphometry and pathological assessment of selected organs of broiler chickens. *Association of Deans of Agriculture in Nigerian Universities*, 5: 105-121.
15. Singh Sharma., Alagbe Olujimi John., Liu Xing., Sharma Ram and Kumar Amita (2022). Comparative analysis of ethanolic Juniperus thurifera leaf, stem bark and root extract using gas chromatography and mass spectroemetry. *International Journal of Agriculture and Animal Production*, 2(6): 18-27.
16. Musa, B., Alagbe, J.O., Adegbite Motunrade Betty, Omokore, E.A. (2020). Growth performance, caeca microbial population and immune response of broiler chicks fed aqueous extract of Balanites aegyptiaca and Alchornea cordifolia stem bark mixture. *United Journal for Research and Technology*, 2(2):13-21.
17. Adewale, A.O., Alagbe, J.O., Adeoye, Adekemi. O. (2021). Dietary Supplementation of Rauvolfia Vomitoria Root Extract as A Phytogenic Feed Additive in Growing Rabbit Diets: Haematology and serum biochemical indices. *International Journal of Orange Technologies*, 3(3): 1-12.
18. Alagbe Olujimi John (2024). Novel phytogenics' impact on weaned pigs growth performance, haematology and serum biochemical indicators. *Black Sea Journal of Agriculture*, 7(2): 82-89.
19. AOAC, (1997). Official Methods of Analysis, 16th ed. Association of Official Analysis Chemists International. AOAC International, Washington DC.
20. Mamza, U.T., Sodipo, O.A. & Khan, I.Z. (2012). Gas Chromatography - Mass spectometry (GC - MS) analysis of bioactive components of Phyllanthus amarus Leaves. *International Research Journal of Plant Science*, 3 (10), 208 - 215.
21. Sumathi, R. 2014. Phytochemical analysis and in-vitro antimicrobial activity of aqueous and solvent extracts of Carica papaya against clinical pathogens. *International Journal of Advanced Research in Biological Science* 1.1:73-77
22. Awa, E.P., Ibrahim, S. & Ameh, D.A. (2012). GC/MS Analysis and Antimicrobial Activity of Dimethyl Ether fraction of Methanolic Extract from stem Bark of Annona senegalensis Pers. *International Journal of Phamaceutical Sciences and Research*. 3 (11), 4213 – 4218.
23. Manilal, A., Sujith, S., Sabarathnam, B., Kiran, G.S., Selvin, J., Shakir, C. & Lipton, A. (2011). Biological activity of the red algae Laurencia brandenii. *Acta Botanica Croatica*, 70 (1), 81-90.
24. Davis L, Kuttan G. (2000). Immunomodulatory activity of Withania somnifera. J Ethnopharmacology, 71:193-200.
25. Bourhia, M.; Alyousef, A.A.; Doumane, G.; Saghrouchni, H.; Giesy, J.P.; et al. (2023). Volatile Constituents in Essential Oil from Leaves of Withania adpressa Coss. Ex Exhibit Potent Antioxidant and Antimicrobial Properties against Clinically-Relevant Pathogens. *Molecules*, 28, 2839.
26. of Iranian Mentha piperita L. and Mentha communis L., essential oils. *Phytochemistry* 67:1249-1255

27. Iuvone T, Esposito G, Capasso F, Izzo A. (2003). Induction of nitric oxide synthase expression by Withania somnifera in macrophages. *Life Science*, 72:1617- 1625
28. Prakash J, Gupta SK, Dinda AK. (2004). Withania somnifera root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice. *Nutr Cancer*, 42:91-97.
29. Jayaprakasam B, Zhang Y, Seeram N, Nair M. (2003). Growth inhibition of tumor cell lines by withanolides from Withania somnifera leaves. *Life Science*, 74:125-132.
30. Sitansu, K.V and Ajay k. (2011). Therapeutic uses of Withania somnifera (ashwagandha) with a note on withanolides and its pharmacological actions. *Asian Journal of Pharmaceutical and Clinical Research*, Vol 4, Suppl 1, 2011, 1-4
31. Bourhia, M.; Laasri, F.E.; Aourik, H.; Boukhris, A.; Ullah, R.; et al. (2019). Antioxidant and Antiproliferative Activities of Bioactive Compounds Contained in Rosmarinus officinalis Used in the Mediterranean Diet. Evid. Based Complement. *Altern. Med*., 7623830
32. Chebbac, K.; Ghneim, H.K.; El Moussaoui, A.; Bourhia, M.; El Barnossi, A.; et al. (2022). Antioxidant and Antimicrobial Activities of Chemically-Characterized Essential Oil from Artemisia aragonensis Lam. against Drug-Resistant Microbes. Molecules, 27, 1136
33. El Moussaoui, F.; Jawhari, Z.; Bourhia, M.; Maliki, I.; Sounni, F.; et al. (2020). Withania frutescens: Chemical characterization, analgesic, anti-inflammatory, and healing activities. *Open Chem*., 18, 927–935
34. Jayaprakasam, B.; Nair, M.G. (2003). Cyclooxygenase-2 enzyme inhibitory withanolides from Withania somnifera leaves. *Tetrahedron*, 59, 841–849.
35. Abdelfattah, E.M.; Aimad, A.; Bourhia, M.; Chebbac, K.; Salamatullah, A.M.; et al. (2022). Insecticidal and Antifungal Activities of Chemically-Characterized Essential Oils from the Leaves of Withania frutescens L. *Life*, 12, 88.
36. Rabhi, G.; Arcile, G.; Le Goff, C.; Da Costa, N.; Ouazzani, J. (2019). Neuroprotective effect of CR-777, a glutathione derivative of Withaferin A, obtained through the bioconversion of Withania somnifera (L.) Dunal extract by the fungus Beauveria bassiana. *Molecules*, 24, 4599
37. El Moussaoui, A.; Mechchate, H.; Bourhia, M.; Es-safi, I.; Salamatullah, A.M.; et al. (2021). Glycemic control potential of chemically characterized extract from Withania frutescens L. Roots in severe diabetes-induced mice. *Appl. Sci*., 11, 3998.
38. Bhattacharya, A.; Ghosal, S.; Bhattacharya, S.K. (2001). Anti-oxidant effect of Withania somnifera glycowithanolides in chronic footshock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J. Ethnopharmacol*., 74, 1–6
39. Bakhtawar, S.; Mughal, T.; Naeem, I. (2010). Chemical Composition of the Essential Oil of Withania coagulans. *Asian J. Chem*., 22, 122–126.
40. Chebbac, A.; Moussaoui, E.; Bourhia, M.; Salamatullah, A.M.; Alzahrani, A.; et al. (2021). Chemical Analysis and Antioxidant and Antimicrobial Activity of Essential oils from Artemisia negrei L. against Drug-Resistant Microbes. *Evid. Based Complement. Altern. Med*., 5902851.
41. Bouslamti, M.; Metouekel, A.; Chelouati, T.; El Moussaoui, A.; Barnossi, A.E.; et al. (2022). Antioxidant, Antibacterial, and Antifungal Activities of Polyphenol-Rich Extracts Chemically Characterized by Use of In Vitro and In Silico Approaches. *Molecules*, 27, 8688.
42. Maškovi´c, P.Z.; Manojlovi´c, N.T.; Mandi´c, A.I.; Mišan, A.C.; Milovanovi´c, I.L. et al. (2012). Phytochemi-cal ˇ screening and biological activity of extracts of plant species Halacsya sendtneri (Boiss.). *Dörfl. Hem. Ind*., 66, 43–51.
43. Chebbac, K.; Benziane Ouaritini, Z.; El Moussaoui, A.; Chebaibi, M.; Salamatullah, A.M.; et al. (2023). In Vitro and In Silico Studies of Antimicrobial, and Antioxidant Activities of Chemically Characterized Essential Oil of Artemisia Flahaultii L. (Asteraceae). *Life*, 13, 779
44. Klavina, L., Springe, G., Nikolajeva, V., Martsinkevich, I., Nakurte, I, et al., (2015). Chemical composition analysis, antimicrobial activity and cytotoxicity screening of moss extracts (moss phytochemistry). Molecule, 20, 17221-17243
45. Harbone, J.B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis. 3rd edn, Chapman and Hall, London, pp302.
46. Sharma, R.R. (2012). Preliminary phytochemical screening of some indigenous medicinal plant leaves extract in regulation of antidiabetic activity. *Science Research Reporter*, 2 (3), 307- 310.
47. Nurdiani, R., Firdaus, M. & Prihanto, A.A. (2012). Phytochemical and pharmacological studies of ethanolic extract from the leaf of mangrove plant (Rhyzophoramucronata) from Porong River Estuary. *Journal of Basic Science and Technology*, 1 (2), 27-29.
48. Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4 (7), 685-688.
49. Simlai, A. & Roy A. (2012). Analysis of and correlation between phytochemical and antimicrobial constituents of Ceriops decandra, a medicinal mangrove plant, from Indian Sundarban estuary. *Journal of Medicinal Plants Research*, 6 (32), 4755-4765.
50. Usman, H. & Usuji, J.C. (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of Newbouldia leavis. *African Journal of Traditional Products*, 4, 476-480
51. Adams, R. P. (2007). Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry, 4th Edition, Allured Publication Corporation, Carol Stream, Illinois

**DOI:10.31579/2768-0487/189**