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# Gas Chromatography-mass Spectrometry Analysis and Antimicrobial Activity of *Withania somnifera* Methanol Extract

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**Across the globe, human civilizations share knowledge about the therapeutic potential of plants. Ashwagandha (*Withania somnifera* (L.) Dunal) is frequently used in traditional medicine, particularly in the Jazan province of Saudi Arabia. This study aims to evaluate the antimicrobial efficacy of the leaf extract from this plant. *Withania somnifera* was collected from Jazan Province. Methanol-water extract was prepared and tested for its antimicrobial capacities. *Escherichia coli*, *Staphylococcus aureus*, *methicillin-resistant Staphylococcus aureus*, and *Candida albicans* fungus were the subjects of this investigation. In addition, its chemical composition was explored through Gas chromatography-mass spectrometry. Results proved that the extract has an important antimicrobial effect against the tested microorganisms, compared to tetracycline. Besides, Gas chromatography-mass spectrometry analysis revealed several bioactive molecules within this extract. Amongst, an Amphetamine-like compound was detected. This may explain the antidepressant effect of this plant. These findings illustrate the effectiveness of this plant in treating a variety of illnesses, including skin conditions, in the Jazan community. The outcomes of this study argue the necessity of further research on *Withania somnifera*, endemic to the province of Jazan, to identify its bioactive compounds and convert them into pharmaceutical products.**

**Keywords:** Antibacterial, Amphetamine-like, effectiveness, Jazan, *Withania somnifera*.

Biomedical research has been dominated in recent decades by the hunt for bioactive chemicals. Plants are among the major biomasses that contain these active components[1]. Plant bioactive chemicals provide a wide range of biological actions essential for preserving human health, including antibacterial, anticarcinogenic, antiallergenic, anti-inflammatory, and antimutagenic qualities. Numerous non-communicable diseases, (such as autoimmune, inflammatory, cardiovascular, cancer, metabolic, and neurodegenerative disorders), can also be prevented or treated with the use of

these qualities. Identifying these components and demonstrating their beneficial effects on health are two of the most popular scientific endeavours[2-4].

The plant *Withania somnifera* is a member of the Solanaceae family. Dunal. It was first reported in A.P.de Candolle, Prodr. In 1852. This plant is a subshrub or shrub that grows in the subtropical biome. It is native to several countries including Saudi Arabia and Yemen[5]. Most people refer to it as ashwagandha. This plant's roots and leaves are used in traditional medicine in various ways. They provide care for a wide range of disorders, infectious and non-infectious.

Scientific research reported *Withania somnifera* pharmacotherapeutics values. Ayurvedic and Unani medical systems utilized ashwagandha root as an adaptogen. Their extract helps to adjust and cope with several stressors[6-9].

Furthermore, a comparative review of pertinent in-vitro, in-vivo, and clinical studies demonstrated the powerful bioactivity of *W. somnifera* phytochemicals as antimicrobial, anti-diabetic, hepatoprotective, hypoglycaemic, hypolipidemic, cardio-protective, spermatogenic agents as well as immunomodulatory, anti-inflammatory, apoptotic, and anti-cancer capacities[10].

In Jazan, this plant is called ABAB. In this study, investigators noted the efficacy of ashwagandha leaves in treating wounds and acne in their local community. Therefore, their current study aims to analyze the *Withania somnifera* leaf methanol extract's antibacterial activity and its chemical makeup.

## MATERIALS AND METHODS

The plant extract preparation, antimicrobial activity, and GCMS analysis were carried out according to a previous study[11].

**Plant extract preparation**

In November 2023, whole plants were gathered from Jazan, Saudi Arabia. These plants were identified by a botanist from the Jazan University Faculty of Science's Biology Department. After removing the suspended dirt, the herbs were dried in the shade. Next, an electrical grinder was used to ground the dried materials. 240 g of dried powder matter was extracted by constantly blending 440 ml water-methanol (v:v) for 72 hours at room temperature while shaking it periodically. The filtrate's methanol evaporated, and the leftover methanol extract was stored at 4°C.

**Gas chromatography mass-spectroscopy (GC-MS) analysis**

The chemical constituents in the *Withania somnifera* were detected utilizing a Thermo Scientific GC-MS fitted with an AS 3000 auto sampler, trace Ultra GC and ISQ detector. Methanolic extract of the sample (10 mg; wet weight) was weighed, diluted with 10 mL of methanol, vortex-mixed (10 min), filtered through 0.2 µ nylon filter, and injected 2 µL of sample in the GC-MS for analysis with injection port set at 2500C in split less mode. The separation was achieved in TR-5MS capillary column (30 m x 0.25 mm ID x 0.25 µm) using Helium as mobile phase at 1.2 mL/min. The GC was initially configured with a ramping program that was initially set at 70°C for 10 minutes. Subsequently, the temperature was increased to 170°C and 290°C at a rate of 15°C/min, with a holding duration of 20 minutes at each stage. The total run time was 65 minutes. The total ion chromatogram was recorded after 4 minutes of delay to eliminate the solvent signals to detect the molecular masses between 50-650 amu using the ISQ kept at 70 eV with 300°C and 290°C temperatures of ion source and MS transfer line respectively. The chemical constituents of Withania somnifera extract were determined using software tools such as matching and reverse matching factors (SI and RSI) with a set value of above nine hundred units. Xcalibur software was employed to calculate the percent relative area peak, by dividing its peak area by the absolute area of all the peaks.

**Antimicrobial activities**

This study used DMSO to dissolve the plant extract. Its antimicrobial capacities were investigated using the Agar-well diffusion method. *Escherichia coli*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus,* and *Candida albicans* fungus were the subjects of this investigation.

Agar plates and potato dextrose agar medium were used to inoculate the bacterial strain and the fungi, respectively. Wells were pounded into the inoculated agar medium using sterile Pasteur pipettes. Wells were filled with varying amounts of the extract (2.5 ug/mL, 5 ug/mL, and 10 ug/mL). Finally, antimicrobial activity was assessed using

inhibition zone diameters (IZD) during a 24-hour incubation period at 37°C for bacteria and 28°C for fungi, respectively. Tetracycline (30ug Discs) served as the assay's positive control.

## RESULTS

Figure 1 represents the GCMS chromatogram of *Withania somnifera* leaf extract. The analysis of the present study revealed around 140 compounds with different similarity indexes.

Table 1 represents the antimicrobial activity of this extract. Compared to tetracycline, this extract exhibits a high growth inhibition against *Candida albicans* and *Escherichia coli.* However, the extract-induced *Staphylococcus aureus* growth inhibition exceeds tetracycline only at 10 ug/mL. Finally, *Withania somnifera* extract ability against *Methicillin-resistant Staphylococcus aureus* was less than tetracycline activity.

## DISCUSSION

According to this research result, *Withania somnifera* leaf extract contains 140 components. Table 2 enumerates the secondary phytochemical metabolites in this extract and their biological activities. Previous research reports eighty-two distinct phytochemical peaks during this plant's reproductive cycle[12].

The secondary phytochemical metabolites in this extract analysis have a range of significant biological functions. Strong antioxidant capacity is exhibited by 5-hydroxymethylfurfural and 2.3-dihydro-3.5-dihydroxy-6-methyl-4H-Pyran-4-one. 5-Hydroxymethylfurfural should be

**Table 1.** Antimicrobial activity of *Withania somnifera* extract

| | Inhibition Zone (mm) | | | |
|---|---|---|---|---|
| | Tetracycline | 10 ug/mL | 5 ug/mL | 2,5 ug/mL |
| *Escherichia coli* | 15 | 30 | 20 | 18 |
| *Staphylococcus aureus* | 15 | 29 | 15 | 0 |
| Methicillin-resistant *Staphylococcus aureus* | 18 | 18 | 14 | 15 |
| *Candida albicans* | 12 | 35 | 30 | 15 |

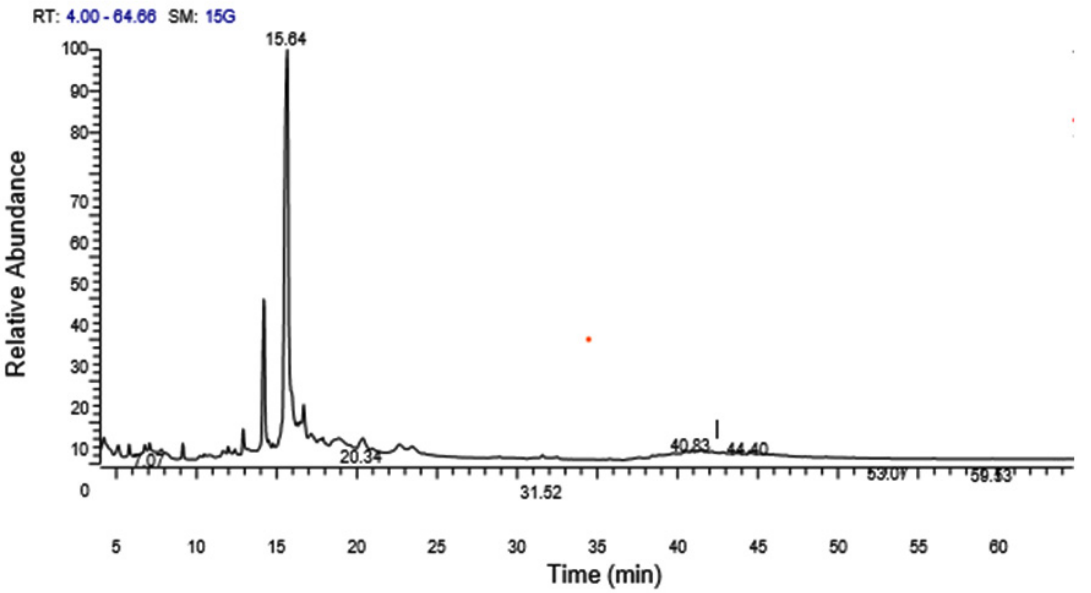


**Fig. 1.** *Withania somnifera* GCMS chromatogram

**Table 2.** Main *Withania somnifera* leaf extract secondary phytochemical metabolites according to their peak surface percentages

| | Area % | RT | Compound Name | Molecular Formula | Biological activity |
|---|---|---|---|---|---|
| 1 | 52.54 | 15.64 | 5-Hydroxymethylfurfural | $C_6H_6O_3$ | Antioxidant and Antiproliferative |
| 2 | 14.48 | 14.19 | 2.3-dihydro-3.5-dihydroxy-6-methyl-4H-Pyran-4-one | $C_6H_8O_4$ | strong antioxidant |
| 3 | 3.33 | 16.67 | 2-Amino-2-cyano-4-methylpentanethioamide | $C_7H_{13}N_3S$ | antibacterial |
| 4 | 2.83 | 4.23 | Furfural | $C_5H_4O_2$ | antifungul |
| 5 | 2.36 | 12.9 | 5-Methyl-2-pyrazinyl | $C_6H_8N_2O$ | Antileishmanial |
| 6 | 2.3 | 18.83 | Desulphosinigrin | $C_{10}H_{17}NO_6S$ | Anti-Inflammatory Hepatoprotective |
| 7 | 1.07 | 9.14 | 2.4-Dihydroxy-2.5-dimethyl-3(2H)-furan-3-one | $C_6H_8O_4$ | flavoring for foods, beverages. and cosmetics |
| 8 | 1.03 | 6.78 | 1-Nitro-2-acetamido-1.2-dideoxy-d-mannitol | $C_8H_{16}N_2O_7$ | antioxidant |
| 9 | 1.03 | 6.78 | Pentanol. 5-amino- | $C_5H_{13}NO$ | Anticancer |
| 10 | 0.91 | 11.96 | 4-Amino-1.5-pentandiol | $C_7H_{13}NO_4$ | Anticancer |
| 11 | 0.91 | 5.79 | methoxy-phenylOxime | $C_8H_9NO_2$ | Antibacterial |
| 12 | 0.83 | 11.64 | Amphetamine | $C_9H_{13}N$ | central nervous system stimulant |
| 13 | 0.55 | 12.38 | ç-Guanidinobutyric acid | $C_5H_{11}N_3O_2$ | anti inflammatory |
| 14 | 0.55 | 12.38 | Furaneol | $C_6H_8O_3$ | flavoring agent |
| 15 | 0.02 | 12.67 | Guanosine | $C_{10}H_{13}N_5O_5$ | Neuromodulator |

developed as a novel natural antioxidant with potential applications in cancer chemoprevention, as demonstrated by Zhao[13]. The antibacterial properties of *Withania somnifera* leaf extract are attributed to furfural, 5-methyl-2-pyrazinyl, and 2-amino-2-cyano-4-methylpentanethioamide[3,14,15]. Additional compounds found in this extract include the central nervous system stimulant amphetamine and neuromodulator guanosine. These results validate the use of *Withania somnifera* to cure neurological and neuropsychological diseases[16].

Furthermore, this study was interested in the antimicrobial activity of this extract. A comparison of the inhibition zones' diameters of the extract and the antibiotic revealed that this extract has strong antimicrobial activities. This result is in line with other studies[17-19]. Previous research explored acetone, water, and ethanol extract of *Withania somnifera* in different parts (roots and leaves). They demonstrated antibacterial activity on the following bacteria; *Escherichia coli*, *Enterobacter sakazakii*, *Klebsiella pneumonia, Staphylocuss aureus, Staphylocuss epidermis, Streptococcus pyogenes, Proteus mirabilis*, and *Pseudomonas aeruginosa*[20-22]. However, only acetone extract had activity on fungal isolate *Trichophyton violaceum*[23]. Taken all together, these results justify the effectiveness of this plant in helping the people of Jazan to treat their dermal problems.

## CONCLUSION

This study was carried out as a student's graduation project. It confirmed the importance of *Withania somnifera.* As presented previously, students collected fresh *Withania somnifera* from their surroundings at Jazan province, southwest of the Kingdom of Saudi Arabia on the coast of the Red Sea. This study elucidates that more research should be carried out to investigate this plant.

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The author(s) do not have any conflict of interest.

**Data Availability Statement**

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**Ethics Statement**

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

**Informed Consent Statement**

This study did not involve human participants, and therefore, informed consent was not required.

**Clinical Trial Registration**

This research does not involve any clinical trials

**Authors' Contribution**

Saida S. Ncibi : Conceptualization, Methodology, Writing – Original Draft; Heba S. Abd-alrahmn - Experiment Conducting; Jawaher A.G. Sahhari - Experiment Conducting; Al Hanouf M. Thubab - Experiment Conducting; Khadijah A.A. Shawk - Experiment Conducting; Weam A. Gharwai - Experiment Conducting; Atyaf A. Hakami - Experiment Conducting; Rehab A. Dawoud - Writing Reviewing; Zia ur Rehman - Experiment Conducting and Writing; Mabrouk Abu Zaid Mabrouk - Experiment Conducting

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