International Journal of Secondary Metabolite
2024, Vol. 11, No. 1, 63–77
https://doi.org/10.21448/ijsm.1256932
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

# GC-MS profiling of anticancer and antimicrobial phytochemicals in the vegetative leaf, root, and stem of *Withania somnifera* (L.) Dunal

**Lali Lingfa**[1], **Aravinda Tirumala**[2], **Srinivas Ankanagari**[1*]

[1]Department of Genetics and Biotechnology, University College of Science, Osmania University, Hyderabad -500 007 (T.S), India
[2]Department of Botany, Nagarjuna Government College, Mahatma Gandhi University, Nalagonda - 508 001 (T.S). India

**ARTICLE HISTORY**
*Received: Feb. 27, 2023*
*Accepted: Dec. 15, 2023*

**Keywords:**
Herbal medicine,
GC-MS,
Antimicrobial,
Anticancer,
*Withania somnifera*.

**Abstract:** *Withania somnifera* has been used for a long time in traditional medicine. Its crude extract, dried powder, and purified metabolites from mature plants have shown promising therapeutic potential. To further investigate its potential, the detection of phytochemicals with anticancer and antimicrobial properties in the vegetative stage is essential. Hence, this study was done to identify phytochemical constituents using GC-MS analysis for anticancer and antimicrobial activities in the vegetative stage from methanolic extracts of stem, leaf, and root in *W. somnifera*. The air-dried plant parts were extracted with methanol at low pressure to concentrate using a rotary evaporator at 40°C. To identify phytochemicals, Shimadzu GCMSQP2010, Japan, was used with the NIST107.LIB database. The GC-MS identified 35 unique phytochemical peaks at the vegetative stage in *W. somnifera*. In leaves, the antibacterial phytochemicals included cyclotrisiloxane, hexamethyl, with a high abundance, and cyclohexasiloxane, dodecamethyl, with the least abundance. In roots, the phytochemicals 2,2-dimethoxybutane, with high abundance, and cathinone, with least abundance, were found to have antibacterial properties, whereas trans-2,3-epoxyoctane, with high abundance, and 2,2-dimethoxybutane, with least abundance, were found to have anticancer properties. In stem, the antibacterial phytoconstituents octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl, and benzenemethanol, alpha.-(1-aminoethyl), were found to be the most abundant and least abundant, respectively, while arabinitol and pentaacetate had both anticancer and antibacterial activities. At the vegetative stage, GC-MS studies of stem, leaf, and root parts revealed the occurrence of potential phytochemicals for antibacterial and anticancer activities in *W. somnifera*.

## 1. INTRODUCTION

*Withania somnifera* (L.) Dunal, a significant Ayurvedic medicinal plant with a variety of therapeutic uses and activities, is a member of the Solanaceae family and is generally referred to as ashwagandha (Dutta *et al.*, 2019). Plant extract and its active constituents from the complete plant, as well as stems, roots, and leaf parts, have been used in the treatment of a broad

*CONTACT: Srinivas Ankanagari ✉ asrinivas@osmania.ac.in Department of Genetics and Biotechnology, University College of Science, Osmania University, Hyderabad-500 007 (T.S), India

e-ISSN: 2148-6905 / © IJSM 2024

range of ailments in humans (Altemimi *et al.*, 2017). Studies indicate that Ashwagandha has been used for a long time as a tonic, in stress management, in improving cognitive health, in decreasing depression, and in lowering cortisol and blood sugar levels (Salve *et al.*, 2019).

This plant has anti-arthritic, anti-inflammatory, anti-epileptic, anti-coagulant, anti-oxidant, anti-depressant, anti-pyretic, and anti-diabetic effects. It also has palliative properties for growth promotion, analgesia, rejuvenation, and regeneration. The plant is found to positively influence metabolism, act as anti-serotogenic, and have other vital properties (Saidulu *et al.*, 2014). Despite its multidrug constituent and medical use since time immemorial, *W. somnifera* still needs to be extensively studied in terms of antimicrobial and anticancer properties, as well as phytochemical correlation (Musher *et al.*, 2021). Moreover, the herbal products of Ashwagandha sold on the market do not adequately explain the phytochemical constituents of the plant and its medicinal properties, as well as the information about the number of bioactive constituents, the reason for harvesting the selected parts of the plant, and the correct developmental stages of the plant that yield the best products (Salemm *et al.*, 2020).

Hence, examining the phytochemical composition of *W. somnifera* at various phases of growth might shed light on how bioactive components alter in plants as they age. Better therapeutic effectiveness may be ensured by using it to help choose the appropriate developmental stage that produces the maximum concentration of the targeted chemicals. Thus, the investigation of *W. somnifera* bioactive components at various developmental stages using GC-MS is a useful method for understanding the therapeutic qualities of plants, refining cultivation techniques, and guaranteeing product quality. According to prior research, *W. somnifera* should be harvested (Kaur *et al*., 2018; Afewerky *et al*., 2021), depending on its dry weight, harvesting period, and mature plant phytochemical profile (Salemm *et al.*, 2020). However, the phytochemical profile of *W. somnifera* based on plant vegetative growth and developmental phases currently has a gap. Thus, in this study, using GC-MS, the phytochemical constituents at the vegetative stage of the *W. somnifera* stem, leaf, and root parts were identified.

## 2. MATERIAL and METHODS

### 2.1. Plant Authentication and Material Collection

*W. somnifera* seeds were obtained from Zooqa Herbs in Chennai, Tamil Nadu. In the natural soil conditions, the seeds were sown and grown at the Department of Genetics, Osmania University, Hyderabad, Telangana. The plant authentication of *W. somnifera* was done by Dr. A. Vijaya Bhaskar Reddy, Botany Department, Osmania University, Hyderabad. The plant material was placed in the Botany Department herbarium, Osmania University, Hyderabad, with the voucher number: GEN/OU/001-2018-HY.

### 2.2. Plant Parts Methanolic Extraction

The *W. sominifera* stem, root, and leaf parts were ground coarsely extracted and in a Soxhlet apparatus for 24 hours with methanol (100 ml), followed by air drying. In a rotary evaporator maintained at 40 °C, the extract was concentrated with reduced pressure to produce a semi-solid viscous mass.

### 2.3. Instrumentation and Sample Analysis in GC-MS

Shimadzu model GCMSQP2010, Japan, was used. The GC-MS system was equipped with the injection port (SPL-1) and injection heat port (INJ-1) to analyse the phytochemicals in the leaf, stem, and root methanolic extracts of *W. somnifera*. An ion source temperature of 230°C and an interface temperature of 250°C were established for a mass-selective detector. A capillary column ZB-5 measuring 30.0 m in length, 0.32 mm in diameter, and 0.25 µm in film thickness was employed in MS analysis running at 70 eV in the electron impact mode. With a 1µl injection volume and a 1.71 mL/min flow rate, the carrier gas 99.9% helium was utilized in a split-less injection (50:1 ratio of split) mode at 250 °C injection temperature. The injection was carried out at a constant linear speed of 47.1 cm/sec, a purge flow of 3 mL/min, and a total flow

of 90.0 mL/min. Initially, 40 °C was maintained in the oven for a duration of one minute. It was elevated to 300 °C gradually at a rate of 10 °C per minute. A total run time of 37 minutes was set for the sample. The mass spectrum range was set at 0–1000 m/z.

### 2.4. Identification of Phytochemicals

The identification of compounds was done by matching spectra with National Institute of Standards and Technology database library (NIST107.LIB) compounds to determine their names, structures, and molecular weights. Retention time (RT) for GC was used to determine components, and MS fragment interpretation was done by comparison with the NIST107.LIB database.

## 3. RESULTS

### 3.1. GC-MS of Vegetative Leaf

In *W. somnifera* vegetative leaf methanol extract, GC-MS identified eighteen unique compounds, as shown in Figure 1. The chromatogram peaks, RT, name of the compound, area of peak, and molecular formulae are presented in Table 1. It indicates presence of compounds ethane, 1,2-dichloro-1-ethoxy- (39.96%); dextroamphetamine (14.79%); 1,3,5-cycloheptatriene (4.29%); propane, 2,2-dimethoxy- (3.87%); alpha.-acetyl-N,N-dinormethadol (3.39%); tartronic acid, 4-(dimethylethylsilyl)phenyl-, dimethyl ester (2.72%); cyclotetrasiloxane, octamethyl- (2.67%); 1,2-Dihydro-2,4-diphenyl-quinazoline (2.4%); 3-ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane (1.6%); 1H-indole-2,3-dione, 3-(O-ethyloxi), 1-(tert-butyldimethylsilyl)-5-chloro-, (1.95%); trimethylsilyl ester, 3-methyl-2-trimethylsilyloxy-, benzoic acid (1.71%); cyclotrisiloxane, hexamethyl-(1.69%); norepinephrine, N-(trifluoroacetyl) -N,O,O',O''- tetrakis (trimethylsilyl)- (1.43%); 1,2,3,5,6,7,8,8a - octahydro-4-trimethyl, 1,2-cinnolinedicarboxylic acid (1.36%); silane, [(1,3-diphenyl-1-butenyl)oxy]trimethyl- (1.3%); cycloheptasiloxane, tetradecamethyl- (1.13%); (+)-2-Aminoheptane (0.2%); cyclohexasiloxane, dodecamethyl- (0.17%); and, N-acetyl-2-methylamphetamine (0.12%).

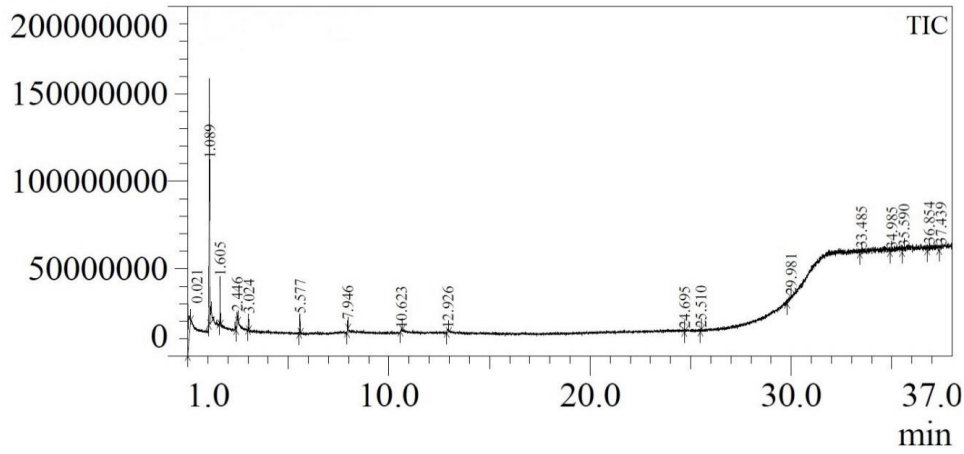


**Figure 1.** GC-MS chromatogram of methanol-extracted vegetative leaves from *Withania somnifera*, where the x-axis represents run time and y-axis represents abundance.

In *W. somnifera* vegetative leaf methanolic extracts, six compounds with antimicrobial activities were identified through a GC-MS study. The chromatogram peaks, area, compound name, RT, molecular formulae, and therapeutic activity are presented in Table 1. These phytochemicals are 1,3,5-cycloheptatriene (4.29%); cyclotrisiloxane, hexamethyl- (1.69%); cyclotetrasiloxane, octamethyl- (2.67%); N- (trifluoroacetyl)-N,O,O',O' tetrakis (trimethylsilyl) norepinephrine (1.43%); dodecamethyl-cyclohexasiloxane (0.17%); cycloheptasiloxane, tetradecamethyl- (1.13%). There were no proven anticancer compounds identified in the vegetative-stage leaf methanolic extracts of *W. somnifera*.

**Table 1.** GC-MS identified phytochemicals in *W. somnifera* vegetative leaf methanol extracts.

| Peaks | RT | Compound name | Formulae | m/z | Peak area% | Phytochemical group | Therapeutic activity | References |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.021 | Dextroamphetamine | $C_9H_{13}N$ | 44.10 | 14.79 | Flavonoid | NA | |
| 2 | 1.089 | Ethane, 1,2-dichloro-1-ethoxy- | $C_4H_8Cl_2O$ | 34.70 | 39.96 | Organohalogen | NA | |
| 5 | 1.605 | Propane, 2,2-dimethoxy- | $C_5H_{12}O2$ | 73.10 | 3.87 | Flavonoid | NA | |
| 6 | 2.446 | 1,3,5-Cycloheptatriene | $C_7H_8$ | 91.15 | 4.29 | Terpenoid | Antibacterial | Yunnikova *et al.*, 2014. |
| 7 | 3.024 | Cyclotrisiloxane, hexamethyl- | $C_6H_{18}O_3Si_3$ | 207.00 | 1.69 | Terpenoid | Antibacterial | Dahpour *et al.*, 2012. |
| 8 | 5.577 | Cyclotetrasiloxane, octamethyl- | $C_8H_{24}O_4Si_4$ | 281.15 | 2.67 | Terpenoid | Antibacterial | Keskin *et al.*, 2012. |
| 9 | 7.946 | N,O,O',O' tetrakis (trimethylsilyl) norepinephrine, N-(Trifluoroacetyl) - | $C_{22}H_{42}F_3NO_4Si_4$ | 73.15 | 1.43 | Alkaloid | Antibacterial | Soliman *et al.*, 2016. |
| 10 | 10.623 | Cyclohexasiloxane, Dodecamethyl-, | $C_{12}H_{36}O_6Si_6$ | 73.10 | 0.17 | Alkaloid | Antibacterial | Moustafa *et al.*, 2013. |
| 11 | 12.926 | Tetradecamethyl-, cycloheptasiloxane, | $C_{14}H_{42}O_7Si_7$ | 73.15 | 1.13 | Alkaloid | Antibacterial | Prasathkumara *et al.*, 2021. |
| 12 | 24.695 | (+)-2-Aminoheptane | $C_7H_{17}N$ | 44.10 | 0.2 | Alkaloid | NA | |
| 13 | 25.51 | N-Acetyl-2-methylamphetamine | $C_{12}H_{17}NO$ | 44.10 | 0.12 | Alkaloid | NA | |
| 14 | 29.981 | 1,2-Dihydro-2,4-diphenyl-quinazoline | $C_{20}H_{16}N_2$ | 207.10 | 2.4 | Alkaloid | NA | |
| 15 | 33.485 | 1-(tert-butyldimethylsilyl)-5-chloro-, 3-(O-ethyloxi, 1H-Indole-2, 3-dione, | $C_{16}H_{23}ClN_2O_2Si$ | 208.05 | 1.95 | Alkaloids | NA | |
| 16 | 33.79 | Alpha.-Acetyl-N,N-dinormethadol | $C_{21}H_{27}NO_2$ | 44.10 | 3.39 | Terpenoids | NA | |
| 17 | 34.985 | [(1,3-diphenyl-1-butenyl)oxy]trimethyl-, silane | $C_{19}H_{24}OSi$ | 281.10 | 1.3 | Terpenoids | NA | |
| 20 | 35.48 | Trimethylsilyl ester, benzoic acid, 3-methyl-2-trimethylsilyloxy- | $C_{14}H_{24}O_3Si_2$ | 281.10 | 1.71 | Phenolic | NA | |
| 21 | 35.59 | 3-Ethoxy-1,1,1,5,5,5 - hexamethyl -, 3 - (trimethylsiloxy) trisiloxane | $C_{11}H_{32}O_4Si_4$ | 208.05 | 1.6 | Phenolic | NA | |

### 3.2. GC-MS of Vegetative Root

In *W. somnifera* vegetative root methanol extracts, GC-MS identified seventeen compounds, as shown in Figure 2. In Table 2, the peaks RT, compound name, area, and molecular formulae are presented. The predominant compounds found to be were: trans-2,3-epoxyoctane (84.78%); - 3,5,5-tris (trimethylsiloxy) tetrasilo, 3 – isopropoxy - 1,1,1,7,7,7- hexamethyl (2.09%); 2, 2-dimethoxybutane (0.44%); hexadecamethyl-, cyclooctasiloxane, (0.4%); 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-, hexasiloxane (0.26%); ethyl ester, benzeneacetic acid, 3-methoxy-4-[(trimethylsilyl)oxy]- (0.19%); 3,5-dimethyl-, (3,5-dimethylphenyl)methyl ester, benzoic acid (0.18%); benzoxazolinone, 4,5 - dibromo - 6 -chloro - 2 - (0.18%); 1,1,3,3,5,5-hexamethyl-, trisiloxane (0.17%); silane, methyltripropoxy- (0.14%); pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl- (0.14%); 2-propanone, 1-methoxy-(0.07%); cyclooctasiloxane, hexadecamethyl- (0.4%); allyl(methoxy)dimethylsilane (0.03%); cathinone (0.03%); 5-chloro-6-methyl-, spiro[2.3]hexan-4-one (0.02%); and, 1,3,2-dithiaborinane, 2-ethyl- (0%).

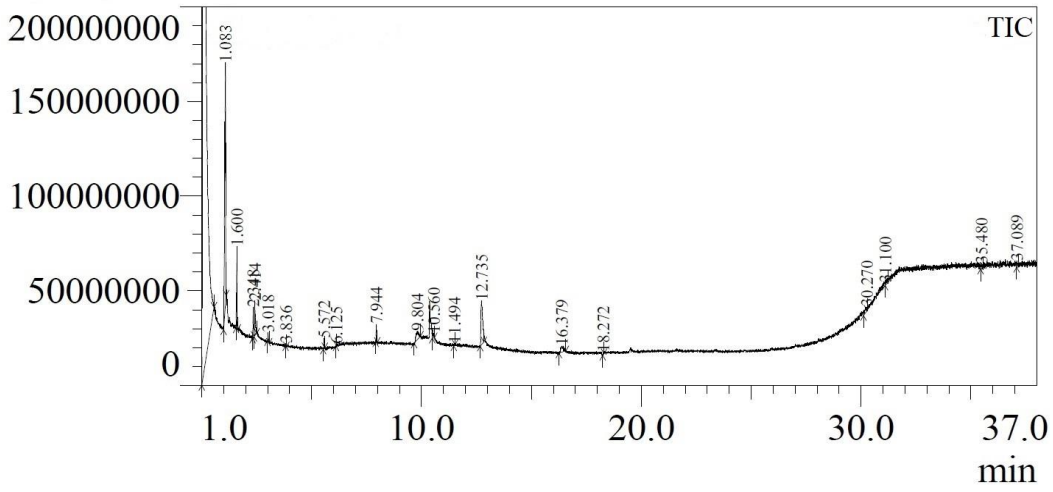


**Figure 2.** GC-MS chromatogram of methanol-extracted vegetative roots from *Withania somnifera*, where the x-axis represents run time and the y-axis represents abundance.

In *W. somnifera* vegetative root methanolic extracts, twelve compounds with antimicrobial activities were identified through GC-MS. The chromatogram peaks area, molecular formulae RT, name of the compound, and its antimicrobial and anticancer bioactivities are presented in Table 2. The phytochemicals found to be 2,2-dimethoxybutane (0.44%); cyclotrisiloxane, hexamethyl- (0.14%); octamethyl-, cyclotetrasiloxane (0.13%); cyclooctasiloxane, hexadecamethyl- (0.4%); N,O,O',O' – tetrakis (trimethylsilyl) norepinephrine, N-(trifluoroacetyl) - (0.23%); cyclohexasiloxane, dodecamethyl- (0.48%); cathinone (0.03%); 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-, hexasiloxane (0.26%); ethyl ester, 3-methoxy-4-[(trimethylsilyl)oxy]-, benzeneacetic acid (0.19%); 3,5-dimethyl-, benzoic acid (3,5-dimethylphenyl)methyl ester (0.18%); pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl- (0.14%) and benzoic acid, 3-methyl-2-trimethylsilyloxy-, trimethylsilyl ester (0.19%). The trans-2,3-epoxyoctane (84.78%) was identified as a phytochemical with anticancer properties, and the 2,2-dimethoxybutane (0.44%) was identified as a phytochemical with both anticancer and antibacterial properties.

**Table 2.** GC-MS identified phytochemicals in *W. somnifera* vegetative root methanolic extracts.

| Peaks | RT | Compound name | Formulae | m/z | Peak area% | Phytochemical groups | Therapeutic activity | References |
|---|---|---|---|---|---|---|---|---|
| 1 | 0.082 | Trans-2,3-Epoxyoctane | $C_8H_{16}O$ | 53.15 | 84.78 | Fatty acids | Anticancer | Akter *et al.*, 2022. |
| 3 | 1.138 | 2-Propanone, 1-methoxy- | $C_4H_8O_2$ | 73.15 | 0.07 | Flavonoid | NA | |
| 7 | 2.348 | 2,2-Dimethoxybutane | $C_6H_{14}O_2$ | 43.15 | 0.44 | Fatty acids | Antibacterial & Anticancer | Hajjar *et al.*, 2017. |
| 10 | 3.836 | 5-chloro-6-methyl-, spiro[2.3]hexan-4-one | $C_7H_9ClO$ | 40.05 | 0.02 | Terpenoids | NA | |
| 12 | 6.125 | 1,3,2-Dithiaborinane, 2-ethyl- | $C_5H_{11}BS_2$ | 40.05 | 0 | Organohalogen | NA | |
| 18 | 11.49 | 2 - (Dimethylaminomethyl) - 3 - nitrophenol | $C_9H_{12}N_2O_3$ | 44.10 | 0.02 | Alkaloids | NA | |
| 19 | 12.74 | Hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasilo, 3 - Isopropoxy-1,1,1,7,7,7 - | $C_{18}H_{52}O_7Si_7$ | 73.10 | 2.09 | Phenolic | NA | |
| 20 | 16.38 | Cyclooctasiloxane, hexadecamethyl- | $C_{16}H_{48}O_8Si_8$ | 73.15 | 0.4 | Alkaloids | Antibacterial | Dahpour *et al.*, 2012. |
| 8 | 5.572 | Cyclotetrasiloxane, octamethyl- | $C_8H_{24}O_4Si_4$ | 281.20 | 0.13 | Alkaloids | Antibacterial | Keskin *et al.*, 2012. |
| 21 | 18.27 | Cathinone | $C_9H_{11}NO$ | 44.10 | 0.03 | Alkaloids | Antibacterial | |
| 22 | 30.27 | 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-, hexasiloxane | $C_{12}H_{38}O_5Si_6$ | 207.10 | 0.26 | Alkaloids | Antibacterial | Majumder *et al.*, 2019. |
| 23 | 31.1 | 3-methoxy-4-[(trimethylsilyl)oxy]-, ethyl ester | $C_{14}H_{22}O_4Si$ | 208.10 | 0.19 | Terpenoids | Antibacterial | Kaviya *et al.*, 2021. |
| 25 | 31.61 | Trisiloxane, 1,1,3,3,5,5-hexamethyl- | $C_6H_{20}O_2Si_3$ | 207.10 | 0.17 | Alkaloids | NA | |
| 28 | 35.48 | Benzoic acid, dimethylphenyl)methyl ester | $C_{18}H_{20}O_2$ | 133.10 | 0.18 | Terpenoids | Antibacterial | EL-Zawawy and Mona, 2021. |
| 31 | 37.09 | 1,1,3,3,5,5,7,7,9,9-decamethyl-, pentasiloxane, | $C_{10}H_{32}O_4Si_5$ | 191.05 | 0.14 | Alkaloids | Antibacterial | Amrati *et al.*, 2021. |

### 3.3. GC-MS of Vegetative Stem

In *W. somnifera* vegetative stem methanolic extracts, eighteen unique phytochemicals were identified by GC-MS analysis, as shown in Figure 3. In Table 3, peak area, RT, compound name, and molecular formulae are presented. The phytochemicals identified included benzenemethanol, alpha.-(1-aminoethyl)- (28.07%); 2-butynoic acid (1.47%); 2 hydroxybenzophenone, 3 - tert - butyl - 5 - chloro - (4.96%); 5 - (ethyl-1-amine) bicyclo [2.2.1], heptane (3.25%); 3-amino-1,2-propanediol (2.24%); 2-hydroxy-5-[N,N-dimethylaminomethyl]benzoic acid (0.88%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-, heptasiloxane (0.04%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, octasiloxane (0.05%); Arabinitol, pentaacetate (0.05%); cyclononasiloxane, octadecamethyl- (0.15%); 2,4,6,8,10-tetradecapentaenoic acid, 9a-(acetyloxy)-1a,1b,4,4a,5,7a,7b (0.22%); heptasiloxane, hexadecamethyl-(8.88%); northiaden (0.03%); tert-butyld, benzenepropanoic acid, beta.-[(tert-butyldimethylsilyl)oxy]-, (1.45%); benzoic acid, 4-methyl-2-trimethylsilyloxy-, trimethylsilyl ester, (0.96%); silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy]phenyl]-1,1,1-trimet, (0.65%); 1-propanone, 1,3-diphenyl-3-(trimethylsilyl)- (0.05%); and dihydropyrimidinyl-4 uracil, 5-2-Oxo-6-phenyl-1,2 (0.87%).

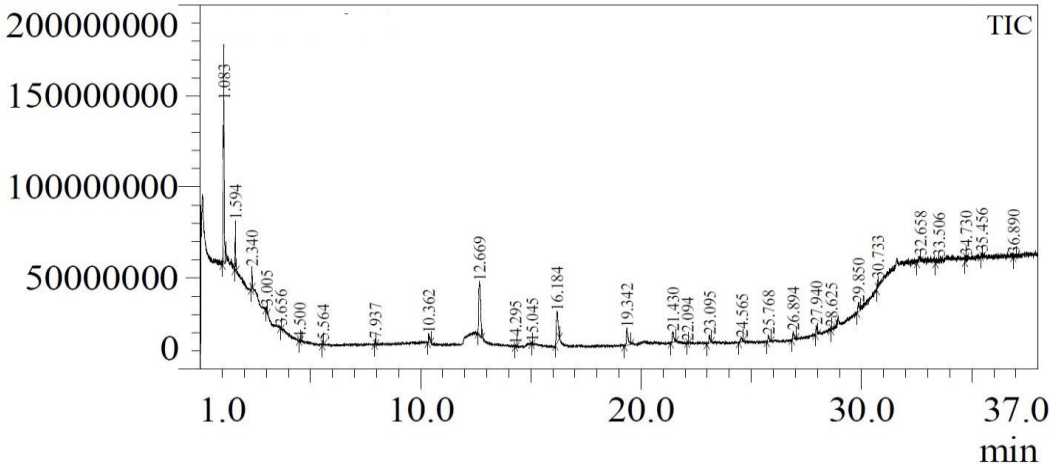


**Figure 3**. GC-MS chromatogram of methanol-extracted vegetative stems from *Withania somnifera*, where the x-axis represents run time and the y-axis represents abundance.

In *W. sominifera* vegetative stem methanolic extracts, phytochemicals identified with antimicrobial properties are given in Table 3. These included benzeneethanamine, N - (3-methylbutylidene)- (28.07%); 2-pyridinecarboxamide N-oxide (2.3%); 2,2-dimethoxybutane (0.88%); cyclotrisiloxane, hexamethyl- (0.19%); benzenemethanol, .alpha.-(1-aminoethyl)- (0.04%); 2 butynoic acid (0.05%); bicyclo[2.2.1], heptane-5-(ethyl-1-amine) (0.02%); 3-tert-Butyl-5-chloro-2-hydroxybenzophenone (0.05%); cyclotetrasiloxane, octamethyl- (0.15%); cyclohexasiloxane, dodecamethyl- (0.99%); tetrakis (trimethylsilyl) norepinephrine, N-(trifluoroacetyl) - N,O,O',O' – (0.22%); cycloheptasiloxane, tetradecamethyl- (8.88%); 3-amino-1,2-propanediol (0.06%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-, cyclooctasiloxane, hexadecamethyl- 5 heptasiloxane (2.8%); 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, octasiloxane (1.45%); hexadecamethyl, heptasiloxane (0.75%) and, cyclononasiloxane, octadecamethyl- (0.65%). Whereas arabinitol, pentaacetate (0.07%) was identified as a phytochemical with both anticancer and antimicrobial properties.

**Table 3.** GC-MS identified phytochemicals in *Withania somnifera* vegetative stem methanolic extracts.

| Peaks | RT | Compound name | Formulae | m/z | Peak area% | Phytochemical groups | Therapeutic activity | References |
|---|---|---|---|---|---|---|---|---|
| 2 | 0.095 | Benzenemethanol, .alpha.-(1-aminoethyl)- | $C_9H_{13}NO$ | 44.05 | 28.07 | Alkaloid | Antibacterial | Diale *et al*., 2021. |
| 3 | 0.185 | 2-Butynoic acid | $C_4H_4O_2$ | 40.05 | 1.47 | Carbohydrate | Antibacterial | Sanabria-Ríos *et al*., 2020. |
| 4 | 0.195 | 3-tert-Butyl-5-chloro-2-hydroxybenzophenone | $C_{17}H_{17}ClO_2$ | 40.05 | 4.96 | Alkaloid | Antibacterial | Fagbemi *et al*., 2021. |
| 5 | 0.235 | Bicyclo [2.2.1], heptane – 5 - (ethyl-1-amine) | $C_9H_{17}N$ | 44.05 | 3.25 | Amine | Antibacterial | Zielińska-Błajet *et al*., 2020. |
| 6 | 2.34 | 2,2-Dimethoxybutane | $C_6H_{14}O_2$ | 43.05 | 0.88 | Fatty acids | Antibacterial | Hajjar et al., 2017. |
| 10 | 1.594 | 3-Amino-1,2-propanediol | $C_3H_9NO_2$ | 44.05 | 2.24 | Alcohol | Antifungal | Chirumamilla *et al*., 2022. |
| 11 | 2.34 | 2-Hydroxy-5-[N,N-dimethylaminomethyl]benzoic acid | $C_{10}H_{13}NO_3$ | 44.05 | 0.88 | Alkaloid | NA | |
| 13 | 3.656 | 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- Heptasiloxane | $C_{14}H_{44}O_6Si_7$ | 73.05 | 0.04 | Organosilicon | Antibacterial | Mukesi *et al*., 2019. |
| 14 | 4.045 | 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-octasiloxane | $C_{16}H_{50}O_7Si_8$ | 73.05 | 0.05 | Alkaloid | Antibacterial | Falowo *et al*., 2017. |
| 15 | 4.154 | Arabinitol, pentaacetate | $C_{15}H_{22}O_{10}$ | 38.30 | 0.05 | Terpenoids | Antibacterial & Anticancer | Khan *et al*., 2021. |
| 16 | 3.005 | Cyclotrisiloxane, hexamethyl- | $C_6H_{18}O_3Si_3$ | 73.10 | 0.19 | Organosilicon | Antibacterial | Dahpour *et al.*, 2012. |
| 17 | 5.564 | Cyclononasiloxane, octadecamethyl- | $C_{36}H_{46}O_8$ | 73.05 | 0.15 | Fatty acids | Antibacterial | Keskin *et al*., 2012. |
| 18 | 7.937 | 2,4,6,8,10-Tetradecapentaenoic acid, 9 a - (acetyloxy)-1a,1b,4,4a,5,7a,7b- | $C_{18}H_{54}O_9Si_9$ | 44.05 | 0.22 | Fatty acids | ND | |
| 20 | 12.669 | Heptasiloxane, hexadecamethyl- | $C_{36}H_{46}O_8$ | 73.07 | 8.88 | Organosilicon | Antibacterial | Hassan, 2016. |
| 22 | 15.045 | Northiaden | $C_{18}H_{19}NS$ | 44.10 | 0.03 | Flavonoids | NA | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 25 | 21.43 | Beta.-[(tert-butyldimethylsilyl)oxy]-, tert-butyld, benzenepropanoic acid, | $C_{21}H_{38}O_3Si_2$ | 96.05 | 1.45 | Alkaloid | NA |
| 27 | 23.095 | Trimethylsilyl ester, benzoic acid, 4-methyl-2-trimethylsilyloxy- | $C_{14}H_{24}O_3Si_2$ | 280.95 | 0.96 | Phenolic | NA |
| 28 | 24.565 | Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl)oxy]phenyl]-1,1,1-trimet | $C_{14}H_{27}NOSi_2$ | 73.05 | 0.65 | Alkaloid | NA |
| 29 | 24.75 | 1-Propanone, 1,3-diphenyl-3-(trimethylsilyl)- | $C_{18}H_{22}OSi$ | 281.95 | 0.05 | Fatty acids | NA |
| 30 | 25.768 | 5 - (2 – Oxo - 6 – phenyl - 1,2-dihydropyrimidinyl - 4)- Uracil | $C_{14}H_{10}N_4O_3$ | 281.90 | 0.87 | Alkaloid | NA |

## 4. DISCUSSION and CONCLUSION

*W. somnifera*-based medicines have been used to treat various human illnesses (Altemimi *et al*., 2017). Most often, the *W. somnifera* roots of mature plants are favoured for a variety of therapeutic uses (Afewerky *et al.*, 2021). The root, stem, and leaf extracts of *W. somnifera* have been shown to be active against several cancers (Yadav *et al*., 2010; Dutta *et al.*, 2019). Also, aqueous and alcoholic extracts of *W. somnifera* leaf, stem, and root have been shown to have antimicrobial properties against a variety of microorganisms (Bisht and Rawat, 2014; Singariya *et al.*, 2011). These phytochemicals with anticancer and antimicrobial properties in *W. somnifera* were found to vary depending on the organ (Lingfa *et al*., 2022). Moreover, their phytochemical status during the vegetative stage is not yet studied in root, stem, and leaf. Hence, it is crucial to understand the bioactive phytochemical distribution in various parts of the plant at various developmental stages, including the vegetative stage of the leaf, stem, and root, for the further development of effective herbal-based formulations.

GC-MS analysis was used in this work to characterize the phytochemicals in *W. somnifera.* The plant parts investigated included the stem, leaf, and root, which were extracted using methanol as the solvent. The choice of methanol as the extraction solvent was based on previous studies that have shown that it yields the highest concentration of alkaloid, flavonoid, phenolic, and terpenoid components in *W. somnifera* (Ruiz-Ruiz *et al*., 2017; Kuppusamy *et al*., 2015; and Chao *et al*., 2014). The extracted samples were then subjected to GC-MS analysis, which involved chromatographic separation, quantification, and identification of the phytochemical compounds. The distribution and existence of antibacterial and anticancer phytochemicals in *W. somnifera* vegetative stage were determined using the GC-MS. The use of GC-MS analysis is advantageous as it is a quick and economical method for evaluating herbal products and provides detailed information on the chemical composition of the plant extracts.

The identified phytochemicals in *W. somnifera* vegetative leaf methanolic extracts, such as 1,3,5-cycloheptatriene (Yunnikova *et al.*, 2014) and cyclotrisiloxane, hexamethyl- have been reported in *Sedum pallidum* (Dahpour *et al*., 2012) to have antibacterial properties. The phytochemical cyclotetrasiloxane, octamethyl-, is also found in *W. somnifera* methanolic stem extracts of reproductive stage (Lingfa *et al*., 2023). In *W. somnifera* vegetative stage leaf methanolic extract: N,O,O',O'- tetrakis (trimethylsilyl), N- (trifluoroacetyl) – norepinephrine phytochemicals were found in red sea cucumber (Soliman *et al*., 2016). cyclohexasiloxane, dodecamethyl in *Argemone ochroleuca* (Moustafa *et al*., 2013). Cycloheptasiloxane, tetradecamethyl- in *Senna auriculaita* (Prasathkumara *et al*., 2021) and reported to have antibacterial properties.

The bioactive phytochemicals benzenemethanol, alpha-(1-aminoethyl)- in *Ribwort plantain* (Haghighi *et al*., 2022), 3-tert-butyl–5–chloro-2-hydroxybenzophenone in the *Tamarindus indica* (Fagbemi *et al*., 2021), cyclotetrasiloxane, octamethyl- in *Olea europaea* (Keskin *et al.*, 2012), N-(Trifluoroacetyl)- N,O,O',O'-tetrakis (trimethylsilyl) norepinephrine in the red sea cucumber *Holothuria atra* (Soliman *et al*., 2016), cyclohexasiloxane, dodecamethyl- in *Argemone ochroleuca* (Moustafa *et al.*, 2013), cycloheptasiloxane, tetradecamethyl- in *Senna auriculata* (Prasathkumara *et al.*, 2021), cyclooctasiloxane, hexadecamethyl- in *Sedum pallidum* (Dahpour *et al*., 2012), heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- in *Olea europaea* (Mukesi *et al*., 2019), 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-octasiloxane in *Moringa oleifera* (Falowo *et al*., 2017) identified in previous studies to have antibacterial properties are identified in the methanolic extracts of *W. somnifera* vegetative stem. The phytochemicals in methanolic extracts of stem from *W. somnifera*, Cyclononasiloxane, octadecamethyl-, were found to be antibacterial as well as antifungal in *Salvadora persica* (Bratty *et al*., 2020), 3-amino-1,2-propanediol as antifungal in *Solanum khasianum* (Chirumamilla *et al*., 2022). Additionally, the identified phytochemicals in this

study in *W. somnifera,* heptasiloxane, hexadecamethyl- (Bratty *et al.,* 2020), benzeneethanamine, N-(3-methylbutylidene)- (Diale *et al*., 2021), 2-butynoic acid (Sanabria-Ríos *et al*., 2020), and bicyclo [2.2.1] heptane-5-(ethyl-1-amine) (Zielińska-Błajet *et al.*, 2020) have been previously reported to be antibacterial. The antibacterial activity of these compounds' ability may be ascribed to disrupt membranes in bacterial cell or inhibit enzymes that are essentially involved in bacterial growth and replication (Barbieri *et al.,* 2017).

In *W. sominfera,* vegetative root methanolic root extracts, the trans-2,3-epoxyoctane, has been reported to be anticancer (Akter *et al.*, 2022). It is found in the herbal remedy known as Triphala, which is made from the fruits of three different herb species: *Terminalia bellirica*, *T. chebula*, and *Phyllanthus emblica* (Akter *et al.,* 2022). Another phytochemical, 2,2-Dimethoxybutane has been reported to be both anticancer and antibacterial, and it is found in the Saudi Arabian herbal fraction, i.e., in JUN_C2_60% (Hajjar *et al*., 2017). The phytochemical arabinitol pentaacetate identified in vegetative stem methanolic extracts of *W. somnifera* has been shown to have both antibacterial and anticancer properties and was reported in *Abutilon indicum* (Khan *et al*., 2021). The mechanisms by which these anticancer substances work include triggering apoptosis, or programmed cell death, in cancer cells, preventing tumor development and metastasis, and adjusting immune responses against cancer cells (Rahman *et al.,* 2021). To fully understand these phytochemicals' unique modes of action and their uses in antibacterial and anticancer treatments, further studies are required.

The phytochemicals responsible for anticancer and antimicrobial properties in *W. somnifera* at the vegetative stage reveal that phytochemicals found in the leaf, stem, and root parts were trivial in the vegetative stage compared to the reproductive stage (Lingfa *et al*., 2023). In the vegetative stage, the total of phytochemicals with antibacterial and anticancer properties was found to be highest in stem methanolic extracts and least in leaf methanolic extracts. To our knowledge, this is the first GC-MS profiling of stem, leaf, and root methanolic extracts based on the developmental stage, *viz.*, the vegetative stage in *W. somnifera*. This study identified phytochemicals that were not previously reported in the GC-MS analysis of *W. somnifera*.

Most of the phytochemical compounds identified at the vegetative stage from the *W. somnifera* methanolic leaf, root, and stem extracts in this study are commercialised as antibiotics or anticancer medications, such as 1,3,5-cycloheptatriene and cathinone. The identified phytochemical compound 1,3,5-cycloheptatriene from the vegetative leaf methanolic extract of *W. somnifera* is clinically used as an antibacterial compound and is commercially known as triprolidine (Manikandan *et al*., 2019). A study shows that the unsubstituted seven-membered ring structure (triprolidine) has antibacterial activity, and the substitution with a keto group in the 2-position (tropolone) enhanced the bacteriostatic and bactericidal activity (Trust and Bartlett 1975). The identified phytochemical compound from the vegetative root methanolic extract of *W. somnifera* at the vegetative stage, cathinone, is used to develop a commercially available drug known as ciprofloxacin, which is useful to treat a variety of infections, including chancroid, anthrax, respiratory tract infections, and urinary tract infections (Piddock *et al*., 2010). It is a quinolin-4(1H)-one and has substitutions at positions 1, 6, 3, and 7 for piperazin-1-yl, cyclopropyl, carboxylic acid, and fluoro, respectively. Its structural functions are similar to those of a topoisomerase IV inhibitor (ASHP, 2015).

Traditional herbal medicines developed from *W. somnifera* play a significant role in the healthcare system. However, further quality-based product formulation is needed to maximize consumer benefits. Furthermore, since the distribution and amount of bioactive chemicals in plants can change throughout the course of their life cycle, the results emphasize the need of taking the developmental stage into account when analyzing its phytochemical composition. The identification of bioactive phytochemicals with anticancer and antimicrobial properties in the vegetative stage extracts of *W. somnifera* has important implications for the development

of herbal-based products. These phytochemicals could serve as potential leads for the development of new antibiotics and anticancer medications. To separate and purify these substances for usage in therapeutic settings, more investigation is required.

In conclusion, while this study provides valuable insights into the phytochemical composition of *W. somnifera* at the vegetative stage, it is important to note that the study focused on methanolic extracts and specific phytochemicals. Further investigations could explore other extraction methods and analyze a broader range of phytochemicals. Additionally, future studies could investigate the biological activities of the identified phytochemicals and their potential mechanisms of action. Understanding the pharmacological properties of these compounds could pave the way for the development of novel herbal-based therapies.

## Plant Authentication

The plant material (*Withania somnifera*) was identified and authenticated by Dr. A. Vijaya Bhaskar Reddy, Assistant Professor, Department of Botany, Osmania University, Hyderabad. The plant was deposited in the herbarium of the Department of Botany, Osmania University, Hyderabad, with the voucher number GEN/OU/001-2018-HY.

## Acknowledgements

LL acknowledges the National Fellowship for Higher Education of ST Students (NFST), Ministry of Tribal Affairs, Government of India, for fellowship. AS acknowledges the Rashtriya Uchchatar Shiksha Abhiyan (RUSA) 2.0 Program, under the Ministry of Human Resources Development, Government of India, and CAS, DST-PURSE-II, UPE-FAR, and DST-FIST, for funding and providing the necessary facilities to carry out the present work.

## Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

## Authorship Contribution Statement

**Lali Lingfa**: Investigation, resources, formal analysis, statistics, and writing the draft. **Aravinda Tirumala**: Resources, visualisation, writing, formal analysis**. Ankanagari Srinivas**: Concept, editing and writing, investigation, resources, supervision, and validation.

## Orcid

Lali Lingfa https://orcid.org/0000-0002-3060-1603

Aravinda Tirumala https://orcid.org/0009-0001-0839-6690

Srinivas Ankanagari https://orcid.org/0000-0001-7631-6536

## REFERENCES

Afewerky, H., Ayodeji, A., Tiamiyu, B., Orege, J., Okeke, E., Oyejobi, A., Bate, P., & Adeyemi. (2021). Critical review of the *Withania somnifera* (L.) Dunal: ethnobotany, pharmacological efficacy, and commercialization significance in Africa. *Bulletin of the National Research Centre*, *45*(1), 176. https://doi.org/10.1186/s42269-021-00635-6

Akter, R., Khan, S., Kabir, M., & Halder, S. (2022). GC-MS-employed phytochemical characterization, synergistic antioxidant, and cytotoxic potential of Triphala methanol extract at non-equivalent ratios of its constituents. *Saudi Journal of Biological Sciences*, *29*(6), 103287. https://doi.org/10.1016/j.sjbs.2022.103287

Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D., & Lightfoot, D. (2017). Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. *Plants* (Basel), *6*(4), 42. https://doi.org/10.3390/plants6040042

Amrati, F., Bourhia,M., Saghrouchni, H., Slighoua, M., Grafov, A., Ullah, R., Ezzeldin, E., Mostafa, G., Bari, A., Ibenmoussa, S., & Bousta, D. (2021). *Caralluma europaea* (Guss.) N.E.Br.: Anti-Inflammatory, Antifungal, and Antibacterial Activities against Nosocomial Antibiotic-Resistant Microbes of Chemically Characterized Fractions. *Molecules*, *26*(3), 636. https://doi.org/10.3390/molecules26030636

Barbieri, R., Coppo, E., Marchese, A., Daglia, M., Sobarzo-Sánchez, E., Nabavi, S.F., & Nabavi, S.M. (2017). Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research*, *196*, 44-68. https://doi.org/10.1016/j.micres.2016.12.003

Bisht, P., & Rawat, V., (2014). Antibacterial activity of *Withania somnifera* against Gram-positive isolates from pus samples. *Ayu*, *35*(3), 330-332. https://doi.org/10.4103/0974-8520.153757

Bratty, M., Makeen, H., Alhazmi, H., Syame, S., Abdalla, A., Homeida, H., Sultana, S., Ahsan, W., Khalid, A., (2020). Phytochemical, Cytotoxic, and Antimicrobial Evaluation of the Fruits of Miswak Plant, *Salvadora persica* L. *Journal of Chemistry*, *2020*(4), 521951. https://doi.org/10.1155/2020/4521951

Chao, S.-Y P. Lin,Y.. Lin K.-H., *et al*., (2014). Antioxidant activity in extracts of 27 indigenous Taiwanese vegetables. *Nutrients*, *6*(5), 2115–2130. https://doi.org/10.3390/nu6052115

Chirumamilla, P., Dharavath, S., and Taduri, S. (2022). GC–MS profiling and antibacterial activity of *Solanum khasianum* leaf and root extracts. *Bulletin of the National Research Centre*, *46*(1), 127. https://doi.org/10.1186/s42269-022-00818-9

Ciprofloxacin Hydrochloride. The American Society of Health-System Pharmacists (ASHP). Archived from the original on 23 September 2015. Retrieved on 17 July 2023.

Dahpour, A., Rahdari, P., & Sobati, Z., (2012). Chemical composition of essential oil, antibacterial activity and brine shrimp lethality of ethanol extracts from *Sedum pallidum*. *Academic Journals*, *6*(16), 3105-3109. https://doi.org/10.5897/JMPR11.1270

Diale, M., Kayitesi, E., and Serepa-Dlamini, M. (2021). Genome In Silico and In Vitro Analysis of the Probiotic Properties of a Bacterial Endophyte, *Bacillus paranthracis* Strain MHSD3. *Frontiers in Genetics*, *12*(2021), 672149. https://doi.org/10.3389/fgene.2021.672149

Dutta, R., Khalil, R., Green, R., & Mohapatra, S. ((2019). *Withania somnifera* (Ashwagandha) and Withaferin A: Potential in Integrative Oncology. *International Journal of Molecular Sciences*, *20*(21), 5310. https://doi.org/10.25081/cb.2021.v12.6867

EL-Zawawy, N., & Mona, M. (2021). Antimicrobial efficacy of Egyptian *Eremina desertorum* and *Helix aspersa* snail mucus with a novel approach to their anti-inflammatory and wound healing potencies. *Scientifi Reports*, *11*(2021), 24317. https://doi.org/10.1038/s41598-021-03664-3

Fagbemi, K., Aina, D., & Olajuyigbe, O. (2021). Soxhlet Extraction versus Hydrodistillation Using the Clevenger Apparatus: A Comparative Study on the Extraction of a Volatile Compound from *Tamarindus indica* Seeds. *Hindawi*, *2021*(5961586), 1- 8. https://doi.org/10.1155/2021/5961586

Falowo, A., Muchenje, V., Hugo, A., Aiyegoro, O., & Fayemi, P. (2017). Antioxidant activities of *Moringa oleifera* L. and *Bidens pilosa* L. leaf extracts and their effects on oxidative stability of ground raw beef during refrigeration storage. *CyTA - Journal of Food*, *15*(2), 249 – 256. https://doi.org/10.1080/19476337.2016.1243587

Haghighi, S., Yazdinezhad, A., Bagheri, K., & Sharafi, A. (2022). Volatile Constituents and Toxicity of Essential Oils Extracted From Aerial Parts of Plantago Lanceolata and Plantago Major Growing in Iran. *Pharmaceutical and Biomedical Research*, *8*(3), 205-224 https://doi.org/10.1515/znc-2006-7-801

Hajjar, D., Kremb, S., Sioud, S., Emwas, A., Voolstra, C., & Ravasi, T. (2017). Anti-cancer agents in Saudi Arabian herbals revealed by automated high-content imaging. *PLoS One*, *12*(6), e0177316. https://doi.org/10.1371/journal.pone.0177316

Hassan, S. (2016). Antibacterial, Anticoagulant and Anti-inflammatory Activities of Marine *Bacillus cereus* S1. *Journal of Pure and Applied Microbiology*, *10*(4), 2593–2606. https://doi.org/10.21608/ejabf.2020.86046

Kaur, A., Singh, B., Ohri, P., Wang, J., Wadhwa, R., Kaul, C., Pati, K. (2018). Organic cultivation of Ashwagandha with improved biomass and high content of active Withanolides: Use of Vermicompost. *PloS One*, *13*(4), e0194314. https://doi.org/10.1371/journal.pone.0194314

Kaviya, M., Balasubramanian, B., Bharathi, K., Malaisamy, A., Al-Dhabi, N., Mariadhas, V., Anand, A., Liu, W. (2021). Evaluation of Nutritional Substances and Investigation of Antioxidant and Antimicrobial Potentials of *Boerhavia diffusa* with *in silico* Molecular Docking. *Molecules*, *27*(4), 1280. https://doi.org/10.3390/molecules27041280

Keskin, D., Guvensen, N., & Dbeys, A. (2012). Antimicrobial activity and chemical constitutions of West Anatolian olive (*Olea europaea* L.) leaves. *Journal of Food Agriculture and Environment*, *10*(2), 99-102.

Khan, S., Shahid, S., Ayaz, A., Alkahtani, J., Elshikh, S., Riaz, T. (2021). Phytomolecules-Coated NiO Nanoparticles Synthesis Using *Abutilon indicum* Leaf Extract: Antioxidant, Antibacterial, and Anticancer Activities. *International Journal of Nanomedicine*, *16*, 1757-1773. https://doi.org/10.2147/IJN.S294012

Kuppusamy P., Yusoff M. M., Parine N. R., and Govindan N. (2015). Evaluation of in-vitro antioxidant and antibacterial properties of *Commelina nudiflora* L. extracts prepared by different polar solvents. *Saudi Journal of Biological Sciences*, *22*(3), 293–301. https://doi.org/10.1016/j.sjbs.2014.09.016

Lingfa, L., & Ankanagari, S. (2023). GC-MS Profiling of reproductive stage *Withania somnifera* for antimicrobial and anticancer phytochemicals. *Biomedical and Pharmacology*, *13*(1), 71-78. https://dx.doi.org/10.13005/bpj/1862

Lingfa, L., Gugulothu, B., Jagtap, S., & Ankanagari, S. (2022). HPTLC fingerprinting reveals leaf and roots phytochemical variability in developmental stages of *Withania somnifera*. *Journal of Pharmacognosy and Phytochemistry*, *11*(4), 283-294. https://doi.org/10.22271/phyto.2022.v11.i4d.14473

Majumder, R., Dhara, M., Adhikari, L., Ghosh, G., & Pattnaik, S. (2019). Evaluation of in vitro Antibacterial and Antioxidant Activity of Aqueous Extracts of *Olax psittacorum*. *Indian Journal of Pharmaceutical Sciences*, *81*(1), 99-109. https://doi.org/10.4172/pharmaceutical-sciences.1000484

Manikandan, M., Gowdaman, V., Duraimurugan, K., Prabagaran, R. (2019). Taxonomic characterization and antimicrobial compound production from Streptomyces chumphonensis BDK01 isolated from marine sediment. *3 Biotech.*, *9*(5), 167. https://doi.org/10.1007%2Fs13205-019-1687-7

Moustafa, M., Alamri, S., Taha, T., & Alrumman, S. (2013). *In vitro* antifungal activity of *Argemone ochroleuca* Sweet latex against some pathogenic fungi. *African Journal of Biotechnology*, *12*(10), 1132-1137. https://doi.org/10.5897/AJB12.2649.

Mukesi, M., Iweriebor, B., Obi, L., Nwodo, U., Moyo., S.x & Okoh, A. (2019). The activity of commercial antimicrobials, and essential oils and ethanolic extracts of *Olea europaea* on *Streptococcus agalactiae* isolated from pregnant women. *BMC Complementary Medicine and Therapies*, *19*(1), 34. https://doi.org/10.1186/s12906-019-2445-4

Musher, M., Saenz, C., & Griffith, P. (2021). Unravelling the bioprospects of mycoendophytes residing in *Withania somnifera* for productive pharmaceutical applications. *Biocatalysis and Agricultural Biotechnology*, *37*, 102172. https://doi.org/10.1016/j.bcab.2021.102172

Piddock, J., Garvey, I., Rahman, M., Gibbons, S (2010). Natural and synthetic compounds such as trimethoprim behave as inhibitors of efflux in Gram-negative bacteria. *J Antimicrob Chemother, 65*, 1215–1223. https://doi.org/10.1093/jac/dkq079

Prasathkumara., M, Raja., K, Vasanth., K, Khusro., A, Sadhasivam., S, Sahibzada., M, Gawwad., M, Farraj., D., & Elshikhg., M. (2021). Phytochemical screening and in vitro antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of *Senna auriculata* (L.) *Roxb. leaves. Arabian Journal of Chemistry, 14*(9), 103345. https://doi.org/10.1016/j.arabjc.2021.103345

Rahman, A., Hannan, A., Dash, R., Rahman, H., Islam, R., Uddin, J., Sohag, M., Rhim, H. (2021). Phytochemicals as a Complement to Cancer Chemotherapy: Pharmacological Modulation of the Autophagy - Apoptosis Pathway. *Front Pharmacol*, 12, 1-20. https://doi.org/10.3389/fphar.2021.639628

Ruiz-Ruiz J.C., Matus-Basto A.J., Acereto-Escoffié P., and Segura-Campos M.R. (2017). Antioxidant and anti-inflammatory activities of phenolic compounds isolated from *Melipona beecheii* honey. *Food and Agricultural Immunology*, *28*(6), 1424-1437. https://doi.org/10.1080/09540105.2017.1347148

Saidulu, C., Venkateshwar, C., Rao, S., & Vardhan., T. (2014). In-vitro Antimicrobial Activity of *Withania somnifera* Leaf and Root Extracts grown in Heavy Metal toxic soils. *International Journal of Advances in Pharmacy, Biology and Chemistry, 3*(4), 872-879.

Salemm, S., Muhammad, G., Hussain, M., & Bukhari, S. (2020). *Withania somnifera* L.: Insights into the phytochemical profile, therapeutic potential, clinical trials, and future prospective. *Iranian Journal of Basic Medical Sciences*, *23*(12), 1501-1526. https://doi.org/10.22038/IJBMS.2020.44254.10378

Salve, J., Pate, S., Debnath, K., & Langade, D. (2019). Adaptogenic and Anxiolytic Effects of Ashwagandha Root Extract in Healthy Adults: A Double-blind, Randomized, Placebo-controlled Clinical Study. *Cureus*, *11*(12), e6466. https://doi.org/10.7759/cureus.6466

Sanabria-Ríos, S., Morales-Guzmán, C., Mooney, J., Medina, S., Pereles-De-León., T, Rivera-Román, A., Ocasio-Malavé, C., Díaz, D., Chorna, N. & Carballeira, N. (2020). Antibacterial activity of hexadecynoic acid isomers towards clinical isolates of multidrug-resistant *Staphylococcus aureus*. *Lipids*, *55*(2), 101–116. https://doi.org/10.1002/lipd.12213

Singariya, P., Kumar, P., & Mourya, K.K. (2011). *In-vitro* Bio-efficacy of Stem extracts of Ashwagandha against Some Pathogens. *Journal of Current Pharmaceutical Research, 8*(1), 25-30.

Soliman, Y., & Hermine, T. (2016). Antifouling and Antibacterial Activities of Marine Bioactive Compounds Extracted from some Red Sea Cucumber. *International Journal of Contemporary Applied Sciences*, 3(9), 83-103. https://doi.org/10.1186/s10152-020-0536-8

Trust, J., Bartlett, H. (1975). Antibacterial activity of tropilidine and tropone. *Antimicrob Agents Chemother, 8*, 381–383. https://doi.org/10.1128/aac.8.3.381

Yadav, B., Bajaj, A., Saxena, M., Saxena K. (2010). In Vitro Anticancer Activity of the Root, Stem and Leaves of *Withania somnifera* against Various Human Cancer Cell Lines. *Indian J Pharm Sci.*, 72(5), 659-63. https://doi.org/10.4103%2F0250-474X.78543

Yunnikova, L., Akenteva, T., Aleksandrova, G., Mikhailova, L., & Eliseev, S. (2014). Synthesis and Antimicrobial Activity of Anilines with 1,3,5-Cycloheptatriene and 5H-Dibenzo[a,d]Annulene Fragments. *Pharmaceutical Chemistry Journal*, *48*(1), 26-29. https://doi.org/10.1007/s11094-014-1038-2

Zielińska-Błajet., M & Feder-Kubis., J. (2020). Monoterpenes and Their Derivatives-Recent Development in Biological and Medical Applications. *International Journal of Molecular Sciences*, *21*(19), 7078. https://doi.org/10.3390/ijms21197078