

GC-MS and HPLC Analyses of Phytochemical Compounds from *Withania somnifera* L. Leaves Extract

Mervat EL-Hefny¹, Mamoun S.M. Abd El-Kareem², Mohamed Z.M. Salem^{3*}

¹Department of Floriculture, Ornamental Horticulture and Garden Design, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt

²Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt

³Forestry and Wood Technology Department, Faculty of Agriculture (EL-Shatby), Alexandria University, Alexandria, Egypt

*Corresponding author: mohamed-salem@alexu.edu.eg

ABSTRACT

In the present work, the shrub *W. somnifera* was used as a source for the extraction of bioactive molecules. Leaves were extracted by ethanol and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) and High-Performance Liquid Chromatography (HPLC). By GC-MS analysis, the highest identified compounds were 2,6-dimethyl-N-(2-methyl- α -phenylbenzyl)aniline, 4-Aminoheptane, n-undecanophenone, 2-cyclohexyl-4-hydroxymethyl-octahydrobenzo[e][1,2]oxazine-3-carbonitrile, and octadecanoic acid 3-octadecyloxypropyl ester, with percentages of 19.43%, 7.51%, 4.47%, 4.34%, and 3.89%, respectively. HPLC analysis identified quercetin and 7-hydroxyflavone with concentrations of 12.36 and 15.14 mg/mL, respectively, as two flavonoid compounds. This work showed that *W. somnifera* leaf extract owns several bioactive compounds that could be useful and have different applications, such as antimicrobial activities.

Key words: *Withania somnifera* leaves; HPLC; GC-MS; quercetin; 7-hydroxyflavone.

INTRODUCTION

Ashwagandha or winter cherry (*Withania somnifera* L. Dunal, family Solanaceae), the evergreen shrub, is originated to the Middle East, India, and parts of Africa (Bellanger; Seeger 2021; Gupta et al., 2021). *W. somnifera* in traditional medicine has many applications such as antitumor, immunomodulatory, antiarthritic, antioxidant, hepatoprotective and anti-inflammatory activities (Bhattacharya et al., 2002; Harikrishnan et al., 2008; Jacobs; Pierce 2018).

W. somnifera L. Dunal, (Ashwagandha or winter cherry), belongs to the family Solanaceae, the evergreen shrub, which has several uses in medicine. The plant has many bioactive compounds and in Egypt, there is a lack of information about this plant in terms of its bioactive compounds. *Withania somnifera* has promising medicinal properties as a therapeutic agent for addressing anxiety, microbial infection, cancer, neurodegenerative disorders and immunomodulation (A Dar et al., 2016). Biochemical constituents of *W. somnifera* like withanolide A, withanolide D, withaferin A and withaniamides play an important role in its pharmacological properties.

Some bioactive compounds like withanamides, and tropine were identified in *W. somnifera* extracts (Chatterjee et al., 2010; Singh et al., 2001; Sivanandhan et al., 2012). The extract showed antioxidant activity *in vitro* with inhibition of lipid peroxidation (Bhatnagar et al., 2009). Phenolic and flavonoid compounds were analyzed by HPLC from

fruit extract, and the most compounds were salicylic acid, vanillic acid, rutin, and myricetin with values of 9.49, 4.78, 4702.58, 1386.62 mg/100 g extract, respectively (El-Hefny et al., 2020). Other chemical compounds related to fatty acids, such as linoleic acid, palmitic acid, oleic acid, and linolenic acid were isolated from leaf and root extracts (Chatterjee et al., 2010). Six different extracts of *W. somnifera* root, prepared in a sequential manner starting from non-polar (hexane) to polar (water) solvents and alkaloids, terpene ansteroid, hydroxybenzene, saponin, organic acids and flavone were identified in methanol extract with thin layer chromatography (Pal et al., 2012).

Extracts from root and leaves of *W. somnifera* observed promising antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluorescens* and *Xanthomonas axonopodis* pv. *malvacearum* and antifungal activity against *Aspergillus flavus*, *Dreschlera turcica* and *Fusarium verticillioides* (Mahesh; Satish 2008).

Therefore, the present work aimed to identify the chemical compounds present in leaf extract using GC-MS and HPLC analyses.

MATERIAL AND METHODS

1. *Withania somnifera* leaf sample

Leaves of *Withania somnifera* were collected from Alexandria, Egypt, 2021. The leaves were washed with tap water to remove the dust and then air-dried under shade at room temperature for 10 days. The air-dried leaves were ground to powder using a small laboratory mill and kept dry. About 50

g of the powdered leaf sample were put in a 1-L flask, and 150 ml of ethanol (70%) was poured inside the flask for the extraction by soaking method (Salem et al., 2019), then filtered through filter paper (Whatman no.1) to separate the dissolved extracts from the solid residue. The extracts were air-dried using Petri dishes, and then stored in sealed tubes until use.

2. GC-MS analysis

The chemical composition of *W. somnifera* leaf ethanol extract was analyzed by GC-MS at the Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt (Anäs et al., 1983; Ekman; Holmbom 1989; Mohareb et al., 2017). The MS library searches (NIST and Wiley) were used as the base for the identification of the chemical compositions in the extract by comparing with the MS literature data (Oberacher et al., 2013).

3. HPLC analysis of phytochemicals

HPLC (Agilent 1100), composed of binary LC pump, a UV/Vis detector, and a C18 column, was used. The separation conditions set for the mobile phase were acetonitrile (A), and 0.2% (v/v) aqueous formic acid (B) with an isocratic elution (70:30) program and the wavelength for the detection was set at 360 nm (Hassan et al., 2021). Standard compounds rutin, 7-hydroxyflavone, naringin, quercetin, kaempferol, hesperidin, and catechin were used.

RESULTS AND DISCUSSION

1. GC-MS of chemical compounds

The peaks of the identified chemical compounds from *W. somnifera* leaf ethanol extract are shown on Figure 1, and the chemical compounds are listed in Table 1 with their retention times, area %, molecular formula, and molecular weight. The most abundant compounds were 2,6-dimethyl-N-(2-methyl- α -phenylbenzyl)aniline (19.43%), 4-Aminoheptane (7.51%), *n*-undecanophenone (4.47%), 2-cyclohexyl-4-hydroxymethyl-octahydrobenzo[e][1,2]oxazine-3-carbonitrile (4.34%), octadecanoic acid 3-octadecyloxypropyl ester (3.89%), (, 13-Phenylpentacosane

Furthermore, minor compounds such as methyl 3-hydroxyhexanoate (1.93%), isomexoticin (1.72%), 1,1-dibutoxy-butane (1.66%), nivalenol (1.49%), 5-phenyleicosane (1.44%), 2-ethyl-1-hexanol (1.40%), dimethoxyglycerol docosyl ether (1.32%), 10,13-Octadecadiynoic acid, methyl ester (1.21%), and 3-acetoxy-7,8-epoxylanostan-11-ol (1.16%) were found.

These chemical compounds could be useful in several biological activities. Previous works showed that a potent biological activity of fruit extract against several bacteria and fungi was found as the extract was applied to wood samples (El-Hefny et al., 2020). *W. somnifera* methanol extract was effective against *C. albicans* and other microbes (A Dar et al., 2016; Kambizi; Afolayan 2008). Monomeric glycoprotein isolated from *W. somnifera* root tubers revealed protease inhibitors as antimicrobial activities against some bacteria and fungi (Girish et al., 2006).

RT: 0.00 - 42.12

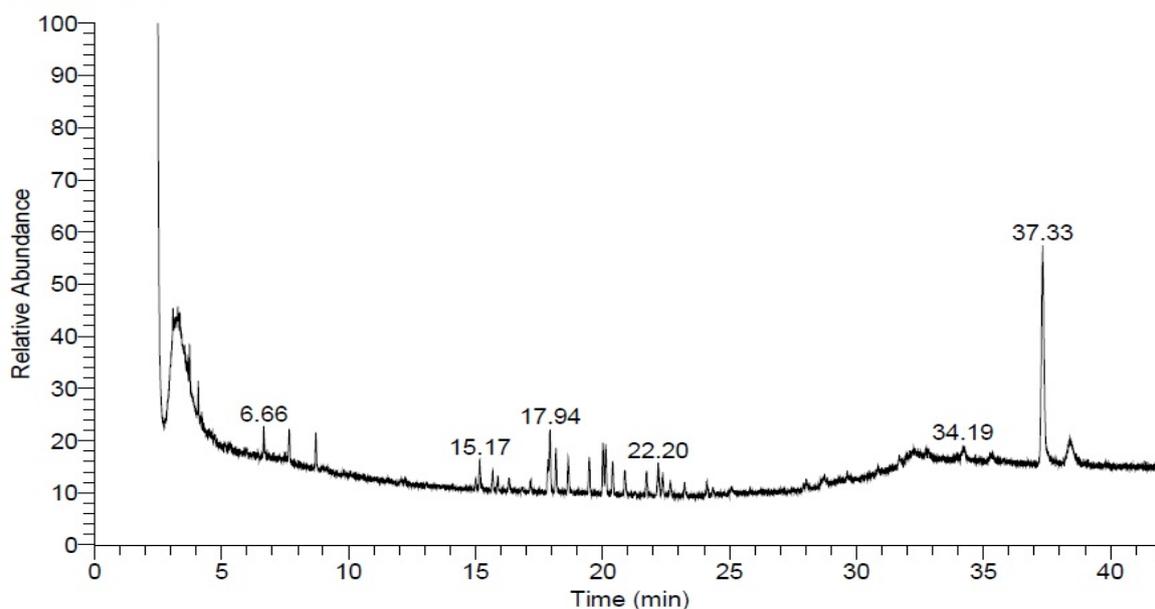


Figure 1: GC-MS chromatogram of the identified chemical compounds in the ethanol extract from *Withania somnifera* leaves.

Table 1: Chemical constituents of *W. somnifera* leaf ethanol extract as analyzed by GC-MS

RT (min)	Compound Name	Area %	Molecular Formula	Molecular Weight
3.09	4-Aminoheptane	7.51	C ₄ H ₈ O	72
3.18	2-Cyclohexyl-4-hydroxymethyl-octahydrobenzo[e][1,2]oxazine-3-carbonitrile	4.34	C ₁₆ H ₂₆ N ₂ O ₂	278
3.28	n-Undecanophenone	4.47	C ₁₇ H ₂₆ O	246
3.55	9,12,15-Octadecatrienoic acid (2-phenyl-1,3-dioxolan-4-yl)methyl ester	0.44	C ₂₈ H ₄₀ O ₄	440
3.65	Docosane	0.56	C ₂₂ H ₄₆	310
4.09	2-Ethylhexanol	1.40	C ₈ H ₁₈ O	130
4.75	(4-Carbomethoxy)benzyl p-toluate	0.47	C ₁₇ H ₁₆ O ₄	284
5.36	1,1'-Bis(2-anthraquinone carboxylic acid)	0.38	C ₃₀ H ₁₄ O ₈	502
5.91	2-hydroxy-5,6-epoxy-15-methyl-Pregan-20-one	0.32	C ₂₂ H ₃₄ O ₃	346
6.00	1,5,8-para-menthatriene	0.44	C ₁₀ H ₁₄	134
6.66	1,1-Dibutoxy-Butane	1.66	C ₁₂ H ₂₆ O ₂	202
8.71	Methyl 3-hydroxyhexanoate	1.93	C ₇ H ₁₄ O ₃	160
15.01	2,4-Di-tert-butylphenol	0.57	C ₁₄ H ₂₂ O	206
15.17	Nivalenol	1.49	C ₁₅ H ₂₀ O ₇	312
15.67	10,13-Octadecadienoic acid, methyl ester	1.21	C ₂₅ H ₃₄	334
22.20	13-Phenylpentacosane	2.32	C ₃₁ H ₅₆	428
22.37	5-Phenyleicosane	1.44	C ₂₆ H ₄₆	358
24.35	Cyclopentanetricadecanoic acid methyl ester	0.54	C ₁₉ H ₃₆ O ₂	292
25.07	Myristyl Oleate	0.57	C ₃₂ H ₆₂ O ₂	478
27.92	Ethyl iso-allocholate	0.37	C ₂₆ H ₄₄ O ₅	436
28.04	12-O-acetyl-8-O-tigloylingol	0.76	C ₂₇ H ₃₈ O ₈	490
28.74	Dimethoxyglycerol Docosyl ether	1.32	C ₂₇ H ₅₆ O ₅	460
29.64	6-[7-nitrobenzofurazan-4-yl]amino- Morphinan-4,5-poxy-3,6-di-ol	0.46	C ₂₆ H ₂₇ N ₅ O ₆	505
31.68	3-acetoxy-7,8-epoxyanostan-11-ol	1.16	C ₃₂ H ₅₄ O ₄	502
32.24	18,19-Didehydro-10-methoxycorynan-17-ol	0.74	C ₂₀ H ₂₆ N ₂ O ₂	326
32.30	Gomezine	0.44	C ₁₈ H ₂₀ N ₂	264
32.74	Arachidyl oleate	0.35	C ₃₈ H ₇₄ O ₂	562
34.23	Isomexoticin	1.72	C ₁₆ H ₂₀ O ₆	308
35.31	9-Desoxo-9-x-acetoxy-3,8,12-tri-O-acetylingol	0.85	C ₂₈ H ₄₀ O ₁₀	536
35.37	Lycopene	0.41	C ₄₀ H ₅₆	536
37.33	2,6-dimethyl-N-(2-methyl- α -phenylbenzyl)aniline	19.43	C ₂₄ H ₃₈ O ₄	390
38.42	Octadecanoic acid 3-octadecyloxypropyl ester	3.89	C ₃₉ H ₇₈ O ₃	594

RT: Retention time (min)

2. HPLC analysis of extract

The HPLC analysis confirmed the identification of two flavonoid compounds quercetin and 7-hydroxyflavone at the retention times of 7.00 and 11.10 min (Figure 2), with concentrations of 12.36 and 15.14 mg/mL, respectively.

Previously works showed that 6,8-dihydroxykaempferol 3-rutinoside, quercetin and its 3-O-rutinoside and 3-rutinoside-7-glucoside, were isolated and identified from the leaves of *W. somnifera* (Kandil et al., 1994). Gallic acid, chlorogenic acid, caffeic acid, sinapic acid, rutin hydrate, quercetin-3-rhamnoside and quercetin were identified from leaf extract of *W. somnifera* (Pal et al., 2015). In humans, *W. somnifera* showed no in vitro CYP3A4 and CYP2D6 enzyme interactions

(Savai et al., 2015). Moreover, 17 phytochemicals from *W. somnifera* have the higher affinity for S1'-specificity pocket of the MMP-9 catalytic domain than two inhibitors reverse hydroxamate and quercetin (Kumar et al., 2017). Flavonoids and phenolic acids such as gallic acid, vanillic acid, rutein, quercetin, kaempferol were identified using TLC from *W. somnifera* leaves extract (Sivamani et al., 2014). Reverse-phase high pressure liquid chromatography (RP-HPLC) was used for the identification epicatechin (3.21 μ g/g), quercetin-3-rhamnoside (1.12 μ g/g), gallic acid (0.05 μ g/g) and rutin hydrate (0.01 μ g/g) from *W. somnifera* root (Pal et al., 2012).

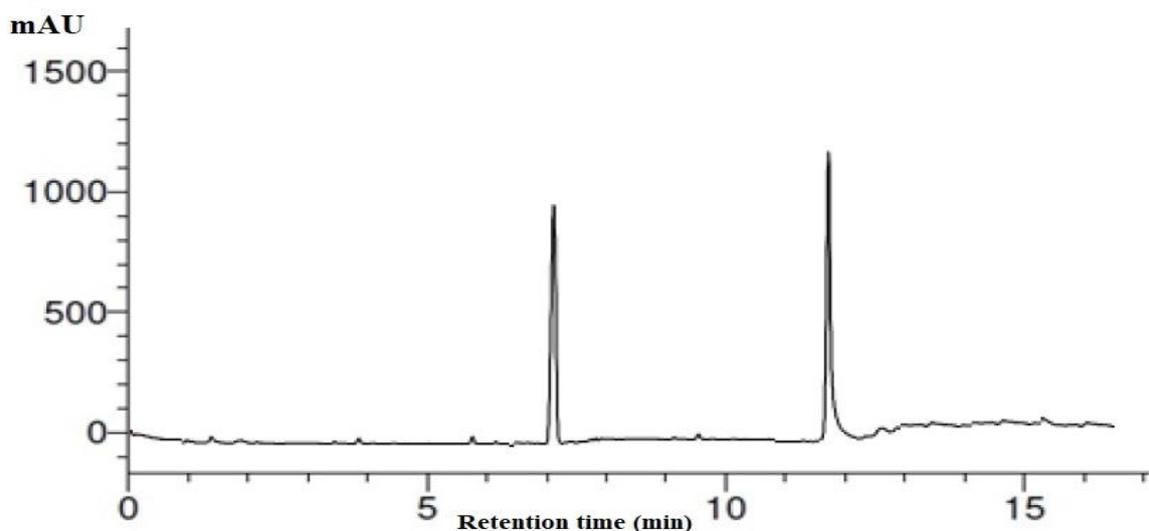


Figure 2: HPLC chromatogram of the identified compounds; quercetin and 7-hydroxyflavone.

As a nutritional supplement, quercetin, a plant-derived aglycone form of flavonoid glycosides, may be beneficial against various diseases such as osteoporosis, certain forms of cancer, pulmonary and cardiovascular diseases (Boots et al., 2008; Zhang et al., 2011). Quercetin has been reported to have several biological activities such as anticancer, antioxidant and antimicrobial (Baghel et al., 2012), and is one of the most prominent dietary antioxidants (Boots et al., 2008). Quercetin has numerous antioxidant activities, including its effects on enzymatic activity, glutathione (GSH), reactive oxygen species (ROS) and signal transduction pathways caused by environmental and toxicological factors (Xu et al., 2019). Six quercetin derivatives of tamarixetin, isorhamnetin, quercetin-3-O-glucuronide, quercetin-3,4'-di-O-glucoside, isorhamnetin-3-O-glucoside, and quercetin-3,5,7,3',4'-pentamethylether have been observed good anti-inflammatory activities compared to butylated hydroxytoluene and aspirin (Lesjak et al., 2018). Quercetin was found in Ashwagandharishta-T, M and its marketed formulation at 0.0021, 0.00192 and 0.00197% w/w, respectively while rutin was found at 0.00469, 0.00441 and 0.00464% w/w respectively (Tiwari; Patel 2012). Another study showed that the leaves of *W. somnifera* have a higher content of flavonoids and polyphenols, with total antioxidant activity of 7.52 mg eq. GA g-1 d.w., 2.30 mg eq. quercetin g-1 d.w. and 38.2 mg eq. GA g-1 d.w., respectively (Molchanova et al., 2019).

7-Hydroxyflavone is identified in leas extract of *W. somnifera*, where this compound showed medicinal values such as anti-glycation activity (Chinchansure et al., 2015). Mixtures containing flavone and 7-hydroxyflavon caused potential

fungal growth inhibition (Örner; Jha 1993). 7-hydroxyflavone showed good antifungal activity against against *Cladosporium herbarum* and *Penicillium glabrum* (Martini et al., 1997), and against five storage fungi of the genus *Aspergillus* (Weidenbörner et al., 1990).

CONCLUSION

Withania somnifera leaf extract showed several bioactive compounds as analyzed by chromatographic tools of GC-MS and HPLC. These compounds (2,6-dimethyl-N-(2-methyl- α -phenylbenzyl) aniline, 4-aminoheptane, n-undecanophenone, quercetin and 7-hydroxyflavone) could be used for several biological and medical purposes.

REFERENCES

- A Dar P.; R Singh L.; A Kamal M.; A Dar T. (2016). Unique medicinal properties of *Withania somnifera*: Phytochemical constituents and protein component. *Current pharmaceutical design*. **22**: 535-540.
- Anäs E.; Ekman R.; Holmbom B. (1983). Composition of nonpolar extractives in bark of Norway spruce and Scots pine. *Journal of Wood Chemistry and Technology*. **3**: 119-130.
- Baghel S. S.; Shrivastava N.; Baghel R. S.; Agrawal P.; Rajput S. (2012). A review of quercetin: antioxidant and anticancer properties. *World J Pharm Pharmaceutical Sci*. **1**: 146-160.
- Bellanger R. A.; Seeger C. M. (2021) Chapter 40 - Complementary and alternative medicine. In: Ray S. D. (ed) Side Effects of Drugs Annual, vol **43**. Elsevier, pp 493-502. doi:https://doi.org/10.1016/bs.seda.2021.07.003

- Bhatnagar M.; Sharma D.; Salvi M. (2009). Neuroprotective Effects of *Withania somnifera* Dunal.: A Possible Mechanism. *Neurochemical Research*. **34**: 1975-1983.
- Bhattacharya S. K.; Bhattacharya D.; Sairam K.; Ghosal S. (2002). Effect of *Withania somnifera* glycowithanolides on a rat model of tardive dyskinesia. *Phytomedicine*. **9**: 167-170.
- Boots A. W.; Haenen G. R. M. M.; Bast A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology*. **585**: 325-337.
- Chatterjee S.; Srivastava S.; Khalid A.; Singh N.; Sangwan R. S.; Sidhu O. P.; Roy R.; Khetrpal C. L.; Tuli R. (2010). Comprehensive metabolic fingerprinting of *Withania somnifera* leaf and root extracts. *Phytochemistry*. **71**: 1085-1094.
- Chinchansure A. A.; Korwar A. M.; Kulkarni M. J.; Joshi S. P. (2015). Recent development of plant products with anti-glycation activity: a review. *RSC Advances*. **5**: 31113-31138.
- Ekman R.; Holmbom B. (1989). Analysis by gas chromatography of the wood extractives in pulp and water samples from mechanical pulping of spruce. *Nordic Pulp & Paper Research Journal*. **4**: 16-24.
- El-Hefny M.; Salem M. Z. M.; Behiry S. I.; Ali H. M. (2020). The Potential Antibacterial and Antifungal Activities of Wood Treated with *Withania somnifera* Fruit Extract, and the Phenolic, Caffeine, and Flavonoid Composition of the Extract According to HPLC. *Processes*. **8**: 113.
- Girish K. S.; Machiah K. D.; Ushanandini S.; Harish Kumar K.; Nagaraju S.; Govindappa M.; Vedavathi M.; Kemparaju K. (2006). Antimicrobial properties of a non-toxic glycoprotein (WSG) from *Withania somnifera* (Ashwagandha). *Journal of Basic Microbiology*. **46**: 365-374.
- Gupta R. C.; Lall R.; Srivastava A. (2021) Nutraceuticals: efficacy, safety and toxicity. Academic Press,
- Harikrishnan B.; Subramanian P.; Subash S. (2008). Effect of *Withania Somnifera* Root Powder on the Levels of Circulatory Lipid Peroxidation and Liver Marker Enzymes in Chronic Hyperammonemia. *E-Journal of Chemistry*. **5**: 589394.
- Hassan H. S.; Mohamed A. A.; Felefel M. N.; Salem M. Z. M.; Ali H. M.; Akrami M.; Abd-Elkader D. Y. (2021). Natural Plant Extracts and Microbial Antagonists to Control Fungal Pathogens and Improve the Productivity of Zucchini (*Cucurbita pepo* L.) In Vitro and in Greenhouse. *Horticulturae*. **7**: 470.
- Jacobs B.; Pierce S. (2018) Chapter 14 - Multiple Sclerosis. In: Rakel D. (ed) Integrative Medicine (Fourth Edition). Elsevier, pp 133-142.e132. doi:https://doi.org/10.1016/B978-0-323-35868-2.00014-1
- Kambizi L.; Afolayan A. (2008). Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. *African Journal of Biotechnology*. **7**: 012-015.
- Kandil F. E.; El Sayed N. H.; Abou-Douh A. M.; Ishak M. S.; Mabry T. J. (1994). Flavonol glycosides and phenolics from *Withania somnifera*. *Phytochemistry*. **37**: 1215-1216.
- Kumar G.; Paliwal P.; Patnaik R. (2017). *Withania somnifera* Phytochemicals Confer Neuroprotection by Inhibition of the Catalytic Domain of Human Matrix Metalloproteinase-9. *Letters in Drug Design & Discovery*. **14**: 718-726.
- Lesjak M.; Beara I.; Simin N.; Pintač D.; Majkić T.; Bekvalac K.; Orčić D.; Mimica-Dukić N. (2018). Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *Journal of Functional Foods*. **40**: 68-75.
- Mahesh B.; Satish S. (2008). Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogens. *World Journal of Agricultural Sciences*. **4** (2008): 839-843.
- Martini H.; WeidenbÖrner M.; Adams S.; Kunz B. (1997). Increased antifungal activity of 3- and 7-hydroxyflavone against *Cladosporium herbarum* and *Penicillium glabrum* through ester formation. *Mycological Research*. **101**: 920-922.
- Mohareb A. S.; Kherallah I. E.; Badawy M. E.; Salem M. Z.; Yousef H. A. (2017). Chemical composition and activity of bark and leaf extracts of *Pinus halepensis* and *Olea europaea* grown in AL-Jabel AL-Akhdar region, Libya against some plant phytopathogens. *J Appl Biotechnol Bioeng*. **3**: 331-342.
- Molchanova A.; Kekina H.; Logvinenko L.; Caruso G.; Golubkina N.; Shevchuk O.; Khlipenko L. (2019). Antioxidant properties and elemental composition of *Withania somnifera* L. *Agriculture and Food*. **7**: 93-101.
- Oberacher H.; Whitley G.; Berger B. (2013). Evaluation of the sensitivity of the 'Wiley registry of tandem mass spectral data, MSforID' with MS/MS data of the 'NIST/NIH/EPA mass spectral library'. *Journal of Mass Spectrometry*. **48**: 487-496.
- Örner M. W.; Jha H. C. (1993). Antifungal activity of flavonoids and their mixtures against different fungi occurring on grain. *Pesticide Science*. **38**: 347-351.

- Pal A.; Kumar M.; Saharan V.; Bhushan B.; CCS HAU H. (2015). Anti-oxidant and free radical scavenging activity of ashwagandha (*Withania somnifera* L.) leaves. *J Glob Biosci.* **4**: 1127-1137.
- Pal A.; Naika M.; Khanum F.; Bawa A. S. (2012). In-vitro studies on the antioxidant assay profiling of root of *Withania somnifera* L.(Ashwagandha) Dunal: Part 2. *Agriculturae Conspectus Scientificus.* **77**: 95-101.
- Salem M. Z. M.; Mansour M. M. A.; Elansary H. O. (2019). Evaluation of the effect of inner and outer bark extracts of sugar maple (*Acer saccharum* var. *saccharum*) in combination with citric acid against the growth of three common molds. *Journal of Wood Chemistry and Technology.* **39**: 136-147.
- Savai J.; Varghese A.; Pandita N.; Chintamaneni M. (2015). Investigation of CYP3A4 and CYP2D6 Interactions of *Withania somnifera* and *Centella asiatica* in Human Liver Microsomes. *Phytotherapy Research.* **29**: 785-790.
- Singh B.; Saxena A. K.; Chandan B. K.; Gupta D. K.; Bhutani K. K.; Anand K. K. (2001). Adaptogenic activity of a novel, withanolide-free aqueous fraction from the roots of *Withania somnifera* Dun. *Phytotherapy Research.* **15**: 311-318.
- Sivamani S.; Joseph B.; Kar B. (2014). Anti-inflammatory activity of *Withania somnifera* leaf extract in stainless steel implant induced inflammation in adult zebrafish. *Journal of Genetic Engineering and Biotechnology.* **12**: 1-6.
- Sivanandhan G.; Arun M.; Mayavan S.; Rajesh M.; Mariashibu T. S.; Manickavasagam M.; Selvaraj N.; Ganapathi A. (2012). Chitosan enhances withanolides production in adventitious root cultures of *Withania somnifera* (L.) Dunal. *Industrial Crops and Products.* **37**: 124-129.
- Tiwari P.; Patel R. K. (2012). Quantification of Quercetin and Rutin in Ashwagandharishta by Validated HPTLC Densitometry. *Asian Journal of Research in Chemistry.* **5**: 441-445.
- Weidenbörner M.; Hindorf H.; Jha H. C.; Tsotsonos P. (1990). Antifungal activity of flavonoids against storage fungi of the genus *Aspergillus*. *Phytochemistry.* **29**: 1103-1105.
- Xu D.; Hu M.-J.; Wang Y.-Q.; Cui Y.-L. (2019). Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. *Molecules.* **24**: 1123.
- Zhang M.; Swarts S. G.; Yin L.; Liu C.; Tian Y.; Cao Y.; Swarts M.; Yang S.; Zhang S. B.; Zhang K.; Ju S.; Olek D. J.; Schwartz L.; Keng P. C.; Howell R.; Zhang L.; Okunieff P. Antioxidant Properties of Quercetin. In: LaManna J. C.; Puchowicz M. A.; Xu K.; Harrison D. K.; Bruley D. F. (eds) *Oxygen Transport to Tissue XXXII*, Boston, MA, 2011// 2011. Springer US, pp 283-289.

الملخص العربي

التحليل الكروماتوجرافي الغازي المتصل بالمطياف الكتلي والتحليل الكروماتوجرافي
السائل عالي الكفاءة لتحليل المركبات الكيميائية من مستخلص أوراق نبات ال
Withania somnifera L.

ميرفت الحفنى^١، مأمون سرحان عبد الكريم^٢، محمد زيدان محمد سالم^٣

^١ قسم الزهور ونباتات الزينة وتنسيق الحدائق - كلية الزراعة- جامعة الأسكندرية

^٢ قسم الفيزياء النووية التجريبية - وحدة الفيزياء الذرية والجزيئية، مركز البحوث النووية،
هيئة الطاقة الذرية المصرية، انشاص، القاهرة، مصر

^٣ قسم الغابات وتكنولوجيا الأخشاب - كلية الزراعة- جامعة الأسكندرية

في العمل، تم استخدام شجيرة ععب نوم *Withania somnifera* كمصدر لاستخلاص وتعريف المكونات الكيميائية النشطة بيولوجيًا. تم استخلاص الأوراق بواسطة الإيثانول وتحليلها بواسطة كروماتوجرافيا الغاز- مطياف الكتلة (GC-MS) والكروماتوجرافيا السائلة عالية الكفاءة (HPLC). من خلال تحليل GC-MS، كانت أعلى المركبات المعرفة هي ٢،٦-ثنائي ميثيل-N- (٢-ميثيل-ألفا-فينيل بنزيل) أنيلين، ٤-أمينو هيبنتان، ن-أونديكانوفينون، ٢-سيكلوهكسيل-٤-هيدروكسي ميثيل-أوكتاهايدروبنزو [1] [e]، oxazine-3-carbonitrile [2] ، وحمض 3-octadecanoic octadecyloxypropyl ester، بنسب ١٩،٤٣٪، ٧،٥١٪، ٤،٤٧٪، ٤،٣٤٪، و ٣،٨٩٪ على التوالي. حدد تحليل HPLC كيرسيتين و٧ هيدروكسي فلافون بتركيزات ١٢،٣٦ و ١٥،١٤ مجم/مل، على التوالي، كمركبين من مركبات الفلافونويد. أظهر هذا العمل أن مستخلص أوراق *W. somnifera* يمتلك العديد من المركبات النشطة بيولوجيًا التي يمكن أن تكون مفيدة ولها تطبيقات مختلفة، مثل الأنشطة المضادة للميكروبات.

الكلمات المفتاحية: أوراق *Withania somnifera*، HPLC، GC-MS، كيرسيتين، ٧ هيدروكسي فلافون.