March 21, 2017

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American Journal of Quantum Chemistry and Molecular Spectroscopy
2017; 2(1): 1-10
http://www.sciencepublishinggroup.com/j/ajqcms
doi: 10.11648/j.ajqcms.20170201.11

Liquid Chromatography – Mass Spectrometry (LC-MS) Analysis of *Withania somnifera* (Ashwagandha) Root Extract Treated with the Energy of Consciousness

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To cite this article:

Mahendra Kumar Trivedi, Alice Branton, Dahryn Trivedi, Gopal Nayak, Cathryn Dawn Nykvist, Celine Lavelle, Daniel Paul Przybylski, Dianne Heather Vincent, Dorothy Felger, Douglas Jay Konersman, Elizabeth Ann Feeney, Jay Anthony Prague, Joanne Lydia Starodub, Karan Rasdan, Karen Mie Strassman, Leonid Soboleff, Maire Anne Mayne, Mary M. Keesee, Padmanabha Narayana Pillai, Pamela Clarkson Ansley, Ronald David Schmitz, Sharyn Marie Sodomora, Kalyan Kumar Sethi, Parthasarathi Panda, Snehasis Jana. Liquid Chromatography – Mass Spectrometry (LC-MS) Analysis of *Withania somnifera* (Ashwagandha) Root Extract Treated with the Energy of Consciousness. *American Journal of Quantum Chemistry and Molecular Spectroscopy*. Vol. 2, No. 1, 2017, pp. 1-10. doi: 10.11648/j.ajqcms.20170201.11

Received: February 6, 2017; **Accepted**: February 27, 2017; **Published**: March 21, 2017

Abstract: *Withania somnifera* (ashwagandha) root extract is very popular ancient herbal medicine. The objective of the study was to characterize and evaluate the impact of The Trivedi Effect®-Biofield Energy Healing Treatment (Energy of Consciousness) on phytoconstituents present in the ashwagandha root extract using LC-MS. Ashwagandha root extract was divided into two parts. One part was denoted as the control, while the other part was defined as The Trivedi Effect® - Biofield Energy Treated sample, which received Energy of Consciousness Healing Treatment remotely from eighteen renowned Biofield Energy Healers. The LC-MS analysis of the control and treated samples showed a very close retention time (R_t), indicated that the polarity of the phytoconstituents present in the root extract are same. The numbers of peaks observed in the total ion chromatograms were 28 and 29 in the control and treated samples, respectively. The change in the peak height% of the phytoconstituents in the treated sample was altered significantly within the range of -50.91% to 118.12% compared with the control sample. Similarly, the change in the peak area% of most of the phytoconstituents in the treated ashwagandha was significantly altered within the range of -54.95% to 66.95% compared with the control sample. An additional peak was appeared in the treated sample at R_t of 5.72 minutes, which was not found in the control sample. The LC-MS spectra indicated the presence of possible withanolides like β-hydroxy-2,3-dihydro-withanolide F, withanolide A, withaferine A, withanone, withanolide D, ixocarpalactone A, withanolide S, thiowithanolide, *etc.* in both the samples. The peak are percentage (%) was altered in the identified withanolides, but withanolide sulfoxide was increased significantly by 12.44% in the treated sample compared with the control sample. These results indicated that The Trivedi Effect® - Biofield Energy Treatment might have an impact on the intrinsic physicochemical properties of the phytoconstituents present in the ashwagandha root extract. This could be the probable cause of alteration in the peak height, peak area, and appearance of a new peak in the treated sample. As a result, the concentrations of the phytoconstituents altered in the treated sample compared with the control sample. The treated ashwagandha root extract would be helpful for designing better pharmaceutical/nutraceutical formulations which might be providing a better therapeutic response against autoimmune diseases, nervous and sexual disorders, infectious diseases, antiaging, diabetes, cancer, ulcer, immunological disorders, stress, arthritis, etc.

Keywords: *Withania somnifera,* Biofield Energy Healing Treatment, Biofield Energy Healers, Consciousness Energy Healing Treatment, The Trivedi Effect®, LC-MS, Retention time, Withanolides

1. Introduction

Now-a-days herbal medicines have been getting exploring throughout the world for the prevention and treatment of various diseases because of their impressive therapeutic effects and fewer side effects compared with the modern medicines [1]. The roots of *Withania somnifera* is an ancient Rasayana herb and is popularly known as 'Ashwagandha' or winter cherry or 'Indian ginseng' [2, 3]. *W. somnifera* is mostly used in the herbal drugs and nutraceuticals for the prevention and treatment of various diseases such as nervous and sexual disorders, infectious diseases, diabetes, cancer, ulcer, immunological disorders, stress, arthritis, etc. As a tonic, it is useful to arrest the aging process, rejuvenate the body and boost the defense system against infectious disorders as well as to promote the longevity [2-6]. The major active phytoconstituents of *W. somnifera* root extract contains highly oxygenated withanolides. Besides withanolides, ashwagandha root contains alkaloids, numerous withanamides, sitoindosides, starch, reducing sugars, peroxidases, glycosides, dilcitol, withanicil, benzoic acid phenyl acetic acid, benzyl alcohol, 2-phenyl ethanol,3,4,5-trihydroxy cinnamic acid, etc. [7-9]. Isolated withanolides from *W. somnifera* possess various pharmacological activities includes antioxidant, anticancer, immunomodulating, hepatoprotective, neuroprotective, anti-inflammatory, antiarthritic, antimicrobial, hypoglycaemic, etc. [10-12]. Therefore, a new proprietary herbomineral formulation was formulated that consisted of the herbal ashwagandha root extract along with zinc, magnesium, and selenium minerals. This herbomineral formulation was designed as a nutraceutical supplement and can be used for the prevention and treatment of various human disorders.

Every living organism preserves some kind of unique quality, an élan vital or vital force, which contributes the 'life'. From the ancient-time, this living force is known as Prana by the Hindus, *qi* or *chi* by the Chinese, and *ki* by the Japanese and is usually believed to create the source of life that is related with soul, spirit, and mind. Now-a-days, this hypothetical vital force is considered as the Bioenergetics Field. This energy field is a dynamic electromagnetic field surrounding the human body. The Biofield Energy is infinite and paradimensional. It can freely flow between the human and the environment that leads to the continuous movement or matter of energy [13, 14]. Thus, the human can harness energy from the earth, the "universal energy field" and transmit it to any living or non-living object(s) around the globe. The objects always receive the energy and respond in a useful way. This process is known as Biofield Energy Healing Treatment [15-17]. Biofield (Putative Energy Fields) based Energy Therapies are used worldwide to promote health and healing [18]. The National Center of Complementary and Integrative Health (NCCIH) has been recognized and accepted Biofield Energy Healing as a Complementary and Alternative Medicine (CAM) health care approach in addition to other therapies, medicines and practices such as natural products, deep breathing, yoga, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupressure, acupuncture, relaxation techniques, healing touch, movement therapy, hypnotherapy, pilates, rolfing structural integration, mindfulness, Ayurvedic medicine, traditional Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, Reiki, cranial sacral therapy and applied prayer (as is common in all religions, like Christianity, Hinduism, Buddhism and Judaism) [19]. The Biofield Energy Treatment (The Trivedi Effect®) has been extensively studied with significant outcomes in many scientific fields such as cancer research [20], altered antimicrobial sensitivity of pathogenic microbes in microbiology [21-23], biotechnology [24, 25], genetics [26, 27], changing the structure of the atom in relation to the various metals, ceramics, polymers and chemicals materials science [28-30], altered physical and chemical properties of pharmaceuticals [31, 32], nutraceuticals [33, 34], organic compounds [35-37], and improved overall growth and yield of plants in agricultural science [38, 39].

Modern, sophisticated techniques such as high-performance liquid chromatography (HPLC) with photodiode array and evaporative light scattering detection, ultra-performance liquid chromatography (UPLC) electrospray ionization (ESI) normally hyphenated with mass spectrometry is very useful for the metabolite profiling and identification of the crude herbal extract [8, 40-42]. The LC-MS/MS, GC-MS and NMR analysis of *W. somnifera* root hydro-alcoholic extract revealed the presence of several known withanolides including withaferin A, withanolide D, withanoside IV or VI, withanolide sulfoxide, etc., along with two new withanolides *i.e.* dihydrowithanolide D and ixocarpalactone A [43]. Therefore, this study was designed for the characterization of the phytoconstituents present in the ashwagandha root hydro-alcoholic extract and to evaluate the influence of The Trivedi Effect®-Biofield Energy Healing on the phytoconstituents with the help of LC-MS.

2. Materials and Methods

2.1. Chemicals and Reagents

Withania somnifera (ashwagandha) hydro-alcoholic root extract was purchased from Sanat Product Ltd., India. All the other chemicals used in this experiment were analytical grade procured from the local vendors.

2.2. Energy of Consciousness Treatment Strategy

Ashwagandha root extract powder was one of the components of the new proprietary herbomineral formulation, developed by our research team, and it was used *per se* as the test sample for the current study. The test sample was divided into two parts, one part of the test sample was treated with The Trivedi Effect® - Biofield Energy Treatment by renowned Biofield Energy Healers and defined as The Trivedi Effect® treated sample, while the second part of the test sample did not receive any sort of treatment and defined as untreated or control ashwagandha root extract sample. The group of eighteen Biofield Energy Healers who participated in this study performed The Trivedi Effect® treatment remotely to the test sample. Eleven of the Biofield Energy Healers were located in the U.S.A., four in Canada, one in Ireland, one in the United Kingdom, and one in Russia performed The Trivedi Effect® - Biofield Energy Treatment on the test sample that was located in the research laboratory of GVK Biosciences Pvt. Ltd., Hyderabad, India. This treatment was provided for 5 minutes through Healer's Unique Energy Transmission process remotely to the test sample under the laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the sample. Similarly, the control sample was subjected to "sham" healers for 5 minutes, under the same laboratory conditions. The sham healer did not have any knowledge about The Trivedi Effect® - Biofield Energy Treatment. After that, the treated and untreated samples were kept in similar sealed conditions and characterized thoroughly by LC-MS.

2.3. Method of Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

The LC-MS analysis of the control and treated samples were conducted by following the almost same method as mentioned in the recent literature [43] using The Waters® ACQUITY UPLC, Milford, MA, USA equipped with a binary pump (The Waters® BSM HPLC pump), autosampler, column heater and a photo-diode array (PDA) detector. A Triple Quad (Waters Quattro Premier XE, USA) mass spectrometer equipped with an electrospray ionization (ESI) source was used for the mass spectrometric analysis. The control and treated extract powders were dissolved in dimethylsulfoxide to afford a 1 mg/mL stock solution. An aliquot of 2 µL of the stock solution was used for LC-MS analysis with a total run time of 25 minutes. Mass spectra were recorded in the positive ionization mode and with the full scan (*m/z* 50-1400). Percent change in peak height, peak area, and peak area% were calculated using following equation (1):

$$\%\ \text{change in peak height/peak area} = \frac{[P_{Treated} - P_{Control}]}{P_{Control}} \times 100 \quad (1)$$

Where, $P_{Control}$ and $P_{Treated}$ are the peak height, peak area, and peak area% of the control and treated samples, respectively.

3. Results and Discussion

The total ion chromatograms (TIC) of the control and treated samples of ashwagandha root extract are shown in Figure 1. The TIC of the control and treated samples exhibited several peaks along with their retention time (R_t) (Table 1 and Figure 1) indicating the presence of numerous phytoconstituents in the ashwagandha root extract. The control sample shown the 28 definite peaks in the chromatogram at R_t of 5.39, 5.59, 5.76, 5.95, 6.08, 6.26, 6.39, 6.55, 6.63, 6.76, 6.88, 6.99, 7.05, 7.25, 7.29, 7.66, 7.78, 7.92, 8.03, 8.12, 8.27, 8.48, 8.61, 8.73, 8.99, 9.10, 9.16, and 9.30 minutes. Similarly, the treated sample showed 28 peaks in the chromatogram at R_t of 5.35, 5.55, 5.79, 5.91, 6.03, 6.22, 6.35, 6.51, 6.58, 6.71, 6.84, 6.93, 7.00, 7.19, 7.23, 7.61, 7.73, 7.87, 8.00, 8.08, 8.23, 8.44, 8.55, 8.70, 8.96, 9.10, 9.15, and 9.29 minutes, along with one additional peak appeared at R_t of 5.72 minutes (Figure 1). Each of the corresponding R_t represents the presence of one phytoconstituent from the ashwagandha root extract. The R_t of both the control and treated samples were very close to each other (Table 1). The peak heights/areas were very important for the measurement of the relative quantities of the compounds present in the sample. The height/area under the peak is directly proportional to the amount of each compound, which had passed the detector, and these areas can be calculated [41].

The concentration of the sample was indicated the peak height/area. The peak height/area% (normalization) calculation procedure reported the area of each peak in the chromatogram as a percentage of the total area of all peaks [44, 45]. The peak height% and peak areas% were calculated with the help of the following equation (2) and the results are presented in Table 1.

$$Peak\ height/Area\% = \frac{Individual\ Peak\ height/Area}{Total\ Peak\ height/Area}\ X\ 100 \quad (2)$$

The peak height/area% did not require prior calibration and did not depend upon the amount of sample injected within the limits of the detector (normalization) [44, 45]. The phytoconstituents of the samples responded in the detector and were eluted, then peak height/area% provided the relative amounts of phytoconstituents (i.e. withanolides, alkaloids, flavonoids, etc.) present in the ashwagandha root extract.

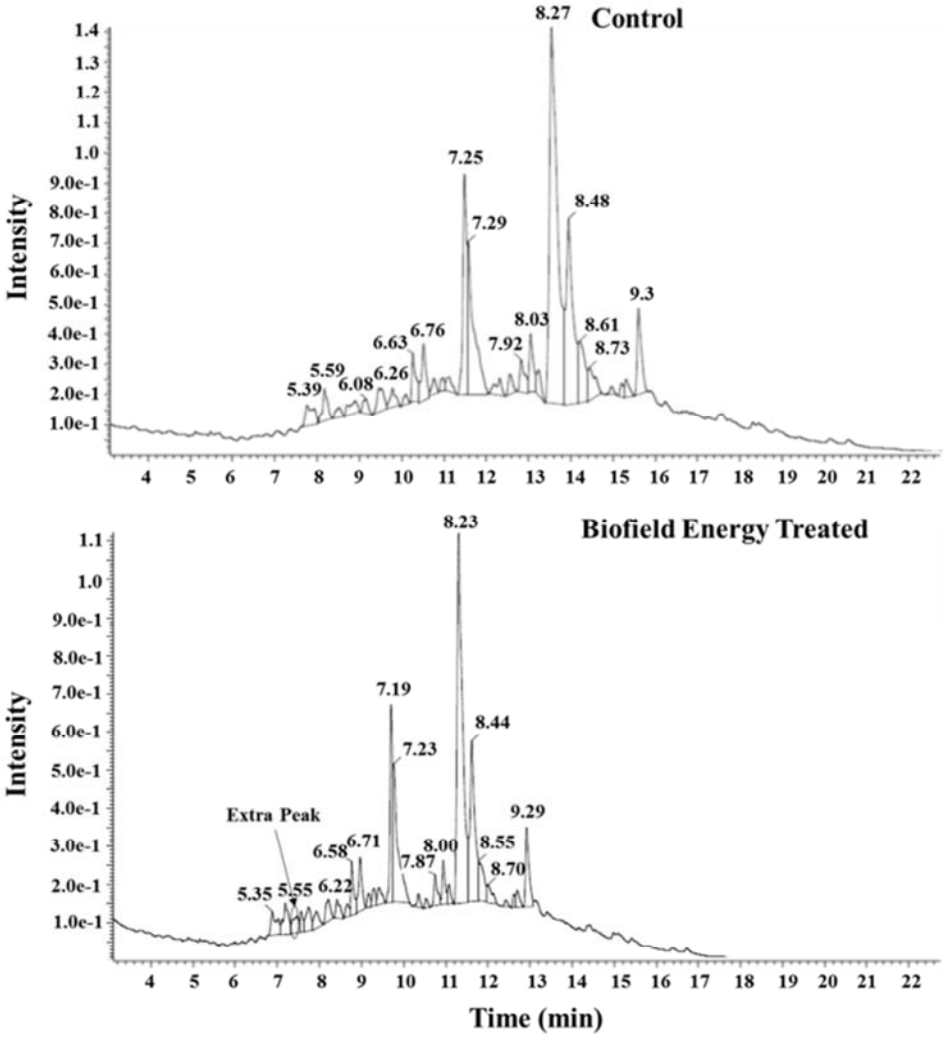

Figure 1. *Total ion chromatograms (TIC) of the control and Biofield Energy Treated ashwagandha root extract using The Waters® ACQUITY UPLC.*

The peak height% of phytoconstituents present in the treated ashwagandha root extract was increased by 16.54%, 4.98%, 118.12%, 99.41%, 20.53%, 3.04%, 6.09%, 38.95%, 18.94%, 0.98%, 33.88%, 17.41%, 2.26%, and 5.23% at R_t of 5.39, 5.59, 5.76, 5.95, 6.08, 6.26, 6.39, 6.55, 6.63, 6.76, 6.99, 7.05, 7.92, and 8.27 minutes, respectively compared with the control sample (Table 1). Similarly, the peak height% of some of the other phytoconstituents in the treated ashwagandha was decreased by 8.79%, 3.39%, 3.47%, 15.86%, 50.91%, 20.09%, 17.55%, 7.65%, 32.36%, 41.10%, 12.24%, 2.24%, 0.48%, and 1.42% at R_t of 6.88, 7.25, 7.29, 7.66, 7.78, 8.03, 8.12, 8.48, 8.61, 8.73, 8.99, 9.10, 9.16, and 9.30 minutes, respectively compared with the control sample (Table 1). The peak height% of the phytoconstituents in the treated ashwagandha root extract was altered significantly in the range of -50.91% to 118.12% compared with the control sample.

Table 1. *Retention times, peak heights, peak areas, peak areas%, and percentage change in the peak areas% of both the control and Biofield Energy Treated samples of ashwagandha root extract.*

Withania somnifera-Control					**Withania somnifera-BE Treated**					**% Change in PH%#**	**% Change in PA%#**
R_t	**PH**	**PA**	**PH%**	**PA%**	**R_t**	**PH**	**PA**	**PH%**	**PA%**		
5.39	71367	7917.81	1.35	1.85	5.35	61571	6251.58	1.57	2.08	16.54	12.43
5.59	107254	7276.48	2.03	1.70	5.55	83350	7855.83	2.13	2.61	4.98	53.53
Extra peak appeared in the BE Treated sample					5.72	42580	5170.35	1.09	1.72	NA	NA
5.76	34040	2652.73	0.64	0.62	5.79	54964	2538.87	1.40	0.84	118.12	35.48
5.95	41399	5065.37	0.78	1.18	5.91	61113	5930.63	1.56	1.97	99.41	66.95
6.08	49966	3680.07	0.94	0.86	6.03	44583	4071.84	1.14	1.35	20.53	56.98
6.26	74763	7205.78	1.41	1.68	6.22	57028	4733.37	1.46	1.57	3.04	-6.55

Withania somnifera-Control					Withania somnifera-BE Treated					% Change in PH%[#]	% Change in PA%[#]
R_t	PH	PA	PH%	PA%	R_t	PH	PA	PH%	PA%		
6.39	61443	4642.95	1.16	1.08	6.35	48255	3790.41	1.23	1.26	6.09	16.67
6.55	32582	1956.15	0.62	0.46	6.51	33514	2184.83	0.86	0.73	38.95	58.70
6.63	159434	8827.08	3.01	2.06	6.58	140387	7020.34	3.59	2.33	18.94	13.11
6.76	186242	11816.94	3.52	2.76	6.71	139230	9008.77	3.56	3.00	0.98	8.70
6.88	52047	3003.03	0.98	0.70	6.84	35144	1901.93	0.90	0.63	-8.79	-10.00
6.99	44279	2315.92	0.84	0.54	6.93	43885	2583.15	1.12	0.86	33.88	59.26
7.05	50827	3825.28	0.96	0.89	7.00	44176	3446.29	1.13	1.15	17.41	29.21
7.25	729764	37673.13	13.80	8.80	7.19	521922	25474.71	13.33	8.47	-3.39	-3.75
7.29	503994	42507.57	9.53	9.93	7.23	360166	29136.59	9.20	9.69	-3.47	-2.42
7.66	56528	4892.71	1.07	1.14	7.61	35210	1931.51	0.90	0.64	-15.86	-43.86
7.78	65181	3884.48	1.23	0.91	7.73	23689	1233.91	0.60	0.41	-50.91	-54.95
7.92	105613	7479.50	2.00	1.75	7.87	79954	4988.71	2.04	1.66	2.26	-5.14
8.03	191753	10425.25	3.63	2.43	8.00	113437	5939.13	2.90	1.97	-20.09	-18.93
8.12	83596	5167.76	1.58	1.21	8.08	51025	2649.64	1.30	0.88	-17.55	-27.21
8.27	1242286	133910.92	23.49	31.27	8.23	967773	92349.31	24.71	30.71	5.23	-1.79
8.48	615223	59363.30	11.63	13.86	8.44	420615	38611.41	10.74	12.84	-7.65	-7.36
8.61	203182	18234.33	3.84	4.26	8.55	101737	9287.03	2.60	3.09	-32.36	-27.47
8.73	109056	10857.75	2.06	2.54	8.70	47554	4430.56	1.21	1.47	-41.10	-42.13
8.99	25915	1293.78	0.49	0.30	8.96	16836	965.25	0.43	0.32	-12.24	6.67
9.10	44063	1992.12	0.83	0.47	9.10	31890	1430.54	0.81	0.48	-2.24	2.13
9.16	59519	3806.37	1.13	0.89	9.15	43850	2796.27	1.12	0.93	-0.48	4.49
9.30	288226	16523.68	5.45	3.86	9.29	210349	13039.13	5.37	4.34	-1.42	12.44

PA: Peak Area; PH: Peak Height; R_t: Retention time; NA: Not applicable; BE: Biofield Energy. [#] denotes the percentage change in the peak height% and peak area% of the Biofield Energy Treated sample with respect to the control sample.

The peak area% of phytoconstituents in the treated ashwagandha was increased by 12.43%, 53.53%, 35.48%, 66.95%, 56.98%, 16.67%, 58.70%, 13.11%, 8.70%, 59.26%, 29.21%, 6.67%, 2.13%, 4.49%, and 12.44% compared with the control sample at R_t of 5.39, 5.59, 5.76, 5.95, 6.08, 6.39, 6.55, 6.63, 6.76, 6.99, 7.05, 8.99, 9.10, 9.16, and 9.30 minutes, respectively (Table 1). On the other hand, the peak area% of some of the phytoconstituents present in the treated ashwagandha was decreased by 6.55%, 10.00%, 3.75%, 2.42%, 43.86%, 54.95%, 5.14%, 18.93%, 27.21%, 1.79%, 7.36%, 27.47%, and 42.13% compared with the control sample at R_t of 6.26, 6.88, 7.25, 7.29, 7.66, 7.78, 7.92, 8.03, 8.12, 8.27, 8.48, 8.61, and 8.73 minutes, respectively (Table 1). The peak area% of the phytoconstituents in the treated ashwagandha root extract were altered in the range of -54.95 to 66.95% compared with the control sample. An additional peak was appeared in the treated ashwagandha with the peak height% and peak area% of 1.09 and 1.72, respectively at R_t of 5.72 minutes, which was not detected in the control sample (Figure 1 and Table 1).

The ESI-MS spectra of the control and treated ashwagandha root extract are shown in Figures 2 and 3, respectively. Some of the important possible withanolides were identified with the help of the ESI-MS spectral analysis (Table 2 and Figure 4), which were already reported in literature [40-43]. The possible withanolides identified both in control and treated samples were β-hydroxy-2,3-dihydro-withanolide F (W1) at R_t of 7.3 minutes and *m/z* 489 $[M + H]^+$ (calculated for $C_{28}H_{40}O_7$, 489). Similarly, the other possible withanolides like withanolide A (W2), withaferine A (W3), withanone (W4), withanolide D (W5), 27-hydroxy withanolide B (W6), 5,7α-epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (W7), 5α,17β-dihydroxy-6α,7α-epoxy-1-oxo-witha-2,24-dienolide (W8) were identified at R_t of 8.6 minutes. These compounds showed the molecular ion peak at *m/z* 471 $[M + H]^+$ (calcd for $C_{28}H_{39}O_6$, 471) and 488 $[M + NH_4]^+$ (calcd for $C_{28}H_{42}O_6N$, 800) along with fragment ions at *m/z* 459 and 120 in the ESI-MS spectra of the control and treated samples, respectively (Figures 2 and 3). The withanolides like ixocarpalactone A (W9) and withanolide S (W10) identified at R_t of 8.7 minutes and *m/z* 505 $[M + H]^+$ (calculated for $C_{28}H_{41}O_8$, 505). Consequently, the ESI-MS spectra of the control and treated samples at R_t of 9.3 minutes (Figure 2 and 3) revealed that withanolide sulfoxide (W**11**) (Figure 4) showed the molecular ion peak at *m/z* 992 $[M + H]^+$ (calculated for $C_{56}H_{79}O_{13}S$, 992) along with the fragmented ions at *m/z* 975, 437 and 120. Withanolide sulfoxide has the pharmacological properties such as antitumor and COX-2 enzyme inhibition activities [43].

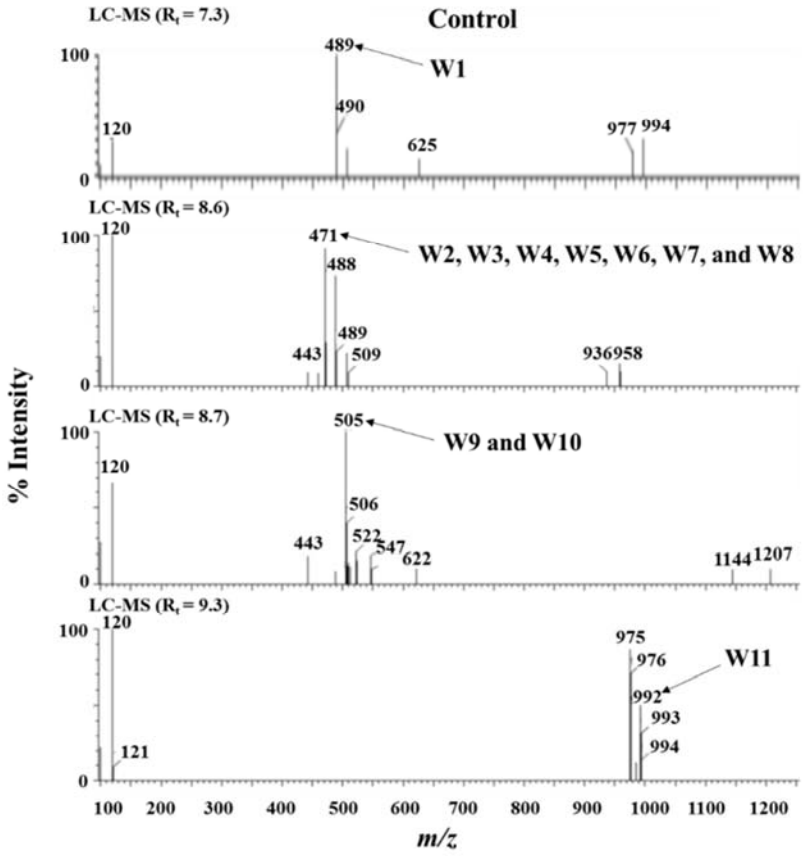

Figure 2. *ESI-MS spectra of the control sample of ashwagandha root extract.*

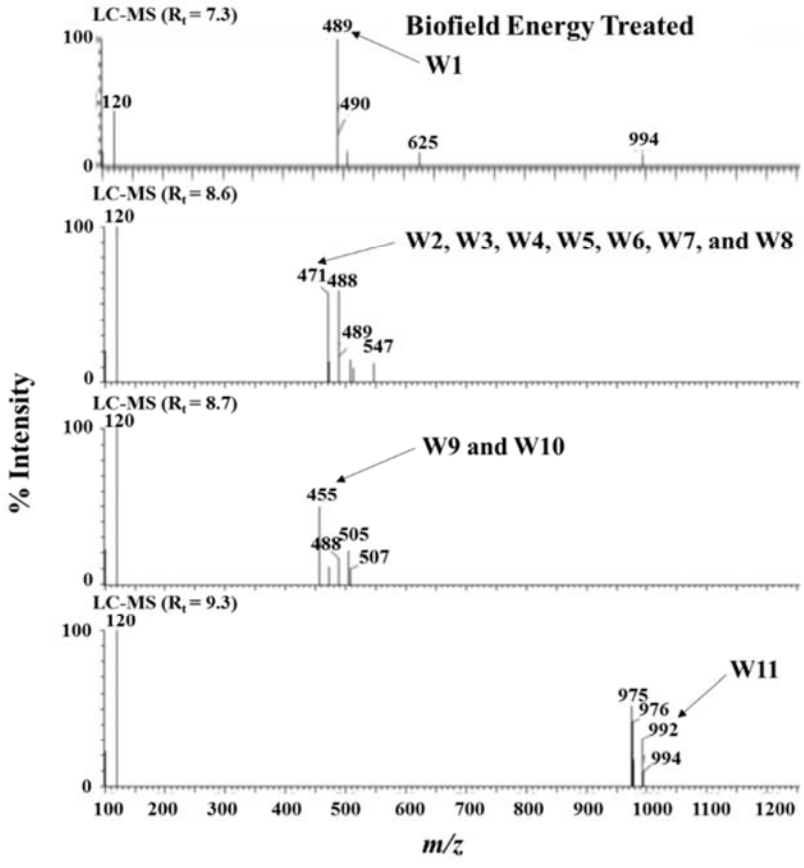

Figure 3. *ESI-MS spectra of Biofield Energy Treated sample of ashwagandha root extract.*

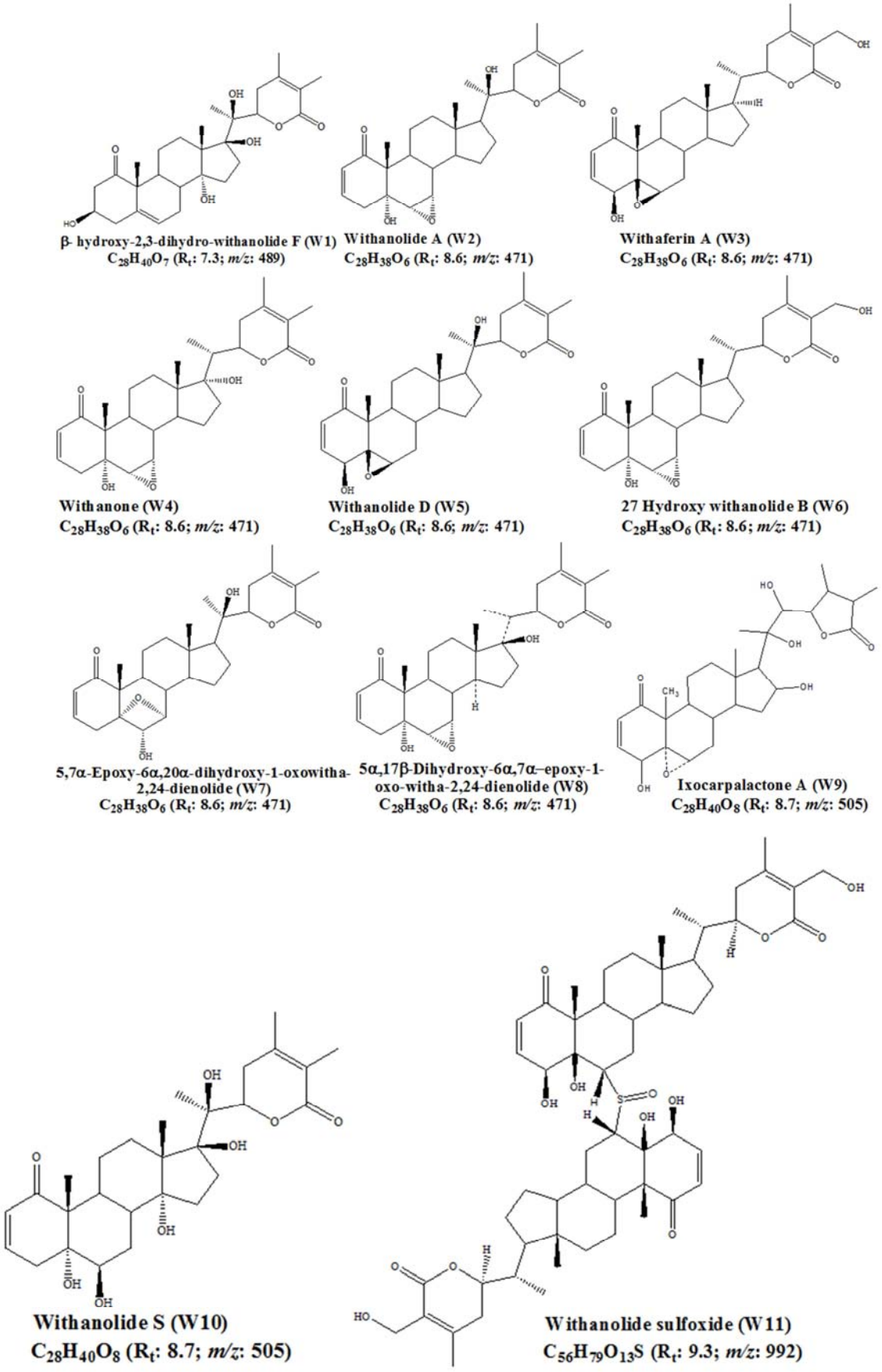

Figure 4. *Possible important withanolides (W) identified from the control and Biofield Energy Treated root extract of ashwagandha.*

Table 2. *Retention times, molecular weights, and possible withanolides identified in both the control and Biofield Energy Treated samples of ashwagandha root extract.*

R_t (min)	*m/z*	Possible Withanolide (W)
7.3	489	β-Hydroxy-2,3-dihydro-withanolide F (W1)
		Withanolide A (W2)
		Withaferine A (W3)
		Withanone (W4)
		Withanolide D (W5)
8.6	471	27-Hydroxy withanolide B (W6)
		5,7α-Epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (W7)
		5α,17β-Dihydroxy-6α,7α-epoxy-1-oxo-witha-2,24-dienolide (W8)
8.7	505	Ixocarpalactone A (W9)
		Withanolide S (W10)
9.3	992	Withanolide sulfoxide (W11)

R_t: Retention time; W: Withanolide; Mol. Wt.: Molecular weight.

Overall, the LC-MS analysis indicated that the peak height% and peak area% of most of the phytoconstituents present in the treated sample was significantly altered compared with the control sample. The Trivedi Effect®-Biofield Energy Healing Treatment assumed to be having a significant role in the alteration of the peak height/area of the phytoconstituents in the ashwagandha root extract. Various literature reported that the Biofield Energy Treatment (The Trivedi Effect®) significantly altered the intrinsic physicochemical properties of many compounds, which have the influence on its solubility profile [28-34]. The increase in the solubility of a compound increase the concentration in the solvent system [46-48]. It can be assumed that, the relative peak height and peak area of the phytoconstituents in the Biofield Energy Treated sample was altered by altering its solubility profile due to the Biofield Energy Healing Treatment. The solubility of an analyte in the solvent system (diluent) has a direct effect on the peak height and peak area. The peak height% and peak area% are directly proportional to the analyte concentration [49, 50]. The Table 1 revealed that the Biofield Energy Treatment might have the significant effect on the relative amount of the phytoconstituents due to the Biofield Energy Healing Treatment.

4. Conclusions

The LC-MS analysis of the control and The Trivedi Effect® (Energy of Consciousness) Biofield Energy Healing treated ashwagandha root extract showed 28 and 29 peaks, respectively in the chromatograms. The peak height and peak area were significantly altered in the treated sample compared with the control sample. The change in the peak height% of the phytoconstituents in the treated ashwagandha was altered significantly within the range of -50.91% to 118.12% compared with the control sample. Similarly, the change in the peak area% of most of the phytoconstituents in the treated ashwagandha was significantly altered within the range of -54.95% to 66.95% compared with the control sample. In the treated sample, an additional peak was appeared at R_t of 5.72 minutes, which was not found in the control sample. The LC-MS spectra indicated the presence of possible withanolides like β-hydroxy-2, 3-dihydro-withanolide F, withanolide A, withaferine A, withanone, withanolide D, ixocarpalactone A, withanolide S, thiowithanolide, *etc.* in both the samples. The peak area% was altered in the identified withanolides, but withanolide sulfoxide was increased significantly by 12.44% in the treated sample compared with the control sample. From the results, it can be hypothesized that The Trivedi Effect® - Biofield Energy Treatment might have an impact on the intrinsic physicochemical properties of the phytoconstituents present in the ashwagandha root extract. This could be the probable cause of alteration in the peak height, peak area, and appearance of a new peak in the treated sample. As a result, the relative amount of the phytoconstituents assumed to be altered in the treated sample compared with the control sample. This treated ashwagandha root extract would be helpful for designing better pharmaceutical/nutraceutical formulations which might be providing a better therapeutic response against various diseases such as diabetes mellitus, allergies and septic shock; stress-related disorders like sleep disorder, anxiety, insomnia, depression, Attention Deficit Disorder (ADD), Attention Deficit Hyperactive Disorder (ADHD), mental restlessness (mind chattering), low libido, brain frog, impotency, lack of motivation, mood swings, fear of the future, confusion, headaches, migraines, forgetfulness, overwhelm, worthlessness, loneliness, indecisiveness, frustration, chronic fatigue, irritability, obsessive/compulsive behavior and panic attacks; inflammatory diseases and immunological disorders like Lupus, Systemic Lupus Erythematosus, Hashimoto Thyroiditis, Type 1 Diabetes, Asthma, Hepatitis, Chronic peptic ulcers, Tuberculosis, Chronic active hepatitis, Celiac Disease (gluten-sensitive enteropathy), Crohn's disease, Addison Disease, Graves' Disease, Pernicious and Aplastic Anemia, Sjogren Syndrome, Irritable Bowel Syndrome (IBS), Multiple Sclerosis, Rheumatoid arthritis, Chronic periodontitis, Ulcerative colitis, Myasthenia Gravis, Chronic sinusitis, Atherosclerosis, Vasculitis, Dermatitis, Diverticulitis, Reactive Arthritis, Rheumatoid Arthritis, Alopecia Areata, Psoriasis, Scleroderma, Fibromyalgia, Chronic Fatigue Syndrome and Vitiligo; aging-related diseases like cardiovascular disease, arthritis, cancer, Alzheimer's disease, dementia, cataracts, osteoporosis, diabetes, hypertension, glaucoma, hearing loss, Parkinson's Disease, Huntington's Disease, Spinocerebellar Ataxia, Prion Disease, Motor Neurone Disease, Spinal muscular atrophy, Amyotrophic lateral sclerosis, Friedreich's Ataxia, Lewy Body Disease, chronic infections and much more.

Abbreviations

DMSO: dimethyl sulfoxide; EI: Electron ionization; ESI: Electrospray ionization; LC-MS: Liquid chromatography-mass spectrometry; PDA: Photodiode array; R_t: Retention

time; UPLC: Ultra-performance liquid chromatography.

Acknowledgements

The authors are grateful to GVK Biosciences Pvt. Ltd., Hyderabad, India, Trivedi Science, Trivedi Global, Inc., and Trivedi Master Wellness for their support throughout the work.

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