

# Study of the Consciousness Energy Healing Treated Ashwagandha Root Extract by LC-MS, GC-MS, and NMR Spectroscopy

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**Abstract:** *Withania somnifera* (ashwagandha) contains many biologically active constituents used for the prevention and treatment of various diseases. The aim of the current study was to evaluate the impact of Energy of Consciousness Healing Treatment (The Trivedi Effect<sup>®</sup>) on the characteristic properties of the ashwagandha root extract using LC-MS, GC-MS, and NMR spectroscopy. Ashwagandha root extract was divided into two parts – one part was control (without treatment), while another part was treated with the Energy of Consciousness Healing Treatment remotely by twenty renowned Biofield Energy Healers and defined as the Biofield Energy Treated sample. LC-MS data revealed that the retention time of the phytoconstituents remained same in the control and Biofield Energy Treated samples, whereas the peak area% *i.e.* the relative amount of the phytoconstituents was significantly altered. The peak area% at  $R_t$  of 5.1, 5.7, 6.4, 6.6, 6.9, 7.1, 7.4, 7.8, 7.9, 8.0, 8.2, 8.5, and 8.9 minutes of the treated sample were increased in the range of 0.92% to 14.67% compared to the control sample. On the contrary, the peak area% of the treated sample at  $R_t$  of 5.2, 5.3, 5.4, 5.6, 6.8, 8.1, 8.4, 8.6, 9.0, 9.1, and 9.2 minutes were decreased by 1.59% to 36.69% with respect to the control sample. A total of 14 withanolides such as sitoindoside IX, dihydrowithanolide D, withanolide A, withaferin A, ixocarpalactone A, withanolide S, withanolide sulfoxide, etc. were proposed with their structure from the molecular mass at  $m/z$  605, 489, 473, 471, 505, and 992 at retention times of 6.9, 7.1, 7.9, 8.2, 8.5, and 9.3 minutes with the help of LC-MS, GC-MS and NMR data of both the control and Biofield Energy Treated samples. Consequently, the mass peak intensities of the treated sample were significantly changed in the range of -36.67% to 106% compared with the control sample at the same retention time. These findings suggest that Energy of Consciousness Healing Treatment could be advantageous for altering the concentration of the phytoconstituents in the ashwagandha root extract by modifying their intrinsic physicochemical properties, which might be helpful to improve the bioavailability of active constituents of ashwagandha extract that might provide better therapeutic response against inflammatory diseases, immunological disorders, arthritis, stress, cancer, diabetes, sexual disorders, aging, and other chronic infections.

**Keywords:** Ashwagandha, Biofield Energy Healers, The Trivedi Effect<sup>®</sup>, Energy of Consciousness Healing Treatment, Biofield Energy Healing Treatment, LC-MS, Withanolides, GC-MS

## 1. Introduction

*Withania somnifera* is an ancient herb commonly known as ashwagandha, winter cherry and ‘Indian ginseng’ used worldwide as herbal medicines for the prevention and treatment of many diseases and for the good health [1-3]. Ashwagandha is mostly used in the herbal drugs and nutraceuticals for the treatment sexual and nervous disorders, infectious diseases, diabetes, cancer, immunological disorders, ulcer, stress, arthritis, etc. It is used as a tonic to rejuvenate the body, arrest the aging process, and boost the defense system against infectious disorders as well as to promote the longevity of life [2-6]. The major active phytoconstituents of *W. somnifera* root extract are majorly withanolides. Besides withanolides, ashwagandha root contains alkaloids, numerous withanamides, sitoindosides, reducing sugars, peroxidases, glycosides, starch, diltitol, withanilic, benzyl alcohol, 2-phenyl ethanol, 3,4,5-trihydroxy cinnamic acid, phenyl acetic acid, benzoic acid, etc. [7-9]. Isolated withanolides from *W. somnifera* possess various pharmacological activities include antioxidant, neuroprotective, immunomodulating, hepatoprotective, anticancer, anti-inflammatory, hypoglycaemic, antiarthritic, antimicrobial, etc. [10-12]. Thus, ashwagandha root extract was considered as one of the components in a novel proprietary herbomineral formulation, and it can be used for the prevention and treatment of various human disorders.

A unique vital force preserved by every living organisms which is usually believed to create the source of life is correlated with the soul, spirit and mind and is also recognized as prana by the Hindus, *ki* by the Japanese, and *qi* or *chi* by the Chinese, from the ancient-time. Now-a-days, this hypothetical vital force is considered as the Bioenergetics Field. This energy field is infinite, paradimensional and dynamic electromagnetic field surrounding the human body. This is also known as Biofield Energy. It can easily flow between the human and environment that leads to the continuous movement or matter of energy [13, 14]. Thus, the human has the capability to harness energy from the earth, the “Universal Energy Field” and transmit it to any living or nonliving 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 have been practiced worldwide in different health disease profiles [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, Qi Gong, Tai Chi, chiropractic/osteopathic manipulation, meditation, special

diets, massage, homeopathy, progressive relaxation, acupressure, acupuncture, guided imagery, relaxation techniques, hypnotherapy, movement therapy, healing touch, pilates, rolfing structural integration, Ayurvedic medicine, mindfulness, alternative Chinese herbs and medicines, naturopathy, essential oils, aromatherapy, cranial sacral therapy, Reiki, and applied prayer (as is common in all religions, like Hinduism, Christianity, Buddhism and Judaism) [19]. The Biofield Energy Treatment (The Trivedi Effect<sup>®</sup>) has been extensively studied with significant outcomes in many scientific fields such as cancer research [20]; altered antimicrobial sensitivity of pathogenic microbes in genetics [21, 22], biotechnology [23, 24], microbiology [25-27], changing the structure of the atom in various metals, ceramics, polymers and chemicals materials science [28-30], altered chemical and physical properties of organic compounds [31-33], pharmaceuticals [34, 35], nutraceuticals [36, 37], and improved overall growth and yield of plants in agricultural science [38, 39].

Modern sophisticated instrumental techniques such as high-performance liquid chromatography (HPLC) with photodiode array and evaporative light scattering detection, ultra-performance liquid chromatography, electrospray ionization (ESI) normally hyphenated with gas chromatography (GC), mass spectrometry (MS), nuclear magnetic resonance (NMR) are 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* hydroalcoholic root extract revealed the presence of several known withanolides including withanolide D, withaferin A, withanoside IV or VI, withanolide sulfoxide, etc. along with two new withanolides *i.e.* dihydrowithanolide D and ixocarpalactone A [43]. For this reason, LC-MS/MS, GC-MS, and NMR analysis were conducted in this study for the profiling and structure elucidation of the phytoconstituents of the Energy of Consciousness Healing Treatment (The Trivedi Effect<sup>®</sup>) ashwagandha hydroalcoholic root extract.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Ashwagandha (*Withania somnifera*) root hydroalcoholic extract was procured from Sanat Product Ltd., India. The HPLC grade Milli Q water and acetonitrile were purchased from Millipore and Merck. All other chemicals used in the experiment were of analytical grade available in India.

### 2.2. Energy of Consciousness Healing Treatment Strategies

Ashwagandha root extract was one of the components of the new proprietary herbomineral formulation, developed by our research team and it was used *per se* as a test compound

for the current study. The test compound was divided into two parts, one part of the test compound was treated with Energy of Consciousness Healing Treatment (The Trivedi Effect<sup>®</sup>) by renowned Biofield Energy Healers and defined as Biofield Energy Treated sample. The second part of the test compound did not receive any sort of treatment and defined as untreated or control ashwagandha root extract sample. This Biofield Energy Treatment was provided by the group of twenty renowned Biofield Energy Healers who participated in this study and performed the Biofield Energy Treatment remotely. Thirteen Biofield Energy Healers were remotely located in the U.S.A., five were located in Canada, and two were located in Australia, while the test compound was located in the research laboratory of GVK Biosciences Pvt. Ltd., Hyderabad, India. This Biofield Energy Treatment was provided for 5 minutes through Healer's Unique Energy Transmission process remotely to the test compound under the laboratory conditions. None of the Biofield Energy Healers in this study visited the laboratory in person, nor had any contact with the compounds. Similarly, the control compound was subjected to "sham" healers for 5 minutes, under the same laboratory conditions. The sham healer did not have any knowledge about the Biofield Energy Healing Treatment. The Biofield Energy Treated and untreated samples were kept in similar sealed conditions and characterized thoroughly by LC-MS, GC-MS and NMR spectroscopy.

$$\% \text{ change in mass peak intensity} = \frac{I_{\text{Treated}} - I_{\text{Control}}}{I_{\text{Control}}} \times 100 \quad (2)$$

Where,  $I_{\text{Control}}$  and  $I_{\text{Treated}}$  are the mass peak intensity of the control and Biofield Energy Treated samples, respectively.

### 2.3.2. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS analysis of the test samples were analyzed by following the same procedure as mentioned in the recent scientific literature [43] with the help of Agilent 7890B with 5977A Mass selective detector, USA equipped with a Quadrupole detector with pre-filter and flame ionization detector (FID). The control and Biofield Energy Treated extract powders were dissolved in dimethylsulfoxide to afford a 1 mg/mL stock solution. An aliquot of 1.0  $\mu\text{L}$  of the stock solution was injected with a total run time of 44.0 min. The identification of analyte was performed using the retention time with a comparison of the mass spectra of the identified substances with references.

### 2.3.3. Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis

$^1\text{H}$  NMR and  $^{13}\text{C}$  NMR analysis of the test samples extract powders were performed on a 400 MHZ VARIAN FT-NMR spectrometer and 100.00 MHz on a VARIAN FT-NMR spectrometer, respectively using the same procedure as mentioned in the recent literature [43].  $^1\text{H}$  NMR multiplicities were labelled as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m), broad (br), apparent (app). Chemical shifts ( $\delta$ ) were in parts per

## 2.3. Characterization

### 2.3.1. Liquid Chromatography Mass Spectrometry (LC-MS)

The LC-MS analysis of the test samples were conducted by following the almost same method as mentioned in the recent literature [43] using The Waters<sup>®</sup> ACQUITY UPLC, Milford, MA, USA equipped with a binary pump (The Waters<sup>®</sup> 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 Biofield Energy Treated extract powders were dissolved in dimethylsulfoxide to afford a 1 mg/mL stock solution. An aliquot of 2  $\mu\text{L}$  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 area% (P) was calculated using following equation (1):

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

Where,  $P_{\text{Control}}$  and  $P_{\text{Treated}}$  are the peak area (%) of the control and Biofield Energy Treated samples, respectively.

Similarly, the percent change in mass peak intensity (I) was calculated using following equation (2):

million (ppm) relative to the solvent's residual proton chemical shift ( $\text{CD}_3\text{OD}$ ,  $\delta = 3.31, 4.80$  ppm) and solvent's residual carbon chemical shift ( $\text{CD}_3\text{OD}$ ,  $\delta = 49.15$  ppm).

## 3. Results and Discussion

The liquid chromatography chromatograms and the chromatographic data of the control and Biofield Energy Treated samples of ashwagandha root extract are presented in Figure 1 and Table 1. The LC chromatograms of the control and Biofield Energy Treated samples showed several peaks at different retention times. The control and Biofield Energy Treated samples shown the 24 definite peaks in the chromatogram at  $R_t$  of 5.1, 5.2, 5.3, 5.4, 5.6, 5.7, 6.4, 6.6, 6.8, 6.9, 7.1, 7.4, 7.8, 7.9, 8.0, 8.1, 8.2, 8.4, 8.5, 8.6, 8.9, 9.0, 9.1, and 9.2 minutes. The Biofield Energy Treated sample along with 24 peaks, three additional peaks appeared at  $R_t$  of 5.81, 8.58, and 8.81 minutes (Figure 1) which did not detect in the control sample (Figure 1). A change in the retention time along with the appearance and disappearance of some peaks was observed in the Biofield Energy Treated sample compared with the control sample. Thus, the polarity of some of the phytoconstituents in the Biofield Energy Treated ashwagandha root extract was altered compared with the control sample. Each of the corresponding  $R_t$  represents the presence of one phytoconstituents from the ashwagandha root extract. The  $R_t$  of both the control and Biofield Energy

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 relative amount of each compound, which had passed the detector, and these areas can be calculated.

The peak area% of phytoconstituents present in the Biofield Energy Treated ashwagandha root extract was increased in the range of 0.92% to 14.67% at  $R_t$  of 5.1, 5.7, 6.4, 6.6, 6.9, 7.1, 7.4, 7.8, 7.9, 8.0, 8.2, 8.5, and 8.9 minutes compared with the control sample (Table 1). Similarly, the peak area% of some of the other phytoconstituents in the Biofield Energy Treated ashwagandha was decreased in the range of 1.59% to 36.69% at  $R_t$  of 5.2, 5.3, 5.4, 5.6, 6.8, 8.1, 8.4, 8.6, 9.0, 9.1, and 9.2 minutes, respectively compared with the control sample (Table 1). The peak area% of the

phytoconstituents in the Biofield energy Treated ashwagandha root extract was altered significantly in the range of -36.69% to 14.67% compared with the control sample (Table 1). The peak area% provides the relative concentration of components in the chromatogram, when all components respond in the detector and are eluted [43, 44]. It is assumed that all the components in both the samples were equally responded in the detector. Table 1 revealed that Biofield Energy Healing Treatment might have the significant effect on the relative amount/concentration of the phytoconstituents. It is assumed that the intrinsic physicochemical properties of ashwagandha root extract such as morphology, particle size, shape, etc. of the compounds that are related to the solubility of the compounds might have altered due to the Biofield Energy Healing Treatments [28-35].

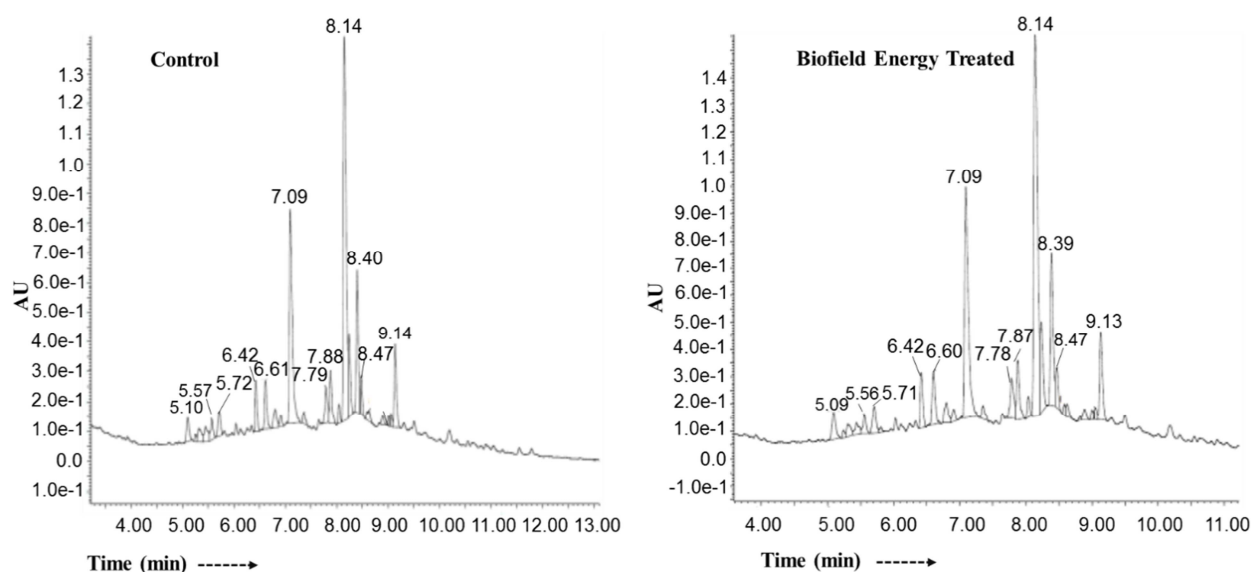


Figure 1. Liquid chromatograms of the control and Biofield Energy Treated *W. somnifera* root extract.

Table 1. Liquid chromatographic data of the control and Biofield Energy Treated *W. somnifera* root extract.

Peak	WS-Control			WS-Biofield Energy		Change in PA%
	$R_t$	PA	PA%	PA	PA%	
1	5.1	4758.53	1.84	6361.95	2.11	14.67
2	5.2	1008.25	0.39	1041.93	0.35	-10.26
3	5.3	3529.39	1.36	3321.84	1.10	-19.12
4	5.4	4389.99	1.69	3224.82	1.07	-36.69
5	5.6	3972.84	1.53	3619.44	1.20	-21.57
6	5.7	5008.86	1.93	6106.93	2.03	5.18
7	6.4	7565.69	2.92	9265.79	3.08	5.48
8	6.6	8618.03	3.33	10471.39	3.48	4.50
9	6.8	4577.27	1.77	5087.22	1.69	-4.52
10	6.9	1855.85	0.72	2416.25	0.80	11.11
11	7.1	47669.68	18.40	55938.64	18.57	0.92
12	7.4	2107.19	0.81	2677.30	0.89	9.88
13	7.8	7198.31	2.78	8645.08	2.87	3.24
14	7.9	8915.69	3.44	11310.46	3.76	9.30
15	8.0	2689.22	1.04	3171.87	1.05	0.96
16	8.1	81752.41	31.56	92861.20	30.83	-2.31
17	8.2	13574.26	5.24	16518.82	5.48	4.58
18	8.4	22861.84	8.83	26186.50	8.69	-1.59
19	8.5	5255.02	2.03	6882.82	2.29	12.81
20	8.6	2626.57	1.01	2258.86	0.75	-25.74

Peak	WS-Control			WS-Biofield Energy		Change in PA%
	R <sub>t</sub>	PA	PA%	PA	PA%	
21	8.9	1691.83	0.65	2115.10	0.70	7.69
22	9.0	1337.64	0.52	1448.85	0.48	-7.69
23	9.1	1977.50	0.76	1755.62	0.58	-23.68
24	9.2	14088.15	5.44	15775.32	5.24	-3.68
Total peak area		259030.01		301200.20		

PA: Peak Area; PH: Peak Height; TIC: Total Ion Chromatogram; R<sub>t</sub>: Retention time; #denotes the percentage change in the peak area (%) of the Biofield Energy Treated sample with respect to the control sample.

**Table 2.** Compounds proposed from ESI-MS spectra of the control and Biofield Energy Treated ashwagandha root extract.

R <sub>t</sub> (min)	ESI-MS (m/z)	Proposed Compounds	m/z peak Intensity		% Change in Intensity*
			Control	Treated	
6.9	650 [M + NH <sub>4</sub> ] <sup>+</sup> 471 [M - 163] <sup>+</sup>	Sitoindoside IX (1)	1.81e6	2.27e6	25.41
7.1	489 [M + H] <sup>+</sup>	β-hydroxy-2,3-dihydro-withanolide F (2)	2.33e7	1.63e7	-30.04
7.9	473 [M + H] <sup>+</sup>	24,25-dihydrowithanolide D (3) 2,3-dihydrowithaferin A (4) Withanolide A (5) Withaferin A (6) Withanone (7)	1.23e7	7.79e6	-36.67
8.2	471 [M + H] <sup>+</sup>	Withanolide D (8) 27-Hydroxy withanolide B (9) 5,7,α-Epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (10) 5α,17-Dihydroxy-6α,7α-epoxy-1-oxo-witha-2,24-dienolide (11)	6.78e7	7.12e7	5.02
8.5	505 [M + H] <sup>+</sup> 488 [M - 16] <sup>+</sup>	Ixocarpalactone A (12) Withanolide S (13)	9.97e6	9.74e6	-2.31
9.2	992 [M + H] <sup>+</sup>	Withanolide sulfoxide (14)	1.16e6	2.39e6	106

\*denotes the percentage change of the Biofield Energy Treated sample with respect to the control sample.

Among these 24 peaks, only six peaks at the R<sub>t</sub> of 6.9, 7.1, 7.9, 8.2, 8.5, and 9.3 minutes having higher peak area% than other R<sub>t</sub> responded to the mass spectrometric analysis and afforded the respective ESI-MS spectra (Table 2). From the ESI-MS spectra, a total of 14 withanolides shown in the Figure 2 were proposed along with the help GC-MS, NMR data and according to the approach described in our recent literature [43].

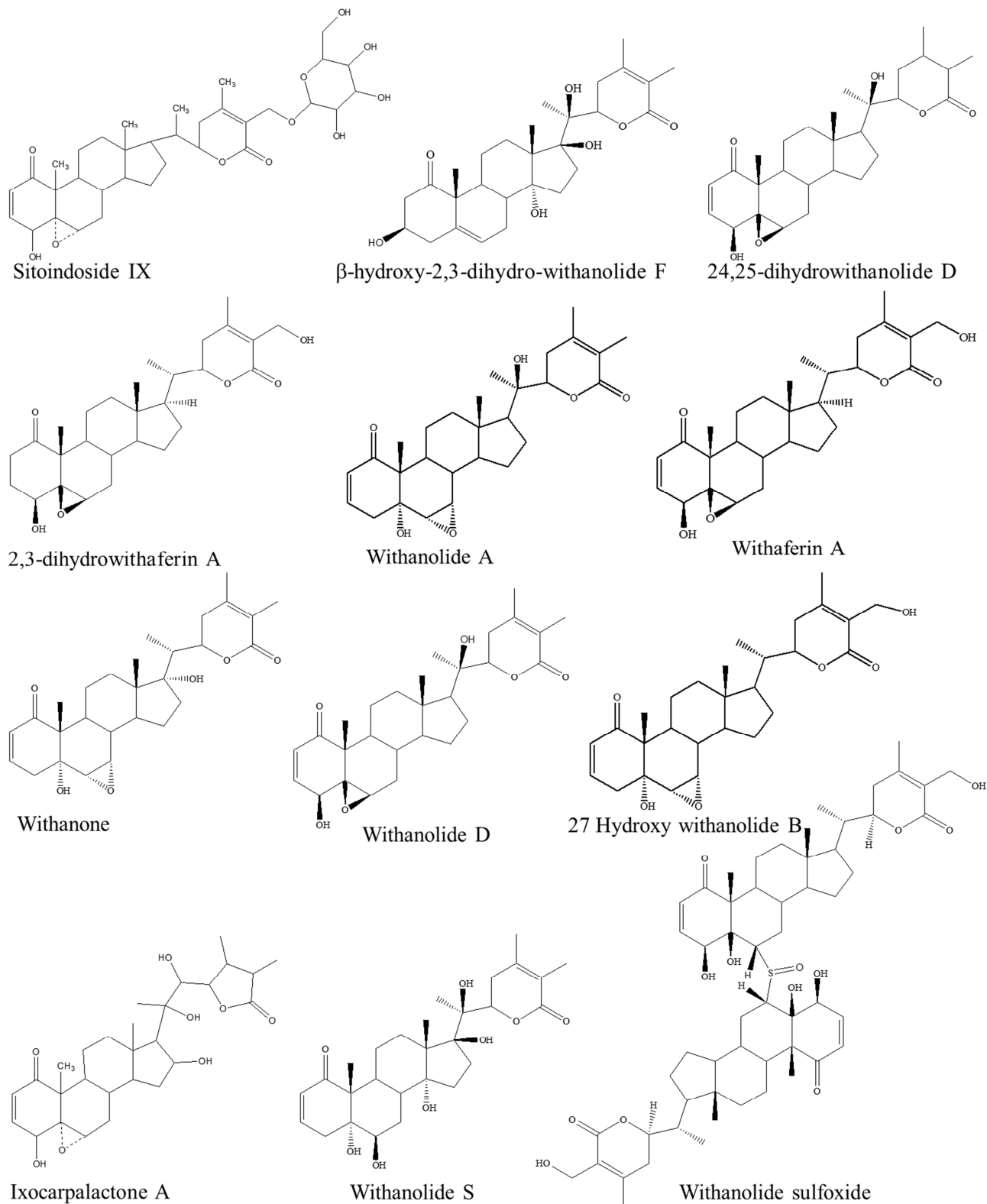
The Sitoindoside IX (1) (Figure 2) was proposed from the ammonium adduct ion peak at m/z 650 [M + NH<sub>4</sub>]<sup>+</sup> (calcd for C<sub>34</sub>H<sub>52</sub>NO<sub>11</sub>, 650) along with daughter ion mass m/z 471 [M - 163]<sup>+</sup> in the mass spectra of the control and Biofield Energy Treated sample at R<sub>t</sub> of 6.9 minutes. The peak area% of sitoindoside IX (1) was significantly increased by 11.11% and the peak intensity at m/z 650 was significantly increased by 25.41% in the Biofield Energy Treated sample compared to the control sample (Tables 2). Similarly, at R<sub>t</sub> of 7.1 minutes 3β-hydroxy-2,3-dihydro-withanolide F (2) was identified with molecular ion peak m/z 489 [M+H]<sup>+</sup> (calculated for C<sub>28</sub>H<sub>40</sub>O<sub>7</sub>, 489) (Table 2 and Figure 2) in the mass spectra of the control and Biofield Energy Treated sample. The peak area% was increased slightly by 0.92%, but the peak intensity at m/z 489 was decreased by 30.04% in the Biofield Energy Treated sample compared to the control sample (Tables 1 & 2). Similarly, the withanolides like 24,25-dihydrowithanolide D (3) or 2,3-dihydrowithaferin A (4) identified with molecular ion peak at m/z 473 (calculated for C<sub>28</sub>H<sub>40</sub>O<sub>6</sub>, 473) were proposed [43] at the R<sub>t</sub> of 7.9 min (Table 2 and Figure 2). The peak area% was increased significantly by 9.30%, but the peak intensity at m/z 473 was

decreased by 47.52% in the Biofield Energy Treated sample compared to the control sample (Tables 1 & 2).

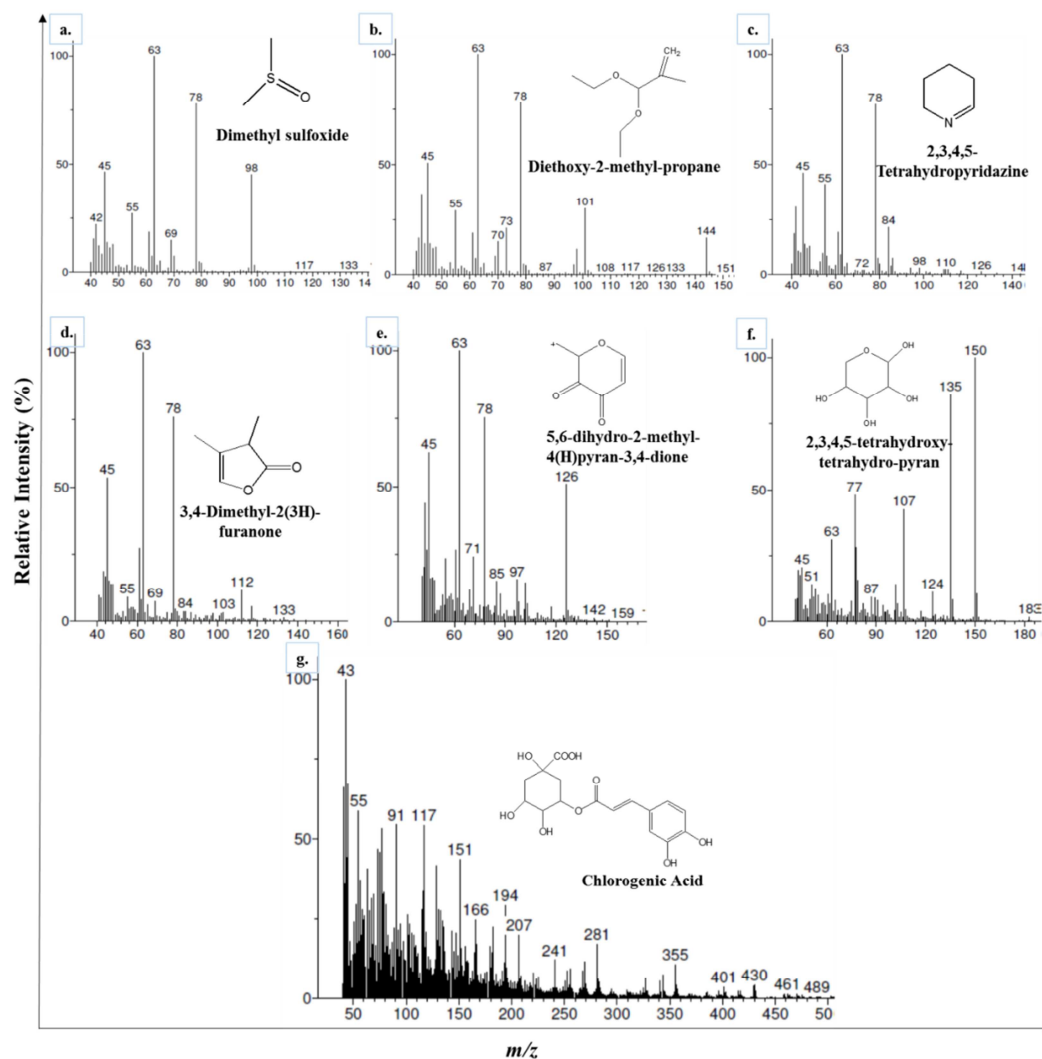
With the help of the recent literature [43], withanolide A (5) or withaferin A (6) or withanone (7) or withanolide D (8) or 27-hydroxy withanolide B (9) or 5,7,α-epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (10) or 5α,17-dihydroxy-6α,7α-epoxy-1-oxo-witha-2,24-dienolide (11) (Figure 2) can show the molecular ion peak with m/z 471 [M + H]<sup>+</sup> (calcd for C<sub>28</sub>H<sub>39</sub>O<sub>6</sub>, 471) in the ESI-MS spectra of both the samples at R<sub>t</sub> of 8.2 minutes. The GC-MS (Figure 3) and NMR data (Figure 4) also supported the presence of any of compounds 11-14. The peak area% and mass peak intensity at m/z 471 were increased by 4.58% and 5.02%, respectively in the Biofield Energy Treated sample compared with the control sample (Tables 1 & 2). At R<sub>t</sub> of 8.55 minutes, ixocarpalactone A (12) or withanolide S (13) were proposed with m/z 505 [M + H]<sup>+</sup> (calculated for C<sub>28</sub>H<sub>40</sub>O<sub>8</sub>, 505) (Table 2 and Figure 2). The peak area% of ixocarpalactone A (12) or withanolide S (13) was increased significantly by 12.81%, but peak intensity at m/z 505 was decreased by 2.31% in the Biofield Energy Treated sample compared to the control sample (Tables 1 & 2). Ixocarpalactone A reported to be promising anti-tumor agent [43]. The withanolide identified at R<sub>t</sub> of 9.2 min and at m/z 992 [M + H]<sup>+</sup> (calculated for C<sub>56</sub>H<sub>79</sub>O<sub>13</sub>S, 992) was withanolide sulfoxide (14) (Table 2 and Figure 2). The change in peak area% of withanolide sulfoxide (14) was decreased slightly by 3.68%, but peak intensity at m/z 992 was significantly increased by 106% in the Biofield Energy Treated sample compared to the control sample (Tables 1 & 2).

The current LC-MS data revealed that the mass fragmentation pattern of both the control and Biofield Energy Treated samples were found almost similar. However, the mass peak intensities of the Biofield Energy Treated sample were significantly altered from the range of -36.67% to 106%

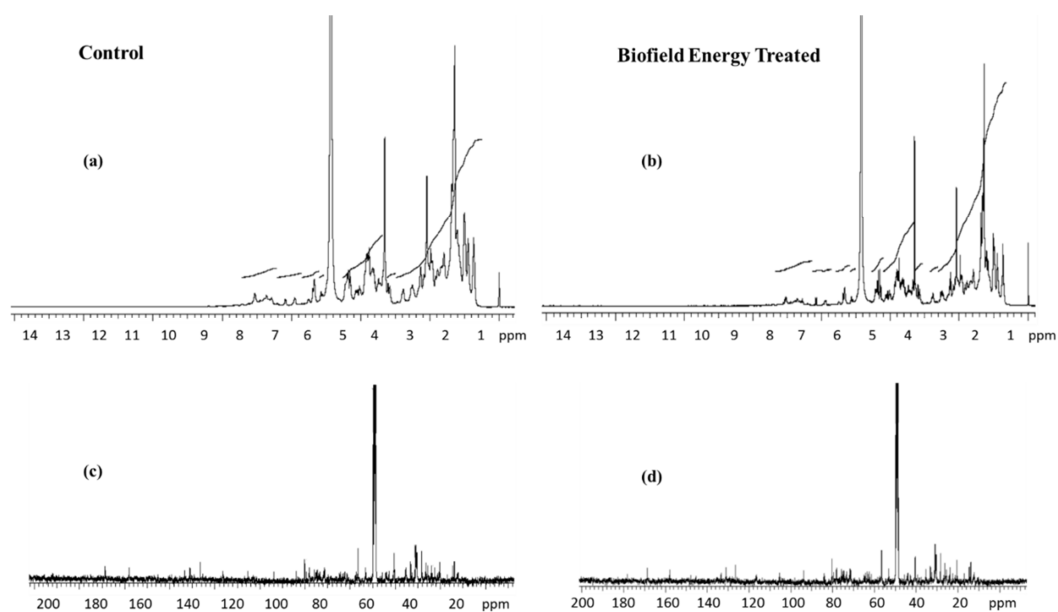
compared with the control sample at the same retention time. This finding suggests that the natural isotopic abundance ratio of the identified phytoconstituents in the ashwagandha root extract might be altered due to The Trivedi Effect® - Consciousness Energy Healing Treatment.



**Figure 2.** Structure of the proposed compounds from *W. somnifera* root extract.



**Figure 3.** GC-MS spectra of the control and Biofield Energy Treated *W. somnifera* root extract with the proposed fragmentation of compounds.



**Figure 4.**  $^1\text{H}$  NMR spectra of the control (a), Biofield Energy Treated (b);  $^{13}\text{C}$  NMR spectra of the control (c), and Biofield Energy Treated (d) *W. somnifera* root extract.

## 4. Conclusions

The LC-MS, GC-MS, and NMR study on *W. somnifera* root extract concluded that The Trivedi Effect® - Energy of Consciousness Healing Treatment has the significant effect on the peak area% *i.e.* the relative concentration of the phytoconstituents without affecting their structural properties. The LC-MS data revealed that the retention time ( $R_t$ ) of the phytoconstituents remained same in the control and Biofield Energy Treated samples, whereas the peak area% *i.e.* the relative amount of the phytoconstituents was significantly altered. The peak area% at  $R_t$  of 5.1, 5.7, 6.4, 6.6, 6.9, 7.1, 7.4, 7.8, 7.9, 8.0, 8.2, 8.5, and 8.9 minutes of the Biofield Energy Treated sample were increased in the range of 0.92% to 14.67% compared to the control sample. In the contrary, the peak area % of the Biofield Energy Treated sample at  $R_t$  of 5.2, 5.3, 5.4, 5.6, 6.8, 8.1, 8.4, 8.6, 9.0, 9.1, and 9.2 minutes were decreased by 1.59% to 36.69% with respect to the control sample. A total of 14 withanolides such as sitoindoside IX, dihydrowithanolide D, withanolide A, withaferin A, ixocarpalactone A, withanolide S, withanolide sulfoxide, etc. were proposed with their structure from the molecular mass at  $m/z$  605, 489, 473, 471, 505, and 992 at retention times of 6.9, 7.1, 7.9, 8.2, 8.5, and 9.3 minutes with the help of LC-MS, GC-MS and NMR data of both the control and Biofield Energy Treated samples. The peak area% of the Biofield Energy Treated sample was altered significantly in the range of -36.69% to 14.67% compared with the control sample. Similarly, the mass peak intensities of the Biofield Energy Treated sample were significantly changed in the range of -36.67% to 106% compared with the control sample at the same retention time. The Biofield Energy Healing Treatment could be valuable for altering the concentration of the phytoconstituents in the ashwagandha root extract by modifying their intrinsic physicochemical properties, which might be helpful to improve the bioavailability of its phytoconstituents. Thus, Biofield Energy Treated *W. somnifera* root extract might provide better therapeutic response against various diseases such as diabetes mellitus, allergies and septic shock; stress-related disorders like sleep disorder, insomnia, depression, anxiety, Attention Deficit Disorder (ADD), Attention Deficit Hyperactive Disorder (ADHD), mental restlessness (mind chattering), low libido, brain fog, impotency, lack of motivation, mood swings, confusion, fear of the future, migraines, headaches, forgetfulness, overwhelm, loneliness, irritability, worthlessness, indecisiveness, frustration, chronic fatigue, obsessive/compulsive behavior and panic attacks; inflammatory diseases and immunological disorders like Lupus, Hashimoto Thyroiditis, Systemic Lupus Erythematosus, Type 1 Diabetes, Asthma, Chronic peptic ulcers, Tuberculosis, Chronic active hepatitis, Hepatitis, Celiac Disease (gluten-sensitive enteropathy), Graves' Disease, Addison Disease, Crohn's disease, Pernicious and Sjogren Syndrome, Aplastic Anemia, Irritable Bowel Syndrome (IBS), Multiple Sclerosis, Rheumatoid arthritis,

Chronic periodontitis, Ulcerative colitis, Chronic sinusitis, Myasthenia Gravis, Vasculitis, Atherosclerosis, Dermatitis, Diverticulitis, Alopecia Areata, Rheumatoid Arthritis, Reactive Arthritis, Psoriasis, Fibromyalgia, Scleroderma, Chronic Fatigue Syndrome and Vitiligo; aging-related diseases like cardiovascular disease, diabetes, arthritis, cancer, Alzheimer's disease, dementia, cataracts, osteoporosis, hearing loss, hypertension, glaucoma, Parkinson's Disease, Prion Disease, Huntington's Disease, Motor Neuron Disease, Amyotrophic lateral sclerosis, Spinocerebellar Ataxia, Spinal muscular atrophy, Friedreich's Ataxia and 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, GC-MS: Gas chromatography-mass spectrometry,  $m/z$ : Mass-to-charge ratio, NMR: Nuclear magnetic resonance spectroscopy.

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