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Chromatographic and Spectroscopic Evaluation of the Consciousness Energy Healing Treated Ashwagandha Root Extract

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Research Article

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Abstract

Ashwagandha (*Withania somnifera*) extract is a popular health supplement, used in many nutraceutical and pharmaceutical formulations for good health benefits along with the prevention and treatment of various human diseases. The purpose of this study was to evaluate the impact of The Trivedi Effect®-Consciousness Energy Healing Treatment on the characteristic properties of the phyto-compounds present in the ashwagandha root extract using LC-MS, GC-MS, and NMR spectroscopy. Ashwagandha root extract was divided into two parts, one part of the sample was termed as untreated/control sample, while the other part of the sample received The Trivedi Effect®-Biofield Energy Healing Treatment remotely by a renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi and termed as Biofield Energy Treated sample. The LC-ESI-MS data revealed that the chromatographic peak area% of the phytoconstituents present in the treated sample was significantly altered in the range of 31.13% to 20.61% compared to the control sample. Similarly, the average peak area of the treated sample was significantly improved by 20.61% compared to the control sample. A total of 14 withanolides such as viscosa lactone B, dihydrowithanolide D, withanolide A, withanolide B, *etc*. were proposed with *m/z* 489, 473, 471, and 455 at the retention times (R_t) of 13.2, 14.5, 14.8, and 18.2 minutes, respectively. At R_t 13.2 minutes (*m/z*: 489), the peak area% and mass peak intensity of the phyto-compound in the treated ashwagandha were significantly increased by 2.57% and 14.83%, respectively compared with the control sample. Similarly, at R_t 14.5 minutes (*m/z*: 473), peak area% was increased by 3.76%, but the mass peak intensity was significantly decreased by 26.48% in the treated sample compared with the control sample. At R_t 14.8 minutes (*m/z*: 471), peak area % was same, but the mass peak intensity was increased by 2.74% in the treated ashwagandha compared with the control sample. Subsequently, at R_t 18.2 minutes (*m/z*: 455), peak area% was increased 1.01%, whereas the peak intensity was significantly decreased by 10.90% in case of the treated sample compared to the control sample. These findings intensify to assume that The Trivedi Effect®-Consciousness Energy Healing Treatment might be responsible for the overall improvement of relative concentrations and altered relative abundance of the phytoconstituents in the ashwagandha root extract compared to the control sample. This Biofield Energy Treated ashwagandha root extract would be helpful to design better nutraceutical/pharmaceutical formulations against inflammatory diseases, immunological disorders, sexual disorders, arthritis, stress, cancer, diabetes, aging, and other chronic infections.

Keywords: Ashwagandha root extract; The Trivedi Effect®; Consciousness Energy Healing Treatment; Withanolides; LC-MS; GC-MS

Introduction

Withania somnifera (also known as ashwagandha and Indian ginseng) and its extract is a popular nutraceutical/ pharmaceutical supplement, with purported good health benefits including prevention and treatment of various diseases [1-3]. Ashwagandha root extract mostly used for the treatment of nervous and immunological disorders, adaptogen /stress, sexual disorders, cancer, diabetes, infectious diseases, ulcer, arthritis, etc. It acts as a tonic to arrest the aging process, rejuvenate the body, and boosts the immune system against infection as well as to promote the longevity of life [2-6]. Ashwagandha root extract contains the major active phytoconstituents like withanolides, withanamides, alkaloids, sitoindosides, reducing sugars, starch, peroxidases, glycosides, withanicil, benzyl alcohol, dilcitol, 2-phenyl ethanol, 3,4,5-trihydroxy cinnamic acid, phenyl acetic acid, benzoic acid, etc [7-9]. Withanolides have various pharmacological activities in the body include immunomodulating, anti-inflammatory, neuroprotective, memory loss, hepatoprotective, antioxidant, hypoglycaemic, gastrointestinal issues, constipation, antiarthritic, antimicrobial, anticancer, insomnia, skin conditions, Alzheimer's, Huntington's, and Parkinson's disorders, etc. [3-6,10-12]. Therefore, ashwagandha root extract used in many nutraceutical and pharmaceutical formulations as a supplement for the prevention and treatment of various human disorders. Over dose or long-term excess use of ashwagandha should not prefer during pregnancy and lactating period, hypothyroidism, and auto-immune diseases.

Recent study revealed that The Trivedi Effect®-Energy of Consciousness Healing Treatment significantly down-regulated the proinflammatory cytokines and potentiated the immunosuppressive effect, along with altered the crystallite size, particle size, surface area, and thermal properties with the help of The Trivedi Effect® Treated formulation containing ashwagandha root extract as a major constituent [13-15]. The Trivedi Effect®-Consciousness Energy Healing Treatment uses the Biofield Energy, which is a type of electromagnetic field around the human body [16,17]. The Biofield Energy Healers has the unique quality to harness the energy from the Universe and can transmit into any living or non-living object(s), which respond to a useful way, and this is known as the Biofield Energy Healing Treatment. Worldwide several Biofield based Energy Healing Therapies are used against various disease conditions [18,19]. Biofield Energy Healing therapy has been recognized worldwide as a Complementary and Alternative Medicine (CAM) health care approach by National Center of Complementary and Integrative Health (NCCIH) with other therapies, medicines and practices such as Ayurvedic medicine, homeopathy, traditional Chinese herbs and medicines, aromatherapy, yoga, meditation, Qi Gong, Tai Chi, chiropractic/osteopathic manipulation, acupressure, acupuncture, healing touch, Reiki, hypnotherapy, movement therapy, naturopathy, cranial sacral therapy, etc [20]. The Trivedi Effect® is a natural and only scientifically proven phenomenon in which a person can harness this inherently intelligent energy and transmit it anywhere on the planet through the possible mediation of neutrinos [16]. The Trivedi Effect®-Consciousness Energy Healing Treatment has scientifically established and transformed the characteristic properties of organic compounds [21,22], metals and ceramic [23,24], nutraceuticals [25, 26], pharmaceuticals [27,28], improve the overall productivity of crops [29,30], and alteration of the isotopic abundance of the organic compounds [31,32]. Similarly, the bioavailability profile of several drugs, *i.e.,* 25-hydroxyvitamin D_3, resveratrol, berberine, etc. are significantly improved by The Trivedi Effect® [33-35]. Therefore, a study has been designed to analyze the impact of The Trivedi Effect®-Consciousness Energy Healing Treatment on the metabolites of ashwagandha hydroalcoholic root extract with the help of the sophisticated Gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS), and Nuclear Magnetic Resonance (NMR) techniques, which are very useful for the metabolite profiling and identification of the crude herbal extract [8,36-39].

Materials and Methods

Chemicals and Reagents

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

Energy of Consciousness Healing Treatment Strategies

Ashwagandha root extract was equally divided into two parts. One part of the test sample was treated with The Trivedi Effect®-Consciousness Energy Healing Treatment by a renowned Biofield Energy Healers, Mr. Mahendra Kumar Trivedi (USA) and defined as Biofield Energy Treated ashwagandha, while the second part of the ashwagandha test sample did not receive any sort of such treatment and defined as untreated/control sample. This Consciousness Energy Healing Treatment was provided for 3 minutes through Healer's Unique Energy Transmission process remotely to the test compound under the standard laboratory conditions. Similarly, the control test sample was subjected to "sham" healers for 3 minutes, under the same laboratory conditions. The "sham" healer did not have any knowledge about the Consciousness 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.

Characterization

➢ **Liquid Chromatography Mass Spectrometry (LC-MS)**

The LC-MS (LC-Dionex Ultimate 3000, MS-TSQ Endura) analysis of the control and Biofield Energy Treated test samples were performed. The column performed on a reversed phase Eclipse Plus-C18 50 × 4.6 mm, 1.8 µm in gradient mode in liquid chromatograph. The mobile phase was 2 mM ammonium acetate and acetonitrile at a constant flow rate of 0.3 mL/min. The column temperature was kept constant at 40˚C. The injection volume was 20 µL and the total run time was 25 min. Chromatographic separation was achieved using gradient condition as follow: 0 min-5%B, 1 min-5%B, 15 min-97%B, 20 min-97%B, 21 min-5%B, and 25 min-5%B. Peaks were monitored using the PDA detector. The control and Biofield Energy Treated extract powders were dissolved in dimethyl sulfoxide (DMSO) 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. A Triple Quad (Thermo Scientific-Endura, USA) mass spectrometer equipped with an electrospray ionization (ESI) source was used for the mass spectrometric analysis. Mass spectra were recorded in the positive ionization mode and with the full scan (*m/z* 50-1400) [39].

Percent change in peak area% (T) was calculated using following equation (1):

$$\%\ \text{change in peak area}\% = \frac{[T_{\text{Treated}} - T_{\text{control}}]}{T_{\text{control}}} \times 100 \quad (1)$$

Where, $T_{Control}$ and $T_{Treated}$ are the peak area (%) of the control and Biofield Energy Treated ashwagandha, respectively. Similarly, the percent change in mass peak intensity (I) was calculated using equation (2):

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

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

➢ **Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

GC-MS Analysis of the control and Biofield Energy Treated ashwagandha were performed on Agilent 7890B with 5977B Mass selective detector, USA. The GC/MS was performed in a silica capillary column. It was equipped with a quadrupole detector with pre-filter. The mass spectrometer was operated in an electron ionization (EI) positive/negative mode at the electron ionization energy of 70 eV. Mass range: 40-1050 Daltons (amu), stability: ± 0.1 *m/z* mass accuracy over 48 hours. The control and Biofield Energy Treated ashwagandha root extract powder were dissolved in dimethyl sulfoxide to afford a 1 mg/mL stock solution. An aliquot of 5.0 µL of the stock solution was injected with a total run time of 44.0 min. Oven temperature was programmed from 50°C (1 min hold) to 150°C@ 20°C/min to 200°C (6 min hold) @ 25°C/min to 280°C@ 20°C/min (12 min hold). Temperatures of the injector, detector (FID), auxiliary, ion source, and quadrupole detector were 230, 250, 280, 230, and 150°C [39]. The identification of analyte was performed using the retention time with a comparison of the mass spectra of the identified substances with references.

➢ **Nuclear Magnetic Resonance (NMR) Spectroscopy Analysis**

^{1}H NMR spectra of ashwagandha were recorded at 400 MHz on Agilent-MRDD2 FT-NMR. Approximately 3 mg of the sample was dissolved in DMSO-d6. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual proton chemical shift {$(CD_3)_2SO$, δ = 2.5}. ^{1}H NMR multiplicities were designated as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q), multiplet (m), broad (br), apparent (app). Similarly, ^{13}C NMR spectra of ashwagandha were measured at 100 MHz on Agilent-MRDD2 FT-NMR spectrometer at room temperature. Approximately 25 mg of the sample was dissolved in DMSO-d6. Chemical shifts (d) were in parts per million (ppm) relative to the solvent's residual carbon chemical shift {$(CD_3)_2SO$, δ = 39.52} [39].

Results and Discussion

The LC-MS chromatograms of the control and Biofield Energy Treated samples of ashwagandha root extract are presented in Figure 1. Several peaks with different retention times (R_t) were observed in the chromatograms of the control and Biofield Energy Treated samples. The chromatographic peaks of both the control and Biofield Energy Treated ashwagandha were found at R_t 10.5, 12.2, 12.5, 12.8, 13.2, 13.4, 14.2, 14.5, 14.8, 15.4, 15.9, 16.1, 16.8, 17.3, 17.5, 17.9, 18.2, 18.9, and 19.5 minutes (Table 1). This indicated that the polarity of Biofield Energy Treated ashwagandha was similar to the control sample. Every chromatographic peak of the chromatogram with the concern R_t represents one phyto-compound present in the ashwagandha root extract. Therefore, several peaks in the chromatogram represent many phyto-compound likely to be present in both the samples of ashwagandha root extract (Table 2). Similarly, the chromatographic peak height and peak areas% is the

qualitative indication of the relative concentration of the phytoconstituents present in the root extract (Table 1). The peak area is directly proportional to the relative concentration of each compound with reference to the standard [39,40]. The overall peak area % of the phytoconstituents present in the Biofield Energy Treated ashwagandha root extract were significantly altered in the range of 31.13% to 20.61% compared to the control sample (Table 1). Similarly, the average peak area of the Biofield Energy Treated ashwagandha was significantly improved by 20.61% compared to the control sample (Table 1). Therefore, it can be assumed that The Trivedi Effect®-Consciousness Energy Healing Treatment might have the significant impact on the relative amount/concentration of the phytoconstituents present in the Biofield Energy Treated ashwagandha root extract. Practically, it could be only possible if there would the improvement of solubility of Biofield Energy treated root extract. So, it can be presumed that the intrinsic physicochemical properties of ashwagandha root extract, *i.e.* particle size, surface area, crystalline structure, *etc.* of the phytoconstituents might have altered due to the The Trivedi Effect®-Consciousness Energy Healing Treatment *via* the possible mediation of neutrino [13-16].

Among many chromatographic peaks, only a few peaks at R_t of 13.2, 14.5, 14.8, and 18.2 minutes having significant peak area were able to characterized with the help of corresponding ESI-MS spectra (Table 2). Along with the LC-MS, GC-MS and NMR spectral information (Figures 1-5) and standard published literature [36-40] fourteen withanolides are proposed from the control, and Biofield Energy Treated samples (Table 2 & Figure 6). The chromatographic peak at R_t 13.2 minutes, produced the corresponding mass peak at *m/z* 489 (calculated for $C_{28}H_{40}O_7$, 489) along with ammonium adducts mass *m/z* 506 in the mass spectrum. This molecular ion peak *at m/z* 489 $[M+H]^+$ proposed to be of 3β-hydroxy-2,3-dihydro-withanolide F (**1**) or viscosa lactone B (**2**) or 27-hydroxy withanolide A (**3**) from the control and Biofield Energy Treated ashwagandha root extract (Table 2 & Figure 6). The peak area% and mass peak intensity of the Biofield Energy Treated ashwagandha were significantly increased by 2.57% and 14.83%, respectively compared with the control sample (Tables 1 & 2).

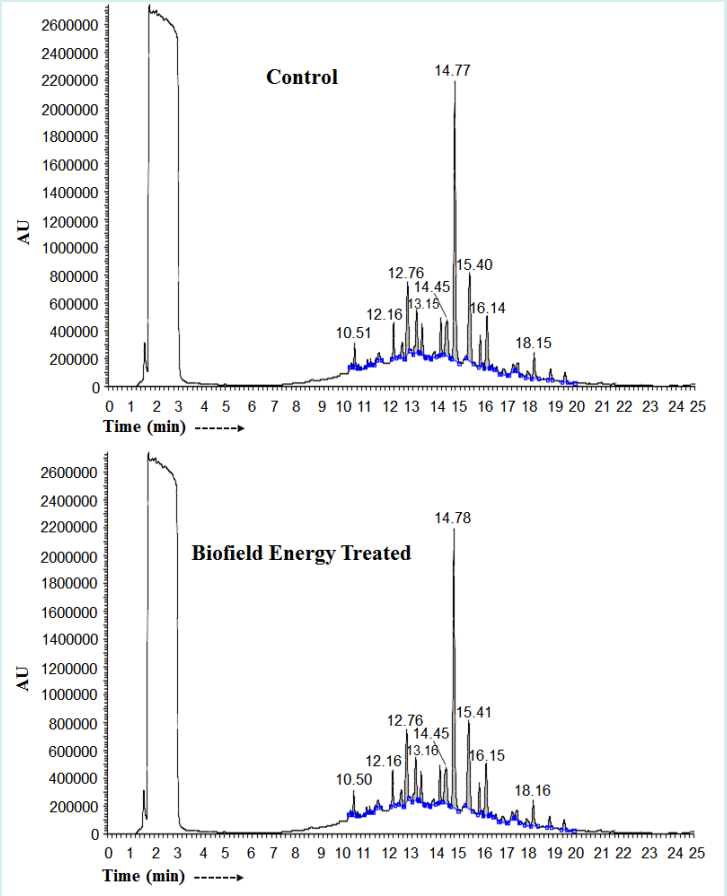

Figure 1: Liquid chromatograms of the control and Biofield Energy Treated ashwagandha root extract.

Peak	Control			Biofield Energy Treated		% Change in PA%*
	R_t	PA	PA%	PA	PA%	
1	10.5	616859.91	1.81	718208.2	1.97	8.84
2	12.2	1011845.71	2.96	1055219.38	2.9	-2.03
3	12.5	622256.71	1.82	734903.53	2.02	10.99
4	12.8	2848615.58	8.34	2989445.08	8.22	-1.44
5	13.2	1728123.98	5.06	1889556.6	5.19	2.57
6	13.4	1061333	3.11	1142034.4	3.14	0.96
7	14.2	1000098.96	2.93	1084008.97	2.98	1.71
8	14.5	2087087.6	6.11	2305782.6	6.34	3.76
9	14.8	10925575	32.01	11636768.8	31.98	-0.09
10	15.4	413893.79	12.12	4435128.5	12.19	0.58
11	15.9	872675.47	2.56	894304.6	2.46	-3.91
12	16.1	2050251.6	6.01	2175317.3	5.98	-0.5
13	16.8	361204.3	1.06	370859.96	1.02	-3.77
14	17.3	361011.45	1.06	267000.15	0.73	-31.13
15	17.5	471734.56	1.38	544786.25	1.5	8.7
16	17.9	410023.85	1.2	387776.7	1.07	-10.83
17	18.2	1017794.72	2.98	1093883.2	3.01	1.01
18	18.9	416614.5	1.22	457141.5	1.26	3.28
19	19.5	423596.08	1.24	434583.24	1.19	-4.03
Average peak area		1510557.73		1821932.05		20.61

Table 1: Liquid chromatographic data of the control and Biofield Energy Treated ashwagandha root extract.
PA: Peak Area; WS: *Withania somnifera*; R_t: Retention time; *denotes the percentage change in the peak area (%) of the Biofield Energy Treated sample with respect to the control sample.

R_t (min)	ESI-MS (*m/z*)	Proposed Compounds	*m/z* peak Intensity		% Change
			Control	Treated	in Intensity*
13.2	489 $[M+H]^+$	β–Hydroxy–2,3– dihydro-withanolide Φ (1)	12897353	14809780	14.83
	506 $[M+NH_4]^+$	Viscosa lactone B (**2**)			
		27-Hydroxy withanolide A (**3**)			
14.5	473 $[M+H]^+$	24,25-Dihydrowithanolide D (**4**)	17370910	12754329	-26.58
		2,3-Dihydrowithaferin A (**5**)			
14.8	471 $[M+H]^+$	Withanolide A (6)	93348576	95907976	2.74
		Withaferine A (**7**)			
		Withanone (**8**)			
		Withanolide D (**9**)			
		27-Hydroxy withanolide B (**10**)			
		5,7,α-Epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (**11**)			
18.2	455 $[M+H+NH_4]^+$	Withanolide B (**12**)	36706200	32704016	-10.9
		Withanolide G (**13**)			
		Withasomidienone (**14**)			

Table 2: Compounds proposed from ESI-MS spectra of the control and Biofield Energy Treated ashwagandha root extract.
R_t: retention time; *m/z*: mass-to-charge ratio; *denotes the percentage change of the Biofield Energy Treated sample with respect to the control sample.

Similarly, at R_t 14.5 minutes 24,25-dihydrowithanolide D (4) or 2,3-dihydrowithaferin A (5) were proposed [39] with molecular ion peak at *m/z* 473 (calculated for $C_{28}H_{40}O_6$, 473) (Table 2 & Figure 6). The peak area% was increased by 3.76%, but the mass peak intensity at *m/z* 473 was significantly decreased by 26.48% in the Biofield Energy Treated ashwagandha compared with the control sample (Tables 1 & 2). Likewise, withanolide A (6) or withaferin A (7) or withanone (8) or withanolide D (9) or 27-hydroxy withanolide B (10) or 5,7,α-epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide (11) (Table 2 & Figure 6) were proposed at R_t 14.8 minutes with the molecular ion peak of *m/z* 471 $[M+H]^+$ (calcd for $C_{28}H_{39}O_6$, 471) in the mass spectra of both the samples. The peak area% was same but the mass peak intensity at *m/z* 471 increased by 2.74% in the Biofield Energy Treated ashwagandha compared with the control sample (Tables 1 & 2). At R_t of 18.2 minutes withanolide B (12) or withanolide G (13) or withasomidienone (14) were proposed with *m/z* 455 $[M+H+NH_4]^+$ (calculated for $C_{29}H_{45}NO_3^+$, 455.34) (Table 2 & Figure 6). The peak area% was increased 1.01%, whereas the peak intensity at *m/z* 505 was significantly decreased by 10.90% in case of Biofield Energy Treated sample (compound 12 or 13 or 15) compared to the control sample (Tables 1 & 2). The fragmented mass observed in the GC-MS, i.e., 3-tert-Butyl-4- hydroxyanisole and 4-phenyl-1,5-trimethylene pyrazole (withasomnine) support the presence of withanolides and related compound present in the ashwagandha root extract (Figure 4). Overall GC-MS and NMR data (Figures 3-5) also supported the presence of the compounds 1-14 (Table 2).

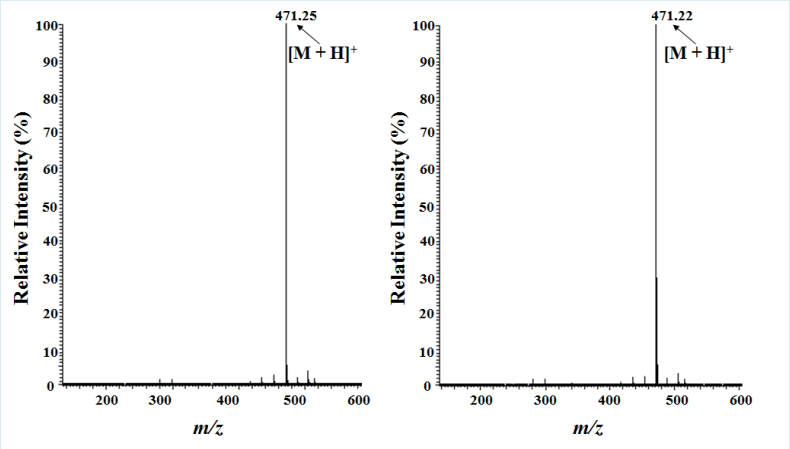

Figure 2: LC-ESI-MS Spectra of control and Biofield Energy Treated ashwagandha root extract at R_t 14.8 minutes.

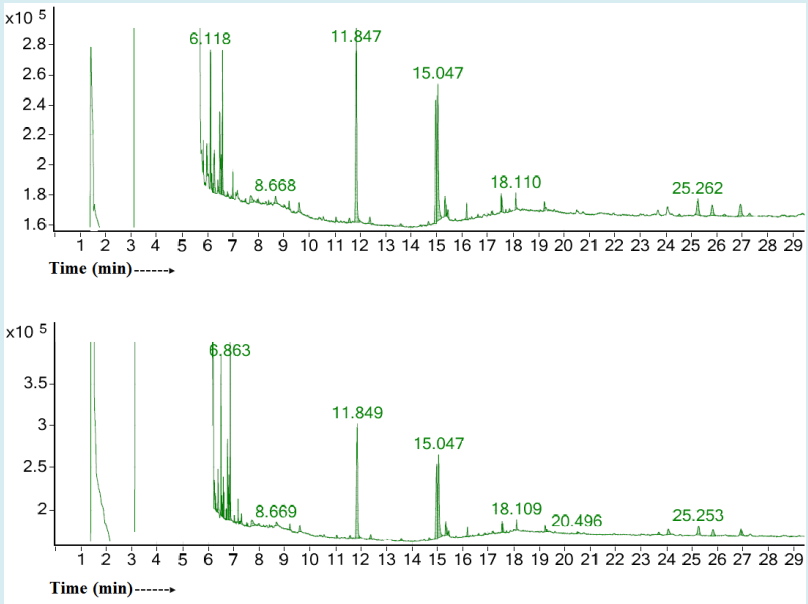

Figure 3: Gas chromatograms of the control and Biofield Energy Treated ashwagandha root extract.

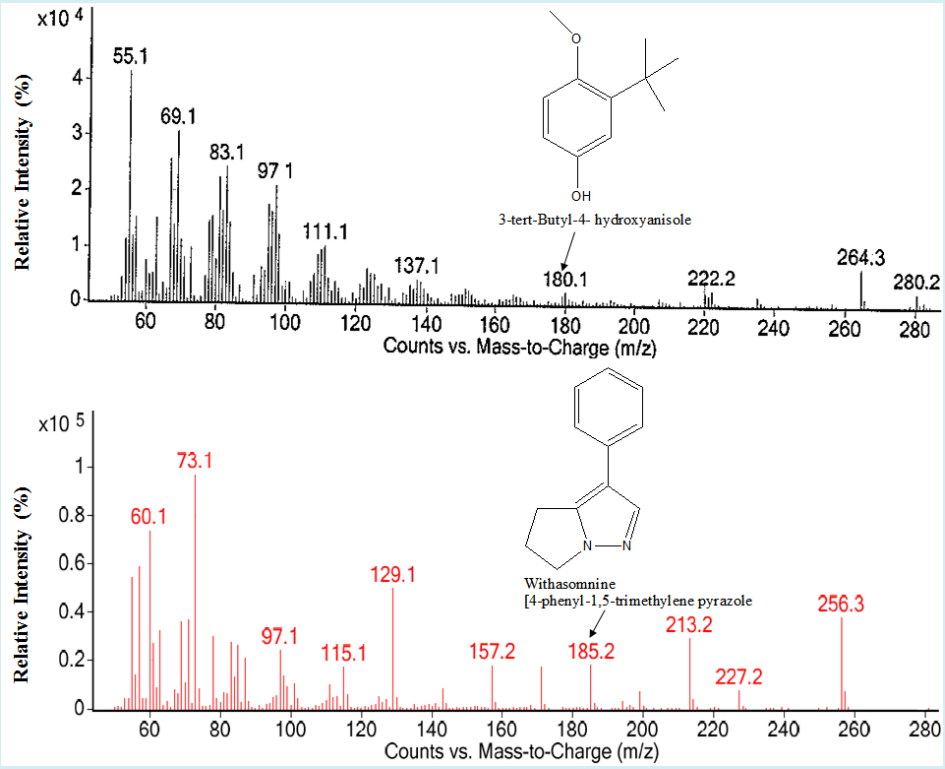

Figure 4: GC-MS spectra of the ashwagandha root extract.

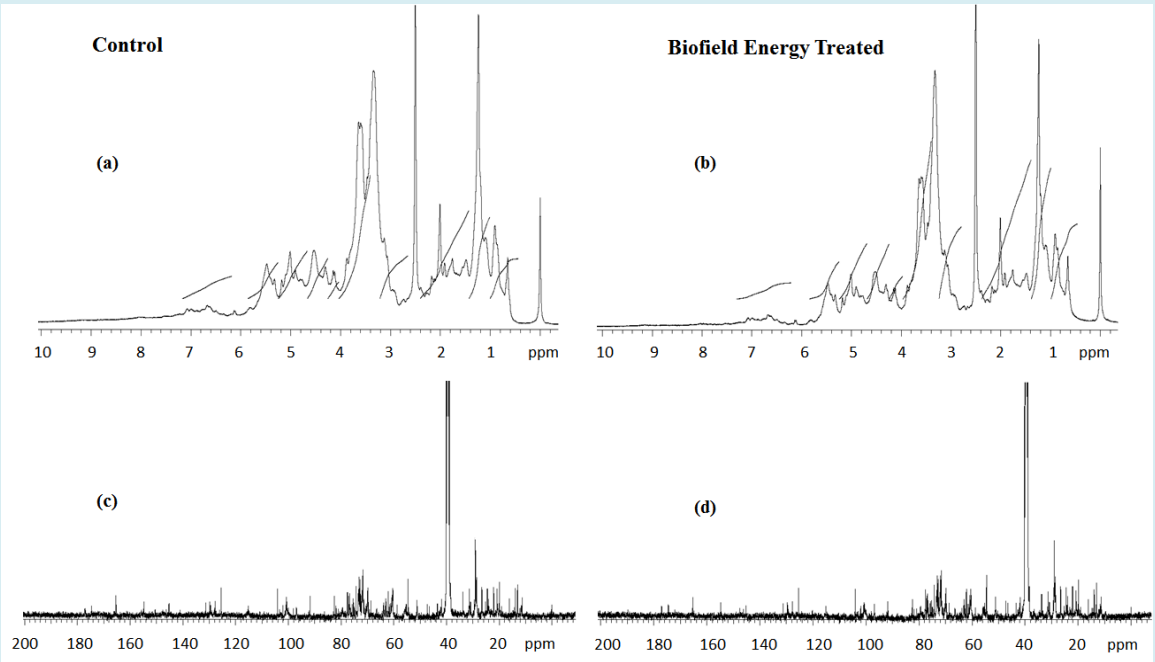

Figure 5: ^{1}H NMR spectra of the control (a), Biofield Energy Treated (b); ^{13}C NMR spectra of the control (c), and Biofield Energy Treated (d) ashwagandha root extract.

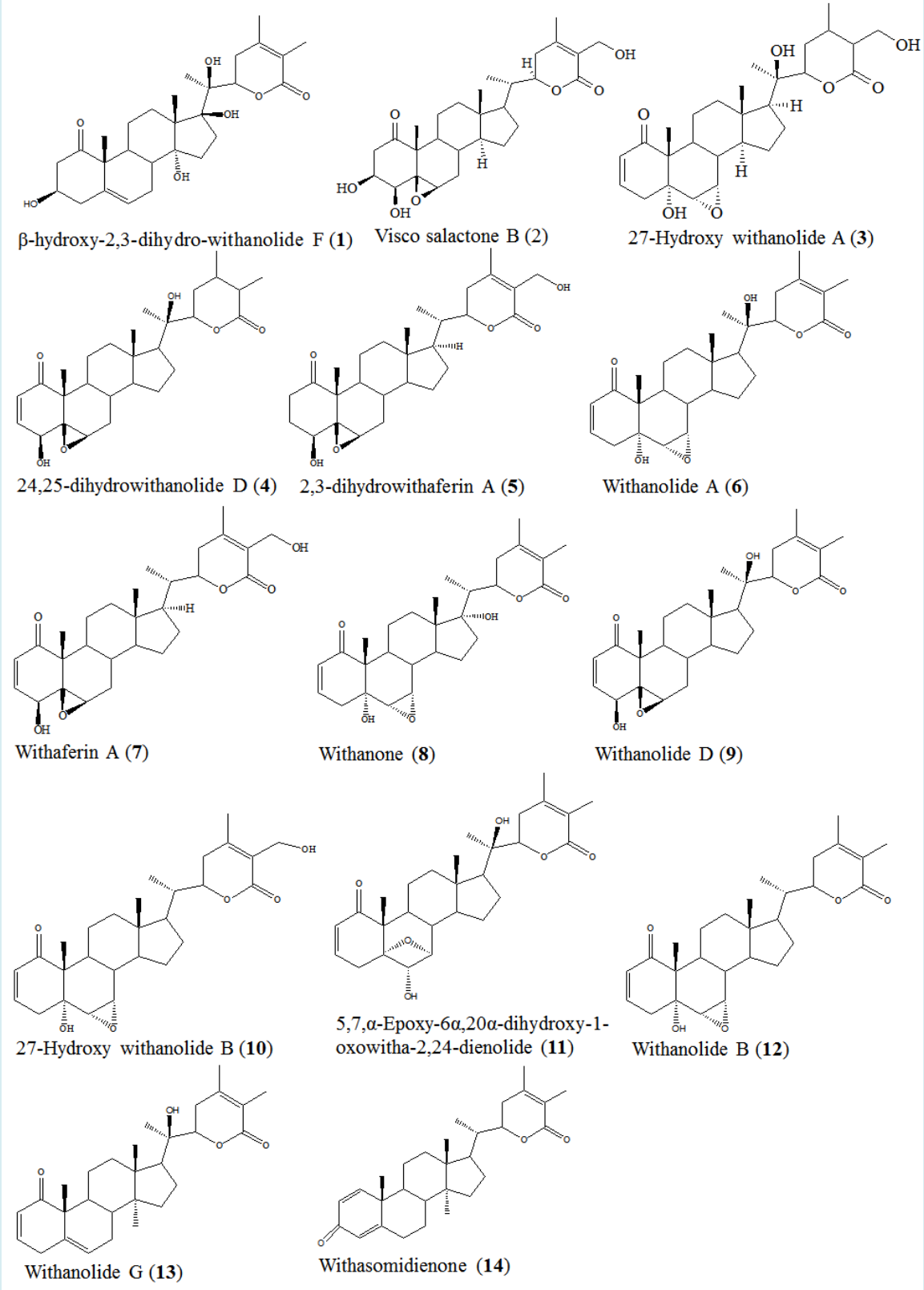

Figure 6: Structure of the proposed compounds from the ashwagandha root extract.

The LC-MS and GC-MS spectroscopic data revealed that the mass fragmentation pattern of the control and Biofield Energy Treated ashwagandha root extract samples were almost similar pattern. But, the relative concentrations and relative abundance have been significantly altered in the phyto-compounds present in the Biofield Energy Treated ashwagandha root extract compared with the control sample. These results suggested that the natural isotopic abundance of the identified phytoconstituents in the ashwagandha root extract might have altered due to The Trivedi Effect®-Consciousness Energy Healing Treatment *via* the possible mediation of neutrino [13-16].

Conclusion

The Trivedi Effect®-Consciousness Energy Healing Treatment on ashwagandha root extract by the renowned Biofield Energy Healer, Mr. Mahendra Kumar Trivedi showed the astonishing significant impact on the relative concentrations and relative abundance of the phyto-compounds present in the extract. The LC-ESI-MS data revealed that the chromatographic peak area% of the phytoconstituents present in the Biofield Energy Treated sample was significantly altered in the range of 31.13% to 20.61% compared to the control sample. Similarly, the average peak area of the Biofield Energy Treated sample was significantly improved by 20.61% compared to the control sample. A total of 14 withanolides such as β-hydroxy-2,3-dihydro-withanolide F, viscosa lactone B, 27-hydroxy withanolide A, dihydrowithanolide D, dihydrowithaferin A, withanolide A, withaferine A, withanone, withanolide D (9), 27-hydroxy withanolide B, 5,7,α-epoxy-6α,20α-dihydroxy-1-oxowitha-2,24-dienolide, withanolide B, withanolide G, and withasomidienone were proposed with *m/z* 489, 473, 471, and 455 at the retention times (R_t) of 13.2, 14.5, 14.8, and 18.2 minutes, respectively. At R_t 13.2 minutes (*m/z*: 489), the peak area% and mass peak intensity of the phyto-compound in the Biofield Energy Treated sample were significantly increased by 2.57% and 14.83%, respectively compared with the control sample. Similarly, at R_t 14.5 minutes (*m/z*: 473), peak area% was increased by 3.76%, but the mass peak intensity was significantly decreased by 26.48% in the Biofield Energy Treated sample compared with the control sample. At R_t 14.8 minutes (*m/z*: 471), peak area% was same, but the mass peak intensity was increased by 2.74% in the Biofield Energy Treated ashwagandha compared with the control sample. Subsequently, at R_t 18.2 minutes (*m/z*: 455), peak area% was increased 1.01%, whereas the peak intensity was significantly decreased by 10.90% in case of the Biofield Energy Treated sample compared to the control sample. These findings intensify to assume that The Trivedi Effect®-Consciousness Energy Healing Treatment might be responsible for the overall improvement of relative concentrations and altered relative abundance of the phytoconstituents in the ashwagandha root extract compared to the control sample. This Biofield Energy Treated ashwagandha root extract might be helpful to design better nutraceutical/pharmaceutical formulations for inflammatory diseases, ulcer, immunological disorders, sexual disorders, arthritis, stress, memory loss, cancer, diabetes, aging, constipation, hepatoprotection, insomnia, skin conditions, Alzheimer's, Huntington's, and Parkinson's disorders, etc.

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