Journal of Diseases and Medicinal Plants
2017; 3(2): 23-32
http://www.sciencepublishinggroup.com/j/jdmp
doi: 10.11648/j.jdmp.20170302.11
ISSN: 2469-8202 (Print); ISSN: 2469-8210 (Online)

Effect of the Energy of Consciousness (The Trivedi Effect®) on *Withania somnifera* Root Extract Using Gas Chromatography – Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy

Mahendra Kumar Trivedi[1], Alice Branton[1], Dahryn Trivedi[1], Gopal Nayak[1], Cathryn Dawn Nykvist[1], Celine Lavelle[1], Daniel Paul Przybylski[1], Dianne Heather Vincent[1], Dorothy Felger[1], Douglas Jay Konersman[1], Elizabeth Ann Feeney[1], Jay Anthony Prague[1], Joanne Lydia Starodub[1], Karan Rasdan[1], Karen Mie Strassman[1], Leonid Soboleff[1], Maire Anne Mayne[1], Mary M. Keesee[1], Padmanabha Narayana Pillai[1], Pamela Clarkson Ansley[1], Ronald David Schmitz[1], Sharyn Marie Sodomora[1], Kalyan Kumar Sethi[2], Parthasarathi Panda[2], Snehasis Jana[2, *]

[1]Trivedi Global, Inc., Henderson, Nevada, USA

[2]Trivedi Science Research Laboratory Pvt. Ltd., Bhopal, Madhya Pradesh, India

Email address:

publication@trivedieffect.com (S. Jana)

[*]Corresponding author

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. Effect of the Energy of Consciousness (The Trivedi Effect®) on *Withania somnifera* Root Extract Using Gas Chromatography – Mass Spectrometry and Nuclear Magnetic Resonance Spectroscopy. *Journal of Diseases and Medicinal Plants.* Vol. 3, No. 2, 2017, pp. 23-32.
doi: 10.11648/j.jdmp.20170302.11

Received: February 6, 2017; **Accepted**: February 18, 2017; **Published**: March 14, 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® - Energy of Consciousness Healing Treatment (Biofield Energy Healing) on phytoconstituents present in the ashwagandha root extract using GC-MS and NMR. 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 The Trivedi Effect® - Energy of Consciousness Healing Treatment remotely from eighteen renowned Biofield Energy Healers. The GC-MS data indicated that the peak height and peak area of The Trivedi Effect® treated sample were found to be altered compared with the control sample. The peak height of the phytoconstituents present in the treated ashwagandha sample was altered significantly in the range of -8.32% to 89.25% compared with the control sample. Similarly, the peak area of the treated sample was altered significantly in the range of -4.28% to 216.30% compared with the control sample. Overall, the change in the peak area% of the treated sample was significantly altered in the range of -18.29% to 170.18% compared with the control sample. The GC-MS and NMR analysis results identified the presence of withanolides such as glyco-withanolides, alkaloids, and sugars in the root extract in both the sample. The peak area of 2,3,4,5-tetrahydropyridazine (1), methyl ethyl sulfoxide (2), 5,6-dihydro-2-methyl-4(H)pyran-3,4-dione (4), diethoxy-2-methyl-propane (5), 2,3,4,5-tetrahydroxy-tetrahydro-pyran (6), and 3,4-dimethyl-2(3H)-furanone (7) were significantly increased by 170.18%, 58.21%, 7.74%, 139.50%, 23.16%, and 45.63%, respectively in the treated sample compared with the control sample. On the contrary, the peak area% of 2-hydroxy-γ-butyrolactone (3) was decreased by -14.96% in the treated ashwagandha compared with the control sample. From the results, it can be hypothesized that The Trivedi Effect® - Biofield Energy Treatment might have the impact on the intrinsic physicochemical properties of the

phytoconstituents present in the ashwagandha root extract and responsible for the alteration in the relative peak height/area of treated sample compared with the control sample. As a result, the concentrations of the phytoconstituents assumed to be increased in treated sample compared with the control sample. This treated ashwagandha root extract would be helpful for designing better nutraceutical/pharmaceutical formulations which might be providing a better therapeutic response against autoimmune diseases, nervous and sexual disorders, infectious diseases, antiaging, diabetes, cancer, immunological disorders, stress, arthritis, etc.

Keywords: Biofield Energy Healing Treatment, Biofield Energy Healers, Consciousness Energy Healers, The Trivedi Effect®, *Withania somnifera,* Withanolides, GC-MS, NMR

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 allopathic 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, immunological disorders, infectious diseases, diabetes, cancer, ulcer, 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 promote the overall quality of life (QOL) [2-6]. The major active phytoconstituents of *W. somnifera* root extract contains highly oxygenated withanolides, alkaloids, numerous sitoindosides, withanamides, starch, reducing sugars, peroxidases, glycosides, dilcitol, withanicil, benzyl alcohol, 2-phenyl ethanol, benzoic acid phenyl acetic acid, 3,4,5-trihydroxy cinnamic acid, etc. [7-9]. Isolated withanolides from *W. somnifera* possess various pharmacological activities includes antioxidant, anticancer, immunomodulating, neuroprotective, hepatoprotective, 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 minerals like zinc, magnesium, and selenium. 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, hypnotherapy, healing touch, movement therapy, 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, gas chromatography (GC), 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* hydro-alcoholic root extract revealed the presence of several known withanolides, alkaloids, glycosides, sugar derivatives, etc. [43]. Therefor, this study was designed for the characterization of the phytoconstituents present in the hydro-alcoholic ashwagandha root extract and to evaluate the influence of The Trivedi Effect® - Energy of Consciousness

Healing Treatment on the phytoconstituents with the help of GC-MS and NMR.

2. Materials and Methods

2.1. Chemicals

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 and procured from Sigma Aldrich, Bangalore, India.

2.2. Energy of Consciousness Treatment Strategies

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 The Trivedi Effect® - Biofield Energy Treatment by renowned Biofield Energy Healers and defined as The Trivedi Effect® - Biofield Energy 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® - Energy of Consciousness Healing 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 Biofield Energy Treatment on the test sample that was located in the research laboratory of GVK Biosciences Pvt. Ltd., Hyderabad, India. This Biofield Energy Treatment (The Trivedi Effect®) 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 Biofield Energy Treatment. After that, the treated and untreated samples were kept in similar sealed conditions and characterized thoroughly by GC-MS and NMR.

2.3. Characterization

2.3.1. 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 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 µL of the stock solution was injected with a total run time of 44.0 min. The identification of analytes was performed using the retention time with a comparison of the mass spectra of the identified substances with references. Percent change in peak height, peak area, and peak area% were calculated using following equation 1:

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

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

2.3.2. Nuclear Magnetic Resonance (NMR) Analysis

^{1}H NMR and ^{13}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}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 (δ) were in parts per million (ppm) relative to the solvent's residual proton chemical shift (CD_3OD, δ = 3.31, 4.80 ppm) and solvent's residual carbon chemical shift (CD_3OD, δ = 49.15 ppm) [44].

3. Results and Discussion

3.1. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The control and treated *W. somnifera* root extract were analyzed by GC-MS. The total ion chromatograms (TIC) having the chromatographic peaks with the retention times are shown in Figure 1, which further helped for the qualitative comparison between the treated and untreated samples. The mass of the proposed metabolites corresponding to retention time (R_t) is described in Table 1. The metabolites are carefully identified with the help of reported published literature [43, 45-48] and mass spectrometry Data Centre, NIST (http://www.nist.gov) in order to overcome the complex and overlapping of spectra to identify the correct molecule.

The TIC of the control sample of ashwagandha root extract showed the peak at R_t of 11.36, 11.57, 12.19, 12.85, 13.26, 13.54, 13.80, 14.16, 14.29, 14.39, 15.77, 15.87, 16.28, 16.84, 30.16, 30.40, 31.60, 32.46, 32.65, and 32.86 min. Similarly, the treated ashwagandha shown the peak at R_t of 11.35, 11.56, 12.21, 12.87, 13.28, 13.56, 13.83, 14.18, 14.31, 14.40, 15.76, 15.87, 16.28, 16.84, 30.16, 30.40, 31.60, 32.46, 32.65, and 32.86. Several R_t in the TIC indicated the presence of numerous metabolites in the root extract. These results revealed that R_t of the treated and control samples of ashwagandha were nearly similar. From the results, it is concluded that, the polarity of the metabolites in the treated ashwagandha was not altered compared with the control sample.

The peak height and peak area of each peak in the TIC were calculated for both the samples and found to be altered in the treated sample compared with the control sample (Table 1). The change in the peak height of metabolites in the

treated ashwagandha was significantly altered in the range of -8.32% to 89.25% compared with the control sample (Table 1). The total peak heights of the metabolites significantly increased by 5.58% compared with the control sample (Table 1). Similarly, the change in the peak area of metabolites in the treated ashwagandha was significantly altered in the range of -4.28% to 216.30% compared with the control sample (Table 1). The total peak area was significantly increased by 10.07% in the treated sample compared with the control sample (Table 1). Overall, the change in the peak area% of the treated sample was significantly altered in the range of -18.29% to 170.18% compared with the control sample.

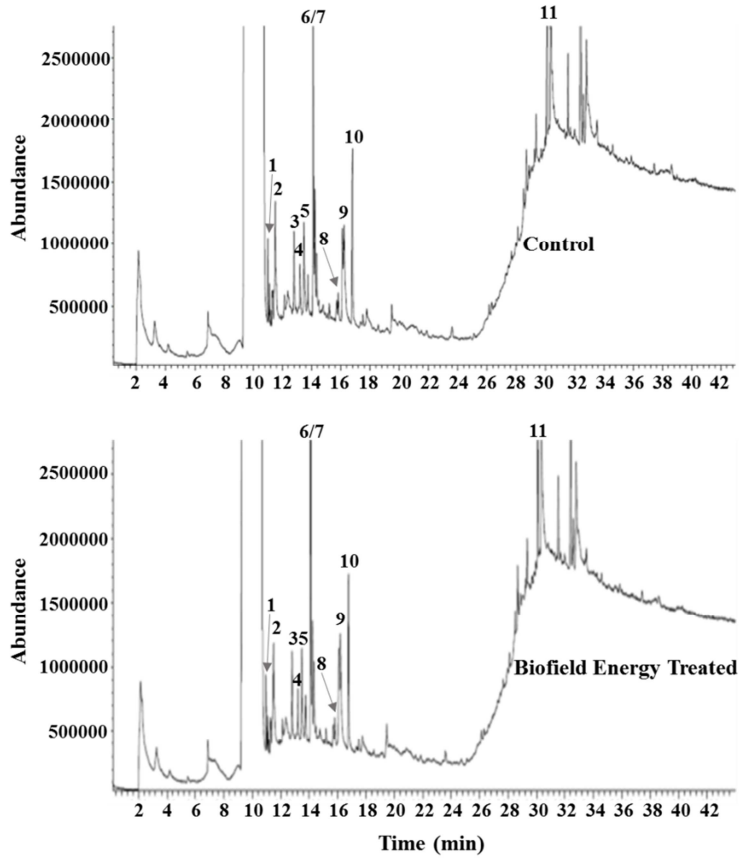

Figure 1. *The total ion chromatograms (TIC) of the control and Biofield Energy Treated sample of W. somnifera.*

The peak area results indicated the relative concentration (amount) of the metabolites present in the sample. In a GC analysis, the area under the peak is proportional to the amount of analyte injected onto the column [49]. Mathematically:

$$A = k\,C$$

Where, A: peak area; C: concentration of sample; k: constant.

In many cases, the peak areas of metabolites of the treated sample were increased compared with the control sample. Therefore, qualitatively the relative concentration of the metabolite in treated ashwagandha was assumed to be increased compared with the control sample, provided the similar experimental condition (*i.e.* 1 mg/mL in DMSO with the injection volume of 1.0 µL).

Table 1. *Retention times, peak heights, peak areas, % change in the peak height and peak area in TIC of Biofield Energy Treated sample compared with the control sample.*

W. somnifera control				W. somnifera Biofield Treated				% change in PH#	% change in PA#	% change in PA%#
R_t (min)	Peak Height	Peak Area	Peak Area%	R_t (min)	Peak Height	Peak Area	Peak Are%			
11.36	13536	213882	1.049	11.35	19241	676515	2.83	42.15	216.30	170.18
11.57	37434	1095743	5.373	11.56	40199	2029477	8.50	7.39	85.21	58.21
12.19	6910	218140	1.070	12.21	6927	211271	0.88	0.25	-3.15	-17.27
12.85	19444	729891	3.579	12.87	20630	726667	3.04	6.10	-0.44	-14.96
13.26	9818	258807	1.269	13.28	9462	250503	1.05	-3.63	-3.21	-17.32
13.54	25468	1152450	5.651	13.56	26238	1102396	4.62	3.02	-4.34	-18.29
13.80	8718	321767	1.578	13.83	10157	405861	1.70	16.51	26.14	7.74
14.16	127383	4078103	19.996	14.18	135376	4189367	17.55	6.27	2.73	-12.25

W. somnifera control				W. somnifera Biofield Treated				% change in PH[#]	% change in PA[#]	% change in PA%[#]
R_t (min)	Peak Height	Peak Area	Peak Area%	R_t (min)	Peak Height	Peak Area	Peak Are%			
14.29	36278	1133228	5.556	14.31	33259	1093686	4.58	-8.32	-3.49	-17.56
14.40	7914	262589	1.288	14.40	14977	736262	3.08	89.25	180.39	139.50
15.77	3113	134680	0.660	15.76	4369	194190	0.81	40.35	44.19	23.16
15.87	5598	187569	0.920	15.87	7299	286526	1.20	30.39	52.76	30.48
16.28	22757	1884422	9.240	16.28	27567	3212774	13.46	21.14	70.49	45.63
16.84	35369	1335390	6.548	16.84	36404	1398322	5.86	2.93	4.71	-10.56
30.16	41842	1002193	4.914	30.16	40164	959288	4.02	-4.01	-4.28	-18.24
30.40	54020	2566975	12.586	30.40	56037	2646508	11.08	3.73	3.10	-11.94
31.60	12484	375676	1.842	31.60	12735	385592	1.61	2.01	2.64	-12.33
32.46	59685	2068451	10.142	32.46	57140	2009360	8.42	-4.26	-2.86	-17.02
32.65	8193	371736	1.823	32.65	8025	363450	1.52	-2.05	-2.23	-16.49
32.86	17611	1003060	4.918	32.86	18272	998374	4.18	3.75	-0.47	-14.98

PH: peak height; PA: peak area. [#] denotes the percentage change in the peak height, peak area, and peak area% of Biofield Energy Treated sample with respect to the control sample.

Table 2. *The identified metabolites from the GC-MS spectra of control and Biofield Energy Treated W. somnifera root extract.*

R_t (min)	Proposed metabolite fragments	Mol. Formula	*m/z*
11.3	2,3,4,5-tetrahydropyridazine (1)	C_5H_9N	84
11.5	methyl ethyl sulfoxide (2)	C_3H_8OS	92
12.8	2-hydroxy-γ-butyrolactone (3)	$C_4H_6O_3$	102
13.8	5,6-dihydro-2-methyl-4(H)pyran-3,4-dione (4)	$C_6H_5O_3$	126
14.4	diethoxy-2-methyl-propane (5)	$C_8H_{16}O_2$	144
15.7	2,3,4,5-tetrahydroxy-tetrahydro-pyran (6)	$C_5H_{10}O_5$	151
16.2	3,4-dimethyl-2(3H)-furanone (7)	$C_6H_8O_2$	112

The GC-MS analysis supported a lot to propose some of the metabolites from the control and treated samples (Table 2 and Figure 2). 2,3,4,5-Tetrahydropyridazine (1) at R_t of 11.3 minutes and *m/z* = 84 $[C_5H_{10}N]^+$ was identified. This fragment suggested the presence of anaferine, anahygrine, and tropine like alkaloids in both the sample [45]. At R_t of 11.5 minutes and *m/z* = 92 $[C_3H_8OS]^+$, methyl ethyl sulfoxide (2) was found, which indicated the presence of withanolide sulfoxide (8) like compound in both the extracts (Figure 3) [46]. Five membered lactone rings 2-hydroxy-gamma-butyrolactone (3) ($C_4H_6O_3^+$; *m/z* = 102) and 3,4-dimethyl-2(3H)-furanone (7) ($C_6H_8O_2^+$; *m/z* = 112) were identified at R_t of 12.8 and 16.2 minutes, respectively (Figure 2). The five membered lactone rings represented the presence of withanolides like ixocarpalactone A (Figure 3) in ashwagandha root extract [47]. Various sugar sub units, *i.e.* 5,6-dihydro-2-methyl-4(H)pyran-3,4-dione (4) ($C_6H_6O_3^+$; *m/z* = 126), and 2,3,4,5-tetrahydroxy-tetrahydro-pyran (6) ($C_5H_{11}O_5^+$; *m/z* = 151) were detected at R_t of 13.8, and 15.7 minutes, respectively (Figure 2 and Table 2). This indicated the presence of O-α-D-glucopyranosyl-β-D-fructofuranosyl-α-D-glucopyranoside and sucrose sugar units were present in the ashwagandha root extract. Further, this is also represented the presence of glyco-withanolides/ withanosides in the root extract [40, 48]. Similarly, diethoxy-2-methyl-propane (5) ($C_8H_{16}O_2^+$; *m/z* = 144) was identified at R_t of 14.4 minutes (Figure 2 and Table 2).

The GC-MS analysis indicated that the peak heights% of the proposed compounds, *i.e.* 2,3,4,5-tetrahydropyridazine (1), methyl ethyl sulfoxide (2), 5,6-dihydro-2-methyl-4(H)pyran-3,4-dione (4), diethoxy-2-methyl-propane (5), 2,3,4,5-tetrahydroxy-tetrahydro-pyran (6), and 3,4-dimethyl-2(3H)-furanone (7) were significantly increased by 170.18%, 58.21%, 7.74%, 139.50%, 23.16%, and 45.63%, respectively in the treated sample compared with the control sample. On the contrary, the peak area% of 2-hydroxy-γ-butyrolactone (3) was decreased by -14.96% in the Biofield Energy Treated ashwagandha compared with the control sample.

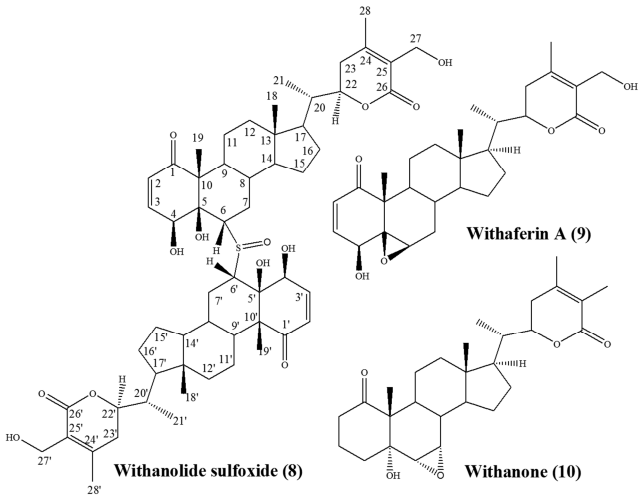

Figure 2. *Proposed withanolides identified by GC-MS and NMR spectral analysis of the hydro-alcoholic root extract of ashwagandha.*

3.2. Nuclear Magnetic Resonance (NMR)

^1H and ^{13}C-NMR spectral values of control and treated ashwagandha are shown in Figure 4 and Table 3, respectively. Some of the metabolites were characterized with the help of the experimental NMR spectral data with Biological Magnetic Resonance Data Bank

(http://www.bmrb.wisc.edu/metabolomics/) and literature. The possible metabolites identified from the ashwagandha root extract are withanolide sulfoxide (WS; 8), withaferin A (WF A; 9), and withanone (WN; 10) (Figure 3), with support of the GC-MS metabolite profiling.

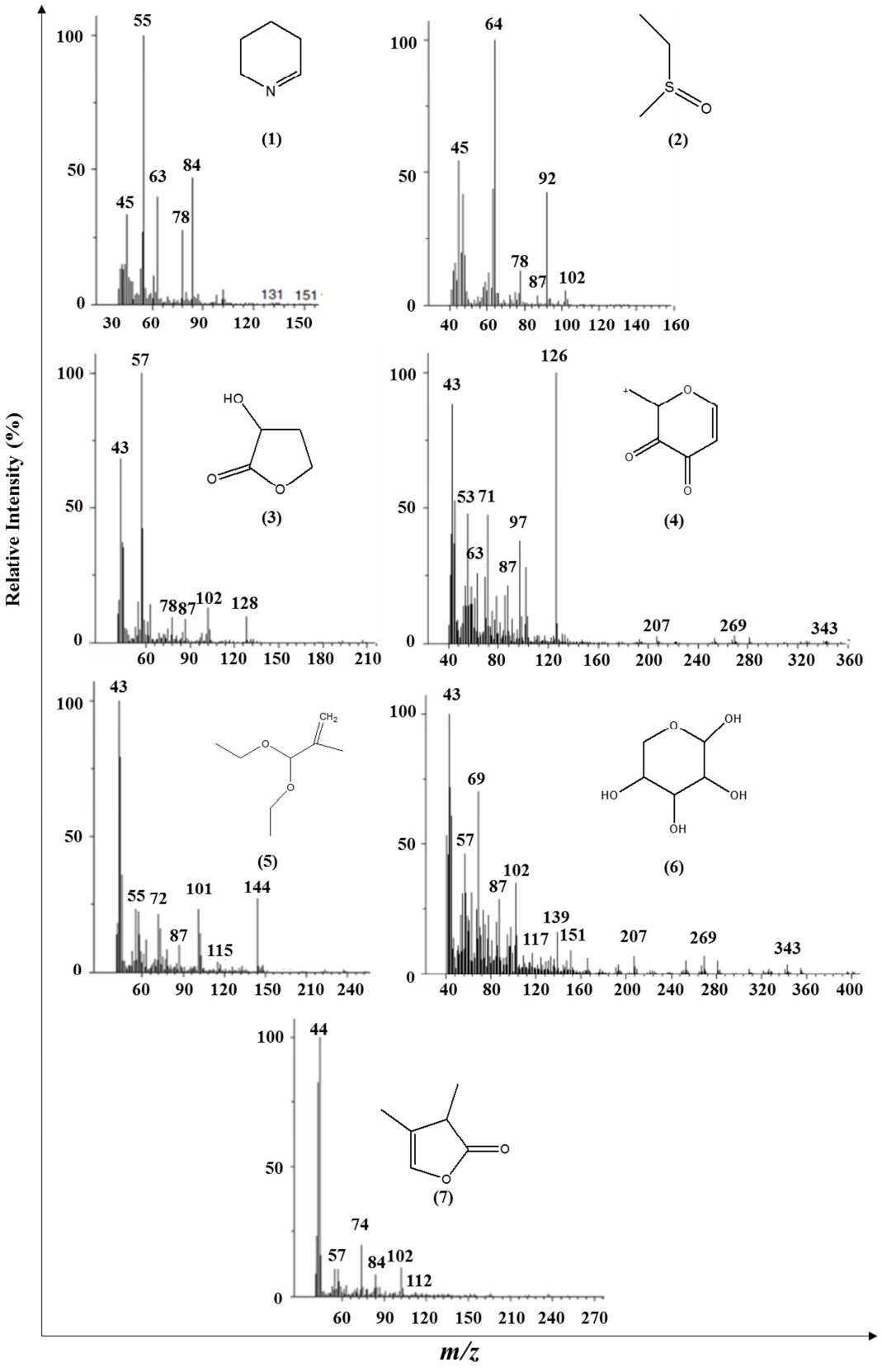

Figure 3. *GC-MS spectra of W. somnifera root extract with proposed fragment of the compounds (1 to 7).*

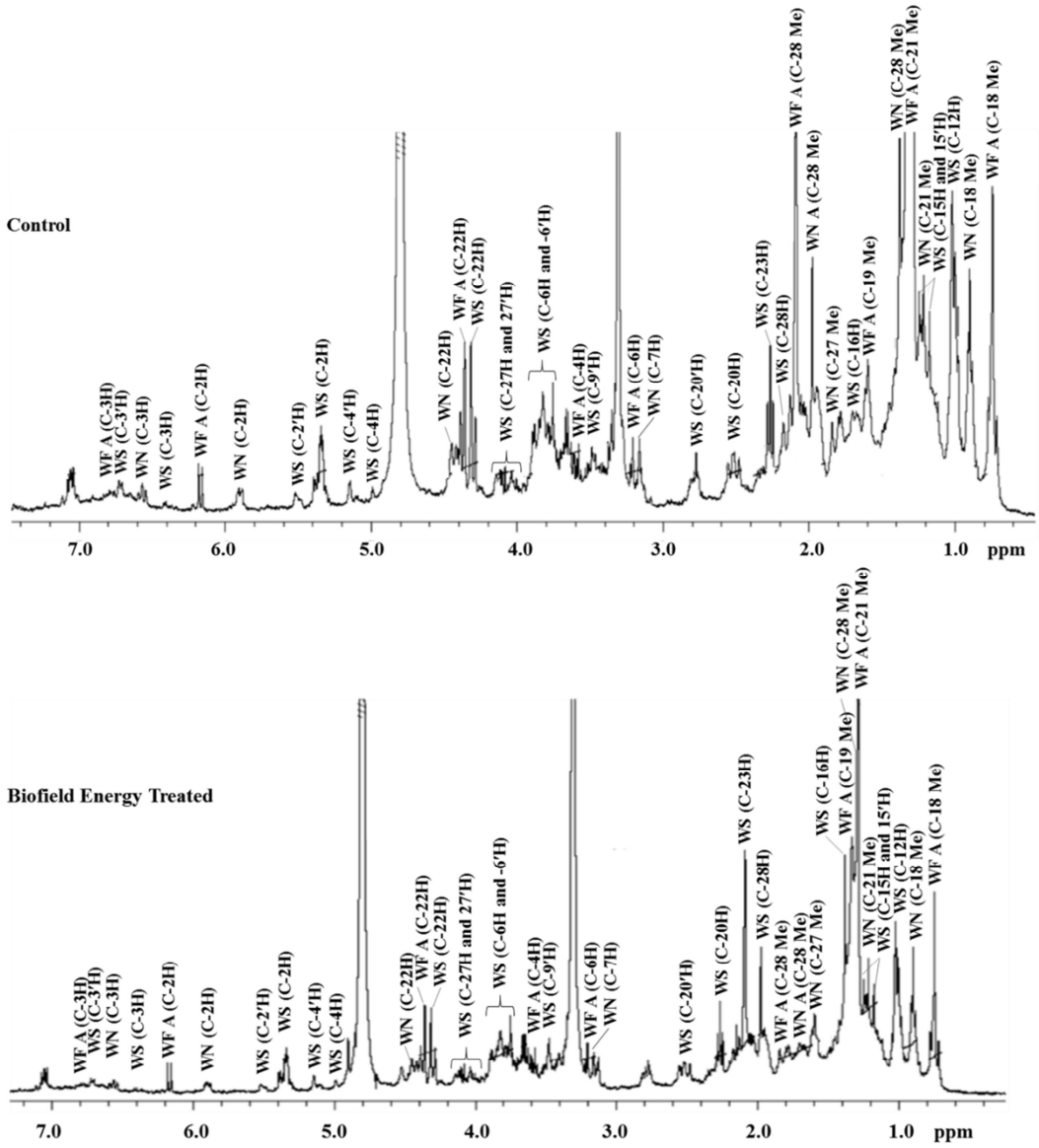

Figure 4. *^{1}H-NMR spectra of control and Biofield Energy Treated sample with proposed metabolites {withanolide sulfoxide (WS), withaferin A (WF A), and withanone (WN)} of W. somnifera root extract.*

^{1}H and ^{13}C NMR spectra of control and treated ashwagandha did not show any significant alteration in the NMR shift value (δ ppm) (Figure 4 and Table 3). These results showed there was no effect of The Trivedi Effect® - Biofield Energy Treatment on the chemical structures of metabolites present in the Biofield Energy Treated ashwagandha root extract compared with the control sample.

Table 3. *^{13}C-NMR data of the control and Biofield Energy Treated ashwagandha root extract.*

^{13}C NMR δ (ppm)	
Control	**Biofield Energy Treated**
12.2, 12.97, 16.8, 22.3, 23.9, 24,.8, 26.7, 28, 9, 29.3, 31.02, 31.6, 38.98, 41.14, 46.9, 47.2, 48.2, 51.6, 54.9, 60.8, 61.9, 62.7, 69.7, 71.8, 72.9, 73.2, 74.3, 76.6, 77.9, 78.6, 82.4, 84.2, 86.4, 92.21, 98.53, 103.91, 124.98, 129.44, 167.04	12.2, 12.9, 18.8, 22.3, 23.9, 24.7, 26.8, 28.8, 29.0, 31.6, 33.7, 38.9, 46.94, 47.15, 48.0, 51.63, 54.96, 58.93, 61.32, 62.9, 65.7, 69.9, 70.2, 71.8, 72.9, 73.2, 74.3, 76.5, 78.0, 81.2, 92.2, 103.9, 114.9, 124.9, 129.5, 156.2, 167.4

The overall analytical observations indicated significant alteration in the peak heights and peak areas of the phytoconstituents present in the treated ashwagandha root extract 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. The Table 1 revealed that The Trivedi Effect® - Energy of Consciousness Healing Treatment might have the significant effect on the relative amount of the phytoconstituents.

4. Conclusions

This study evaluated the impact of The Trivedi Effect® - Energy of Consciousness Healing Treatment (Biofield Energy Treatment) on metabolites of *W. somnifera* root

extract and helped in a qualitative comparison between the treated and untreated ashwagandha sample using GC-MS and NMR. The GC-MS data indicated that the peak height and peak area of the treated sample was found to be altered compared with the control sample. The peak height of the phytoconstituents present in the treated sample was altered significantly in the range of -8.32% to 89.25% compared with the control sample. Similarly, the peak area of the treated sample was altered significantly in the range of -4.28% to 216.30% compared with the control sample. Overall, the change in the peak area% of the treated sample was significantly altered in the range of -18.29% to 170.18% compared with the control sample. The GC-MS and NMR analysis results identified the presence of withanolides such as glyco-withanolides, alkaloids, and sugars in the root extract. Specifically, the peak area of 2,3,4,5-tetrahydropyridazine (1), methyl ethyl sulfoxide (2), 5,6-dihydro-2-methyl-4(H)pyran-3,4-dione (4), diethoxy-2-methyl-propane (5), 2,3,4,5-tetrahydroxy-tetrahydro-pyran (6), and 3,4-dimethyl-2(3H)-furanone (7) were significantly increased by 170.18%, 58.21%, 7.74%, 139.50%, 23.16%, and 45.63%, respectively in the treated sample compared with the control sample. On the contrary, the peak area% of 2-hydroxy-γ-butyrolactone (3) was decreased by -14.96% in the treated ashwagandha compared with the control sample. From the results, it can be hypothesized that The Trivedi Effect® - Energy of Consciousness Healing Treatment might have the 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 relative peak height and peak area of the treated sample. As a result, the concentrations of the phytoconstituents is assumed to be increased 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, insomnia, anxiety, depression, Attention Deficit Disorder (ADD), Attention Deficit Hyperactive Disorder (ADHD), mental restlessness (mind chattering), brain frog, low libido, impotency, lack of motivation, mood swings, fear of the future, confusion, migraines, headaches, forgetfulness, overwhelm, loneliness, worthlessness, indecisiveness, frustration, irritability, chronic fatigue, obsessive/compulsive behavior and panic attacks; inflammatory diseases and immunological disorders like Lupus, Systemic Lupus Erythematosus, Hashimoto Thyroiditis, Type 1 Diabetes, Asthma, Chronic peptic ulcers, Tuberculosis, Hepatitis, Chronic active hepatitis, Celiac Disease (gluten-sensitive enteropathy), Addison Disease, Crohn's disease, Graves' Disease, Pernicious and Aplastic Anemia, Sjogren Syndrome, Irritable Bowel Syndrome (IBS), Multiple Sclerosis, Rheumatoid arthritis, Chronic periodontitis, Ulcerative colitis, Chronic sinusitis, Myasthenia Gravis, Atherosclerosis, Vasculitis, Dermatitis, Diverticulitis, Rheumatoid Arthritis, Reactive 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, Prion Disease, Motor Neurone Disease, Spinocerebellar Ataxia, Spinal muscular atrophy, Amyotrophic lateral sclerosis, Friedreich's Ataxia and Lewy Body Disease, chronic infections and much more.

Abbreviations

GC-MS: Gas chromatography-mass spectrometry; *m/z*: Mass-to-charge ratio; NMR: Nuclear magnetic resonance spectroscopy; R_t: Retention time.

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.

References

[1] Kesarwani K, Gupta R (2013) Bioavailability enhancers of herbal origin: An overview. Asian Pac J Trop Biomed 3: 253-266.

[2] Singh N, Bhalla M, Jager P, Gilca M (2011) An overview on ashwagandha: A rasayana (rejuvenator) of Ayurveda. Afr J Tradit Complement Altern Med 8: 208-213.

[3] Kulkarni SK, Dhir A (2008) *Withania somnifera*: An Indian ginseng. Prog Neuropsychopharmacol Biol Psychiatry 32: 1093-1105.

[4] Dar NJ, Hamid A, Ahmad M (2015) Pharmacologic overview of *Withania somnifera*, the Indian ginseng. Cell Mol Life Sci 72: 4445-4460.

[5] Mir BA, Khazir J, Mir NA, Hasan T-ul, Koul S (2012) Botanical, chemical and pharmacological review of *Withania somnifera* (Indian ginseng): An Ayurvedic medicinal plant. Indian J Drugs Dis 1: 147-160.

[6] Mishra LC, Singh BB, Dagenais S (2000) Scientific basis for the therapeutic use of *Withania somnifera* (Ashwagandha): A review. Altern Med Rev 5: 334-346.

[7] Kumar V, Dey A, Hadimani MB, Marcović T, Emerald M (2015) Chemistry and pharmacology of *Withania somnifera*: An update. Tang (Humanitas Medicine) 5: e1.

[8] Bolleddula J, Fitch W, Vareed SK, Nair MG (2012) Identification of metabolites in *Withania sominfera* fruits by liquid chromatography and high-resolution mass spectrometry. Rapid Commun Mass Spectrom 26: 1277-1290.

[9] Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazón J (2009) Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. Molecules 14: 2373-2393.

[10] Singh A, Duggal S, Singh H, Singh J, Katekhaye S (2010) Withanolides: Phytoconstituents with significant pharmacological activities. Int J Green Pharm 4: 229-237.

[11] Chen LX, He H, Qiu F (2011) Natural withanolides: An overview. Nat Prod Rep 28: 705-740.

[12] Budhiraja RD, Krishan P, Sudhir S (2000) Biological activity of withanolides. J Sci Ind Res 59: 904-911.

[13] Stenger VJ (1999) Bioenergetic fields. Sci Rev Alternative Med 3.

[14] Rogers, M (1989) Nursing: A Science of Unitary Human Beings. In J. P. Riehl-Sisca (ed.) Conceptual Models for Nursing Practice. 3rd edition. Norwark: Appleton & Lange.

[15] Rosa L, Rosa E, Sarner L, Barrett S (1998) A close look at therapeutic touch. JAMA 279: 1005-1010.

[16] Warber SL, Cornelio D, Straughn, J, Kile G (2004) Biofield energy healing from the inside. J Altern Complement Med 10: 1107-1113.

[17] Nelson LA, Schwartz GE (2005) Human biofield and intention detection: Individual differences. J Altern Complement Med 11: 93-101.

[18] Nemeth L (2008) Energy and biofield therapies in practice. Beginnings. Summer 28: 4-5.

[19] Koithan M (2009) Introducing complementary and alternative therapies. J Nurse Pract 5: 18-20.

[20] Trivedi MK, Patil S, Shettigar H, Mondal SC, Jana S (2015) The potential impact of biofield treatment on human brain tumor cells: A time-lapse video microscopy. J Integr Oncol 4: 141.

[21] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Antimicrobial sensitivity, biochemical characteristics and biotyping of *Staphylococcus saprophyticus*: An impact of biofield energy treatment. J Women's Health Care 4: 271.

[22] Trivedi MK, Branton A, Trivedi D, Nayak G, Shettigar H, Mondal SC, Jana S (2015) Effect of biofield energy treatment on *Streptococcus* group B: A postpartum pathogen. J Microb Biochem Technol 7: 269-273.

[23] Trivedi MK, Patil S, Shettigar H, Gangwar M, Jana S (2015) Effect of biofield treatment on antimicrobials susceptibility pattern of *Acinetobacter baumannii* - An experimental study. J Clin Diagn Res 3: 1.

[24] Trivedi MK, Branton A, Trivedi D, Nayak G, Bairwa K, Jana S (2015) Effect of biofield treatment on physical, thermal, and spectral properties of SFRE 199-1 mammalian cell culture medium. Advances in Biochemistry 3: 77-85.

[25] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Bacterial identification using 16S rDNA gene sequencing and antibiogram analysis on biofield treated *Pseudomonas fluorescens*. Clin Med Biochemistry Open Access 1: 101.

[26] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of antibiogram, genotype and phylogenetic analysis of biofield treated *Nocardia otitidis*. Biol Syst Open Access 4: 143.

[27] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2015) Antibiogram, biochemical reactions, and genotypic pattern of biofield treated *Pseudomonas aeruginosa*. J Trop Dis 4: 181.

[28] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Characterization of physical and structural properties of aluminum carbide powder: Impact of biofield treatment. J Aeronaut Aerospace Eng 4: 142.

[29] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) The Potential impact of biofield energy treatment on the atomic and physical properties of antimony tin oxide nanopowder. American Journal of Optics and Photonics 3: 123-128.

[30] Trivedi MK, Patil S, Tallapragada RM (2013) Effect of bio field treatment on the physical and thermal characteristics of vanadium pentoxide powders. J Material Sci Eng S 11: 001.

[31] Trivedi MK, Branton A, Trivedi D, Shettigar H, Bairwa K, Jana S (2015) Fourier transform infrared and ultraviolet-visible spectroscopic characterization of biofield treated salicylic acid and sparfloxacin. Nat Prod Chem Res 3: 186.

[32] Trivedi MK, Patil S, Shettigar H, Bairwa K, Jana S (2015) Effect of biofield treatment on spectral properties of paracetamol and piroxicam. Chem Sci J 6: 98.

[33] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Latiyal O, Jana S (2015) Potential impact of biofield treatment on atomic and physical characteristics of magnesium. Vitam Miner 3: 129.

[34] Trivedi MK, Tallapragada RM, Branton A, Trivedi D, Nayak G, Mishra RK, Jana S (2015) Biofield treatment: A potential strategy for modification of physical and thermal properties of gluten hydrolysate and ipomoea macroelements. J Nutr Food Sci 5: 414.

[35] Trivedi MK, Branton A, Trivedi D, Nayak G, Bairwa K, Jana S (2015) Spectroscopic characterization of disodium hydrogen orthophosphate and sodium nitrate after biofield treatment. J Chromatogr Sep Tech 6: 282.

[36] Trivedi MK, Branton A, Trivedi D, Nayak G, Panda P, Jana S (2016) Gas chromatography-mass spectrometric analysis of isotopic abundance of ^{13}C, ^{2}H, and ^{18}O in biofield energy treated *p*-tertiary butylphenol (PTBP). American Journal of Chemical Engineering 4: 78-86.

[37] Trivedi MK, Branton A, Trivedi D, Nayak G, Sethi KK, Jana S (2016) Gas chromatography-mass spectrometry based isotopic abundance ratio analysis of biofield energy treated methyl-2-napthylether (Nerolin). American Journal of Physical Chemistry 5: 80-86.

[38] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of biochemical marker - glutathione and DNA fingerprinting of biofield energy treated *Oryza sativa*. American Journal of BioScience 3: 243-248.

[39] Trivedi MK, Branton A, Trivedi D, Nayak G, Gangwar M, Jana S (2016) Molecular analysis of biofield treated eggplant and watermelon crops. Adv Crop Sci Tech 4: 208.

[40] Chatterjee S, Srivastava S, Khalid A, Singh N, Sangwan RS, Sidhu OP, Roy R, Khetrapal CL, Tuli R (2010) Comprehensive metabolic fingerprinting of *Withania somnifera* leaf and root extracts. Phytochemistry 71: 1085-1094.

[41] Chaurasiya ND, Uniyal GC, Lal P, Misra L, Sangwan NS, Tuli R, Sangwan RS (2008) Analysis of withanolides in root and leaf of *Withania somnifera* by HPLC with photodiode array and evaporative light scattering detection. Phytochem Anal 19: 148-154.

[42] Musharraf SG, Ali A, Ali RA, Yousuf S, Rahman AU, Choudhary MI (2011) Analysis and development of structure-fragmentation relationships in withanolides using an electrospray ionization quadropole time-of-flight tandem mass spectrometry hybrid instrument. Rapid Commun Mass Spectrom 25: 104-114.

[43] Trivedi MK, Panda P, Sethi KK, Jana S (2016) Metabolite profiling of Withania somnifera roots hydroalcoholic extract using LC-MS, GC-MS and NMR spectroscopy. Chem Biodivers. Article *in press*. doi:10.1002/cbdv.201600280.

[44] Gottlieb HE, Kotlyar V, Nudelman A (1997) NMR chemical shifts of common laboratory solvents as trace impurities. J Org Chem 62, 7512-7515.

[45] Narinderpal K, Junaid N, Raman B (2013) A Review on pharmacological profile of *Withania somnifera* (Ashwagandha). Res Reviews J Bot Sci 2: 6-14.

[46] Mulabagal V, Subbaraju GV, Rao CV, Sivaramakrishna C, Dewitt DL, Holmes D, Sung B, Aggarwal BB, Tsay HS, Nair MG (2009) Withanolide sulfoxide from Aswagandha roots inhibits nuclear transcription factor-Kappa-B, cyclooxygenase and tumor cell proliferation. Phytother Res 23: 987-992.

[47] Kinghorn AD, Su BN, Lee D, Gu JQ, Pezzuto JM (2003) Cancer chemopreventive agents discovered by activity-guided fractionation: An update. Curr Org Chem 7: 213-226.

[48] Uddin Q, Samiulla L, Singh VK, Jamil SS (2012) Phytochemical and pharmacological profile of *Withania somnifera* dunal: A review. Journal of Applied Pharmaceutical Science 02: 170-175.

[49] Hübschmann HJ (2009) Handbook of GC/MS: Fundamentals and Applications. 2nd Edn, John Wiley & Sons, Weinheim, p 33-36.