LC-MS, GC-MS, and NMR Spectroscopy Based Evaluation of the Energy of Consciousness Healing Treated *Withania somnifera* (Ashwagandha) Root Extract

Mahendra Kumar Trivedi¹, Alice Branton¹, Dahryn Trivedi¹, Gopal Nayak¹, Ariadne Esmene Afaganis¹, Barbara Marie Bader¹, Brian A. Weekes¹, Daphne Luisa Dumas¹, Denise Marie Fiedler¹, Dennille Mellesia Smith¹, Desi Panò¹, Donna Felice Galla¹, Donna Maria Alija¹, Elaine Barbara Mullins¹, Elaine M. Scorza¹, Ellia O'Donnell¹, Fabio Massimo Paciucci¹, Frances Goodman Warlick¹, Haddon Norman Salt¹, Inthirani Arul¹, Jacqueline Y. Andrews¹, James Jay McLeRan¹, James Stephen Burnett¹, Jean Caroline White¹, Parthasarathi Panda², Kalyan Kumar Sethi², Snehasis Jana¹, *

¹Trivedi Global, Inc., Henderson, Nevada, USA
²Trivedi 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, Ariadne Esmene Afaganis, Barbara Marie Bader, Brian A. Weekes, Daphne Luisa Dumas, Denise Marie Fiedler, Dennille Mellesia Smith, Desi Panò, Donna Felice Galla, Donna Maria Alija, Elaine Barbara Mullins, Elaine M. Scorza, Ellia O'Donnell, Fabio Massimo Paciucci, Frances Goodman Warlick, Haddon Norman Salt, Inthirani Arul, Jacqueline Y. Andrews, James Jay McLeRan, James Stephen Burnett, Jean Caroline White, Parthasarathi Panda, Kalyan Kumar Sethi, Snehasis Jana. LC-MS, GC-MS, and NMR Spectroscopy Based Evaluation of the Energy of Consciousness Healing Treated *Withania somnifera* (Ashwagandha) Root Extract. *Journal of Drug Design and Medicinal Chemistry*. Vol. 3, No. 2, 2017, pp. 18-26.
doi: 10.11648/j.jddmc.20170302.11

Received: March 14, 2017; Accepted: March 27, 2017; Published: April 18, 2017

Abstract: *Withania somnifera* root extract which contains withanolides as major active constituent and it is used traditionally for the prevention and treatment of several diseases. The objective of the current study was to investigate the impact of The Trivedi Effect® - Consciousness Energy Healing Treatment on the structural 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 other part was treated with the Consciousness Energy Healing Treatment remotely by twenty renowned Biofield Energy Healers and defined as the Biofield Energy Treated sample. The LC-MS analysis 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 at respective retention time was significantly altered. The peak area% at Rₜ of 5.6, 6.8, 6.9, 7.2, 7.9, 8.4, 8.5, 8.6, and 9.2 minutes of the treated sample were increased significantly in the range of 1.46% to 253.06% compared to the control sample. In the contrary, the peak area% of the treated sample at Rₜ of 6.4 and 8.2 minutes were significantly decreased by 12.72% and 17.35%, respectively with respect to the control sample. A total of 16 withanolides such as withanoside IV, coagulin Q, viscous lactone B, dihydrowithanolide D, withanolide A, withaferin A, withanone, withanolide D, ixocarpalactone A, withanolide sulfoxide, withanolide B, etc. were proposed with their structure from the molecular mass at *m/z* 783, 621, 489, 473, 767, 471, 505, and 992 at retention times of 6.4, 6.8, 7.2, 7.9, 8.2, 8.4, and 9.1 minutes, respectively with the help of LC-MS, GC-MS and NMR data of both the control and Biofield Energy Treated samples. The mass peak intensities of the Biofield Energy Treated sample were significantly changed in the range of -81.36% to 1720.90% compared with the control sample at the same retention time. These findings suggest that The Trivedi Effect® - Consciousness Energy Healing Treatment could be beneficial 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 *W. somnifera* extract that might provide better therapeutic response against
inflammatory diseases, immunological disorders, stress, arthritis, cancer, diabetes, sexual disorders, aging, and other chronic infections.

Keywords: Ashwagandha, Biofield Energy Healers, The Trivedi Effect®, Consciousness Energy Healing Treatment, Biofield Energy Healing Treatment, LC-MS, Withanolides, GC-MS

1. Introduction

Herbal medicines have been getting reputation worldwide for the prevention and treatment of the various diseases because of their impressive therapeutic effects and fewer side effects [1]. The roots of *Withania somnifera* is an ancient 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 include sexual and nervous disorders, diabetes, infectious diseases, cancer, ulcer, immunological disorders, stress, arthritis, etc. As a tonic, it is useful to arrest the aging process, rejuvenate the body and boost the defense system against infectious disorders as well as to promote the longevity [2-6]. The major active phytoconstituents of *W. somnifera* root extract are highly oxygenated withanolides. Besides withanolides, ashwagandha root contains alkaloids, numerous sitoindosides, withanamides, reducing sugars, starch, peroxidases, glycosides, dilitol, withanic, benzyl alcohol, 2-phenyl ethanol, 3,4,5-trihydroxy cinnamic acid, benzoic acid, phenyl acetic acid, etc. [7-9]. Isolated withanolides from *W. somnifera* possess various pharmacological activities include antioxidant, anticancer, neuroprotective, immunomodulating, hepatoprotective, anti-inflammatory, antiarthritic, hypoglycaemic, antimicrobial, etc. [10-12]. Therefore, ashwagandha root extract was considered as one of the components in a novel proprietary herbomineral formulation, and 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, *qi* or *chi* by the Chinese, and *ki* by the Japanese 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 The 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, Tai Chi, Qi Gong, chiropractic/osteopathic manipulation, meditation, massage, special diets, homeopathy, progressive relaxation, guided imagery, acupuncture, relaxation techniques, hypnotherapy, healing touch, movement therapy, pilates, rolfling 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 biotechnology [21, 22], genetics [23, 24], microbiology [25-27], 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 organic compounds [31-33], nutraceuticals [34, 35], pharmaceuticals [36, 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* hydroalcoholic root extract revealed the presence of several known withanolides including withaferin A, withanolide D, withanoside IV or VI, withanolide sulfioxide, 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 Biofield Energy Treated (The Trivedi Effect®) *W. somnifera* hydroalcoholic root extract.

2. Materials and Methods

2.1. Chemicals and Reagents

*Withania somnifera* (Ashwagandha) root hydroalcoholic extract was procured from Sanat Product Ltd., India. The HPLC grade acetonitrile and Milli Q water were purchased.
from Merck and Millipore. 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 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 compound for the current study. The test compound was divided into two parts, one part of the test compound was treated with The Trivedi Effect® - Consciousness Energy Healing Treatment (Biofield Energy Treatment) 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. Eighteen Biofield Energy Healers were remotely located in the U.S.A. and two of which were remotely located in Canada, 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. After that, the Biofield Energy Treated and untreated samples were kept in similar sealed conditions and characterized thoroughly by LC-MS, GC-MS and NMR spectroscopy.

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® ACQUITY UPLC, Milford, MA, USA equipped with a binary pump (The Waters® BSM HPLC pump), autosampler, column heater and a photo-diode array (PDA) detector. A Triple Quad (Waters Quattro Premier XE, USA) mass spectrometer equipped with an electrospray ionization (ESI) source was used for the mass spectrometric analysis. The control and 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 analyte was performed using the retention time with a comparison of the mass spectra of the identified substances with references.

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 µL 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) Analysis

1H NMR and 13C 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]. 1H 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,OD, δ = 3.31, 4.80 ppm) and solvent’s residual carbon chemical shift (CD,OD, δ = 49.15 ppm).

3. Results and Discussion

The LC chromatograms and their chromatographic data of the control and Biofield Energy Treated samples of W. somnifera root extract are presented in the Figure 1 and Table 1, respectively. The liquid chromatograms of the control and Biofield Energy Treated samples (Figure 1) showed several peaks at different retention times. Among the obtained chromatographic peaks, only 11 peaks showing almost same retention time and higher peak area in the control and Biofield Energy Treated sample (Table 1). A change in the retention time along with the disappearance and appearance of some peaks was observed in the Biofield Energy Treated sample compared the control sample. Thus, the polarity of the phytoconstituents in the Biofield Energy Treated ashwagandha root extract was altered compared with the control sample.

There was a significant increase in the peak area% of the Biofield Energy Treated sample in the range of 1.46% to 253.06% at Rt of 5.6, 6.8, 6.9, 7.2, 7.9, 8.4, 8.5, 8.6, and 9.2 minutes compared with the control sample (Table 1). Consequently, the peak area% of the Biofield Energy Treated sample at Rrt of 6.4 and 8.2 minutes were significantly decreased by 12.72% and 17.35%, respectively with respect to the control sample (Table 1). The peak area% provides the relative amounts of components in the chromatogram, when all components respond in the detector and are eluted [43].
The liquid chromatographic conditions for the control and Biofield Energy Treated samples were same. It is assumed that all the components in both the samples were equally responded in the detector. Thus, the obtained peak area% are revealed the relative amounts of the phytoconstituents present in the \textit{W. somnifera} root extract. The 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 \textit{W. somnifera} (Ashwagandha) root extract.](image)

| Sl. No. | Retention time (Rₜ, min) | Peak area% | Control | Biofield Treated | % Change* |
|--------|--------------------------|------------|---------|-----------------|-----------|
| 1      | 5.6                      | 1.36       | 1.69    | 24.26           |
| 2      | 6.4                      | 2.83       | 2.47    | -12.72          |
| 3      | 6.8                      | 1.66       | 2.33    | 40.36           |
| 4      | 6.9                      | 0.52       | 1.42    | 173.08          |
| 5      | 7.2                      | 18.49      | 18.76   | 1.46            |
| 6      | 7.9                      | 3.85       | 6.00    | 55.84           |
| 7      | 8.2                      | 31.53      | 26.06   | -17.35          |
| 8      | 8.4                      | 9.31       | 10.38   | 11.49           |
| 9      | 8.5                      | 2.45       | 8.65    | 253.06          |
| 10     | 8.6                      | 1.18       | 1.58    | 33.90           |
| 11     | 9.2                      | 4.98       | 7.00    | 40.56           |

* denotes the percentage change in the peak area (%) of the Biofield Energy Treated sample with respect to the control sample.

Among these 11 peaks, only 7 peaks at the Rₜ of 6.4, 6.8, 7.2, 7.9, 8.2, 8.4, and 9.2 min having higher peak area% than other Rₜ responded to the mass spectrometric analysis and afforded the respective ESI-MS spectra (Table 1). From the ESI-MS spectra, a total of 16 withanolides shown in the Figure 2 were proposed along with the help GC-MS and NMR data (Figure 3 and 4, respectively). Compounds proposed (Figure 2) from the mass of the molecular ion and its fragmentation pattern at corresponding retention time (Table 2) along with the GC-MS (Figure 3) and NMR data (Figure 4) of the ashwagandha root extract according to the approach described in our recent literature [43].

![Figure 2. Liquid chromatograms of the control and Biofield Energy Treated \textit{W. somnifera} (Ashwagandha) root extract.](image)

| Rₜ (min) | Identified compounds | ESI-MS (m/z) | Peak Intensity |
|----------|----------------------|--------------|----------------|
|          |                      | Control      | Biofield Energy Treated | % Change* |
| 6.4      | Withanoside IV (1)   | 783 [M + H]^+ | 9.93e5         | 5.77e5   | -41.89 |
|          | Withanoside VI (2)   | 800 [M + NH₄]^+ | 8.48e6         | 6.78e6   | -20.05 |
|          |                      | 621 [M + H - β-glucose]^+ | 1.22e6 | 6.98e5 | -42.79 |
| 6.8      | Coagulin Q (3), Physagulin D (4) | 621 [M + H]^+ | 1.40e6 | 8.76e5 | -37.43 |
|          |                      | 471          | 2.88e6         | 3.38e6   | 17.36 |
|          |                      | 650          | 3.61e6         | 3.35e6   | -7.20 |
|          |                      | 314          | 2.68e5         | 4.88e6   | 1720.90 |
| 7.2      | Viscosa lactone B (5) | 489 [M + H]^+ | 2.40e7         | 2.09e7   | -12.92 |

Table 1. Liquid chromatographic data of the control and Biofield Energy Treated \textit{W. somnifera} (Ashwagandha) root extract.

Table 2. Compounds proposed from ESI-MS spectra of the control and Biofield Energy Treated ashwagandha root extract.
| R_t (min) | Identified compounds | ESI-MS (m/z) | Peak Intensity |
|-----------|----------------------|-------------|---------------|
|           |                      | Control     | Biofield Energy Treated | % Change |
| 7.9       | 27-hydroxy withanolide A (6) (20S,22R)-3α,6α-epoxy-4β,5β,27-trihydroxy-1-oxowitha-24-enolide (7) (20S,22R)-4β,5β,6α,27-tetrahydroxy-1-oxo-with-2,24-dienolide (8) | 506 [M + NH_4]^+ | 4.94e6 | 3.65e6 | -26.11 |
| 7.9       | Dihydrowithanolide D (9) | 473 [M + H]^+ | 9.38e6 | 1.79e7 | 90.83 |
|           | Withanoside V (10) | 490 [M + NH_4]^+ | N. F. | 2.35e6 | N. D. |
|           | 767 [M + H]^+ | N. F. | 1.94e6 | N. D. |
|           | 784 [M + NH_4]^+ | 6.49e6 | 1.78e7 | 174.27 |
| 8.2       | Withanolide A (11) | 471 [M + H]^+ | 5.56e7 | 1.92e7 | -65.47 |
|           | Withanolone (13) | 488 [M + NH_4]^+ | 1.69e7 | 3.15e6 | -81.36 |
|           | Withanolide D (14) | 958 [2M + NH_4]^+ | 8.36e6 | 1.99e6 | -76.20 |
| 8.4       | Ixocarpalactone A (15) | 505 [M + H]^+ | 3.53e6 | 2.92e6 | 14.47 |
|           | 488 [M - H_2O + 2H]^+ | 3.04e6 | 3.48e6 | 30.26 |
|           | 471 [M - 2 H_2O + 3H]^+ | 9.98e5 | 1.30e6 | -12.04 |
|           | 443 | 5.48e5 | 4.82e5 | -12.73 |
| 9.2       | Withanolide sulfoxide (16) | 992 [M + H]^+ | 4.04e6 | 2.53e6 | -29.54 |
|           | 976 [M - H_2O + H]^+ | 1.08e7 | 7.61e6 | 174.27 |

N. F.- Not found, N. D. – Not determined, * denotes the percentage change of the Biofield Energy Treated sample with respect to the control sample.

The ESI-MS, GC-MS (Figure 3) and NMR (Figure 4) spectral analysis, along with the literature [43] confirmed the presence of viscosa lactone B (5) or 27-hydroxy withanolide A (6) or (20S,22R)-3α,6α-epoxy-4β,5β,27-trihydroxy-1-oxowitha-24-enolide (7) or (20S,22R)-4β,5β,6α,27-tetrahydroxy-1-oxo-with-2,24-dienolide (8) (Figure 2) at m/z 489 [M + H]^+ in the control and Biofield Energy Treated samples at R_t of 7.2 minutes. Consequently, at R_t of 7.9 minutes dihydrowithanolide D (9) or withanoside V (10) was proposed with the molecular ion peak at m/z 473 [M + H]^+ (calcd for C_{28}H_{41}O_{6}, 473) and 767 [M + H]^+ (calcd for C_{40}H_{62}O_{14}, 767), respectively in the ESI-MS spectra of both the samples (Figure 2) [43].

![Figure 2. Structure of proposed compounds 1-16.](image-url)
With the help of the recent literature [43], withanolide A (11) or withaferin A (12) or withanone (13) or withanolide D (14) (Figure 2) can show the molecular ion peak at \( m/z \) 471 \([M + H]^+\) (calcd for C\(_{28}\)H\(_{39}\)O\(_6\), 471) and ammonium adduct ion at \( m/z \) 488 \([M + NH_4]^+\) (calcd for C\(_{28}\)H\(_{42}\)O\(_6\)N, 488) along with fragment ions in the ESI-MS spectra of both the samples at \( R_t \) 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 at \( R_t \) of 8.2 minutes displayed the most intense peak in the LC (Figure 1 and Table 1). Hence, any of the compounds 11-14 was the major phytoconstituents in the control and Biofield Energy Treated samples. The peak area% at \( R_t \) of 8.2 minutes of the Biofield Energy Treated sample was significantly decreased by 17.35% compared with the control sample. The molecular ion peak at \( m/z \) 505 \([M + H]^+\) (calcd for C\(_{28}\)H\(_{41}\)O\(_8\), 505) and 992 \([M + H]^+\) (calcd for C\(_{56}\)H\(_{78}\)O\(_{13}\)S, 992) in the ESI-MS spectra of both the samples at \( R_t \) of 8.4 and 9.2 minutes, respectively indicated the presence of the mass of ixocarpalactone A (15) and withanolide sulfoxide (16), respectively. The GC-MS and NMR data also revealed the presence of ixocarpalactone A (15) [45] and withanolide sulfoxide (16) in the control and Biofield Energy Treated ashwagandha root extract.

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 changed from the range of -81.36% to 1720.90% 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 3. GC-MS spectra of the control and Biofield Energy Treated W. somnifera root extract with the proposed fragmentation of withanolides.](image-url)
Figure 4. $^1$H NMR spectra of the control (a), Biofield Energy Treated (b), $^{13}$C NMR spectra of the control (c), and Biofield Energy Treated (d) W. somnifera (Ashwagandha) root extract.

4. Conclusions

The LC-MS, GC-MS, and NMR study on W. somnifera (Ashwagandha) root extract concluded that The Trivedi Effect® - Consciousness Energy 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-ESI-MS analysis demonstrated that the peak area% at $R_t$ of 5.6, 6.8, 6.9, 7.2, 7.9, 8.4, 8.5, 8.6, and 9.2 minutes of the treated sample were increased significantly in the range of 1.46% to 253.06% compared to the control sample. In the contrary, the peak area% of the treated sample at $R_t$ of 6.4 and 8.2 minutes were significantly decreased by 12.72% and 17.35%, respectively with respect to the control sample. A total of 16 withanolides such as withanoside IV, coagulin Q, viscosa lactone B, dihydrowithanolide D, withanolide A, withaferin A, withanolone, withanolide D, ixocarpalactone A, withanolide sulfoxide, withanolide B, etc. were proposed with their structure from the molecular mass at $m/z$ 783, 621, 489, 473, 767, 471, 505, and 992 at retention times of 6.4, 6.8, 7.2, 7.9, 8.2, 8.4, and 9.1 minutes with the help of LC-MS, GC-MS and NMR data of both the control and Biofield Energy Treated samples. The mass peak intensities of the Biofield Energy Treated sample were significantly changed in the range of -81.36% to 1720.90% compared with the control sample at the same retention time. The Trivedi Effect® - 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, the 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, anxiety, depression, Attention Deficit Disorder (ADD), Attention Deficit Hyperactive Disorder (ADHD), mental restlessness (mind chattering), brain fog, 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 Neuron Disease, Spinocerebellar Ataxia, Spinal muscular atrophy, Amyotrophic lateral sclerosis, 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.

Acknowledgements

The authors are grateful to GVK Biosciences Pvt. Ltd., Trivedi Science, Trivedi Global, Inc. and Trivedi Master Wellness for their assistance and support during this 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, Bhatta 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 somnifera 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 72: 4445-4460.

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

[12] Bhardwaj BA, Singh S, Singh P, Palit S, Sarker SS (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. Norwalk: 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, Gangwar M, Jana S (2015) Bacterial identification using 16S rDNA gene sequencing and antibiotic analysis on biofield treated Pseudomonas fluorescens. Clin Med Biochemistry Open Access 1: 101.

[22] 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.

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

[24] Trivedi MK, Branton A, Trivedi D, Nayak G, Mondal SC, Jana S (2015) Evaluation of antibiotics, genotype and phylogenetic analysis of biofield treated Nocardia otitidiscaviarum. J Altern Complement Med 11: 1-24.

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

[26] 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.

[27] 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.

[28] Trivedi MK, Patil S, Tallapragada RM (2013) Effect of biofield treatment on the physical and thermal characteristics of vanadium pentoxide powders. J Material Sci Eng S 11: 601.

[29] 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.

[30] 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.
[31] Trivedi MK, Branton A, Trivedi D, Nayak G, Panda P, Jana S (2016) Gas chromatography-mass spectrometric analysis of isotopic abundance of $^{12}$C, $^{2}$H, and $^{18}$O in biofield energy treated $p$-tertiary butylphenol (PTBP). American Journal of Chemical Engineering 4: 78-86.

[32] 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.

[33] 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.

[34] 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.

[35] 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.

[36] 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.

[37] 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.

[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 quadrupole 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] https://www.scribd.com/document/220440935/Theory-of-HPLC-Quantitative-and-Qualitative-HPLC.

[45] 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-kB, cyclooxygenase and tumor cell proliferation. Phytother Res 23: 987-992.