Indian Journal of Traditional Knowledge
Vol 19(3), July 2020, pp 486-493

Study on impact of different climatic zones on physicochemical and phytochemical profile of *Withaniasomnifera* (L.) Dunal

Sneha D Kherde[a,b], Komal M Parmar[a], Mukund G Tawar[b], Satyendra K Prasad[a] & Prakash R Itankar*[,a,+]

[a]Department of Pharmaceutical Sciences, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur, Maharashtra, India
[b]P R Pote Patil College of Pharmacy, Amravati, Maharashtra, India

E-mail:[+]prakashitankar@hotmail.com

Received 02 April 2019; revised 15 July 2020

The main objective of the present investigation included a comparative physicochemical, phytochemical along with chromatographic evaluation of *Withania somnifera* (L.) Dunal roots collected from various Indian climatic zones. The study includes macroscopical, microscopical, physicochemical, phytochemical and chromatographic evaluation of *W. somnifera* roots collected from different climatic zones (desert, plains, coastal and plateau) of India. Further, chromatographic evaluations were carried out taking Withaferine-A and Withanoloid-A as biomarkers with the help of HPTLC and HPLC analysis. The physicochemical parameters evaluated were found to be within the prescribed limits of the WHO, while the phytochemical analysis showed the higher quantities of phytoconstituents obtained from desert followed by coastal regions. The chromatographic analysis revealed the presence of maximum number of phytoconstituents in sample from coastal and desert region. Further, the quantification of Withaferine-A and Withanoloid-A was carried out using HPTLC and HPLC. The results confirmed higher quantity of both the biomarkers in samples collected from desert followed by coastal region. Thus, the study may be helpful in understanding the role of climatic zones in relation to the variability in phytochemical composition of medicinal plants such as *W. somnifera*. This will help in selecting the better quality of medicinal plant for preparation of herbal formulations with best therapeutic value that will serve the society.

Keywords: Climatic zones, Phytochemical standardization, Withaferin A, *Withaniasomnifera*, Withanoloid A

IPC Code: Int. Cl.[20]: A61K 36/00

India is situated between 8°-30° N and 68°-97.5° E and is floristically rich, where around 33% of organic riches is endemic and showing variation in altitude ranging from the Himalaya to ocean level[1]. Due to high distinctive agro-climatic/phyto-geographical zones, India has a rich diversity including both cultivated as well as wild plant. In India, there are more than 1500 medicinal plants and traditional societies use native wild plants as source for various medicinal purposes[2]. *Withania somnifera* (L.) Dunal. (Ashwagandha; Solanaceae) is a well-known medicinal plant due to its high therapeutic value and distribution throughout the world[3]. The plant is utilized as herbal medicine in different forms such as powder, infusions, decoctions, syrup and ointments and has a consistent demand in pharmaceutical industries. *W. somnifera* is a xerophytic plant, observed in the drier sub-tropical areas and in high altitude ascending to 5,500 feet in the Himalayas of India. Punjab, Rajasthan, Haryana, Gujarat, Uttar Pradesh, Madhya Pradesh, and Maharashtra are major states of India where Ashwagandha is mainly cultivated[4]. The secondary metabolites from medicinal plants play a critical role in their survival and are also important for their interactions with pathogen, competitors, with pollinators and seed dispersers. Natural variables like temperature, humidity, light have significant impact on the development of plant secondary metabolite generation[5]. The high medicinal value of *W. somnifera* is mainly attributed to the presence of mainly alkaloids and withanolides (steroidal lactones) present in roots[6].The demand of *W. somnifera* for withanolides throughout the world is increasing at a very high rate and has surpassed the production rate ofplant[7]. However, the production of *W. somnifera* is still limited due to its limited habitat, slow growth rate, large gestation period and low seed germination rate. Hence, the wild variety of Ashwagandha is undergoing a very high over exploration to meet the current demands of

*Corresponding author

pharmaceutical sector. Such unwise and random harvesting in a mass scale of medicinal plants from their native natural habitat has resulted in a great loss of their genetic diversity and has also depleted the plant resources to a great extent. Variation in the content of active molecule in medicinal plants from different geographical locations is of prime importance and should be evaluated[1]. Therefore, present investigation was undertaken to understand the impact of geographical variation on the microscopical, physicochemical and phyto-chemical profile of the medicinally rich plant *W. somnifera* collected from four different climatic locations of India.

Methodology

Plant materials and authentication

Roots of *Withania somnifera* from different climatic zone of India were collected i.e., desert of Rajasthan (Udaipur), plane of Uttar Pradesh (Lucknow), western coastal region of Maharashtra (Mumbai) & from central plateau region of central India (Wardha). Authentication of plant species and root samples were carried out by Dr N Dongarwar, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur (Specimen number-9948). The roots of the plant (500 g) were dried in shade, coarsely grinded and were subjected to extraction with the help of Soxhlet extractor using ethanol (1.5 L) as solvent. Extraction process was continued till the plant material was completely exhausted. Finally, the extract obtained from the extraction was concentrated in a rotary evaporator under reduced pressure and further evaporated to obtain brown colour extract (R-100 BUCHI India Pvt. Ltd.).

Macroscopic and microscopic evaluation

The roots were subjected to morphological studies which comprised of shape, size, taste, colour and odour. Further, theroots were fixed using Formalin, Acetic acid and 70% Ethyl alcohol in the proportion of 5 mL: 90 mL for about 24 h. Following the processes of dehydration, clearing and infiltration, roots were further sectioned using Microtome at a thickness range of 10-12 µm which was followed by dew axing as described previously[8,9]. The sections were then stained and examined under digital microscope.

Physicochemical standardization

The dried roots of the plants were grinded and different physicochemical parameters such as foreign matter, loss on drying, extractive value, total ash, water soluble ash, acid insoluble ash, foaming index, swelling index were evaluated. Further, the study also included determination of pesticide content, crude fibre content and total number of starch grains[10,11].

Phytochemical standardization

Phytochemical screenings of different sample extracts of *W. somnifera* roots collected from different climatic zone of India were performed as per standard procedure[12]. Total phenolic and total tannin contents in extracts were determined by adopting the Folinciocalteu reagent method[13]. The total flavonoid along with total flavanone contents in the extract samples were quantified using the method proposed by Kumaran and Karunakaran[14] while, total saponin content was quantified using diosgenin as a reference standard[15]. Further, total alkaloid content in the plant material was determined following the gravimetric analysis[16], while the method proposed by Yemm and Willis[17]was adopted for determining the total carbohydrates content in the plant extracts.

HPTLC and HPLC quantification of Withanoloid-A and Withaferine-A

Ethanolic extracts of roots collected from different climatic zone of India were standardized taking Withanoloid-A and Withaferine-A (Sigma-Aldrich, St. Louis, MO, USA) as biomarkers, following HPTLC (High Performance Thin Layer Chromatography) method. The stock solution of standards Withanoloid-A (0.5 mg/mL), Withaferine-A (0.1 mg/mL) and sample extracts (5 mg/mL), were prepared using methanol. The solvents used in the mobile phase for chromatographic analysis included toluene, ethyl acetate and formic acid in the ratio15:4.5:1.5 (v/v/v). The study was performed on a Camag- HPTLC instrumentation (Camag, Mutten, Switzerland) which was equipped with a Linomat V sample applicator, Camag TLC visualizer, Camag TLC scanner 3 and WINCATS 4 software. The R_f values of different bands were noted, while screening and photo-documentation ofthe developed plate were carried out at visible range following derivatization with methanolsulphuric acid reagent.

To obtain a comparative chromatographic data, the extracts of *W. somnifera* were further standardized with Withaferine-A and Withanoloid-A using HPLC. Before analysis, the extracts, the standards and the mobile phase mixture solvents were filtered through a 0.45 µm membrane filter (Millipore, Ahmedabad, India). A Shimadzu HPLC system (Japan) attached

with a PDA detector was used, while the separation was carried out on a Cosmosil C_{18} column (150 mm × 4.6 mm, 5 µm particle). A mixture of methanol and water in the ratio of 60: 40 v/v was used as mobile phase for this analysis[18]. The flow rate and injection volume were kept at 0.7 mL/min, 20 µL respectively. The wavelength for data collection was kept at 237 nm while the peaks were identified by comparing its retention time of sample with that of standard (Class VP series software, Shimadzu, Japan).

Results

Macroscopically, the roots collected from desert, plains and plateau region appeared buff yellow in colour with size ranging between 8 to 15 cm in length and 2 to 4 mm in width, while roots from coastal region appeared brownish yellow in colour with size ranging from 4 to 12 cm in length and 0.5 to 1.5 mm in width (Fig. 1). The transverse section of root in general shows exfoliates cork which is nonlignified with 2-4 layers of phellogen and about 15-20 rows of phelloderm. It also shows part of vascular tissue like cambium, consisting of 3-5 layer of tangentially elongated cells, phloem region with parenchyma, sieve tubes and companion cells, secondary xylem is hard which forms a continuous vascular ring interrupted by medullary rays. Cork shows two to six layers of isodiametric non-lignified, suberized parenchymatous cells, followed by cork cambium which is single layered or indistinct, cortex: occupy $1/5^{th}$ part of the T.S few layers of slightly flattened parenchymatous cell. Outer cells are with interspaces while inner cells are arranged compactly. All the cells are heavily loaded with simple, reniformstarch grains, which are oval in shape, normally found in parenchyma of the cortex and vascular region, Phloem are isodiametric parenchymatous cells with intracellular spaces. In matured roots phloem consist of sieve tubes, companion cells with parenchyma, cambium shows few layers of elongated cells. Secondary xylem consists of xylem parenchyma, tracheid. Medullary rays are multiseriate and ground tissue consists of parenchymatous cells loaded with starch grains. From the overall observation of the microscopy, the samples from desert and plains showed larger paranchymatous cells compared to other two samples. The roots from desert region also showed large amount of tanniferous cells, while the vascular tissues were found to be larger in samples from coastal and plateau region (Fig. 2.)

The parameters evaluated under physicochemical analysis are represented in Table 1, where the results demonstrated a significantly higher foreign matter in roots from plateau region, while higher moister content was observed in roots collected from coastal region. Further, the total ash along with acid insoluble ash was also reported to be significantly higher in case of roots from plateau region suggesting higher

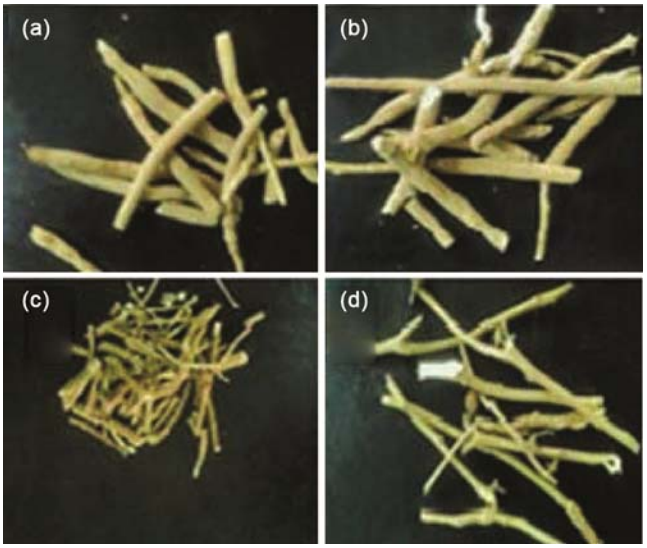

Fig. 1 — Roots of *W. somnifera*In figure A: *W. sonifera* roots from desert region, B: *W. somnifera* roots from plains region, C: *W. somnifera* roots from coastal region and D: *W: somnifera* roots from plateau region.

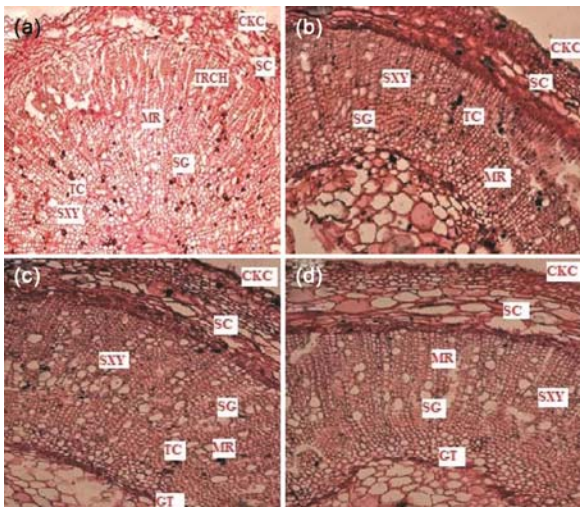

Fig. 2 — Transverse section of *W. Somnifera*, where, A: *W. Somnifera* roots from desert region, B: *W. somnifera* roots from plains region, C: *W. somnifera* roots from coastal region and D: *W somnifera* roots from plateau region. In figure, TRCH Tracheids, MR: Medullary Rays, SG: Starch grains, TC: Tannin cells, SC: Secondary cortex, GT: Ground tissue and SXY: Secondary xylem vessels.

Table 1 — Physicochemical parameters of *W. somnifera* collected from different climatic zones of India

Parameters	Desert Region	Plains Region	Coastal Region	Plateau Region
Foreign matter (%w/w)	0.526 ±0.13	0.566 ±0.05	0.723 ±0.06	0.921 ±0.31[a]
Loss on drying (%w/w)	2.350 ±0.19	2.885 ±0.17	3.397 ±0.13[a]	2.903 ±0.10
Swelling index (mL/g)	2.5 ±0.14	2.7±0.26	3.1 ±0.29	3.3 ±0.35[a]
Bitterness value	Strong bitter	Strong bitter	Strong bitter	Strong bitter
Total ash (%w/w)	4.124 ±0.55	4.995 ±0.21	5.312 ±0.57[a]	6.512 ±0.47[ab]
Acid insoluble ash (%w/w)	0.523 ±0.05	0.605 ±0.05	0.665 ±0.05	1.195 ±0.25[abc]
Water soluble ash (%w/w)	1.110 ±0.29	2.329 ±0.48[a]	2.121±0.75	1.625 ±0.43
Total number of starch grains (per mg of plant material)	126532.25 ±4732.71	124611.57 ±9499.57	132715.95 ±5736.996	251456.43 ±5759.43[abc]
Crude fiber content (%w/w)	1.875 ±0.39	2.14 ±0.44	7.30 ±0.88[ab]	6.44 ±0.61[ab]
Foaming index	Less than 100	Less than 100	Less than 100	Less than 100

Values are mean ±S.E.M. (n = 3). Where, a corresponds to p<0.05 vs. *W. somnifera* collected from desert region, b corresponds to p<0.05 vs. *W. somnifera* collected from plains region and c corresponds to p<0.05 vs. *W. somnifera* collected from coastal region.

Table 2 — Quantitative estimations of *W. somnifera* collected from different climatic zones of India

Test	Desert Region	Plains Region	Coastal Region	Plateau Region
Total phenolic content (mg/g gallic acid equivalent)	95 ±5.16[ab]	90.325 ±4.15	83.3 ±5.09	80.625 ±5.08
Total alkaloid content (% w/w in plant material)	0.50 ±0.02[abc]	0.20 ±0.001	0.21 ±0.12	0.17 ±0.13
Total flavonoid content (mg/g rutin equivalent)	86.153 ±4.35[ab]	80.23 ±5.93	78.459 ±5.74	73.153 ±4.99
Total flavanone content (mg/g naringine equivalent)	35.423 ±2.49[a]	30.16 ±2.23[a]	26.85 ±2.12[a]	15.78 ±2.31
Total carbohydrates (mg/g d-fructose equivalent)	130.11 ±4.75[ac]	122.26 ±5.65[a]	147.46 ±4.35[acd]	100.11 ±4.92
Total tannin content (mg/g tannic acid equivalent)	30.10 ±1.13[abc]	23.02 ±1.12	22.81 ±1.12	21.45 ±1.35

Values are mean ±S.E.M. (n = 3). Where, a corresponds to p<0.05 vs. *W. somnifera* collected from plateau region, b corresponds to p< 0.05 vs. *W. somnifera* collected from coastal region, c corresponds to p < 0.05 vs. *W. somnifera* collected from plains region and d corresponds to p < 0.05 vs. *W. somnifera* collected from desert region.

amount of impurities. In pesticide analysis, the roots did show the presence of chlorinated (range between 0.315 ±0.05 to 0.146 ±0.02 mg/kg of plant material) and phosphated (range between 0.055±0.03 to 0.0122 ±0.02 mg/kg of plant material) pesticides but were present within the standard limits of these pesticides.

Phytochemical analysis of the root samples from desert, plains, coastal and plateau revealed the presence of mainly alkaloids, phenols, steroids, flavonoids, tannins, and carbohydrates as major phytochemicals. The results of the quantitative estimations of all the phytoconstituents is presented in Table 2, where the roots collected from desert region showed significantly higher quantities of phenols, flavonoids, alkaloids and tannins, while carbohydrates was found to be higher in roots from coastal region. The observations from the HPTLC and HPLC analysis confirmed the presence of Withanoloid-A and Withaferine-A in all sample extracts. The HPTLC results showed the presence of Withanoloid-A, which was reported to be 3.22%, 1.41%, 2.40% and 0.63% w/w in the samples from desert, plains, coastal and plateau regions respectively, while Withaferine-A was found to be 1.19%, 0.42%, 0.82% and 0.49% w/win the respective samples (Fig. 3). From the HPLC analysis, the quantity of Withanoloid-A was found to be 4.24%, 1.45%, 2.35% and 1.24% w/w in the samples collected from desert, plains, coastal and plateau regions respectively, whereas the content of Withaferine-A was reported to be 1.35%, 1.28%, 1.35% and 0.75% w/w respectively in the same order of samples (Fig. 4). The HPTLC and HPLC quantification of Withaferin A and Withanoloid A demonstrated a higher quantity of both the markers in sample from desert region followed by sample from coastal areas. In addition, higher quantities of markers were observed in HPLC as compared to HPTLC analysis due to its higher sensitivity.

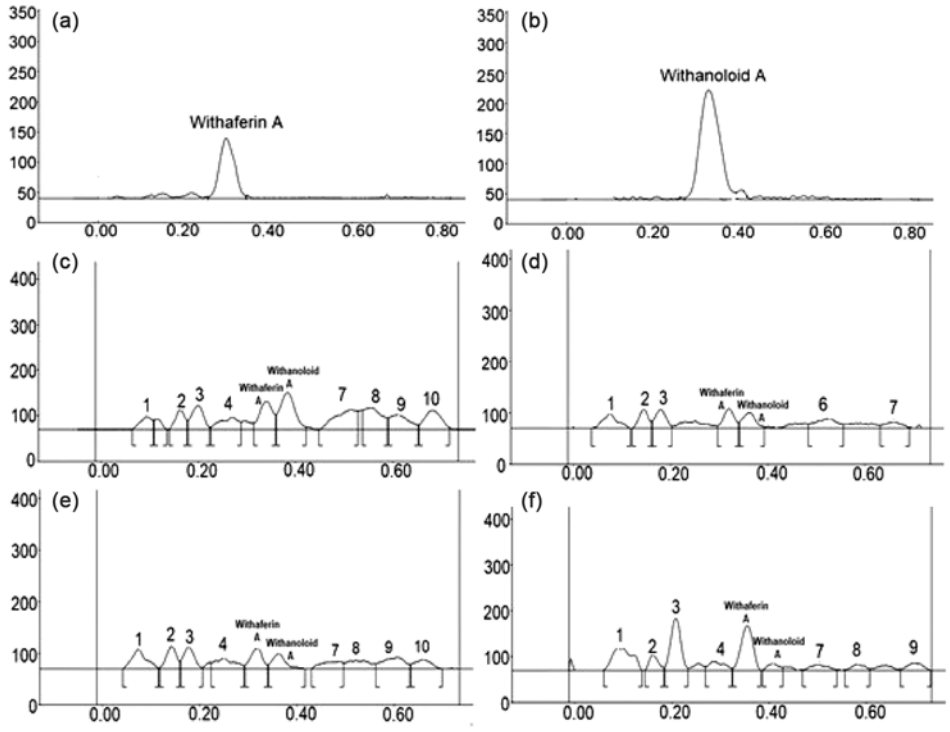

Fig. 3 — HPTLC chromatogram of *W. somnifera* showing the presence of Withaferin A and Withanoloid A. In figure, A: Standard peak of Withferin A, B: Standard peak of Withanoloid A, C: *W somnifera* roots from desert region, D: *W. somnifera* roots from plain region, E: *W somnifera* roots from coastal region and F: *W somnifera* roots from plateau region.

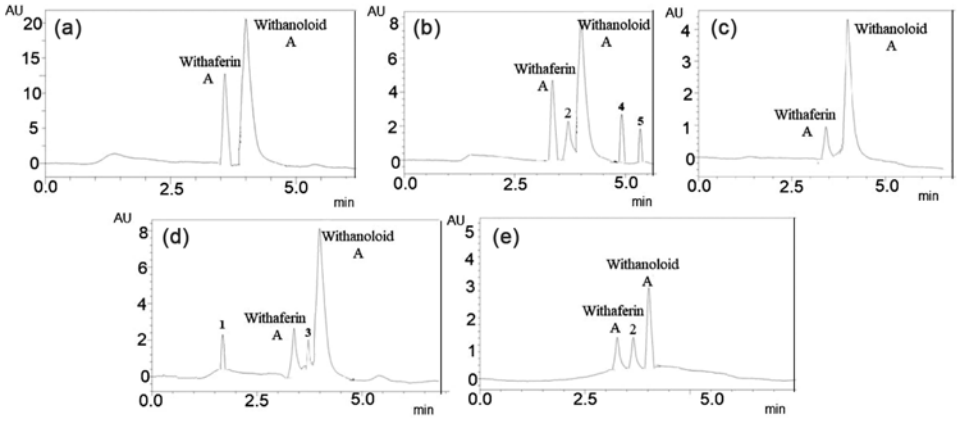

Fig. 4 — HPLC chromatogram of *W. somnifera* showing the presence of Withaferin A and Withanoloid A. In figure, A: Standard peak of Withaferin A and Withanoloid A, B: *W somnifera* roots from desert region, C: *W somnifera* roots from plains region, D: *W. somnifera* roots from coastal region and E: *W. somnifera* from plateau region.

Discussion

India is known to be characterized by wide variations in temperature and rainfall zones accompanied by different environmental and climatic fluctuations in varied seasons[19]. Further, the composition of phytochemical in plants is highly affected due to different agro-climatic conditions. Even though, there have been various studies performed on the phytochemical & pharmacological aspect of *W. somnifera* and its active constituents, there is no scientific data available on its comparative phytochemical and chromatographic evaluation of *W. somnifera* roots collected from different climatic zones of India. As we know, at different climatic conditions, it has been observed that there has been great variation in physical & chemical behaviour of plant which leads to contrast variation in different active constituents present in that particular plant[20].

According to WHO[10], macroscopic and microscopic evaluations are said to be the primary step in confirming the identity and purity of medicinal plants. From the microscopical observation of the roots, the samples from desert and plains showed larger paranchymatous cells compared to other two samples. The roots from desert region also showed large amount of tanniferous cells, while the vascular tissues were found to be larger in samples from coastal and plateau region. Loss on drying is a very important parameter for plant material as it determines the amount of both water and volatile matter of the plant material, which may absorb moisture easily and may get deteriorate quickly[10,21]. The results showed the presence of very low moisture content in the roots obtained from dessert and plains, while it was higher in sample collected from plateau and coastal regions, but were within the prescribed limits. Ash values help us in determining different impurities like carbonates, silicates and oxalates present in the drug. The water soluble ash gives an idea about the amount of inorganic compounds, while the acid insoluble ash provides information on the amount of silica present and also helps in determining the contamination with earthy material[22]. The roots showed the presence of higher amount of water soluble ash as compared to acid insoluble ash. The extractive value helps us in determining the appropriate amount of active ingredients present in the plant materials by treating them in different solvents for a specific period of time in a specific quantity[10]. From the results, a consistent decline in the extractive values was observed as the polarity of the solvent went on decreasing in all four samples. Swelling index is an indicator of the presence of mucilage, gums, pectin and hemicelluloses in a plant material and plays a significant role in judgement of the therapeutic or pharmaceutical value of the plant material[10]. The results showed a lower swelling index of all samples which may be due to low quantity of the above mentioned parameters. The foaming index of a plant material gives an idea about its ability to form foam in an aqueous decoction of that plant material or its extracts, which was reported to be low in all samples[10]. Agricultural protocols such as treatment of soils, spraying, and application of fumigants during storage may result in accumulation of pesticide residues in medicinal plant material. Therefore, it is very essential for herbal drugs to undergo a broad groups testing of these pesticide in general, instead of individual pesticides[23]. The roots showed the presence of both the chlorinated as well asphosphated pesticides, however they were found to be below the standard limits of these pesticides.

The results from the phytochemical analysis revealed the presence of steroids, alkaloids, carbohydrates, amino acid, proteins, saponins, whereas glycoside were found to be absent in all the extracts, which was also evident through the quantitative estimations of phytoconstituents. Alkaloids rank among the most diverse, efficient and wide range of therapeutically significant plant substances[24]. Phenolic compounds have been known for their potential antioxidant properties and also as substrates for lowering oxidative stress and possess anticarcinogenic, anti-inflammatory, antibacterial activities, and also plays significant role in treatment of coronary heart disease[25]. Flavonoids have also been known for their antioxidant and anti-inflammatory activities and contribute in increasing capillary permeability and have also proven effective in treating various cardiovascular diseases[26]. Steroids have been accounted for potent activities such as immunomodulatory, anti-inflammatory, antitumour, anti-stress, adaptogenic, anticonvulsant, neuro-pharmacological and musculotropic activities[27]. Tannins provide astringent and haemostatic properties to a compound and are reported to have anti-inflammatory, anti-bacterial, anti-oxidant and anti-viral activities[9]. The results from preliminary phytochemical screening and quantitative analysis showed the extract of *W. somnifera* from dessert region to be highly rich in, steroid, alkaloids, carbohydrates, phenols and saponins, whereas the sample from plateau region showed least quantity of above constituent. However, the sample from plains and coastal regions showed the moderate quantity of steroid, alkaloid and polyphenols.

With the help of chromatographic studies, one can easily determine purity of sample, examination of reaction, identification of compounds in a mixture and separation of multicomponent in pharmaceutical formulation and in cosmetic industries, etc[28]. Results obtained from the chromatographic studies showed maximum number of components in sample from coastal region compared to other samples. In the present study, we have also quantified the content of Withanoloid A and Withaferine A in *W. somnifera* root extract using HPTLC and HPLC. Withaferin A and Withanoloid A are steroidal lactones present in *W. somnifera*. Withaferin A has been pharmacologically known as potential anti-cancer candidate due to its anti-metastatic, apoptotic, cytotoxic, anti-mitotic and anti-

angiogenesis activities and has also been reported for anti-inflammatory, anti-angiogenesis, anti-parasitic and hepatoprotective potential. Withanolide-A has shown its potency against Parkinson's disease, Alzheimer disease, convulsions and cognitive function impairment due to its ability to reconstruct neural networks[29]. From the results, it was observed that, the sample from dessert region showed higher quantity of both Withaferin A as well as Withanoloid A. However, the sample from coastal region also showed higher quantity of Withaferin A and Withanoloid A, which was quite similar to the sample from dessert region.

Thus, from the overall observation, it may be presumed that, the sample from dessert region represented a better phytochemical profile as compared to the other sample and therefore can be preferred for preparation of herbal medicines, which will be having a higher therapeutic index that may serve the common people with better health benefits.

Acknowledgment

The authors would like to acknowledge the valuable support of Anchrom Enterprises (I) Pvt. Ltd, Mumbai, Maharashtra, India for providing their lab facility for performing the HPTLC analysis. Further, we would also like to acknowledge the valuable contribution of Unijules Life Sciences Ltd. Nagpur, Maharashtra, India for their valuable guidance and for providing the samples of Ashwagandha roots.

Conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the writing and content of the paper.

References

1 Kumar SM, Yadav A & Yadav JP, Impact of spatial and climatic conditions on phytochemical diversity and *in vitro* antioxidant activity of Indian *Aloe vera* (L.) Burm.f, *S. Afr. J. Bot*, 111(2017)50–59.

2 Maheswari JK, Patenting Indian medicinal plants and products, *Ind. J. Sci. Technol,* 4 (3) (2011) 298-301.

3 Singh AK, Dwivedi V, Rai A, Pal S, Reddy SG, *et al*, Virus-induced gene silencing of *Withania somnifera* squalene synthase negatively regulates sterol and defence-related genes resulting in reduced withanolides and biotic stress tolerance, *Plant Biotechnol. J*, 13(2015)1287–1299.

4 Kumar A, Kaul M, Bhan M, Khanna P & Suri K, Morphological and chemical variation in 25 collections of the Indian medicinal plant, *Withania somnifera* (L.) Dunal (Solanaceae), Gen. Resour, *Crop Evol*, 54(2007)655–660.

5 Yang L, Kui-Shan W, Xiao R, Zhao Y, Feng W, et al, Response of Plant Secondary Metabolites to Environmental Factors, *Molecules,* 23(2018)762.

6 Mirjalili MH, Moyano E, Bonfill M, Cusido RM & Palazon J, Streoidal lactones from *Withania somnifera*, an ancient plant for novel medicine, *Molecules,* 14(2009) 2373–2393.

7 Sivanandhan G, Sivanandhan G, Mariashibu T, Arun M, Rajesh M, Kasthurirengan S, Selvaraj N & Ganapathi A, The effect of polyamines on the efficiency of multiplication and rooting of *Withania somnifera* (L.) Dunal and content of some withanolides in obtained plants, *Acta Physiol. Plant*, 33(2011) 2279–2288.

8 Sharma C, Irshad S, Khatoon S & Arya KR. Pharmacognostical evaluation of Indian folk-traditional plants *Coelogynecristata* and *Pholidota articulata* used for healing fractures, *Indian J. Tradit. Knowle*, 55 (2017) 422-127.

9 Prasad SK, Sahu AN & Hemalatha S, Cytomorphological and physicochemical evaluations of *Cryptocorynespiralis* (Retzius) Wydler, *J. Herbs Spices Med. Plants,* 18 (2012)304-307.

10 Anonymous, Quality Control Methods for Medicinal Plant Materials (An Authorized publication of World Health Organization, Geneva) (A.I.T.B.S. Publishers & Distributors (Regd) Delhi), 2002.

11 Rajmohan M, Mohammed F, Kumar S & Ravishankar B. Traditional method of preparation and development of quality standards for an edible vegetable fat Malabar tallow (*Vateria indica* L. seed butter), *Indian J. Tradit. Knowle*, 9 (2018) 60-65.

12 Trease GE & Evans WC, Pharmacognosy, 15th Edition, (W.B. Saunders Company Ltd, London), 2002.

13 Hagerman AE, Riedl KM, Jones, GA, Sovik KN, Ritchard NT, *et al*, High molecular weight plant polyphenolics (tannins) as biological antioxidants, *J. Agri. Food Chem*. 46(2000) 1887–1892.

14 Kumaran A & Karunakaran J, In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India, *LWT - Food Sci. Technol*, 40(2006) 344–352.

15 Baccou JC, Lambert F & Sauvaire Y, Spectrophotometric method for the determination of total steroidal sapogenin, *Analyst,* 102 (1977) 458–465.

16 Wagner H & Bladt S, Plant drug analysis. 2nd Edition, (Springer-Verlog Berlin Heidelberg, Germany), 1996.

17 Yemm EW & Willis AJ, The estimation of carbohydrates in plant extracts by Anthrone, *Biochem J*, 57(1954) 514-518.

18 Mir B, Koul S & Soodan A, Reproductive biology of *Withania* ashwagandha sp. Novo, *Ind. Crops Prod*, 45 (2013)442–446.

19 Krishnarajua AV, Rao TV, Sundararajua D, Vanisreeb M, Tsayb H, et al, Assessment of bioactivity of India medicinal plants using brine shrimp (*Artemiasalina*) lethality assay, *Int. J. Appl. Sci. Eng. Res,* 3(2005) 125–134.

20 Lobell DB, Schlenker W & Costa-Roberts J, Climate trends and global crop production since 1980, *Science,* 333(2011) 616–620

21 Kokate CK, Purohit AP & Gokhale SB, Textbook of Pharmacognosy, (Nirali Prakashan, Pune, Maharashtra, India), 3, 2010.

22 Singh GK & Bhandari A, Text book of Pharmacognosy, 1st Edition, (CBS Publisher & Distributor, New Delhi), 2005, 39-62.

23 Nafiu MO, Hamid AA, Muritala HF & Adeyemi SB, Preparation, Standardization and Quality Control of

Medicinal Plants in Africa, In: Medicinal Spices and Vegetables from Africa, (2017) 171–204

24 RoyA, A Review on the Alkaloids an Important Therapeutic Compound from Plants, *Int. J. Plant Biotechnol*, 3(2017) 1-10.

25 Shahidi F & Ambigaipalan P, Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects – A review, *J. Funct. Foods*, 18(2015) 820-897.

26 Catarino DM, Alves-Silva J, Pereira RO & Cardoso MS, Antioxidant Capacities of Flavones and Benefits in Oxidative-Stress Related Diseases, *Curr. Topics Med. Chem*, 15(2015) 105–119.

27 Uddin Q, Samiulla L, Singh V & Jamil S, Phytochemical and Pharmacological Profile of *Withania somnifera* Dunal: A Review, *J. Appl. Pharm. Sci*, 2(2012)170–175.

28 Kasture AV, Wadodkar SG, Mahadik KR & More HN, Pharmaceutical Analysis Instrumental methods,10th Edition, (Nirali Prakashan, Educational Publishers), 2004, 26-27.

29 Pati D, Gautam M, Mishra S, Karupothula S, Gairola S, *et al*., Determination of withaferin A and withanolide A in mice plasma using high-performance liquid chromatography-tandem mass spectrometry: Application to pharmacokinetics after oral administration of *Withania somnifera* aqueous extract, *J. Pharma. Biomed. Anal*, 80(2013) 203– 212.