International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 6, Issue 11 -2019

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.11.010

Standardization and Physicochemical Evaluation of Indian Traditional Siddha Formulation *Visha Sanjeevi*

T. Subathra*[1], P. Shanmugapriya[2], M. Murugesan[3]

[*1]Assistant Professor, Department of Noi Anuga Vidhi including Research Methodology, Sri Sai Ram Siddha Medical College and Research Centre, Sai Leo Nagar, Poonthandalam, West Tambaram, Chennai 600044, Tamil Nadu, India.

[2] Associate Professor, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.

[3] Former Dean and Head, Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.

Corresponding Author: **Dr. T. Subathra,** Assistant Professor, Department of Noi Anuga Vidhi Ozhukam including Research Methodology, Sri Sai Ram Siddha Medical College and Research Centre, Sai Leo Nagar, Poonthandalam, West Tambaram, Chennai 600044, Tamil Nadu, India.

Abstract

Standardization is a quality measure on ensuring the purity and genunity of the raw drug and finished formulations as well. As per the direction provided by the international authorities, it is now become mandate for the indigenous and traditional medicine to comply with the standards for all the medication that has been supplied with in the country and also the drugs of export values. Heavy metal toxicity has proven to be a major threat and there are several health risks associated with it. The toxic effects of these metals, even though they do not have any biological role, remain present in some or the other form harmful for the human body and its proper functioning. They sometimes act as a pseudo element of the body while at certain times they may even interfere with metabolic processes. Siddha system of traditional medicine has numerous potential formulation but still now most of the formulations has to be validated for its safety and quality. Hence the main aim of the present investigation is to standardize and to establish the monograph for the formulation *Visha Sanjeevi (VS)* as per ASU regulatory guidelines. The results obtained from standardization and physicochemical analysis clearly reveals that the Loss on drying value for VS is 5.86 %, total ash value is about 23.82% in which the acid-Insoluble ash is 3.86%. Water and alcohol soluble extractives are 5.56 and 0.81 % respectively. Sterility profiling of VS reveals the absence of microbes such as *E. coli, Salmonella, Staphylococcus aureus* and *Enterobacteriacae*. Results of phytochemical analysis reveals the presence of alkaloids and heavy metal analysis indicates the presence of lead whose content are within the WHO recommended limit. Further radical estimation analysis reveals the presence of acid (Chlorides and phosphates) and basic (Iron) radicals. From the data obtained from the results of the present investigation, it was evident that the formulation VS complies with the regulatory standard and also possess novel bioactive phytocomponents like alkaloids.

Keywords: Siddha, Visha Sanjeevi, Standardization, Heavy metal, Phytochemical analysis

1. Introduction

Regulation on quality control of traditional formulations streamlined the process of validating the optimized procedure in the process of siddha drug formulation and export of herbal based drugs. India is among the important mega biodiversity centers of the world with nearly 45,000 known plant species [1]. This diversity coupled with a rich heritage of traditional knowledge (TK) has made India home to several important health care systems viz., *Ayurveda*, *Siddha* and *Unani*. Whilst there is increasing demand for Indian TM globally, it is only for products validated by modern scientific research. India exports of around $100 million which were relatively low compared to Chinese figures of $3 billion. Report by Group-II Task Force on "Pharmaceuticals and Knowledge Based Industries" 1999, states that Indian exports of herbal products are low due to several factors, quality being the most important [2]. To be a strong player in global herbal market, it is critical that India develops appropriate quality control systems for standardization of raw materials and finished products and to strengthen the regulatory mechanism.

The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world [3]. *Nicotiana tabacum* contains a number of structurally related alkaloids [4] and is considered as the model plant for alkaloid research. Nicotine is the predominant alkaloid accumulating in the leaves of most *N. tabacum* varieties and represents 90–95% of the total alkaloid content [5-8].

Contamination in siddha formulation may appear in the form of microbial and metal adulterants which may be sourced from raw drugs with inadequate purification.In biological systems, heavy metals have been reported to affect cellular organelles and components such as cell membrane, mitochondrial, lysosome, endoplasmic reticulum, nuclei, and some enzymes involved in metabolism, detoxification, and damage repair [9]. Metal ions have been found to interact with cell components such as DNA and nuclear proteins, causing DNA damage and conformational changes that may lead to cell cycle modulation, carcinogenesis or apoptosis [10, 11].

Standardization is a process which maintains consistency in the claimed efficacy of a product and its batch-to-batch reproducibility. The major challenges in terms of scientific standardization to adhere to industry norms are variation in the source, lack of safety evaluations and difficulty in quality control [12]. Hence the main aim of the present investigation is to standardize and to establish the monograph for the formulation *Visha Sanjeevi (VS)* as per ASU regulatory guidelines.

2. Materials and Methods

2.1. Ingredients

The formulation *Visha Sanjeevi* comprises of the following ingredients

1. *Pugaielai (Leaves of Nicotiana tabacum)*
2. *Panaivellam (Palm jaggery)*
3. *Leaves of Agathi (Sesbania grandiflora)*

2.2. Source and authentication of raw drug:

All the raw drugs werebought from Indigenous authentic country drug shop at Chennai, Tamil Nadu, India. All the raw drugs were identified and authenticated by the Botanist of National Institute of Siddha, Chennai-47, Tamil Nadu, India.

2.3. Method of preparation:

The above mentioned purified Pugaielai and Palm Jaggery are blended together and are kept in a shallow earthen plate. This is covered with another shallow earthen plate. The margins are covered with two layers of mud pasted cloth, dried and burnt with cow dung cakes which are 3 times the weight of sealed earthen plates. After cooling, the lid is opened and the processed medicine thus obtained is collected and kept in an air tight container.

Therapeutic dose: *1 varaagan (4.2 gram)*
Adjuvant: *Water*
Indication: *Snake bites*

2.4.Physicochemical Evaluation [13,14]

2.4.1.Percentage Loss on Drying

10gm of test drug was accurately weighed in evaporating dish. The sample was dried at 105^{o}C for 5 hours and then weighed.

Percentage loss in drying = Loss of weight of sample/ Wt of the sample X 100

2.4.2. Determination of Total Ash

3 g of test drug was accurately weighed in silica dish and incinerated at the furnace a temperature 400ºC until it turns white in color which indicates absence of carbon. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.4.3. Determination of Acid Insoluble Ash

The ash obtained by total ash test will be boiled with 25 ml of dilute hydrochloric acid for 6mins. Then the insoluble matter is collected in crucible and will be washed with hot water and ignited to constant weight. Percentage of acid insoluble ash will be calculated with reference to the weight of air-dried ash.

Acid insoluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.4.4. Determination of Water Soluble Ash

The ash obtained by total ash test will be boiled with 25 ml of water for 5 mins. The insoluble matter is collected in crucible and will be washed with hot water, and ignite for 15mins at a temperature not exceeding 450ºC. Weight of the insoluble matter will be subtracted from the weight of the ash; the difference in weight represents the water soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Water Soluble Ash = Weight of Ash/Wt of the Crude drug taken X 100

2.4.5. Determination of Alcohol Soluble Extractive

About 5 g of test sample will be macerated with 100 ml of Alcohol in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Alcohol sol extract = Weight of Extract/ Wt of the Sample taken X 100

2.4.6. Determination of Water Soluble Extractive

About 5 g of the test sample will be macerated with 100 ml of chloroform water in a closed flask for twenty-four hours, shaking frequently during six hours and allowing it to stand and for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tarred flat bottomed shallow dish, and dry at 105ºC, to constant weight and weigh. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Water soluble extract = Weight of Extract/ Wt of the Sample taken X 100

2.4.7. Determination of pH

About 5 g of test sample will be dissolved in 25ml of distilled water and filtered the resultant solution is allowed to stand for 30 mins and the sample subjected to pH evaluation

2.5. Atomic Absorption Spectrometry (AAS) [15]

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

2.5.1. Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO_3.

2.5.2. Standard preparation

As & Hg- 100 ppm sample in 1mol/L HCl
Cd &Pb- 100 ppm sample in 1mol/L HNO_3

2.6. Test for Acid and Basic radicals [16]

Carried out as per the standard procedure for carry out the inorganic elemental analysis as per the standard protocol.

Procedure	Observation	Inference
Test for Potassium: Sample was treated with sodium nitrate solution and then treated with 2ml of cobalt nitrate in 30% of glacial acetic acid.	Formation of Yellow colour precipitate	Presence of Potassium
Test for Calcium: Sample was treated with acetic acid and potassiumchromate solution were added	No Yellow precipitate	Presence of Calcium
Test for Magnesium: Sample was treated with few drops of Magnason reagentwas added in drops.	Formation of Blue colour precipitate	Presence of Magnesium
Test for Ammonium: Sample was treated with few ml of Nessler's reagent.	Appearance of Brown colour	Presence of Ammonium
Test for Sodium: Sample was treated with HCl and made it into paste. And introduced into the blue flame of Bunsen burner.	Appearance of intense Yellow colour	Presence of Sodium
Test for Iron (Ferrous): Sample was treated with conc. HNO_3 and ammonium thiocyanate were added.	Appearance of Blood red colour	Presence of Ferrous iron
Test for Zinc: Sample was treated with potassiumferro cyanide solution was added.	Formation of White colour precipitate	Presence of Zinc
Test for Aluminium: Sample was treated with sodium hydroxide drops were added to it.	White precipitate obtained	Presence of Aluminium
Test for Lead: Sample was treated with 2ml of potassium iodide solution	Formation of yellow colour precipitate	Presence of Lead
Test for Copper: Sample wastreated with dilute hydrochloric acid was added and then hydrogen sulphide gas is passed through the solution.	Black precipitate	Presence of Copper
Test for Mercury: Sample was treated With 2ml of sodium hydroxide solution.	Formation of Yellow precipitate	Presence of Mercury
Test for Arsenic: Sample was treated with 2ml of sodium hydroxide solution.	Formation of brownish red precipitate	Presence of Arsenic

2.6.1. Test for Acidic Radical

Procedure	Observation	Inference
Test for Sulphate: 2 ml of the extract was taken in clean, dry test tube and 5 % barium chloride solution was added to it.	Formation of white precipitate	Presence of Sulphate
Test for Chloride: The extract was taken in a test tube and then treated with Silver nitrate solution.	Formation of White precipitate	Presence of Chloride
Test for Phosphate: The extract was taken in a test tube and treated with ammonium molybdate and conc. HNO_3.	Formation of Yellow precipitate	Presence of Phosphate
Test for Carbonate: The substance was taken in a clean dry test tube and then treated with Conc. HCl.	Formation of Effervescence	Presence of Carbonate
Test for fluoride &oxalate: Sample was added with 2ml of dil. acetic acid, 2ml calcium chloride solution and then heated.	Formation of cloudy appearance	Presence of Fluoride & Oxalate
Test For Nitrate: 1gm of the samplewas heated with copper turnings and concentrated H2SO4 and observed the test tube vertically down.	Characteristic changes	Presence of Nitrate

2.7. Preliminary Qualitative Phytochemical Analysis [17]

Test for steroids: To the test sample, 2ml of chloroform was added with few drops of conc. Sulphuric acid (3ml), and shaken well. The upper layer in the test tube was turns into red and sulphuric acid layer showed yellow with green fluorescence. It showed the presence of steroids.

Test for alkaloids: Mayer's Test: To the test sample, 2ml of mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Test for flavonoids: To the test sample about 5 ml of dilute ammonia solution were been added followed by addition of few drops of conc. Sulfuric acid. Appearance of yellow color indicates the presence of Flavonoids.

Test for glycosides- Born Trager's Test: Test drug is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

Triterpenoids: Liebermann–Burchard test: To the chloroform solution, few drops of acetic anhydride was added then mixed well. 1 ml concentrated sulphuric acid was added from the sides of the test tube, appearance of red ring indicates the presence of triterpenoids

Test for tannins: To the test sample, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

Test for phenols: To the test sample; 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Proteins (Biuret Test): To the extracts 1% solution of copper sulphate was added followed by 5% solution of sodium hydroxide, formation of violet purple colour indicates the presence of proteins.

Test for saponin: To the test sample, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of Saponins.

2.8. Determination of Microbial Load [18,19]

2.8.1. Pre-treatment of the sample

10 gms of the sample was suspended in a Soybean – caesin digest medium proven to have no antimicrobial activity, and was diluted to 100 ml with the same medium. The suspension was mechanically homogenized. The filtrate was collected aseptically in a clean conical flask. The rate of dilution obtained was 1/10

2.8.2. Detection of total bacterial count

The required numbers of approximately 15 – 20 ml of Soybean – caesin digest agar plates were prepared. 0.1 ml of the pretreated sample (Dilution – I) was added over the plate, and spreaded uniformly on the surface using a L-rod. Serial dilutions of the sample were performed if necessary to obtain an expected colony of not more than 300. Duplicates for each dilution were maintained. Negative control plates without the addition of sample were also maintained. All the plates were incubated at 37°C for 24hours. The number of colonies formed was counted and the results were calculated.

2.8.3. Detection of total fungal count

The required numbers of approximately 15 – 20 ml of Sabourads glucose Agar plates were prepared. 0.1 ml of the pretreated sample was added over the plate, and spreaded uniformly on the surface using a L-rod. Serial dilutions of the sample were performed if necessary to obtain an expected colony of not more than 100. Duplicates for each dilution were maintained. Negative control plate without the addition of sample was also maintained .All the plates were incubated at 20 - 25°C for 5 days. The number of colonies formed was counted and the results were calculated.

2.8.4. Detection of *Enterobacteriaceae*

The required numbers of approximately 15 – 20 ml of Macconkey agar plates were prepared. 0.1 ml of the pretreated sample was added over the plate, and spreaded uniformly on the surface using a L-rod. Serial dilutions of the sample were performed if necessary to obtain an expected colony of not more than 300. Duplicates for each dilution were maintained. Negative control plate without the addition of sample was also maintained. All the plates were incubated at 37°C for 24 hours. The number of colonies formed was counted and the results were calculated.

2.8.5. Detection of *Escherichia coli*

The required numbers of approximately 15 – 20 ml of Eosin Methylene Blue agar plates were prepared. 0.1 ml of the pretreated sample was added over the plate, and spreaded uniformly on the surface using a L-rod. Duplicates were maintained. Negative control plate without the addition of sample was also maintained. All the plates were incubated at 37°C for 24 hours. Appearance of small, red, metallic sheen producing colonies indicates the presence of *E. coli*. The material passes the test, if no colonies are detected.

2.8.6. Detection of *Salmonella*

The required numbers of approximately 15 – 20 ml of Brilliant Green Agar plates were prepared. 0.1 ml of the pretreated sample was added over the plate, and spreaded uniformly on the surface using a L-rod. Duplicates were maintained. Negative control plate without the addition of sample was also maintained. All the plates were incubated at 37°C for 24 hours. Appearance of small, transparent, colourless, pink or white (frequently surrounded by pink zone) colour indicates the presence of *Salmonella*. The material passes the test if no colonies are detected.

2.8.7. Detection of *Staphylococcus aureus*

The required numbers of approximately 15 – 20 ml of Baird parker agar plates were prepared. 0.1 ml of the pretreated sample was added over the plate, and spreaded uniformly on the surface using a L-rod. Duplicates were maintained. Negative control plate without the addition of sample was also maintained. All the plates were incubated at 37°C for 24 hours. Appearance of black coloured colonies indicates the presence of *Staphylococcus aureus.* The material posses the test of no growth appears.

3. Results

3.1. Results of Organoleptic Evaluation of *Visha Sanjeevi*

Organoleptic Character of VS described in Table 1. The study reveals the color of the drug appears black with sparing solubility and no characteristic color change upon action on heat and flame. As shown in Table 1.

Table 1: Organoleptic evaluation of *Visha Sanjeevi*

S.No	Parameter	Observation
1	Appearance	Amorphous in nature
2	Solubility	Sparingly Soluble
3	Action of heat	No White fumes evolved
4	Flame test	No Bluish green flame appeared
5	Ash test	No Yellow colour flame appeared

3.2. Results of Physicochemical Analysis

The results obtained from physiochemical analysis clearly reveals that the Loss on drying value for VS is 5.86 %, total ash value is about 23.82% in which the acid-Insoluble ash is 3.86%. Water and alcohol soluble extractives are 5.56 and 0.81 % respectively. As shown Table 2.

Table 2: physicochemical evaluation of *Visha Sanjeevi*

S. No.	Parameters	Results
1	pH	5.3 - 5.5
2	Loss on Drying at 105^{o}C (%w/w)	5.86
3	Total Ash Content (%w/w)	23.82
4	Acid-Insoluble Ash (%w/w)	3.86
5	Water Soluble Extractive (%w/w)	5.56
6	Alcohol Soluble Extractive (%w/w)	0.81

3.3. Results of Sterility and Specific pathogen analysis of *Visha Sanjeevi*

The results obtained from sterility reveals the presence of TBC within the permissible limit further there is no presence of fungus upon incubation in nutritive medium. Similarly results of specific pathogen indicate the absence of microbes such as *E. coli*, *Salmonella*, *Staphylococcus aureus* and *Enterobacteriaceae* as shown Table 3.

Table 3: Sterility and Specific pathogen analysis of *Visha Sanjeevi*

S.No	Parameters	Results	Permissible Limit
1	Total Bacterial Count (TBC)	4×10^3 CFU/g	10^5 CFU/g
2	Total Fungal Count (TFC)	Absent	10^3 CFU/g
3	*Enterobacteriaceae*	Absent	10^3 CFU/g
4	Escherichia coli	Absent	10CFU/g
5	Salmonella Spp	Absent	Absent
6	Staphylococcus aureus	Absent	Absent

3.4. Phytochemical Investigation *Visha Sanjeevi*

Phytochemical investigation report justifies the presence of alkaloid a valid bioactive components responsible for wide range of pharmacological activity. Results were tabulated in table 4.

Table 4: physicochemical evaluation of *Visha Sanjeevi*

S. No.	Procedures	Results
1	Test For Starch	-
2	Test For Reducing Sugar	-
3	Test For Alkaloids	+
4	Test For Tannic acid	-
5	Test For Unsaturated Compound	-
6	Test For Amino acid	-
7	Test For Type of Compound	-

(+) Denotes "Presence"; (-) Denotes "Absence"

3.5. Result Analysis of heavy metal analysis of *Visha Sanjeevi* by AAS technique

Heavy metal analysis of VS shows that presence of heavy metal Lead with concentration 0.043 ppm. Further other metals such as Cadmium, Arsenic and Mercury were not detected and some are below the quantitative level. As shown in Table 5.

Table 5: Heavy metal analysis of *Visha Sanjeevi*

S. No.	Name of the elements	Results	Permissible limit
1	Lead	0.0430 ppm	10 ppm (WHO)
2	Cadmium	ND	0.3 ppm (WHO)
3	Arsenic	BDL	3 ppm (WHO)
4	Mercury	BDL	1 ppm (WHO)

ND – Not Detected; BDL – Below Detectable

3.6. Result of Biochemical Analysis – Basic radical

Results of the present investigation on test of basic radical reveals the presence of iron and all other radicals like lead, copper, zinc, calcium, magnesium, sodium etc were found absent. As shown in Table 6.

Table 6: Result Analysis of Test for Basic radicals

S. No.	Procedures	Results
1	Test For Lead	-
2	Test For Copper	-
3	Test For Aluminium	-
4	Test For Iron	+
5	Test For Zinc	-
6	Test For Calcium	-
7	Test For Magnesium	-
8	Test For Ammonium	-
9	Test For Potassium	-
10	Test For Sodium	-
11	Test For Mercury	-
12	Test For Arsenic	-

(+) Denotes "Presence"; (-) Denotes "Absence"

3.7. Result of Biochemical Analysis – Acid radical

Results of the investigating test of acid radical reveals the presence of chlorides and phosphates, other radicals like sulphate, carbonate, nitrite etc were found absent. As shown in Table 7.

Table 7: Result Analysis of Test for Acid radicals

S. No.	Procedures	Results
1	Test for Sulphate	-
2	Test For Chloride	+
3	Test For Phosphate	+
4	Test For Carbonate	-
5	Test For Nitrate	-
6	Test For Sulphide	-
7	Test For Fluoride & Oxalate	-
8	Test For Nitrite	-
9	Test For Borate	-

(+) Denotes “Presence”; (-) Denotes “Absence”

4. Discussion

Siddha system of medicine has holistic and traditional approach on treating certain life threatening disease. Further ancient siddhar has unique formulation on healing poisoning, *Visha Sanjeevi* is one of the novel siddha preparation indicated for treating snake bites. This formulation majorly comprises of Leaves of *Nicotiana tabacum* and palm jaggery. Medicinal plants are one of the main resources of therapeutic agents. Indeed, 80% of the world's population uses plants in health care [20]. Recently, the interest in the search for natural substances has considerably increased, because these substances are intended for use in foods or drugs to replace synthetic compounds, which are limited because of their side effects [21]. Phytochemical investigation report justifies the presence of alkaloid a valid bioactive components responsible for wide range of pharmacological activity.

Presence of microbes in the study drug may indulge septicemia and organ dysfunction. The results obtained from sterility reveals the presence of TBC within the permissible limit, further there is no presence of fungus upon incubation in nutritive medium. Similarly results of specific pathogen indicate the absence of microbes such as *E.coli*, *Salmonella*, *Staphylococcus aureus* and *Enterobacteriacae*.

Physicochemical evaluation of the preparation plays vital role in establishing the monograph of the formulation, as it becomes the documentary evidence to substantiate the standards of the preparation. It renders the useful information like genuinity, stability, selective characteristic feature and nature of the compound’s present in the drug. WHO and other regulatory authorities in collaboration with government agency setup a bench mark for proper standardization of the raw drug as well the finished formulations. The results obtained from physiochemical analysis clearly reveals that the Loss on drying value for VS is 5.86 %, total ash value is about 23.82% in which the acid-Insoluble ash is 3.86%. Water and alcohol soluble extractives are 5.56 and 0.81 % respectively.

Emergence of advance techniques advances the process of validation and standardization process of several traditional preparations, presence of heavy metal in drugs may cause potential liver, kidney and brain damages upon long term applications. It’s always ideal if drugs are free from some toxic heavy metals such as cadmium, mercury and arsenic.

In recent years, there has been an increasing ecological and global public health concern associated with environmental contamination by the heavy metals. Also, human exposure has risen dramatically as a result of an exponential increase of their use in several industrial, agricultural, domestic and technological applications [22]. Reported sources of heavy metals in the environment include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources [23]. In the present study, heavy metal analysis of VS shows that presence of heavy metal Lead with concentration 0.043 ppm. Further other metals such as Cadmium, Arsenic and Mercury were not detected and some are below the quantitative level.

5. Conclusion

There is an increasing interest in using medicinal plants and their phytoconstituents as natural sources because of their well-known ability in treating specific condition. Effectively, plants are sources of natural antioxidants compounds that possess various pharmacological properties with little or no side effects and protect human health from many diseases. Studies have shown that chemical components like alkaloids possess several biological applications.From the data obtained from the results of the present investigation, it was evident that the formulation *Visha Sanjeevi* complies with the regulatory standard and also possess novel bioactive phytocomponents like alkaloids.

Acknowledgments

I wish to acknowledge my thanks to Director and Faculties of Department of Nanju Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, Chennai 600047, Tamil Nadu, India.

6. References

1. Myers N. Threatened biotas: Hotspots in tropical forests. Environmentalist. 1988;8:187–208.
2. Bhowmik D, Dubey P, Chandira M, Kumar KP. Herbal drug toxicity and safety evaluation of traditional medicines. Archives of Applied Science Research. 2009;1:32–56.
3. Seth S.D., Sharma B. Medicinal plants of India. Indian J. Med. Res. 2004;120:9–11.
4. Cai J.B., Liu B.Z., Lin P., Su Q.D. Fast analysis of nicotine related alkaloids in tobacco and cigarette smoke by megabore capillary gas chromatography. J. Chromatogr. A. 2003;1017: 187–193.
5. Wang J.M., Sheehan M., Brookman H., Timko M.P. Characterization of cDNAs differentially expressed in roots of tobacco (*Nicotiana tabacum* cv Burley 21) during the early stages of alkaloid biosynthesis. Plant Sci. 2000;158:19–32.
6. Shoji T., Kajikawa M., Hashimoto T. Clustered transcription factor genes regulate nicotine biosynthesis in tobacco. Plant Cell. 2010;22:3390–3409.
7. Hibi N., Higashiguchi S., Hashimoto T., Yamada Y. Gene expression in tobacco low-nicotine mutants. Plant Cell. 1994;6:723–735.
8. Siminszky B., Gavilano L., Bowen S.W., Dewey R.E. Conversion of nicotine to nornicotine in *Nicotiana tabacum* is mediated by CYP82E4, a cytochrome P450 monooxygenase. Proc. Natl. Acad. Sci. USA. 2005;102:14919–14924.
9. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem. 2001;222:3–9.
10. Chang LW, Magos L, Suzuki T, editors. Toxicology of Metals. Boca Raton. FL, USA: CRC Press; 1996
11. Beyersmann D, Hartwig A. Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol. 2008;82 (8):493–512.
12. Wang Zhen-Gang, Ren Jun. Current status and future direction of Chinese herbal medicine. Trends Pharmacol Sci. 2002;23(8):347–8.
13. The Ayurvedic Pharmacopoeia of India. Part II. Volume II, Fifth Edition, Department of AYUSH, New Delhi, 2008.
14. Department of AYUSH. The Ayurvedha Pharmacopoeia of India. Vol I, Ministry of Family health and welfare .2008; 59: 83.
15. Samudralwar DL, Garg AN. Minor and trace elemental determination in the Indian herbal and other medicinal preparations. Biol Trace Elem Res. 199g; 54:11–21.
16. Elisabeth Mohammed. Optimization of instrument conditions for the analysis for mercury, arsenic, antimony and selenium by atomic absorption spectroscopy. Methods X. 2018; 5: 824–833.
17. Brain KR, Turner TD. The Practical Evaluation of Phytopharmaceuticals. Bristol: Wright Scientechnica; 1975:36-45
18. Akhtar S., Riaz M., Ismail T., et al. Microbiological Safety of street vended fresh fruit juices, drinks and conventional blends in Multan-Pakistan. Pak. J. Agri. Sci. 2013;50: 255–260.
19. Prescott L.M., Harley J.P., Kleen D.A. Microbiology. 5th ed. New York: McGraw Hill; 2002:965–972.
20. Oliveira S. G. D., de Moura F. R. R., Demarco F. F., Nascente P. D. S., Pino F. A. B. D., Lund R. G. An ethnomedicinal survey on phytotherapy with professionals and patients from basic care units in the brazilian unified health system. Journal of Ethnopharmacology. 2012;140(2):428–437.
21. Velioglu Y. S., Mazza G., Gao L., Oomah B. D. Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. Journal of Agricultural and Food Chemistry. 1998;46(10):4113–4117.

22. Bradl H, editor. Heavy Metals in the Environment: Origin, Interaction and Remediation Volume 6. London: Academic Press; 2002.
23. He ZL, Yang XE, Stoffella PJ. Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol. 2005; 19(2–3):125–140.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Siddha Medicine
DOI:10.22192/ijarbs.2019.06.11.010	

How to cite this article:
T. Subathra, P. Shanmugapriya, M. Murugesan. (2019). Standardization and Physicochemical Evaluation of Indian Traditional Siddha Formulation *Visha Sanjeevi*. Int. J. Adv. Res. Biol. Sci. 6(11): 68-78.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.11.010