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Comparative elemental analysis of siddha Raw drug Thalagam (Arsenic trisulphide) by Atomic absorption spectrometry technique

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Abstract

Metals such as zinc, arsenic, copper, iron, manganese, and chromium are essential nutrients; they are important for the physiological and biological functions of the human body. However, an increase in their intake above certain permissible limits can become toxic. Manufacturing of traditional medicines requires extensive quality control, including the control of all manufacturing phases until the final product. Some countries have set strict quality control regulations and many others failed. Several regulatory agencies highlighted that some dietary supplements may induce health problems with regard to their quality, effectiveness and safety for human consumption. Poor quality control increases the risk of contamination of the preparations. Metal based siddha formulation offering therapeutic benefits to the mankind since ancient times. According to siddha metals and minerals act as chelator, detoxifier, mediator and also cures several dreadful disorders. In siddha system of medicine thalagam (Arsenic trisulphide) plays a vital role as it was claimed for treating range of indications as per the vedic literatures. But most of time elements and metals undergo change in transitional state upon subjecting to purification process. Present study aimed at comparing the change in elemental nature of unpurified (T1) and purified (T2 & T3) thalagam using atomic absorption spectrometry (AAS) methods of analysis.Biochemical analysis for the basic radicals reveals that both unpurified thalagam (T1) and purified thalagam (T2, T3) contains ferrous iron, Potassium, Calcium, Arsenic, Mercury and Lead. At the same time Calcium contains only in T2 samples. sample T1, T2 and T3 contain chloride and sulphate. Other acidic radicals were absent. Unpurified (T1) and purified thalagam (T2) contains flouride& oxalate. Results of elemental analysis reveals the absence of cadmium in the sample T3 and also significant decrease in the level of arsenic in purified sample T2 and T3. It was concluded that the purification process had significantly reduced the level of arsenic and also notable level of calcium observed exclusively in purified form.

Keywords: Siddha, Elemental nature, Quality control, Atomic absorption spectrometry, Thalagam, Heavy metals.

1. Introduction

In India, it has been estimated that about 14% sick persons utilizes Indian system of medicine [1]. A report by the World Health Organization (WHO) indicates that many people in developing countries still rely on traditional preparations [2]. Majority of people believe that traditional medicines are safe and nontoxic, unlike modern chemotherapeutic agents. Individuals generally use herbal medicine for prolonged periods to achieve a desirable effect. On contrarily, it has been reported that herbal drugs used in the Indian subcontinent and China contain higher concentration of heavy metals than in other areas [3-5].Another study showed that one of five herbal medical products, produced in south asia contains high levels of lead, mercury, and arsenic [6,7]. However, heavy metals are integral to some formulations and are been used for centuries [8].

Heavy metals are toxic, but their oxides are usually not. Food and Drug Administration has approved arsenic trioxide to be used in acute promyelocytic leukemia (APL) [9]. There are some reports published on the harmful effects of Indian system of medicine. Actually the metallic preparationss can be toxic or harmful to humans only if they are not prepared in the correct manner [10]. The preparations are then prescribed with certain accompaniments, e.g., ginger or cumin water, tulsi extract, etc. that have been shown to protect against unwanted toxicity due to varied reasons, [11,12] including high proportions of trace elements and synergistic or protective effects due to buffering between various constituents.

Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds. Oxides of arsenic recently approved by FDA is a popular therapeutic agent for various types of cancer and has been demonstrated to have a high therapeutic effect in APL. Present study aimed at comparing the change in elemental nature of unpurified (T1) and purified (T2 & T3) thalagam using atomic absorption spectrometry (AAS) methods of analysis.

2. Materials and Methods

2.1. Atomic Absorption Spectrometry (AAS) [13]

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.

2.1.1. 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 HNO3.

2.1.2. Standard reparation

As & Hg- 100 ppm sample in 1mol/L HCl Cd & Pb- 100 ppm sample in 1mol/L HNO₃

2.2.Test for Acid and Basic radicals [14]

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 were treated with sodium nitrate	Formation of Yellow	Presence of
solution and then treated with 2ml of cobalt nitrate in 30% of	colour precipitate	Potassium
glacial acetic acid.		
Test for Calcium: Sample were treated with acetic acid and	No Yellow precipitate	Presence of
potassiumchromate solution were added		Calcium
Test for Magnesium:Sample were treated with few drops of	Formation of Blue	Presence of
Magnason reagentwas added in drops.	colour precipitate	Magnesium
Test for Ammonium: Sample were treated with few ml of	Appearance of Brown	Presence of
Nessler's reagent.	colour	Ammonium
Test for Sodium:Sample were treated with HCl and made it	Appearance of intense	Presence of
into paste. And introduced into the blue flame of Bunsen	Yellow colour	Sodium
burner.		
Test for Iron (Ferrous): Sample were treated with conc.	Appearance of Blood	Presence of
HNO ₃ and ammonium thiocyanate were added.	red colour	Ferrous iron
Test for Zinc:Sample were treated with potassiumferro cyanide	Formation of White	Presence of
solution was added.	colour precipitate	Zinc
Test for Aluminium:Sample were treated with sodium	White precipitate	Presence of
hydroxide drops were added to it.	obtained	Aluminium
Test for Lead:Sample were treated with 2ml of potassium	Formation of yellow	Presence of
iodide solution	colour precipitate	Lead
Test for Copper:Sample were treated with dilute hydrochloric	Black precipitate	Presence of
acid was added and then hydrogen sulphide gas is passed		Copper
through the solution.		
Test for Mercury:Sample were treated With 2ml of sodium	Formation of Yellow	Presence of
hydroxide solution.	precipitate	Mercury
Test for Arsenic: Sample were treated with 2ml of sodium	Formation of brownish	Presence of
hydroxide solution.	red precipitate	Arsenic

2.2.1. Test for Acidic Radical

Procedure	Observation	Inference
Test for Sulphate: 2 ml of the extract was taken in clean,	Formation of white	Presence of
dry test tube and 5 % barium chloride solution was added	precipitate	Sulphate
to it.		
Test for Chloride: The extract was taken in a test tube	Formation of White	Presence of
and then treated with Silver nitrate solution.	precipitate	Chloride
Test for Phosphate: The extract was taken in a test tube	Formation of Yellow	Presence of
and treated with ammonium molybdate and conc. HNO _{3.}	precipitate	Phosphate
Test for Carbonate: The substance was taken in a clean	Formation of	Presence of
dry test tube and then treated with Conc. HCl.	Effervescence	Carbonate
Test for fluoride &oxalate:Sample were added with	Formation of cloudy	Presence of Fluoride
2ml of dil.acetic acid, 2ml calcium chloride solution and	appearance	& Oxalate
then heated.		
Test For Nitrate: 1gm of the samplewas heated with	Characteristic changes	Presence of Nitrate
copper turnings and concentrated H2SO4 and observed		
the test tube vertically down.		

3. Results

3.1. Result Analysis of heavy metal analysis by AAS technique

Heavy metal analysis of T2and T3 shows that presence of heavy metals such as Lead with concentration 332,271 mg/kg and Cadmium (T2) with concentration 8 mg/kg and Arsenic with concentration 3.8, 3, 5mg/kg (T2,T3). Where is the result further show that the heavy metal such as lead Mercury(T1,T2 and T3) is found below the detection limit and Cadmium (T3). Heavy metal analysis of T2 shows that presence of heavy metals such as cadmium with concentration 0.001 mg/kg and mercury with concentration 143.18 mg/kg. Whereas the result further shows that the heavy metal such as lead and Arsenic are found below the detection limit. As shown in Table 1.

S.no	Name of the Element	T1 mg/Kg	T2 mg/Kg	T3 mg/Kg
1.	Lead	586	332	271
2.	Cadmium	71	8	BLQ
3.	Mercury	BLQ	BLQ	BLQ
4.	Arsenic	4.2	3.8	3.5

Table 1:Heavy metal analysis of unpurified and purified by AAS technique

BDL: Below Limit of Quantification

3.2. Result of Biochemical Analysis – Basic radical

Results of the present investigation on test of basic radical reveals that both Unpurified Thalagam (T1) and Purified Thalagam (T2, T3) contain ferrousiron, Potassium, Calcium, Arsenic, Mercury and Lead. At the same time Calcium contains only in after purification (T2). As shown in Table 2.

Table 2: Result Analysis of Test for Basic radicals

S.no	Procedures	T1	T2	Т3
1.	Test for Ammonium	+	+	+
2.	Test for Sodium	-	-	-
3.	Test for Magnesium	+	+	+
4.	Test for Aluminium	+	+	+
5.	Test for Potassium	-	-	-
6.	Test for Calcium	-	+	-
7.	Test for Ferrous iron	+	+	+
8.	Test for Copper	-	-	-
9.	Test for Zinc	-	-	-
10.	Test for Arsenic	+	+	+
11.	Test for Mercury	+	+	+
12.	Test for Lead	+	+	+

"+" present, "-"absent.

3.3. Result of Biochemical Analysis – Acid radical

Results of the present investigation on test of acid radical shows the sample T1, T2 and T3 contain Chloride and Sulphate. Other acidic radicals were absent. Unpurified (T1) and Purified Thalagam(T2) Contains Flouride& Oxalate. As shown in Table 3.

S.no	Procedures	T1	T2	T3
1.	Test for Sulphate	+	+	+
2.	Test for Chloride	+	+	+
3.	Test for Phosphate	-	-	-
4.	Test for Flouride&Oxalate	+	-	+
5.	Test for Nitrate	-	-	-
"⊥ "Present " "absent				

Table 3: Result Analysis of Test for Acid radicals

"Present, "-"absent

4. Discussion

AYUSH medicine in India is too sporadic and dispersed to facilitate an understanding of AYUSH care utilization [15]. It may be noted that much of the earlier evidence on use of traditional medical services comes from small area studies [16] and there are only a few studies based on a sample large enough to generate any evidence [17]. Clearly, there is a need to undertake more systematic analysis to examine regional. AYUSH care utilization across socioeconomic and demographic groups [18]. Also, in a country like India which is home to many traditional medicine systems, it is also essential to understand these patterns in conjunction with allopathic medicine.

All metals are toxic at higher concentrations [19]. Excessive levels can be damaging to the organism. Other heavy metals such as mercury, plutonium, and lead are toxic metals that have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of animals can cause serious illness. Certain elements that are normally toxic are for certain organisms or under certain conditions, beneficial. Examples include vanadium, tungsten, and even cadmium [20-22].

The usage of arsenicals in Indian system of medicine has a very long history of treating various diseases including gonorrhea, epilepsy, syphilis, asthma, psoriasis, chronic fever, cancer, tuberculosis and other respiratory diseases. Biochemical analysis for the basic radicals reveals that both Unpurified Thalagam (T1) and Purified Thalagam (T2, T3) contain ferrous iron, Potassium, Calcium, Arsenic, Mercury and Lead. At the same time Calcium contains only in after purification (T2). Acid radical analysis reveals that sample T1, T2 and T3 contain Chloride and Sulphate. Other acidic radicals were absent. Unpurified (T1) and Purified Thalagam(T2) Contains Flouride& Oxalate.

Highly sensitive spectroscopic techniques such as flame absorption, graphite furnace and atomic absorption spectrometry's are mainly applied for elemental analysis in various samples. Such techniques require aqueous samples. Thus, solid samples need to be regularly converted into solutions using an appropriate dissolution method [23]. Acid digestion methods are generally used for the dissolution of herbal product samples prior to elemental analysis. AAS analysis of the present investigation have clearly shows that the sample has T1, T2 and T3 has no traces of heavy metal Mercury. Further the results show the presence of cadmium in sample T2 at the concentration of 71 ppm and in sample T3 at 8 ppm level. The level of cadmium is much higher than the allowed recommended limit of 0.3 ppm in both of these samples. Similarly, the allowable limit of lead as per AYUSH guideline is 10 in ppm whereas results of the present analysis show the presence of Lead, in T1 at 586 ppm, in sample T2 at 322 ppm and in sample T3 with 271 ppm level.

5. Conclusion

Traditional, alternative and complementary systems of medicines account for a major part of the healthcare being provided worldwide. However, little attention has been paid to understand the current scenario in which dynamism in the choice of purification and detoxification process. It was concluded from the results of the present investigation that the siddha purification process had significantly reduced the level of arsenic and also notable level of calcium observed exclusively in purified form.

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