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Determination of essential and potentially toxic elements by inductively Coupled Plasma-Optical Emission Spectrometry and in vitro antioxidant evaluation of Shatavaryadi Churna: An Ayurvedic Formulation

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Abstract:
The present study was undertaken to determine the concentrations of twelve elements and in vitro antioxidant activity in two formulations of Shatavaryadi churna and their ingredients. Concentrations of various elements were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). Antioxidant potential of the *Shatavaryadi churna* was studied using different *in vitro* free radical model i.e. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) Marketed Shatavaryadi churna formulation has shows presence of cadmium, whereas the levels of Cr and Pb are distinctly lower in marketed formulation. The high levels of Ca, Mg, and Fe are present in all the ingredients and formulations of Shatavaryadi churna. Methanolic extract of *Mucuna pruriens* Linn. seed shows significantly higher antioxidant potential with IC_{50} value 7.713µg/ml, as determined by DPPH radical scavenging activity and methanolic extract of *Chlorophytum tuberosum* Baker bulb shows least antioxidant potential amongst all ingredients and formulations of Shatavaryadi Churna. This study indicates the presence of essential and potentially toxic elements are within the limit and formulation can be used on regular basis without any harmful effect. The data obtained in DPPH radical scavenging activity suggest a possible use of Shatavaryadi churna as a natural antioxidant agent.

Keywords: Shatavaryadi churna, Potentially toxic elements, Antioxidant, DPPH, ICP-OES

Introduction

Ayurvedic medicine originated in India several thousand years ago [1, 2, 3]; it is extensively used nowadays in this country and is becoming increasingly popular in western nations. Ayurvedic formulations are easily available from ethnic markets, medical practitioners, health food stores, and the Internet[4, 5]. Generally, Ayurvedic practice involves the use of medications that typically contain herbs, metals, minerals and other materials [2, 3, 6]. Ayurvedic practitioners usually make up their own medicines, but several companies manufacture and sell such formulations for the Indian market and/or other countries. Shatavaryadi churna is the composition of *Asparagus racemosus* Willd. Tubers -1part, *Tribulus terrestris* Linn. Fruits -1part, *Mucuna pruriens* (L.) DC seeds -1part, *Withania somnifera* Dunal. roots -1part and *Chlorophytum tuberosum* Baker bulbs -1part[7]. Dargan (2008) et al.[8] reported the risks of heavy metal poisoning associated with the use of Ayurvedic medicines. Several literature reports have also demonstrated lead poisoning from these formulations[9, 10, 11, 12, 13]. The effects of elements like Cd, Hg and Pb on humans are well known; these elements have no known biological function in the human body and are simply

tolerated at low levels, but become toxic above certain concentrations. Other elements, such as Cr, Cu, Fe, Mn, Zn and surprisingly As, are essential to human life at adequate levels, but they can have negative effects if their concentrations exceed certain threshold limits [14]. Hence it is very interesting to determine the element content in traditional Ayurvedic medicines, taking into account their role as nutrients and/or toxins. In this study we assessed the levels of twelve elements (Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Pb, Ni, Ti, and V) in a Shatavaryadi churna and its ingredients. Concentrations were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES) after sample mineralization in a microwave oven and the technique had the advantage of being multi-elementary; hence it provides the concentrations of the analytes of interest in a single run.

It is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between formation and neutralization of free radicals[15]. Recently, much attention has been directed toward the development of "Ethno medicine" that posses strong antioxidant properties and beneficially less toxicity[16].

We examined in vitro antioxidant activity of Shatavarydi churna formulations (i.e. In house formulation and Marketed formulation) and its ingredients; and compared the estimated daily intake of each element with reference values (Table 2), considering maximum tolerable intake levels or recommended nutrient amounts issued by internationally recognized organizations.

Materials and methods

Plant Materials

The plant materials of Shatavaryadi churna were collected from the different sources and authenticated by Dr. E. Roshini Nayar, Principal Scientist, National Herbarium of Cultivated Plants, National Bureau of Plant Genetic Resources, Indian Council of Agricultural Research, Pusa Campus, New Delhi (India) i.e. Shatavari *(Asparagus racemosus* Willd.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-7/); Goksura *(Tribulus terrestris* Linn.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/2011-9/); Atmagupta *(Mucuna pruriens* (L.) DC) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-6/); Ashwagandha *(Withania somnifera* Dunal.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGHR/2011-10/) and Safed *Musli (Chlorophytum tuberosum* Baker.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGHR/2011-8/).

Parts of the ingredients were crushed and powdered using grinder and passed through sieve number#85. In-house Shatavaryadi churna was prepared from these powders by mixing them in one part for each ingredient and named as IH. Marketed Shatavaryadi churna was also procured from local market and named as M.

Chemicals and instrumentation

1, 1-diphenyl-2-picryl-hydrazyl and ascorbic acid were purchased from Sigma-Aldrich Pvt. Ltd.; methanol, concentrated Nitric acid, concentrated hydrogen peroxide and concentrated hydrochloric acid were purchased from Rankem RFCL Limited. Weighing balance (Mettler Toledo AB265-S), UV-Visible

Spectrophotometer (Shimadzu/UV-1700), Multiwave 3000 SOLV (Anton Paar) and Optical 2100DV inductively coupled plasma optical emission spectrometry (Perkin Elmer) were used for weighing, spectrophotometric analysis, digestion and elemental analysis respectively.

Elemental Analysis

0.5 gm of the powdered sample was digested in Multiwave 3000 SOLV 3000 SOLV at 1400 watt for 3 hours in the solvent system of concentrated Nitric acid, concentrated hydrogen peroxide and concentrated hydrochloric acid in the ratio of 4:2:1; diluted to 100 ml and filtered. The heavy metals present in the sample were estimated quantitatively with the help of instrument ICP-OES. Calculations of the elements were done in mg/kg. (Table 1)

In vitro Antioxidant activity

Preparation of extracts: Powdered samples were macerated in methanol for 72 hours, with occasional shaking. Macerate was decanted and filtered through whatman filter paper 1. The methanol extract was concentrated *in vacuo* and kept in a vacuum desiccator for complete removal of solvent. DPPH scavenging activity was measured by spectrophotometric method.

Preparation of reference standard solution: 1ml of different concentrations of stock solution of ascorbic acid (50 µg/ml dissolved in methanol) i.e. 0.8, 1.6, 2.4, 3.2, 4.0, 4.8, 5.6, 6.4, 7.2 & 8.0 µg/ml; 2 ml of DPPH (100 µM) solution were taken and finally make up the volume up to 5.0 ml with methanol.

Preparation of sample solution and dilutions: 10 mg of extract was dissolved in 10 ml of methanol to make stock solution and the series of dilutions 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 & 100.0 µg/ml for *Asparagus racemosus* Willd. tuber, *Tribulus terrestris* Linn. fruit, *Withania somnifera* Dunal. root, *Chlorophytum tuberosum* Baker bulb, *In-house*(IH) formulation of Shatavaryadi churna and Marketed formulation of Shatavaryadi churna; 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 9.0 & 10.0 µg/ml for *Mucuna pruriens* Linn. seed were prepared from stock solution (methanolic extract).

DPPH assay: The antioxidant activity of methanolic extract of all samples were determined by using a method based on the reduction of methanolic solution of colored-free radical 1,1di phenyl-1-2picryl hydrazyl (DPPH). The radical scavenging activity of tested sample was expressed as an inhibition percentage. Ascorbic acid was used as reference standard. In 5.0 ml volumetric flasks added 2.0 ml of DPPH solution, 1.0 ml of final dilutions of different concentrations range prepared from Methanolic extract of sample stock solutions and made up the volume to 5.0 ml with methanol. In same way prepared the control dilutions of DPPH, replacing 1.0 ml of prepared dilutions (the drug solution under investigation) with methanol. The absorbance of all the dilutions was taken after 30 minutes at wavelength (λmax) 517nm using methanol as blank.

Statistical Analysis: The percentage inhibition was calculated using the formula: % inhibition=(Ac-As/A^0)x100. Where Ac = Absorbance of control and As = Absorbance of sample. IC_{50} value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC_{50} value was calculated by using the equation of line (Papuc *et al,* 2008). Results of antioxidant data of ascorbic acid and methanolic extract of *Asparagus racemosus* Willd. tuber, *Tribulus terrestris* Linn. fruit, *Withania somnifera* Dunal. root, *Chlorophytum tuberosum* Baker bulb, *In-house*(IH) formulation of Shatavaryadi churna, Marketed formulation of

Shatavaryadi churna and *Mucuna pruriens* Linn. seed are given in Tables 3, 4, 5 and 6. IC_{50} values were also calculated for all the samples and presented in Figures (1-8)

Results

Elemental analysis

Element concentrations in the investigated samples are reported in Table 1. In formulation; IH has a remarkably high concentration of cadmium, whereas the levels of Cr and Pb are distinctly lower in formulation M. The high levels of Ca, Mg, and Fe are present in all the ingredients and formulations of Shatavaryadi churna.

Daily intake and reference values: The daily intake of each investigated element upon consumption of Ayurvedic medicines was calculated taking into account the posology reported in the product packages, when present, or indications from the literature. Minimum and maximum amounts ingested daily are reported in Table 2.

Table 1: Amount of different elements in various samples of Shatavaryadi churna and its ingredients

S. No.	Name of Element	Amount of Elements (mg/kg)						
		AR	TT	MP	WS	CT	IH	M
1	Ca	2946.412	32316.251	2768.866	16607.568	3302.619	11838.739	14537.392
2	Cd	Nd	0.169	nd	Nd	nd	nd	0.959
3	Co	Nd	0.337	nd	Nd	0.188	0.228	1.198
4	Cr	4.166	8.429	4.557	4.541	3.769	8.908	5.273
5	Cu	19.504	20.566	30.077	9.514	15.640	25.354	19.415
6	Fe	170.044	318.442	163.143	231.784	175.617	303.563	570.470
7	Mg	1048.854	4519.555	1629.603	1545.730	1363.294	2581.087	3815.916
8	Mn	23.480	65.577	39.920	20.973	25.815	39.059	49.377
9	Ni	1.704	0.506	5.104	2.162	1.319	2.513	2.157
10	Pb	10.225	10.789	9.661	13.838	9.610	20.329	15.580
11	Ti	3.408	5.732	0.547	2.162	1.884	5.939	12.464
12	V	1.136	nd	0.182	1.297	nd	0.457	nd

Asparagus racemosus (AR), *Tribulus terrestris* (TT), *Mucuna pruriens* (MP), *Withania somnifera* (WS), *Chlorophytum tuberosum* (CT), *In-house* formulation (IH), Marketed formulation (M), not detected (nd)

Table 2: Estimation of elements daily intake upon consumption of Shatavaryadi churna formulation (mg/day, minimum–maximum)

S. No.	Element	IH formulation	M formulation	Reference	
				Dosage	Parameter
1	Ca	34.5218-69.0435	42.3910-84.7821	1000.00	RLNI (SINU)
2	Cd	0.0000-0.0000	0.0028-0.0056	0.06	PTDI (JECFA)
3	Co	0.0007-0.0013	0.0035-0.0070	0.05-1.00	RLNI (ATSDR)
4	Cr	0.0260-0.0520	0.0154-0.0308	0.05-0.20	RLNI (SINU)
5	Cu	0.0739-0.1479	0.0566-0.1132	1.20	RLNI (SINU)
6	Fe	0.8852-1.7704	1.6635-3.3270	10.00	RLNI (SINU)
7	Mg	7.5264-15.0529	11.1272-22.2544	150.00-500.00	RLNI (SINU)
8	Mn	0.1139-0.2278	0.1440-0.2880	1.00-10.00	RLNI (SINU)
9	Ni	0.0073-0.0147	0.0063-0.0126	3.00-7.00	PSL (ATSDR)
10	Pb	0.0593-0.1186	0.0454-0.0909	0.21	PTDI (JECFA)
11	Ti	0.0173-0.0346	0.0363-0.0727	0.30 µg	PSL (IPCS)
12	V	0.0013-0.0027	0.0000-0.0000	0.01-0.02	PSL (EFSA)

PTDI: Provisional Tolerable Daily Intake, RLNI: Recommended Level of Nutrient Intake, PSL: Prescribed Safety Limit, EFSA: European Food Safety Authority[17, 18], JEFCA: Joint FAO/WHO Expert Committee on Food Additive[17], SINU: Italian Society for Human Nutrition[17], EVM: Expert group on Vitamins and Minerals. ATSDR: Agency for Toxic Substances and Disease Registry [19, 20], IPCS: International Programme on Chemical Safety[21].

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Antioxidant activity:

Methanolic extract of all the ingredients and formulations of Shatavaryadi churna were evaluated for antioxidant properties by using DPPH method. Results of antioxidant activity were compared with ascorbic acid, a standard antioxidant. As observed in Figures (1-8), DPPH screening has shown the IC_{50} values of 1.258µg/ml, 89.149µg/ml, 74.180µg/ml, 7.713µg/ml, 78.936µg/ml, 98.761µg/ml, 68.882µg/ml and 70.300µg/ml for ascorbic acid, methanolic extract of *Asparagus racemosus* Willd. tuber, *Tribulus terrestris* Linn. fruit, *Mucuna pruriens* Linn. seed, *Withania somnifera* Dunal. root, *Chlorophytum tuberosum* Baker bulb, *In-house* formulation of Shatavaryadi churna and *Marketed* formulation of Shatavaryadi churna respectively. Methanolic extract of *Mucuna pruriens* Linn. seed shows potent antioxidant activity, both the Shatavvaryadi churna formulations shows the moderate antioxidant activity, while remaining samples shows less antioxidant activity as compared to IC_{50} value of ascorbic acid.

Table 3: Values of absorbance and % Inhibition with increase in concentration of methanolic solution of Ascorbic Acid (Standard Antioxidant)

Conc. (µg/ml)	Absorbance	% Inhibition
0.80	0.227±0.003	41.26%
1.60	0.186±0.003	52.02%
2.40	0.177±0.000	54.26%
3.20	0.136±0.004	64.94%
4.00	0.097±0.006	74.85%
4.80	0.068±0.009	82.52%
5.60	0.056±0.000	85.53%
6.40	0.045±0.000	88.37%
7.20	0.044±0.013	88.72%
8.00	0.040±0.022	89.79%
IC_{50} (µg/ml)	1.258	

Table 4: Values of absorbance and % Inhibition with increase in concentration of methanolic solution

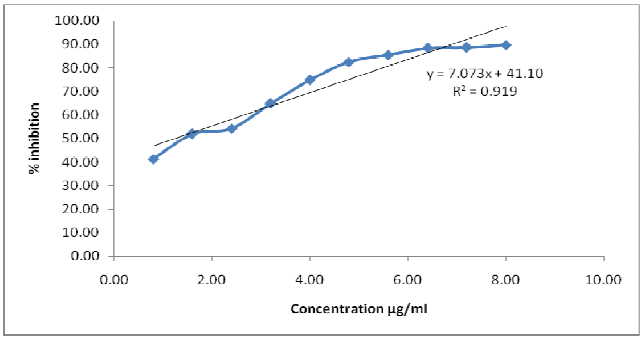
Conc. (µg/ml)	*A. racemosus*		*T. terrestris*		*W. somnifera*		*C. tuberosum*	
	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition	Absorbance	% Inhibition
10.00	0.349±0.002	1.32%	0.340±0.019	10.67%	0.371±0.002	3.30%	0.364±0.002	3.36%
20.00	0.342±0.002	3.30%	0.312±0.000	18.11%	0.350±0.002	8.77%	0.349±0.018	7.34%
30.00	0.327±0.009	7.53%	0.292±0.000	23.36%	0.325±0.002	15.45%	0.334±0.003	11.41%
40.00	0.306±0.002	13.47%	0.276±0.002	27.65%	0.272±0.002	29.08%	0.320±0.002	15.21%
50.00	0.298±0.002	15.91%	0.227±0.003	40.33%	0.248±0.002	35.50%	0.296±0.002	21.40%
60.00	0.222±0.005	37.29%	0.218±0.003	42.87%	0.222±0.005	42.19%	0.272±0.002	27.76%
70.00	0.206±0.003	41.71%	0.206±0.003	46.02%	0.206±0.003	46.27%	0.255±0.002	32.27%
80.00	0.190±0.003	46.23%	0.194±0.003	48.99%	0.182±0.000	52.60%	0.221±0.000	41.38%
90.00	0.178±0.003	49.81%	0.163±0.004	57.13%	0.168±0.003	56.16%	0.197±0.003	47.66%
100.00	0.160±0.004	54.71%	0.114±0.005	69.99%	0.165±0.000	57.03%	0.187±0.000	50.40%
IC_{50} (µg/ml)	89.149		74.180		78.936		98.761	

Table 5: Values of absorbance and % Inhibition with increase in concentration of methanolic solution of *Mucuna pruriens* Linn. Seed.

Conc. (µg/ml)	Absorbance	% Inhibition
1.00	0.321±0.002	17.82%
2.00	0.301±0.002	23.10%
3.00	0.287±0.002	26.51%
4.00	0.282±0.002	27.79%
5.00	0.257±0.000	34.27%
6.00	0.196±0.003	49.79%
7.00	0.163±0.004	58.23%
8.00	0.133±0.000	65.98%
9.00	0.101±0.000	74.17%
10.00	0.091±0.000	76.73%
IC_{50} (µg/ml)	7.713	

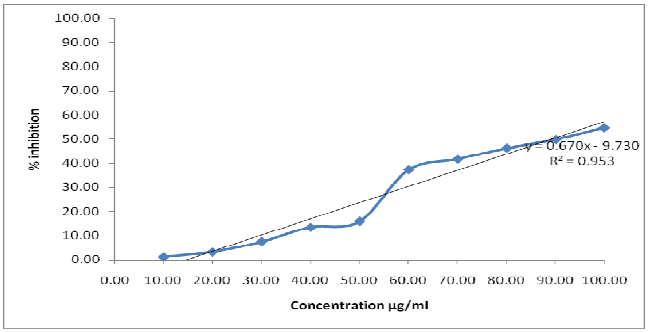
Table 6: Values of absorbance and % Inhibition with increase in concentration of methanolic solution.

Conc. (µg/ml)	IH house formulation		Marketed formulation	
	Absorbance	% Inhibition	Absorbance	% Inhibition
10.00	0.311±0.002	13.76%	0.296±0.002	7.40%
20.00	0.307±0.002	15.05%	0.291±0.000	9.06%
30.00	0.290±0.000	19.67%	0.285±0.002	11.04%
40.00	0.241±0.002	33.15%	0.261±0.000	18.44%
50.00	0.221±0.000	38.78%	0.200±0.003	37.40%
60.00	0.208±0.003	42.47%	0.193±0.003	39.79%
70.00	0.187±0.000	48.20%	0.158±0.004	50.52%
80.00	0.148±0.043	59.10%	0.128±0.000	60.00%
90.00	0.135±0.004	62.51%	0.108±0.005	66.35%
100.00	0.095±0.006	73.78%	0.080±0.007	74.90%
IC_{50} (µg/ml)	68.882		70.300	



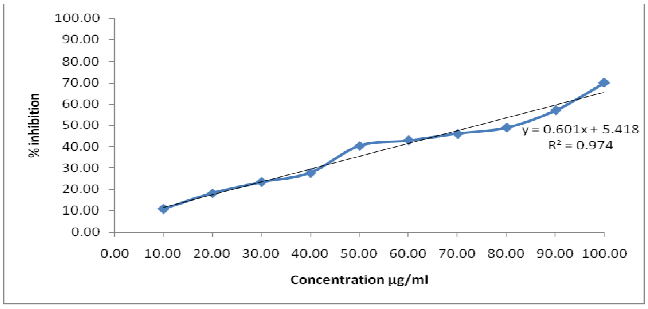
IC_{50} (µg/ml) 1.258

Figure 1: Graphical representation of antioxidant activity of ascorbic acid.



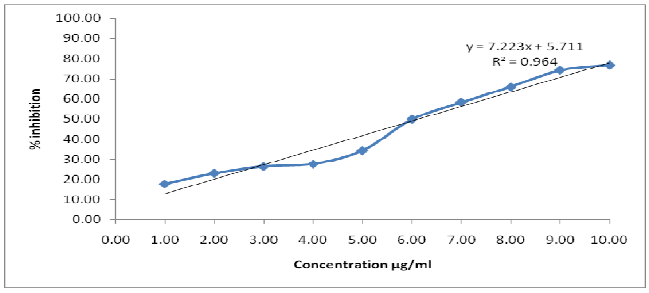
IC_{50} (µg/ml) 89.149

Figure 2: Graphical representation of antioxidant activity of *Asparagus racemosus* Willd. Tuber.



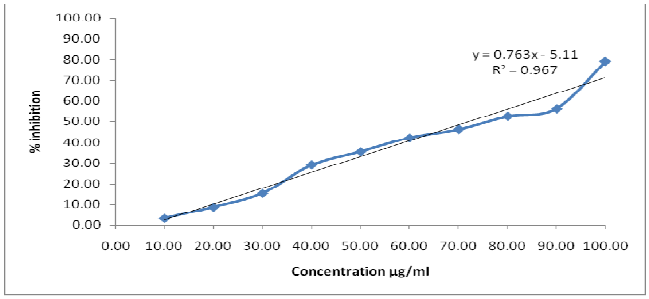
IC_{50} (µg/ml) 74.180

Figure 3: Graphical representation of antioxidant activity of *Tribulus terrestris* Linn. Fruit.



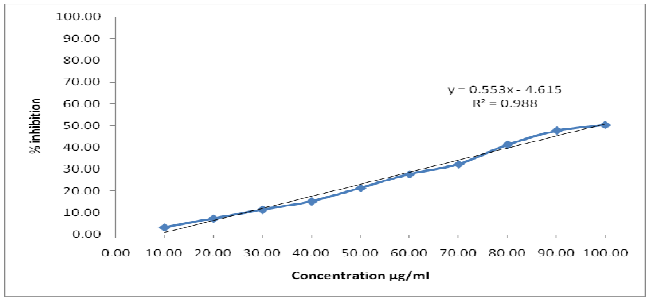
IC_{50} (µg/ml) 7.713

Figure 4: Graphical representation of antioxidant activity of *Mucuna pruriens* Linn. Seed.



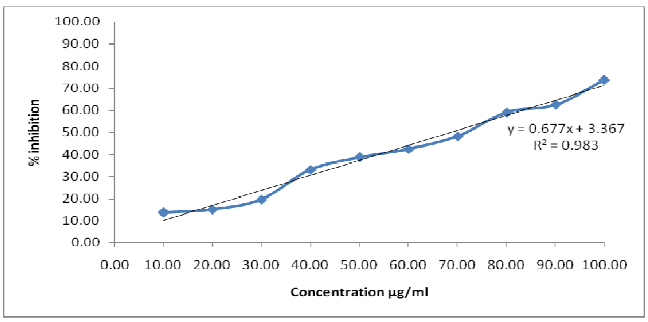
IC_{50} (µg/ml) 78.936

Figure 5: Graphical representation of antioxidant activity of *Withania somnifera* Dunal. root.



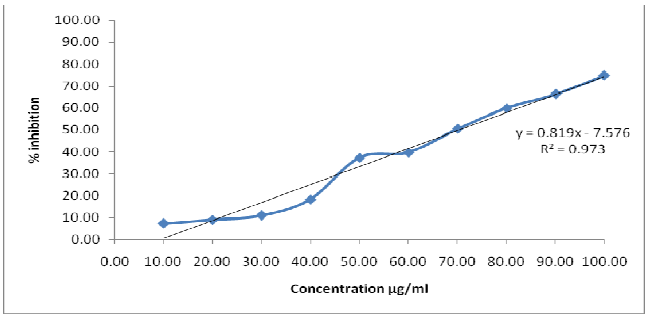
IC_{50} (µg/ml) 98.761

Figure 6: Graphical representation of antioxidant activity of *Chlorophytum tuberosum* Baker bulb.



IC_{50} (µg/ml) 68.882

Figure 7: Graphical representation of antioxidant activity of *In-house* formulation of Shatavaryadi churna



IC_{50} (µg/ml) 70.300

Figure 8: Graphical representation of antioxidant activity of Marketed formulation of Shatavaryadi churna

Discussion

The analysis of ingredients and samples of Shatavaryadi churna showed that they were passed the maximum tolerable limit of the elements. The comparison between the calculated daily intake of each element upon use of the investigated products and reference values showed that the all the elements were present within the limit for both the products.

The present study shows the antioxidant potential of methanolic extract of all the ingredients and formulations of Shatavaryadi churna. All the samples shows antioxidant potential and maximum potential was given by *Mucuna pruriens* Linn. seed; the presence of *Mucuna pruriens* Linn. seed in the formulation may be the major factor for moderate antioxidant potential of Shatavaryadi churna formulation. In house formulations show slightly more antioxidant potential as compared to Marketed Shatavaryadi churna formulation.

Conflict of interest statement

We declare that we have no conflict of statement.

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