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 show 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 IC50 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. 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. Recently, much attention has been directed toward the development of “Ethno medicine” that posses strong antioxidant properties and beneficially less toxicity.

We examined in vitro antioxidant activity of Shatavaryadi 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 chuna 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/NBPGR/2011-7/); Goksura (Tribulus terrestris Linn.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGR/2011-9/); Atmagupta (Mucuna pruriens (L.) DC) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGR/2011-6/); Ashwagandha (Withania somnifera Dunal.) Purchased from local traders of New Delhi (India) (Identification Voucher No.: NHCP/NBPGR/2011-10/) and Safed Musli (Chlorophytum tuberosum Baker.) Collected from the Narayanpur district of Chhatisgarh (India) (Identification Voucher No.: NHCP/NBPGR/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 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. 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/Ao)x100. Where Ac = Absorbance of control and As = Absorbance of sample. IC50 value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC50 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 Wild. 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\textsubscript{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

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of Element</th>
<th>AR</th>
<th>TT</th>
<th>MP</th>
<th>WS</th>
<th>CT</th>
<th>IH</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ca</td>
<td>2946.412</td>
<td>32316.251</td>
<td>2768.866</td>
<td>16607.568</td>
<td>3302.619</td>
<td>11838.739</td>
<td>14537.392</td>
</tr>
<tr>
<td>2</td>
<td>Cd</td>
<td>Nd</td>
<td>0.169</td>
<td>nd</td>
<td>Nd</td>
<td>nd</td>
<td>0.959</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Co</td>
<td>Nd</td>
<td>0.337</td>
<td>nd</td>
<td>Nd</td>
<td>0.188</td>
<td>0.228</td>
<td>1.198</td>
</tr>
<tr>
<td>4</td>
<td>Cr</td>
<td>4.166</td>
<td>8.429</td>
<td>4.557</td>
<td>4.541</td>
<td>3.769</td>
<td>8.908</td>
<td>5.273</td>
</tr>
<tr>
<td>6</td>
<td>Fe</td>
<td>170.044</td>
<td>318.442</td>
<td>163.143</td>
<td>231.784</td>
<td>175.617</td>
<td>303.563</td>
<td>570.470</td>
</tr>
<tr>
<td>7</td>
<td>Mg</td>
<td>1048.854</td>
<td>4519.555</td>
<td>1629.603</td>
<td>1545.730</td>
<td>1363.294</td>
<td>2581.087</td>
<td>3815.916</td>
</tr>
<tr>
<td>9</td>
<td>Ni</td>
<td>1.704</td>
<td>0.506</td>
<td>5.104</td>
<td>2.162</td>
<td>1.319</td>
<td>2.513</td>
<td>2.157</td>
</tr>
<tr>
<td>11</td>
<td>Ti</td>
<td>3.408</td>
<td>5.732</td>
<td>0.547</td>
<td>2.162</td>
<td>1.884</td>
<td>5.939</td>
<td>12.464</td>
</tr>
<tr>
<td>12</td>
<td>V</td>
<td>1.136</td>
<td>nd</td>
<td>0.182</td>
<td>1.297</td>
<td>nd</td>
<td>0.457</td>
<td>nd</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Element</th>
<th>IH formulation</th>
<th>M formulation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dosage</td>
<td>Parameter</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Ca</td>
<td>34.5218-69.0435</td>
<td>42.3910-84.7821</td>
<td>1000.00</td>
</tr>
<tr>
<td>2</td>
<td>Cd</td>
<td>0.0000-0.0000</td>
<td>0.0028-0.0056</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>Co</td>
<td>0.0007-0.0013</td>
<td>0.0035-0.0070</td>
<td>0.05-1.00</td>
</tr>
<tr>
<td>4</td>
<td>Cr</td>
<td>0.0260-0.0320</td>
<td>0.0154-0.0308</td>
<td>0.05-0.20</td>
</tr>
<tr>
<td>5</td>
<td>Cu</td>
<td>0.0739-0.1479</td>
<td>0.0566-0.1132</td>
<td>1.20</td>
</tr>
<tr>
<td>6</td>
<td>Fe</td>
<td>0.8852-1.7704</td>
<td>1.6635-3.3270</td>
<td>10.00</td>
</tr>
<tr>
<td>7</td>
<td>Mg</td>
<td>7.5264-15.0529</td>
<td>11.1272-22.2544</td>
<td>150.00-500.00</td>
</tr>
<tr>
<td>8</td>
<td>Mn</td>
<td>0.1139-0.2278</td>
<td>0.1440-0.2880</td>
<td>1.00-10.00</td>
</tr>
<tr>
<td>9</td>
<td>Ni</td>
<td>0.0073-0.0147</td>
<td>0.0063-0.0126</td>
<td>3.00-7.00</td>
</tr>
<tr>
<td>10</td>
<td>Pb</td>
<td>0.0593-0.1186</td>
<td>0.0454-0.0909</td>
<td>0.21</td>
</tr>
<tr>
<td>11</td>
<td>Ti</td>
<td>0.0173-0.0346</td>
<td>0.0363-0.0727</td>
<td>0.30 µg</td>
</tr>
<tr>
<td>12</td>
<td>V</td>
<td>0.0013-0.0027</td>
<td>0.0000-0.0000</td>
<td>0.01-0.02</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.80</td>
<td>0.227±0.003</td>
<td>41.26%</td>
<td>89.149</td>
<td>74.180</td>
<td>68.882</td>
</tr>
<tr>
<td>1.60</td>
<td>0.186±0.003</td>
<td>52.02%</td>
<td>74.180</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>2.40</td>
<td>0.177±0.000</td>
<td>54.26%</td>
<td>78.936</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>3.20</td>
<td>0.136±0.004</td>
<td>64.94%</td>
<td>98.761</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>4.00</td>
<td>0.097±0.006</td>
<td>74.85%</td>
<td>68.882</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>4.80</td>
<td>0.068±0.009</td>
<td>82.52%</td>
<td>70.300</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>5.60</td>
<td>0.056±0.000</td>
<td>85.53%</td>
<td>89.149</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>6.40</td>
<td>0.045±0.000</td>
<td>88.37%</td>
<td>74.180</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>7.20</td>
<td>0.044±0.013</td>
<td>88.72%</td>
<td>78.936</td>
<td>7.713</td>
<td>68.882</td>
</tr>
<tr>
<td>8.00</td>
<td>0.040±0.022</td>
<td>89.79%</td>
<td>98.761</td>
<td>7.713</td>
<td>68.882</td>
</tr>
</tbody>
</table>

IC$_{50}$ (µg/ml) 1.258

Table 4: Values of absorbance and % Inhibition with increase in concentration of methanolic solution of Shatavaryadi churna formulations.

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

IC$_{50}$ (µg/ml) 89.149

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

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>Absorbance</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00</td>
<td>0.321±0.002</td>
<td>17.82%</td>
</tr>
<tr>
<td>2.00</td>
<td>0.301±0.002</td>
<td>23.10%</td>
</tr>
<tr>
<td>3.00</td>
<td>0.287±0.002</td>
<td>26.51%</td>
</tr>
<tr>
<td>4.00</td>
<td>0.282±0.002</td>
<td>27.79%</td>
</tr>
<tr>
<td>5.00</td>
<td>0.257±0.000</td>
<td>34.27%</td>
</tr>
<tr>
<td>6.00</td>
<td>0.196±0.003</td>
<td>49.79%</td>
</tr>
<tr>
<td>7.00</td>
<td>0.163±0.004</td>
<td>58.23%</td>
</tr>
<tr>
<td>8.00</td>
<td>0.133±0.000</td>
<td>65.98%</td>
</tr>
<tr>
<td>9.00</td>
<td>0.101±0.000</td>
<td>74.17%</td>
</tr>
<tr>
<td>10.00</td>
<td>0.091±0.000</td>
<td>76.73%</td>
</tr>
</tbody>
</table>

IC$_{50}$ (µg/ml) 7.713
Table 6: Values of absorbance and % Inhibition with increase in concentration of methanolic solution.

<table>
<thead>
<tr>
<th>Conc. (µg/ml)</th>
<th>IH house formulation</th>
<th>Marketed formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absorbance</td>
<td>% Inhibition</td>
</tr>
<tr>
<td>10.00</td>
<td>0.311±0.002</td>
<td>13.76%</td>
</tr>
<tr>
<td>20.00</td>
<td>0.307±0.002</td>
<td>15.05%</td>
</tr>
<tr>
<td>30.00</td>
<td>0.290±0.000</td>
<td>19.67%</td>
</tr>
<tr>
<td>40.00</td>
<td>0.241±0.002</td>
<td>33.15%</td>
</tr>
<tr>
<td>50.00</td>
<td>0.221±0.000</td>
<td>38.78%</td>
</tr>
<tr>
<td>60.00</td>
<td>0.208±0.003</td>
<td>42.47%</td>
</tr>
<tr>
<td>70.00</td>
<td>0.187±0.000</td>
<td>48.20%</td>
</tr>
<tr>
<td>80.00</td>
<td>0.148±0.043</td>
<td>59.10%</td>
</tr>
<tr>
<td>90.00</td>
<td>0.135±0.004</td>
<td>62.51%</td>
</tr>
<tr>
<td>100.00</td>
<td>0.095±0.006</td>
<td>73.78%</td>
</tr>
</tbody>
</table>

IC$_{50}$ (µg/ml) 68.882

IC$_{50}$ (µg/ml) 70.300

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

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

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

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

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

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


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