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Evaluation of Nutritive, Non-Nutritive Contents and Antioxidant Activity of Polyherbal Formulations

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Abstract

Efficacy and safety of polyherbal formulations was based on non-nutritive and nutritive contents. Present work deals with estimation of non-nutritive phytochemicals such as total phenolic and flavonoid compounds, antioxidant activity and nutritive elements and heavy metals content of polyherbal formulations of *Sitopaladi* and *Talisadichurna*. Results have revealed that total phenolic and flavonoid concentrations of *Sitopaladichurna* extracts were rich in SC1 followed by SC3, SC2 and SC4, and in case of *Talisadichurna* extracts, the order was TC3 followed by TC2, TC1 and TC4. This specifies that substantial positive linear correlation occurred between total phenolic and flavonoid contents. The herbal formulations disclosed higher scavenging ability with DPPH, $ABTS^{\cdot+}$ and reducing power than standard ascorbic acid. The extracts displayed better antioxidant activity which also contains higher phenolic contents, indicating that there was a correlation. Findings unveil that herbal formulations were found to be rich source of macro and micronutrients. Furthermore, heavy metals such as Arsenic, Chromium, Cadmium and Lead were not detected at noticeable concentrations in herbal medicines and Mercury concentrations were below the detection limit (< 0.3 mg/kg).

Keywords: Antioxidant activity, Macronutrients, Micronutrients, *Talisadichurna*, *Sitopaladichurna*

Introduction

Several years ago the traditional medicine in India was originated as *Ayurveda*. It has gained worldwide attention owing to no side effects, inexpensive, efficacy and remedy for several chronic diseases rather than synthetic drugs. Combination of various plant materials were used to treat different infectious and non-infectious diseases and disorders (1).

Unlike synthetic drugs, herbal medicines quality was highly dependent on chemical composition of plant materials used for formulations. It is difficult to maintain the constant chemical composition of plant materials used in the preparation of herbal products. Thus, the quality of herbal finished products may vary from one manufacturer to other and also batch to batch of the same manufacturer (2)(3). Almost all the manufacturers of herbal medicines have mentioned the major ingredients only on the herbal finished products. Unfortunately, manufacturers did not report the effectiveness and safety of herbal medicines. Therefore, many researchers all over the world studied the essential nutrients and heavy metalcontents in herbal medicines (4)(5).

The present study deals with the estimation of total phenolic and flavonoid contents, antioxidant activities, nutrients and heavy metal contents in *Sitopaladichurna* (*Annona reticulate*, *Saccharumofficinarum*, *Bambusaarundianacea*,

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Piper longum, *Elettariacardamomum*, *Syzygiumaromaticum* and *Cinnamomum-zeylanicum*) and *Talisadichurna*(*Abieswebbiana*, *Piper nigrum*, *Zingiberofficinale*, *Piper longum*, *Bambusaarundianacea*, *Cinnamomum-zeylanicum*, *Elettariacardamomum*, *Saccharum-officinarum* and *Syzygiumaromaticum*). These are widely used polyherbal medicines for the treatment of respiratory diseases (6) such as cough, cold, tuberculosis and sinusitis (7). In addition, *Sitopaladichurna*(SC) was prescribed for treating burning sensation in palm, feet, low digestion power, loss of sensation in tongue and bleeding from nose (8), *Talisadichurna* (TC) was used for the treatment of anaemia and spleen diseases (9).

In view of public health FAO/WHO (10) and other organizations (11), prescribed maximum permissible limit for heavy metals in raw herbal materials and herbal finished products (12). Concentrations of the essential elements and heavy metals in plant samples alters the constituents of the plant material used in the preparation of herbal medicines (13). In most of the cases, the medicinal properties of herbal medicine may be due to its phytochemical constituents mainly, phenolic and flavonoid contents. Numerous studies revealed that antioxidant activity of medicinal plant**s** is linked to its total phenolic content (TPC) and total flavonoid content (TFC) (14)(15). Estimation of antioxidant activity in laboratory formulation of SC from their respective ingredients has been reported in the literature (8). However, simultaneous estimation of antioxidant activity, macro and micronutrients and heavy metals of commercially available SC and TC samples have not been reported. In general, phytochemical contents of herbal medicines differ from one manufacturer to other, based on the plant material used (3)(5).

The current research was considered to evaluate the total phenolic and flavonoid contents, antioxidant activity, essential nutrients and heavy metal contents of SC and TC of popular manufacturers in India and to find out the correlations between phenolic and flavonoid contents, and antioxidant activity.

Materials and Methods

Samples: *Sitopaladichurna* samples (SC1, SC2, SC3 and SC4)and *Talisadichurna*samples (TC1, TC2, TC3 and TC4) of four different manufacturers were purchased from local markets in Vijayawada, Andhra Pradesh, India.

Extract preparation: Ten grams of dried powder of selected polyherbal formulations were macerated individually in 100 mL of water for 16 h at room temperature. The contents were stirred occasionally and then filtered through Whatman filter paper and the residue was again extracted twice and filtered. The combined filtrate was concentrated on a rotary evaporator under reduced pressure to obtain the crude extract. The extract was then dried in vacuum freeze dryer) for 24 h, weighed and stored at 4^{o}C for further experimental use.

Determination of total phenolic content (TPC): The total phenolic content of the extracts were determined by modified spectrophotometric method using Folin-Ciocalteu reagent (16) with slight modifications. TPC was estimated using a standard curve prepared with gallic acid (1-10 ppm) and was expressed as milligrams of gallic acid equivalent per gram of sample extract. 1 mL of extract of each sample was mixed with 0.5 mL of Folin-Ciocalteu reagent (50% v/v) and the mixture was allowed to react for 5min, 2 mL sodium carbonate (20% w/v) solution added and finally diluted to 25 mL with distilled water. After 30 min incubation, the absorbance was measured at 760 nm using UV-Visible spectrophotometer against distilled water as blank.

Determination of total flavonoid content (TFC): Total flavonoid content of sample extracts were assayed as per standard procedure using spectrophotometer (17). TFC was determined using standard rutin curve (5-50 ppm) and expressed as milligrams of rutin equivalent per gram of the sample extract. 2 mL of each sample

extract was mixed with 1 mL of aluminium chloride (10% w/v) and sodium nitrate (5% w/v) solutions each allowed to stand for 10 min at room temperature. Then 2mL of 1M sodium hydroxide was added and made up to 10 mL with distilled water and allowed to stand for 20 min. The absorbance at 510 nm was measured against distilled water as blankusing UV-Visible spectrophotometer.

Evaluation of antioxidant activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) method: The antioxidant activity of herbal extracts were determined by the spectrophotometric method as measurement of radical scavenging using DPPH free radical (18). 2 mL of various concentrations of SC and TC sample extracts (150, 300 450, 500 and 750 μg/mL) were mixed with 2 mL of 0.08 mM DPPH in methanol and allowed to stand at room temperature for 1 h. Absorbance was recorded at 517 nm. Ascorbic acid (AA) was used as a standard. Mixture of 2 mL DPPH solution and 2 mL of methanol was taken as a control. The scavenging ability of extracts were expressed as inhibition percentage (%) using the following equation:

$$\% \text{ inhibition} = (1\text{-}Abs_{sample}/Abs_{control}) \times 100$$

Here, Abs_{sample} is the absorbance of the extract solution at a particular concentration and $Abs_{control}$ is the absorbance of control without extract.

2,2 È-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method : This assay was based on measurement of antioxidant ability of herbal extracts by $ABTS^{\cdot+}$ reduction method (19). $ABTS^{\cdot+}$ solution was prepared by dissolving ABTS and potassium persulfate in distilled water to final concentration of 7 mM and 2.45mM. The mixture was stored in dark room at room temperature for 16 h before use. The $ABTS^{\cdot+}$solution was diluted with 0.1 M potassium phosphate buffer (*p*H=7.4) to an absorbance of (0.7±0.02) at 734 nm. 10 μL of herbal extracts of varying concentrations (150, 300, 450, 600 and 750 μg/mL) were added to 2.99 mL of $ABTS^{\cdot+}$ solution and the measurements were taken immediately at 734 nm after 10 min. Ascorbic acid was used as a standard. $ABTS^{\cdot+}$ solution was taken as a control. The percentage inhibition of samples were determined using following equation:

$$\% \text{ inhibition} = (1\text{-}Abs_{Sample}/Abs_{Control}) \times 100$$

Reducing power: The reducing power of the SC and TC extracts were measured according to a well-known method (19) with some modifications. 1 mL of each sample extract at various concentrations (150, 300, 450, 600 and 750 μg/mL) were mixed with 2.5 mL of 0.2 M sodium phosphate buffer solution (*p*H=6.6) and 2.5 mL of potassium ferricyanide solution (1%w/v). The reaction mixture was incubated for 20 min at 50 ^{o}C and then 2.5 mL of trichloro acetic acid solution (10%w/v) was added. The resulting mixture was centrifuged for 10 min at 3000 rpm. To 3 mL of the supernatant solution, 3 mL of distilled water and 1 mL of ferric chloride solution (0.1%w/v) was added and thoroughly mixed. The absorbance at 700 nm was recorded against phosphate buffer blank.

Determination of elements: Each sample of SC and TC (0.1g) was taken into 100 mL volumetric flask. The samples were digested separately with a mixture of concentrated nitric acid and hydrogen peroxide (9:1 v/v) at room temperature for overnight. The contents were heated on hot plate at 100-150 ^{o}C. The process was continued until a clear solution obtained. The solutions were filtered using filter paper and diluted to 100 mL with distilled water and stored for further use (8).

Acid digested samples of SC and TC were studied for various essential elements and heavy metals using Inductive Coupled Plasma-Optical Emission Spectrometer (I-CAP-6500, Thermo scientific company-UK). Instrumental details and operating conditions as described in(4). Independent analysis of blank solution spiked with

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standard metal at a lower level of concentrations were used to determine the limits of detection (LOD) of elements. The concentrations of metal in the sample solution was determined from the standard calibration curves.

Statistical analysis: All determinations were carried out in triplicates. Experimental data was subjected to ANOVA test and statistical significance was obtained at *ñ*< 0.05. Finally, the data was expressed as mean ± SD.

Results and Discussion

Total phenolic and flavonoid contents: TPC and TFC present in SC and TC samples were quantified as gallic acid equivalent and rutin equivalent were shown in (Figs. 1 and 2). There was noteworthy differences in TPC and TFC of different manufacturers of SC and TC. In this assay, SC1 and TC3 Sample extracts had exceptionally high amounts of phenolic and flavonoid constituents, and the concentrations were 2-3 times greater than the TPC and TFC values of the remaining samples investigated. In case of SC extracts, both TPC (4.75-43.23 GAE/g) and TFC (22.24-82.57 RE/g) assays followed the same order of SC1 > SC3 > SC2 >SC4, whereas in TC extracts, both TPC (23.99-46.36 GAE/g) and TFC (4.36-46.07 RE/g) analyses displayed the similar order of TC3 > TC2 > TC1 >TC4. It was observed that there was a correlation between TPC and TFC.

Antioxidant activity: In general, more than one method was essential to estimate antioxidant activity because the plant material exhibits antioxidant activity through different mechanisms having variety of chemical compounds. Antioxidant substances readily quench DPPH free radical by donating hydrogen radicals or electrons (17). The extent of the decrease in absorbance indicates the strength of the scavenging activity of the samples. (Figs. 3 and 4) represents the DPPH radical scavenging activity of various SC and TC extracts. All the extracts exhibited different levels of DPPH radical scavenging activity. Among all the samples evaluated, TC3 (95.57%) and SC1 (94.34%) samples displayed highest scavenging activity at 750 *µ*g/mL when compared to the positive control AA (90.45%).

$ABTS^{\cdot+}$ is often used as an indicator to identify the hydrogen donating activity of samples, which is an important mechanism of phenolic antioxidant activity (20). The sample extracts of the

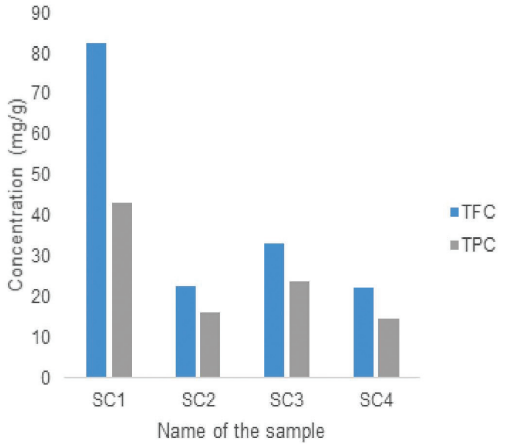

Fig 1. Total phenolic and total flavonoid contents of four different brands of *Sitopaladichurna* extracts expressed as mg GAE/g of extract and mg RE/g of extract (n=3).

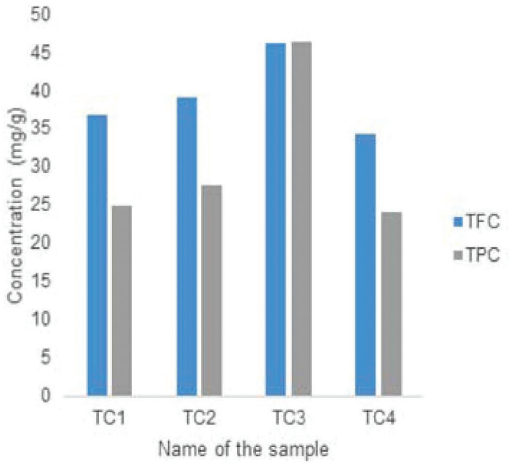

Fig 2. Total phenolic and total flavonoid contents of four different brands of *Talisadichurna* extracts expressed as mg of GAE/ g of extract and mg of RE/ g of extract (n=3).

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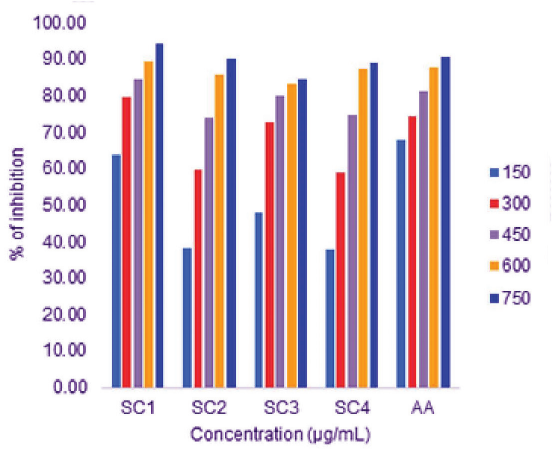

Fig 3. DPPH scavenging activity of *Sitopaladichurna* samples and standard ascorbic acid.

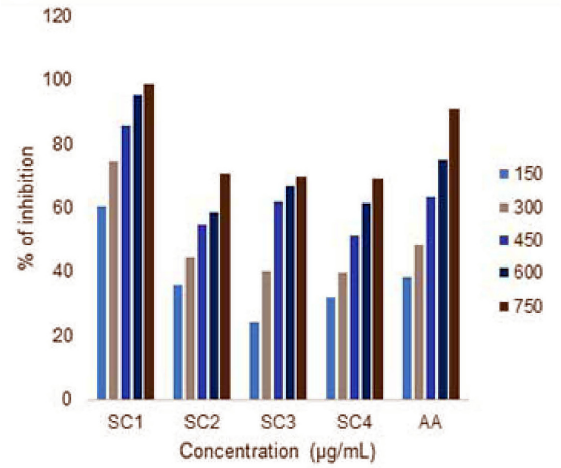

Fig 5. ABTS$^{\cdot+}$scavenging activity of *Sitopaladichurna* samples and standard ascorbic acid.

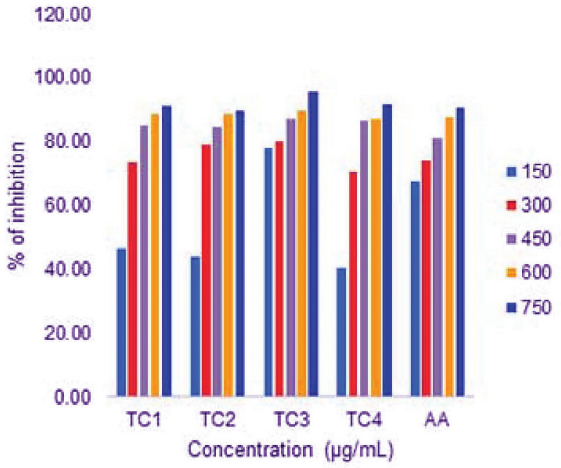

Fig 4. DPPH scavenging activity of *Talisadichurna* samples and standard ascorbic acid

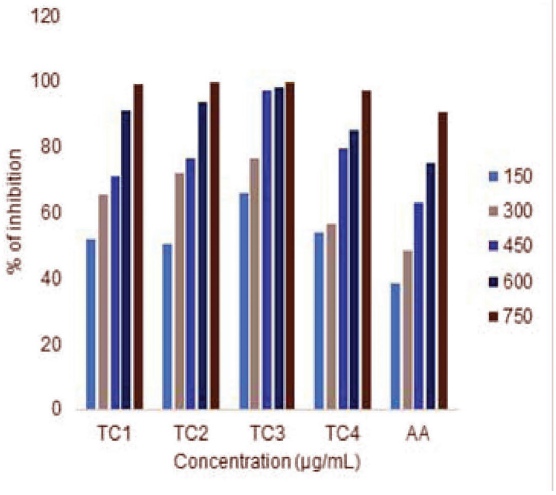

Fig 6. ABTS$^{\cdot+}$scavenging activity of *Talisadichurna* samples and standard ascorbic acid.

scavenging activities with increasing concentrations of the extracts were shown in (Figs. 5 and 6). The ABTS$^{\cdot+}$ scavenging activity of the extracts TC2 (99.57%), TC1 (99.28%), TC3 (99.28%) and SC1 (98.42%) exhibited highest activities at a concentration of 750 µg/mL, when compared to the standard ascorbic acid (90.71%). There are good antioxidants which reduces oxidative stress by acting as metal chelators. The ability of the extract to reduce Fe^{+3} complex to Fe^{+2} was illustrated in the (Figs. 7 and 8). SC1 and TC3 samples demonstrated the maximum intensity and reducing power, which suggests that there was a strong relationship between the

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phenolic and the reducing power ability. The ability of these extracts to scavenge DPPH radical, $ABTS^{\cdot+}$ and reduces Fe^{+3} to Fe^{+2} indicating the possibility of electrons donors which readily quench free radicals and convert them to more stable products and terminate radical chain reactions.

Nutrients and heavy metal contents: In total, fifteen elements were analysed for both SC and TC samples. The essential elements encompass macronutrients (Ca, K, Mg, Na and P) and micronutrients (Co, Fe, Mn, Ni, and Zn). The results of macro and micronutrients analyses were given in (Tables 1 and 2). The studied samples were found to be rich in source of macronutrients. The macronutrients (Ca, K, P and Mg) were predominant in SC2 sample when compared to other samples. In investigated samples, micronutrients (Fe and Mn) concentrations were within the permissible limits, whereas, the concentrations of Nickel in all assessed samples were slightly exceeded than the endorsed limit (1.63 mg/kg) excluding SC4 and TC1. Zinc was observed at higher concentration in all samples except SC1 and TC4 than prescribed limit (27.4 mg/kg) by ASEAN (11). Except SC3 sample, the concentration of Cobalt in all the samples were found within the permissible range (0.14-0.48 mg/kg).

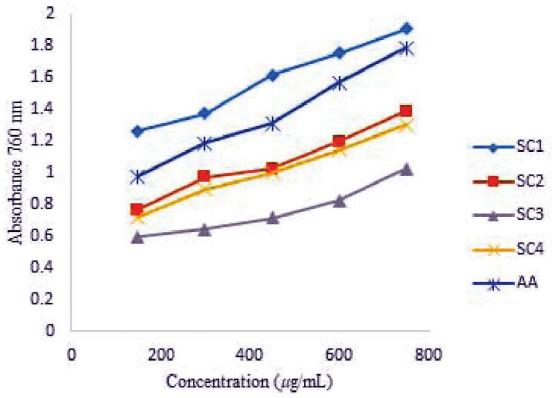

Fig 7. Reducing power ability of *Sitopaladichurna* samples and standard ascorbic acid.

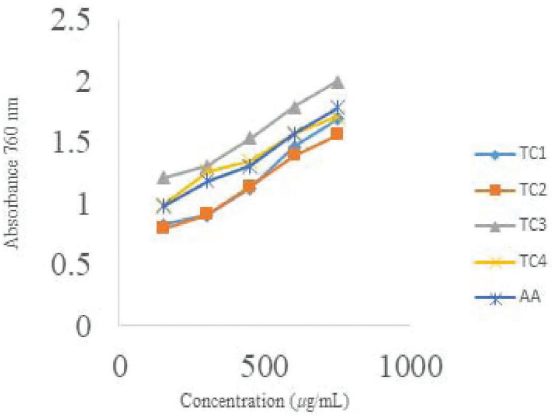

Fig 8. Reducing power ability of *Talisadichurna* samples and standard ascorbic acid.

The concentrations of As, Cd, Cr, Hg and Pb in herbal medicines were associated with the human health complications. Therefore, the content of these heavy metals in the evaluated samples were compared with the maximum permissible limits prescribed by WHO for herbal medicines (10)(12). The profile for As, Cd, Cr, Hg and Pb concentrations in SC and TC samples were presented in (Table 3). Higher concentrations of Arsenic noticed in SC3 (4.57±0.23 mg/kg) and TC4 (4.37±0.93 mg/kg) samples which were lower than the maximum permissible limit of 5 mg/kg. The evaluated samples contain Chromium concentration within the admissible level (2.0 mg/kg), except TC3 sample (2.66±0.46 mg/kg), whereas, Cadmium concentration was within the maximum allowable limit (0.3 mg/kg). Mercury was not observed in all the examined samples and this may be due to the presence of Mercury at lower concentration than its detection limit (0.3 mg/kg). Concentration of Lead was detected at higher level in TC1 (8.32±0.69 mg/kg) sample and this was lower than maximum allowable limit (10 mg/kg). Heavy metal concentrations in the sample TC2 were found to be less than the detection limits.. These findings revealed that the concentrations of the essential elements and

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heavy metals vary considerably from sample to sample due to the changes in environmental conditions and cultivation process, which accordingly alters the constituents of the plant material used in the preparation of herbal medicines (13)(21)(22).

Conclusion

This study has revealed that two samples (SC1 and TC3) showed remarkably higher concentrations of TPC and TFC. TC3 and SC1 displayed higher DPPH inhibition percentage when compared to the standard ascorbic acid. Our findings shown that there was linear relationship between phenolic compounds and antioxidant activity. All the samples were found to be rich in source of macronutrients which plays a vital role in human beings metabolism. The nutrients content differ significantly among the analysed samples and heavy metals concentration of sample TC2 were less than the detection limits. The heavy metal contents of the studied samples were found to be negligible. Current research could shed some light of eliminating low quality products

Table 1: Macro-nutrients concentration (mg/kg) of *Sitopaladi churna* and *Talisadi churna* samples (mean), n=5

Sample	Ca	K	Mg	Na	P
SC1	1327.73±5.09	2043.83±31.86	425.71±1.65	898.82±22.25	246.94±3.81
SC2	2180.32±11.62	7785.12±44.28	900.22±2.99	1246.58±34.39	652.59±4.22
SC3	1112.16±10.27	1348.65±26.85	401.35±1.81	3733.59±84.67	159.07±1.59
SC4	1508.73±14.26	3551.97±31.69	458.96±1.78	392.07±14.94	378.69±3.55
TC1	809.85±11.31	2446.46±27.20	387.56±1.90	341.02±14.11	283.63±1.87
TC2	699.92±6.21	943.2±18.64	390.25±1.93	160.01±6.95	155.29±1.44
TC3	842.82±7.37	1061.6±22.09	373.62±2.06	1043.44±28.98	281.92±1.78
TC4	1188.07±17.40	1969.24±32.361	367.87±1.01	292.26±13.67	267.59±2.18
LOD	1.73	0.45	1.18	4.62	2.86

LOD: Limits of detection

Table 2: Macro-nutrients concentration (mg/kg) of *Sitopaladi churna* and *Talisadi churna* samples (mean), n=3

Sample	Co	Fe	Mn	Ni	Zn
SC1	0.41±0.07	355.25±25.35	32.11±0.63	5.67±0.18	19.13±1.65
SC2	0.39±0.04	452.74±33.80	88.80±2.70	3.16±0.23	46.43±2.05
SC3	0.53±0.11	225.86±15.03	32.81±0.71	2.12±0.09	36.83±1.74
SC4	0.25±0.06	484.90±15.50	37.03±3.91	1.33±0.12	39.33±2.77
TC1	0.21±0.02	228.44±10.48	28.83±0.89	1.06±0.05	30.55±2.30
TC2	0.16±0.03	135.49±9.67	12.31±0.20	4.41±0.11	36.41±1.32
TC3	0.40±0.09	198.44±11.32	44.06±3.25	2.01±0.12	29.55±2.24
TC4	0.37±0.05	151.64±9.01	52.96±2.61	5.41±0.22	21.29±1.45
WHO	0.14-0.48	261-1239	44.6-339	1.63	27.4
LOD	0.11	0.64	0.03	0.27	0.76

LOD: Limits of detection

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Table 3: Heavy metals concentration (mg/kg) of *Sitopaladi churna* and *Talisadi churna* samples (mean), n=3

Sample	As	Cr	Cd	Hg	Pb
SC1	3.15±0.31	≤1.5	0.11±0.02	≤0.3	4.59±0.88
SC2	≤1.1	≤1.5	0.13±0.08	≤0.3	5.94±0.95
SC3	4.57±0.23	≤1.5	≤0.09	≤0.3	≤1.5
SC4	≤1.1	1.79±0.96	0.26±0.11	≤0.3	2.53±0.62
TC1	1.92±0.61	1.83±0.23	0.14±0.02	≤0.3	8.32±0.69
TC2	≤1.1	≤1.5	≤0.09	≤0.3	≤1.5
TC3	≤1.1	2.66±0.46	≤0.09	≤0.3	6.91±0.16
TC4	4.37±0.93	1.62±0.20	≤0.09	≤0.3	1.63±0.07
WHO	5	2.0	0.3	0.1	10
LOD	1.1	1.5	0.09	0.3	1.5

LOD: Limits of detection

in the market. Results propose that it is recommended for the manufacturers to maintain heavy metals content lower than the permissible limits for consumer's health and safety.

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Conflict of Interest

The authors claim that there is no conflict of interest.

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