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Comparative analysis of nutritive and non-nutritive content of *Shatavari* – A commercial Indian herbal medicine

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Shatavari is a plant based product used for treating infertility and improving vitality and immunity in women. The present study was aimed to compare the total phenolic, flavonoid, steroidal saponin and antioxidant activity of *Shatavari* aqueous and methanol extracts which were prepared by agitation extraction (AE) and ultra-sonication extraction (UE) and also to determine the variation of nutrients and heavy metals content of marketed *Shatavari* herbal products in India. Antioxidant activity of *Shatavari* aqueous and methanol extracts were assessed against DPPH and ABTS spectrophotopmetrically. Compared to the aqueous, methanol extracts significantly increased the yields of total phenolic and flavonoid contents, and steroidal saponin. Antioxidant activity was effective by using ABTS compared to DPPH with low IC_{50} values. Among all the samples SH1 has revealed highest total phenolic and flavonoid contents and antioxidant activities. No significant differences were noticed between the two extraction techniques. Studies unveil that samples SH1 and SH2 were found to be rich source of nutrients. Heavy metal contents in the analysed samples were likely to be of negligible concern. In all herbal samples mean concentration of heavy metals were within the limits stated by WHO guidelines for human consumption.

Keywords: Ultra-sonication, anti-oxidant activity, *Shatavari*, heavy metals, nutrients.

Introduction

Now a days, marketing of Ayurvedic medicine has been tremendously increased through exports and online business to meet their demand global level. Traditional plant based medicine exert greater importance due to healing, rejuvenation and lack of side effects.

Most of the women across the world are suffering from infertility[1]. *Shatavari* is a famous Ayurvedic formulation for improving cellular vitality and immunity[2] and widely used for the treatment of stress related immune complaints and hormonal imbalances in women and promotes lactation[3]. *Shatavari* root powder or extract was highly effective in female reproductive disorders, avoiding premature birth and utilized as uterine tonic[4]. *Shatavari* has various nutritive, therapeutic and preventative properties contributed by its various chemical constituents. *Asparagus racemosus* is the major constituent of *Shatavari* products. Their identification was difficult, because roots are in similar shape of *Stemona* plant roots[5]. There is a chance that *Stemona* plant roots may be mistaken for *Asparagus racemosus*.

The activity of *Shatavari* is strongly swayed by both natural and human activities. To enhance its therapeutic activities manufacturers were adding various plant based products with different chemical composition and marketed with different commercial names[6]. The quality of herbal medicine was quantified by presence of phytochemicals, minerals and heavy metal content[7]. Concentrations of the essential elements and heavy metals in plant samples alters the chemi-

cal structure and composition of plant material used in the preparation of herbal medicines. Extraction efficiency was also essential for the preparation of herbal products to maximize health benefits. Therefore, the standardization of herbal formulations is mandatory. Moreover, a lot of survey has been found on phytochemical and therapeutic usage of *Shatavari*, but as per our knowledge no study has been conducted on total phenolic and flavonoid content, steroidal saponin, and antioxidant activity of aqueous and methanol extracts of *Shatavari* products prepared from agitation extraction (AE) and ultra-sonication extraction (UE), and there were no information of nutrients and heavy metal content of selected *Shatavari* products marketed in India[8,9].

Keeping above information in mind, the present study aimed to compare the total phenolic and flavonoid content, steroidal saponin, antioxidant activity of *Shatavari* aqueous and methanol extracts prepared by AE and UE techniques. Antioxidant activity was studied using DPPH and ABTS spectrophotometrically, and moreover, evaluation of minerals and heavy metal content of regularly prescribed and highly marketed *Shatavari* products in India.

Materials and methods

Samples:

Shatavari products SH1, SH2, SH3, SH4 and SH5 are commercially available herbal medicines purchased from local markets in Vijayawada, Andhra Pradesh, India.

Extract preparation:

Agitation extraction:

10 g of dried powder of selected poly-herbal formulations were extracted individually in 100 mL of water and methanol for 8 h at room temperature using shaker in 150 rpm speed and the residue was again extracted twice and centrifuged. The combined filtrate was concentrated on a rotary evaporator under reduced pressure at 40°C to obtain the crude extract. The extract was then dried in a vacuum freeze dryer (Martin Christ freeze dryer; Model: Gamma 2-16 LSC) for 24 h, weighed and stored at 4°C. 50 mg of the each extract was dissolved in respective solvents and diluted to 25 mL and used as stock solution for analysis.

Ultra-sonication extraction:

10 g of dried powder of samples were extracted with 100 mL of water and methanol individually by using electronic ultrasonic bath at room temperature for 8 h set to 35 kHz and filtered. The residue was re-extracted twice. The resultant filtrate was concentrated using rotary evaporator and freeze dried for 24 h. 50 mg of each extract dissolved in water and methanol solvents and diluted to 25 mL and stored for further use.

Determination of total phenolic content:

Total phenolic content (TPC) of the extracts were assayed by modified spectrophotometric method using Folin-Ciocalteu reagent[10–12]. TPC was estimated using a standard curve prepared with gallic acid and expressed as mg of gallic acid equivalent (GAE) per gram of the sample extract. 500 μL of each extract was mixed individually with 500 μL of Folin-Ciocalteu reagent (50% v/v) and the mixture was allowed to react for 5 min, 2000 μL of sodium carbonate (10% w/v) solution was added to the resultant mixture and finally diluted to 10 mL with distilled water. After 30 min of incubation, the absorbance was measured at 760 nm against distilled water as blank using UV-Visible spectrophotometer. The determination of total phenolic compounds in all the sample extracts were carried out in triplicate and the results were averaged.

Determination of total flavonoid content:

Total flavonoid content of sample extracts were determined using spectrophotometric method of earlier studies with slight changes[10,13]. The concentration of total flavonoid was assayed using a standard rutin curve and expressed as mg of rutin equivalent (RE) per gram of the sample extract. 1000 μL of extracts each mixed with 500 μL of aluminium chloride (10% w/v) and 500 μL of sodium nitrate (5% w/v) solutions allowed to stand for 10 min at room temperature. Then 2000 μL of 1 *M* sodium hydroxide was added. Finally the mixture was made up to 10 mL with distilled water. The absorbance was measured at 510 nm against distilled water as blank using UV-Visible spectrophotometer. Flavonoid contents in all the sample extracts were determined in triplicate and average results were considered.

Determination of total steroidal saponin:

Water and methanol extracts of *Shatavari* were washed with diethyl ether and re-extracted with n-butanol, finally washed with 5% aqueous sodium chloride. The remaining solution was evaporated and dried for constant weight to obtain crude saponin[14]. Further the extract was acid hydrolysed for 3 h at 90°C to deglycosylate and the resultant steroidal sapogenin was dissolved with ethyl acetate and made

up to 10 mL of which 2 mL of aliquot was added to one mL of 0.5% anisaldehyde in ethyl acetate (v/v) and one mL of 50% sulphuric acid in ethyl acetate (v/v). The resulting mixture was stirred and maintained at 60°C in a water bath for 20 min to develop chromophore and then allowed to cool at room temperature. The absorbance of the coloured solution was measured at 430 nm against ethyl acetate as blank using UV-Visible spectrophotometer[15]. The steroidal saponin concentration were calculated from standard curve of sarsasapogenin and expressed as mg sarsasapogenin equivalent (SE) per gram of crude extract.

Evaluation of antioxidant activity:

2,2-Diphenyl-1-picryl-hydrazyl method:

The radical scavenging activity of samples were determined by the spectrophotometric method[16,17] using ascorbic acid as standard. 2000 µL of 0.08 mM of DPPH in methanol was mixed with 2000 µL of different concentrations (200, 500, 1000, 1500 and 2000 µg/mL) of extracts and allowed to stand at room temperature for 1 h. Finally the absorbance of each sample was measured at 517 nm. Mixture of 2000 µL of DPPH solution and 2000 µL of methanol was taken as control. 50% Inhibitory concentration (IC 50%) was calculated by linear regression analysis. The results were compared with that of ascorbic acid, the standard antioxidant.

2,2-Azino-bis(3-ethylbenzothiozoline-6-sulfonic acid):

The antioxidant activity of sample extracts were evaluated by ABTS radical cation reduction method[17,18]. ABTS reagent with an absorbance of 0.700±0.02 at 734 nm was prepared by mixing 7 mM of ABTS, 2.45 mM of potassium persulfate and finally diluted with 0.1 *M* potassium phosphate buffer (pH 7.4). 10 µL of various concentrations (200, 500, 1000, 1500 and 2000 µg/mL) of extracts were added to 2990 µL of ABTS reagent and the mixture incubated for 30 min at room temperature. The absorbance was measured at 734 nm and the results were expressed in terms of IC_{50} values.

Determination of elements:

Each sample of *Shatavari* 100 mg were digested 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°C, until a clear solution obtained and filtered, finally diluted to 100 mL with distilled water and stored for further use. Essential elements and heavy metal content of acid digested *Shatavari* samples were determined using Inductive Coupled Plasma-Optical Emission Spectrometer (I-CAP-6500, Thermo scientific company-UK). Operating conditions as described[19,20]. By using standard calibration curve, the concentration of metals in the studied samples were determined. Blank solution spiked with standard metal at a lower concentrations were used to determine the limits of detection (LOD) of elements.

Statistical analysis:

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

Results and discussion

The total amount of phenolic and flavonoid contents present in *Shatavari* samples were quantified as gallic acid equivalent (GAE) and rutin equivalent (RE) were shown in Table 1. Methanol extracts had significantly higher amounts of total phenolics and flavonoids than the aqueous extracts. Both extraction techniques (AE and UE) were effective. There is no considerable variation were observed between the two extraction techniques. SH1 had exceptionally higher amounts of phenolic and flavonoid contents, whereas, SH3 sample

Table 1. Total phenolic and flavonoid contents of different brands of *Shatavari* sample extracts expressed as mg of GAE/g of extract and mg of RE/g of extract (n = 3)

Sample	Total phenolic contents				Total flavonoid contents			
	Aqueous (mg/g)		Methanol (mg/g)		Aqueous (mg/g)		Methanol (mg/g)	
	AE	UE	AE	UE	AE	UE	AE	UE
SH1	6.19±0.14	6.05±0.04	14.64±0.9	15.73±0.18	3.85±0.02	3.81±0.1	13.43±0.32	13.11±0.45
SH2	2.51±0.01	2.76±0.05	4.01±0.07	3.98±0.11	1.46±0.02	1.22±0.16	3.96±0.15	4.18±0.03
SH3	2.58±0.02	2.99±0.01	4.58±0.1	4.78±0.03	1.50±0.01	1.69±0.05	4.25±0.11	4.36±0.05
SH4	1.49±0.02	1.44±0.02	2.59±0.03	2.44±0.01	0.56±0.01	0.98±0.02	0.78±0.01	0.69±0.01
SH5	5.23±0.05	5.37±0.08	3.97±0.05	4.06±0.06	2.23±0.05	2.17±0.02	3.92±0.26	4.01±0.13

showed lower concentrations among the studied samples. The concentrations of total phenolic and flavonoid in the *Shatavari* methanol and aqueous extracts obtained by both techniques were noticeably higher than those previously reported[21,22] for plant raw material. According to the earlier results[11], the serial dilutions of *Shatavari* plant raw material showed higher phenolic and flavonoid contents. The phenolic content varied widely among the samples in methanol and aqueous extracts ranged from 15.73–2.44 mg/g of extract weight and 6.19–1.44 mg/g of extract weight whereas, flavonoid content ranged from 13.43–0.69 mg/g of extract weight and 3.85–0.56 mg/g of extract weight.

The steroidal saponin was determined by using sarasasapogenin standard curve. In the present study, we observed a considerable amount of steroidal saponin in the studied samples as shown in Table 2. Among all these samples, SH1 had revealed highest steroidal saponin content in both aqueous and methanol medium. SH4 had shown least steroidal saponin content. The order of steroidal saponin content of aqueous extracts obtained from both AE and UE techniques as SH1 > SH2 > SH5 > SH3 and SH4. In case of methanol extracts the order as SH1 > SH5 > SH3 > SH2 and SH4.

Table 2. Steroidal saponin concentration of aqueous and methanol extracts of *Shatavari* samples expressed as mg of SE/g of saponin extract (n = 3)

Sample	Steroidal saponin			
	Aqueous (mg/g)		Methanol (mg/g)	
	AE	UE	AE	UE
SH1	26.60±0.6	25.04±1.4	38.89±0.27	38.18±0.02
SH2	20.59±0.02	22.67±1.23	25.62±0.33	26.55±0.86
SH3	17.58±0.53	17.98±0.8	26.01±0.25	27.19±0.74
SH4	13.61±0.40	12.11±1.59	20.66±0.54	18.61±0.16
SH5	19.72±0.01	20.45±0.77	28.67±0.11	27.88±0.63

Antioxidant activity:

The sample extracts are very multifaceted mixture of many distinct compounds of different plant materials with various activities. Therefore, more than one method was essential to estimate antioxidant activity. The scavenging capacity of extracts against DPPH radical was evaluated by determining the decrease in absorbance by increasing the concentration. DPPH scavenging ability of methanol and aqueous extracts of commercial samples which were prepared by both techniques (AE and UE) were listed in Table 3. The methanol extracts had significantly shown lower IC_{50} values with higher activity than the aqueous extracts. Among the methanol extracts the highest antioxidant activity was demonstrated by SH1 followed by SH2, SH5, SH3 and SH4. While the aqueous extracts followed the order of SH1, SH2, SH3, SH5 and SH4 in both the techniques. Previous studies[12,23] reported comparatively low activity with high IC_{50} value in aqueous extracts. Former studies[11] of serial dilution of raw material had shown high antioxidant activity with low IC_{50} value. The ability of sample extracts to scavenge ABTS radical by donating active hydrogen and convert them into more stable products was listed in the Table 3. The methanol extracts are effective towards ABTS radical compared to aqueous extracts. The highest ABTS radical scavenging activity by SH1 in methanol and aqueous extracts. In contrast SH4 shown lowest scavenging ability in methanol and aqueous.

Essential elements and heavy metals:

A wide range of variation in the nutrients and heavy metals content of different commercially available *Shatavari* samples were noticed. The traditional use of samples due to the presence of phytochemicals and nutrients composition. The concentration of macronutrients calcium, potassium, magnesium, sodium and phosphorous were given in the Table

Table 3. The IC_{50} values of aqueous and methanol extracts of *Shatavari* samples in DPPH and ABTS assay

Sample	DPPH assay				ABTS assay			
	Aqueous (μg/mL)		Methanol (μg/mL)		Aqueous (μg/mL)		Methanol (μg/mL)	
	AE	UE	AE	UE	AE	UE	AE	UE
SH1	265.93±1.4	249.67±4.2	193.80±2.4	189.1±0.8	70.23±1.9	72.77±0.9	30.74±0..5	33.45±1.1
SH2	446.67±5.9	449.01±2.5	331.94±4.6	337.5±5.2	173.05±2.1	166.56±1.3	44.56±2.2	43.61±0.8
SH3	511.45±7.8	509.2±1.8	398.12±5.4	400.87±1.5	165.12±3.6	158.32±3.4	58.90±3.7	56.76±1.5
SH4	747.19±8.5	760.11±7.6	535.55±4.9	542.33±2.4	220.90±3.1	212.85±4.9	116.66±2.4	109.39±1
SH5	541.88±8.1	534.6±4.1	356.61±1.1	351.8±2.2	104.20±5.6	103.47±0.5	47.85±1.6	45.17±1.9

Table 4. Macro-nutrients concentration (mg/kg) of *Shatavari* samples (mean), n = 3

Sample	Calcium	Potassium	Magnesium	Sodium	Phosphorous
SH1	8011.50±1.31	9658.99±2.18	1774.29±2.66	2011.93±1.45	1679.45±0.67
SH2	10366.69±2.75	2676.22±5.30	5685.13±1.28	1702.96±0.45	418.06±0.79
SH3	1039.82±0.89	6184.47±1.74	709.06±0.65	396.18±0.49	2386.02±1.25
SH4	693.62±0.67	162.75±0.11	268.83±0.29	1372.74±0.93	190.02±0.95
SH5	1604.38±0.58	1760.65±0.30	510.33±0.13	1786.85±1.53	336.79±1.20
LOD	1.73	0.45	1.18	4.62	2.86

4. Calcium was essentially prime for bone health, the maximum concentration of calcium were noticed as 10366.69±2.75 mg/kg, 8011.50±1.31 mg/kg in SH2 and SH1 respectively. The minimum concentration was reported as 693.62±0.67 mg/kg in SH4. From the earlier reports[24] calcium content of *Shatavari* leafs and roots in the range of 961.0±0.6 to 2115.0±3.2 mg/kg lower than SH1 and SH2. All the samples exhibited higher potassium content except SH4 (162.75±0.11 mg/kg). Potassium was very useful for transmission of nerve impulses and building muscles. In the analysed samples magnesium was detected at high concentration in SH2 (5685.13±1.28 mg/kg) and low concentration in SH4 (268.83±0.29 mg/kg). The concentration of sodium was ranges from 396.18±0.49 mg/kg to 2011.93±1.45 mg/kg. The high concentration of phosphorous in SH3 (2386.02±1.25 mg/kg) and relatively low concentration in SH4 (190.02±0.95mg/kg). Phosphorous plays important structural role in nucleic acids and cell membranes.

The concentration of micronutrients (cobalt, iron, manganese, nickel and zinc) was given in Table 5. Cobalt was detected in SH1 and SH5 only at the concentration of 0.74±0.01 and 5.92±0.17 mg/kg respectively. Manganese was an essential cofactor for many enzymes. Iron was involved in oxygen transport. Both manganese and iron are the nutrients that exhibited the higher concentrations in SH1 as 53.49±0.63 mg/kg and 1981.03±1.81 mg/kg compared to other samples. Various enzymes synthesis was dependent on the concentration of zinc in the human and animal body. Lack of zinc can inhibit the growth and well-being[25]. SH4 showed high concentration of zinc 193.73±0.13 mg/kg than the concentration reported previously. Nickel is used for increasing iron absorption in the body. Nickel concentration ranges from 2.92±0.02 mg/kg to 14.49±0.80 mg/kg.

The concentration of arsenic, cadmium, chromium, mercury and lead in the samples are present in the Table 6. Arsenic consumed in lower dose for a long period cause vascular toxicity, respiratory, liver toxicity and hypopigmentation[25]. Higher concentrations of arsenic noticed in SH2 (1.98±0.01 mg/kg) and SH3 (1.84±0.02 mg/kg) samples which were lower than the maximum permissible limit of 5 mg/kg. Cadmium intake exert toxic effects on kidneys, the respiratory system and the skeletal system[26]. It has ability to substitute the other metal ions like Ca^{2+}, Zn^{2+} and Cu^{2+} leads to negative effect on enzymatic systems. The elevated levels of cadmium was noticed in SH1 of concentration 0.21±0.03 mg/kg whereas, cadmium concentration was within the maximum allowable limit (0.3 mg/kg). Chromium is an essential trace metal in carbohydrate metabolism and protein synthesis. When chromium concentration is higher than 2 mg/kg in herbal medicine, it may cause skin problems

Table 5. Micro-nutrients concentration (mg/kg) of *Shatavari* samples (mean), n = 3

Sample	Cobalt	Iron	Manganese	Nickel	Zinc
SH1	0.74±0.01	1981.03±1.81	53.49±0.63	14.49±0.80	169.33±0.89
SH2	ND	1180.03±3.67	41.08±0.24	7.62±0.24	146.03±0.28
SH3	ND	213.81±0.58	11.37±0.02	2.92±0.02	51.07±0.81
SH4	ND	832.44±0.32	11.69±0.01	13.60±0.13	121.95±0.19
SH5	5.920±0.17	1273.41±0.55	25.52±0.24	6.30±0.08	193.73±0.13
WHO	0.14–0.48	261–1239	44.6–339	1.63	–
LOD	0.10	0.63	0.03	0.26	0.75

Table 6. Heavy metals concentration (mg/kg) of *Shatavari* samples (mean), n = 3

Sample	Arsenic	Chromium	Cadmium	Mercury	Lead
SH1	≤ 1.1	ND	0.21±0.03	≤ 0.1	9.23±0.27
SH2	1.98±0.01	1.33±0.01	0.07±0.02	≤ 0.1	7.46±0.16
SH3	1.84±0.02	0.04±0.01	ND(0.05±	≤ 0.1	4.38±0.03
SH4	≤ 1.1	0.03±0.0	0.09±0.02	≤ 0.1	6.78±0.11
SH5	≤ 1.1	0.02±0.0	0.09±0.01	≤ 0.1	3.96±0.23
WHO	5	2.0	0.3	0.1	10
LOD	1.1	0.01	0.08	0.1	1.5

and damages liver, kidneys, circulatory nerve tissues and respiratory problems[27]. Chromium content was detected only in sample SH2 at level of 1.33±0.01 mg/kg. Lead has adverse effects on various body systems such as reproductive, digestive, cardiovascular and immunological systems[28,29]. Lead was present at concentration ranging from 3.96±0.23 to 9.23±0.27 mg/kg which were lower than the maximum recommended limit 10 mg/kg by WHO[30]. 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 (0.1 mg/kg). The concentration of heavy metals in the studied samples were below the permissible limits of WHO. These findings revealed that the concentrations of the essential elements and heavy metals vary considerably from sample to sample due to differences in plant species environmental conditions and cultivation process, which accordingly alters the constituents of the plant material used in the preparation of herbal medicines.

Conclusion

From this study, SH1 sample displayed more pronounced antioxidant potential than the remaining commercially available samples. Some samples showed linear correlation between phenolic compounds and antioxidant activity, some did not. Both AE and UE techniques were effective for phytochemical extraction. No significant variation was observed between the two extraction techniques. Presence of total phenolic and flavonoid contents, steroidal saponin, antioxidant activity and nutrients contribute a good basis for understanding the curative effects of the samples. The concentration of heavy metals indicated that there was no anthropogenic inputs of the metals in any of the samples. Thus, results of the present study proposes monitoring the levels of heavy metals in herbal medicines was mandatory for manufacturers.

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References

1. S. Patra and S. Unisa, *Glob. J. Med. Public. Health.,* 2017, **6**, 1.
2. N. K. Satti, K. A. Suri, P. Dutt, O. P. Suri, M. Amina and G. N. Oazi, *J. Liq. Chromatogr. Relat. Technol.,* 2006, **29**, 219.
3. R. K. Sharma and B. Dash, "Charaka Samhita", Chowkhamba, Varnasi, India, 2003, **5**, 129.
4. Atreya, "Ayurvedic healing for women", York: Sauel Weiser, Inc., 1999.
5. Y. Kumeta, T. Maruyama, D. Wakana, H. Kamakura and Y. Goda, *J. Nat. Med.,* 2013, **67**, 168.
6. S. Parasuraman, G. S. Thing and S. A. Dhanaraj, *Phcog. Rev*., 2014, **8(16)**, 73.
7. M. Rajani and S. K. Niranjan, "Bioactive Molecules and Medicinal Plants", 2008, Chap. 19, 349.
8. S. Alok, S. K. Jain, A. Verma, M. Kumar, A. Mahor and M. Sabharwal, *Asian. Pac. J. Trop. Dis.,* 2013, **3(3)**, 242.
9. A. K. Singh, A. Srivastava, V. Kumar and K. Singh, *The Natural Products J.,* 2018, **8**, 32.
10. S. Kaur and P. Mondal, *J. Microbiol. Exp*., 2014, **1**, 23.
11. M. I. Hossain, F. A. Sharmin, S. Akhter, M. A. Bhuiyan and M. Shahriar, *Int. Curr. Pharm. J.,* 2012, **1**, 250.
12. L. Taepongsorat and S. Rattana, *Phcog. J.,* 2018, **10**, 1129.
13. L. Subedi, S. Timalsena, P. Duwadi, R. Thapa, A. Paudel and K. Parajuli, *J. Tradit. Chin. Med*., 2014, **34**, 584.
14. B. O. Obadoni and P. O. Ochuko, *Glob. J. Pure. Appl. Sci.,* 2001, **8**, 203.
15. J. C. Baccou, F. Lambert and Y. Sauvaire, *Analyst*, 1977, **102**, 458.

16. K. Devendra, D. kiran, V. Ritesh, B. Satyendra and K. Abhishek, *J. Phcog. Phytochem.,* 2013, **2**, 116.
17. G. Marinova and V. Batchvarov, *Bulgarian. J. Agric. Sci*., 2011, **17**, 11.
18. E. A. Shalaby and S. M. M. Shanab, *Indian. J. Geo-Marine. Sci*., 2013, **42**, 556.
19. K. Puspendra, J. Shivesh and N. Tanveer, *Int. J. Drug Dev. & Res*., 2015, **7(1)**, 23.
20. A. Giacomino, O. Abollino, C. Casanova, C. L. Gioia, E. Magi and M. Malandrino, *Micro. Chem. J*., 2015, **120**, 6.
21. K. Devendra, D. Kiran, V. Ritesh, B. Satyendra, Kmar. Abhishek, *J. Phcog. Phytochem.*, 2013, **2(4)**, 116.
22. S. Velavan, K. Nagulendran, R. Mahesh and V. H. Begum, *Phcog. Mag.,* 2007, **3**, 26.
23. G. V. Jayashree, P. Rachitha, K. Krupashree, K. H. Kumar and F. Khanum, *Phcog. J.,* 2015, **7**, 183.
24. J. S. Negi, P. Singh, G. J. Nee Pant, M. S. M. Rawat and H. K. Pandey, *Biol. Trace. Elem. Res.,* 2010, **135**, 275.
25. N. K. Singh, A. S. Raghubanshi, A. K. Upadhyay and U. N. Rai, *Ecotoxicology and Environmental safety,* 2016, **30**, 224.
26. N. V. V. Jyothi, P. C. Mouli and S. R. J. Reddy, *J. Trace Elem. Med. Biol.*, 2003, **17**, 79.
27. S. Wilbur, H. Abadin, M. Fay, D. Yu, B. Tencza, L.Ingerman, *et al*., "Agency for Toxic Substances and Disease Registry", Toxicological Profiles, Department of Health and Human Services, US, 2012.
28. M. Jalili MD and R. Azizkhani MD, *West J. Emerg. Med.*, 2009, **10(4)**, 244.
29. S. Tokalioglu, *Food Chem.,* 2012, **134**, 2504.
30. WHO, "Guidelines for assessing quality of herbal medicines with reference to contaminants and residues", World Health Organization, Geneva, Switzerland 2007.