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PHARMACEUTICAL EVALUATION AND TOXICOLOGICAL QUANTIFICATION OF HEAVY METALS AND ADULTERATED ALLOPATHIC CONTENTS IN RAW AND FINISHED DOSAGE FORM OF ANTIHYPERTENSIVE HERBAL PRODUCTS

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Abstract

Background: Herbal products of questionable quality create major concern for human population since their production is often not controlled and regulated.
Material and Methods: Antihypertensive herbal products were subjected to pharmaceutical quality control parameters specified in Pharmacopoeias, toxic quantification of heavy metals by flame atomic absorption spectrophotometer and adulterated allopathic contents were quantified using advanced HPLC techniques.
Results: A lot of variations in pharmaceutical parameters like moisture contents and LOD% values were observed. Also deviations to a greater extent in weight variation, (P1, P2, P6, P12, P16, P17, P19, and P20), and hardness of the tablets of products (P1, P3, P8 and P11) were found. Friability of tablets of the Products (P3, P9 and P11) was found failed. Heavy metals i-e Fe (1597.20ppm, 1648ppm) in P5, P9, Pb (61.32ppm, 16.59 ppm) in P5, Cr (96.91ppm ,108.48 ppm) in P4, P14, Cd (39.53ppm, 32.31 ppm) in P11, P12, Cu (28.22ppm, 21.04 ppm) in P15, P17, Zn (80.31ppm,76.27 ppm) in P15, P16, Ni (45.46ppm,22.18ppm) in P9, P13 in toxic concentrations were detected. Adulterated allopathic contents of Amlpdopine in higher quantities, administered according to manufacturer dose were found in P12 (20.30 mg/day), Verapamil in P2 (93.50 mg/day), Nifedipine (38.65 mg/day) in P6. Products P4, P5 and P7 were found to have a combination of Amlodipine and Hydrochlorothiazide and higher concentrations were found in P5 (10.72 mg/day, 24.75 mg/day).
Conclusion:. The antihypertensive herbal products contained different kind of adulterants. Our findings suggest that effective regulatory measures should be put in place to address this problem. This will help to decrease the toxic effects of these remedies and increase the commercialization, internationalization and harmonization of antihypertensive herbal products.

Key words: Herbal products, Pharmaceutical parameters, Heavy metals, allopathic contents

Introduction

The prevalence of hypertension is increasing across the globe and its control rates remains low (Gallagher *et al.*, 2006). Despite the fact that a variety of consistent guidelines are available for the treatment of hypertension, the problem of insufficient management still persists (Selvam *e*t al., 2010). Herbal medicines are widely consumed the world over, for primary healthcare because of their better acceptability within the human body, higher safety margins and less cost (Dasgupta, 2003). Such increase in popularity has also brought concerns and fears regarding the quality, efficacy and safety of herbal and natural sources available in the market (Schuppan *e*t al., 1999). The use of herbal remedies of questionable quality exposes human population to multiple risks and creates major concern for various health agencies on national and international level (Barnes, 2003). The quality of herbal products remains poor because their production is often not controlled or regulated (Rousseaux and Schachter, 2003). Therefore screening of traditional remedies for efficacy and safety has been recommended to protect public health (Calixto, 2000).

These products may be contaminated with excessive or banned pesticides, heavy metals, chemical toxins and adulterated with undeclared allopathic contents (Chan, 2003). Additional contaminants like particulate matters may also be introduced during handling and production of herbal medicines since no conscious efforts are made to decontaminate them (Lenaghan *e*t al., 2009). Therefore, an effective and advanced quantification method is needed to monitor the levels of various adulterants in herbal medicines (Klinsunthorn et al., 2011). Herbal medicines are commonly used and the idea about its potential toxicity is not very well known which often lead to improper use of such remedies (Khalil et al., 2014). Increased reports on side effects and the adulteration of herbal drugs the world over have raised concerns on its use (Niggemann and Grüber, 2003).

Therefore an attempt is made for the identification and quantification of selected antihypertensive herbal products both raw material and finished dosage form, for their pharmaceutical quality control parameters, toxic heavy metals and undeclared adulterated allopathic drug contents.

Material and Methods

Pharmaceutical parameters

The pharmaceutical parameters like melting point, pH value, bulk density, tap density, LOD% and moisture content of raw materials and parameters like gelatin identification test for capsule shells, weight variation, diameter, thickness, hardness and friability of the tablets of finished dosage form were determined by standard methods available in Pharmacopoeia. Melting point was determined by melting point apparatus, pH value by pH meter, bulk density and tap density by density measure equipment, loss on drying by LOD equipment and moisture contents by Carl Fischer. For identification of gelatin the capsule shells were weighed and dissolve in 100 ml of hot water. The solution was then placed in refrigerator (2-10°C) for 4 hours. The gelled solution was then removed and placed in a container at 60°C for 30 minutes, when stirred, the gel reverts to the original liquid state (USP, 2014).

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Weight variation was determined by electronic analytical balance (USP, 2014). Diameter and thickness were calculated by Vernier caliper (USP, 2014). Hardness of tablets was determined by Monsanto tester. Friability of the tablets was determined using friability tester i-e Erweka tablets friability tester (USP, 2014).

Toxic heavy metals evaluation by flame atomic absorption spectrophotometer

The herbal drug products were ground and about one gram (1g) was taken and added in 10 ml of concentrated Nitric acid solution (67%) in a conical flask and kept overnight at room temperature. After 24 hrs about 4 ml perchloric acid was added and heated till one (1ml) solution remained in the flask. It was then cooled, diluted with deionized water and filtered via Whatmann filter paper. Finally 100 ml volume was made by deionized water. The prepared samples were then analyzed by flame atomic absorption spectrophotometer for determination of toxic heavy metals (Saeed et al., 2010).

Adulterated allopathic contents evaluation by HPLC

The liquid chromatography system consisted of an isocratic pump (Model SPD 10AV-VP) UV-Visible detector (Schimadzo), LC-10AT VP, Column C18 (Thermo scientific) was used. For Amlodipine identification, the mobile phase consists of; Methanol, acetonitrile, and Buffer (35:15:50). For Verapamil mobile phase was Acetonitrile, 2-aminoheptane and buffer (60:1:140) while for Nifedipine, Acetonitrile, methanol, and water (25:25:50). Hydrochlorothiazide identification was carried out using mobile phase (0.1 M monobasic sodium phosphate and acetonitrile (9:1 adjust with phosphoric acid to a pH 3.0 ±0.1). UV detection was conducted at 237 nm for Amlodipine, 278 nm for Verapamil, 265 nm for Nifedipine and 254 nm for hydrochlorothiazide (USP, 2014)

Table 1: Pharmaceutical properties of local herbal anti hypertensive raw materials

Product Code	Appearance	Melting Point (C^0)	PH Value (1dose/100ml in D/W	Bulk Density (mg/ml)	Tap Density (mg/ml)	LOD (%)	Moisture Contents (%)
P1	Brown Amorphous	238±0.05	5.96±0.0	0.843±0.0	0.767±0.0	6.79±0.0	6.38±0.04
P2	White Homogenous	242±0.0	6.70±0.01	0.976±0.0	0.846±0.0	5.96±0.03	5.84±0.02
P3	White Granules	279±0.1	5.73±0.0	0.986±0.0	0.887±0.0	6.49±0.02	6.11±0.06
P4	White homogenous Powder	253±0.57	6.11±0.0	0.976±0.0	0.794±0.0	4.37±0.03	4.30±0.02
P5	Brown amorphous Powder	189±0.0	7.02±0.01	0.736±0.0	0.679±0.0	8.00±0.01	7.87±0.04
P6	Brown fibrous Powder	208±0.57	5.88±0.01	0.983±0.0	0.876±0.0	6.11±0.02	5.84±0.03
P7	White Powder	194±0.05	5.46±0.01	0.967±0.0	0.843±0.0	5.35±0.02	5.27±0.03
P8	Brown granules	234±0.0	5.27±0.0	0.739±0.0	0.687±0.0	4.76±0.0	3.96±0.02
P9	Amorphous Powder	273±0.1	6.11±0.0	0.847±0.0	0.769±0.0	5.73±0.01	5.48±0.02
P10	Dark brown Powder	235±0.0	7.30±0.01	0.884±0.0	0.762±0.0	4.88±0.01	4.29±0.01
P11	Dark brown Powder	271±0.5	6.80±0.0	0.936±0.0	0.843±0.0	3.97±0.03	3.88±0.04
P12	White granules	219±0.5	5.70±0.0	0.946±0.0	0.837±0.0	6.76±0.0	6.67±0.0
P13	Brown Powder	247±0.05	5.64±0.0	0.976±0.0	0.939±0.0	5.28±0.01	5.01±0.01
P14	Metallic green powder	236±0.0	6.34±0.02	0.949±0.0	0.913±0.0	4.39±0.02	4.24±0.02
P15	White fine Powder	274±0.0	7.11±0.01	0.984±0.0	0.947±0.0	7.33±0.01	7.04±0.02
P16	Metallic brown Powder	227±0.0	6.84±0.02	0.849±0.0	0.814±0.0	5.76±0.0	5.48±0.01
P17	White Powder	277±0.0	5.94±0.02	0.973±0.0	0.949±0.0	5.14±0.02	5.01±0.01
P18	Greenish granules	264±0.0	5.76±0.03	0.931±0.0	0.916±0.0	6.39±0.0	6.22±0.01
P19	Brown granules	208±0.0	6.84±0.0	0.967±0.0	0.926±0.0	8.00±0.01	7.76±0.0
P20	White homogenous powder	287±0.0	6.73±0.01	0.964±0.0	0.949±0.0	3.96±0.02	3.71±0.0

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Results and Discussion

The herbal remedies indicated for hypertension when subjected to the same physicochemical tests conducted for allopathic drugs, fails a variety of tests (Table 1 and 2). High moisture contents promote microbial growth and may result to infectious diseases. A lot of variation in weight, hardness, thickness, diameter and friability of tablets were found in herbal dosage forms. Also the drug product supplied in capsule dosage form (P16) failed the gelatin identification test. The physicochemical parameters are important given that therapeutic remedies fail such tests which may lead to toxicity or deficient pharmacological effects.

Table 2: Pharmaceutical Properties of local herbal antihypertensive finished dosage forms

Product Code	Dosage Form	Gelatin Test	Average Weight (mg)	Average Diameter (mm)	Average Thickness (mm)	Average Hardness (Kg)	Friability (%)
P1	Tablet	NA	732.10±26.36	9.39±0.32	6.15±0.05	16.0±1.63	0.09
P2	Tablet	NA	604.00±23.16	6.72±0.41	4.37±0.32	15.0±2.62	0.06
P3	Tablet	NA	523.60±7.05	5.95±0.07	3.42±0.06	2.51±0.48	Failed
P4	Tablet	NA	577.60±8.26	4.78±0.22	3.50±0.10	14.0±0.81	0.04
P5	Tablet	NA	409.20±8.43	4.06±0.26	4.65±0.05	11.2±0.71	0.73
P6	Tablet	NA	878.20±14.12	8.9±0.18	5.26±0.06	12.0±0.95	0.98
P7	Tablet	NA	898.70±5.34	9.97±0.13	4.09±0.08	9.0±1.33	0.42
P8	Tablet	NA	264.60±7.63	3.91±0.12	1.08±0.08	5.10±0.14	0.43
P9	Tablet	NA	306.80±4.6	3.57±0.11	3.45±0.05	5.0±0.89	Failed
P10	Capsule	(+)	500.20±15.57	NA	NA	NA	NA
P11	Tablet	NA	462.20±5.05	5.32±0.26	5.43±0.07	3.47±1.05	Failed
P12	Capsule	(+)	897.10±13.93	NA	NA	NA	NA
P13	Capsule	(+)	556.1±8.06	NA	NA	NA	NA
P14	Capsule	(+)	659.70±5.64	NA	NA	NA	NA
P15	Tablets	NA	221.50±6.83	3.09±0.1	5.17±0.06	12.20±2.2	0.76
P16	Capsule	(–)	747.50±15.14	NA	NA	NA	NA
P17	Tablets	NA	649.90±14.04	5.79±0.15	3.43±0.04	7.50±0.67	0.87
P18	Capsule	(+)	849.70±14.59	NA	NA	NA	NA
P19	Capsule	(+)	903.10±15.98	NA	NA	NA	NA
P20	Capsule	(+)	789.30±21.36	NA	NA	NA	NA

NA= Not Applicable, (+) = Pass gelatin Identification Test, (-) = Failed gelatin Identification Test

Pharmaceutical evaluation of raw materials and finished dosage form

Melting Point

Melting points of raw materials of herbal antihypertensive products were determined by melting point apparatus (USP, 2014). Melting points of all the tested samples are given in Table 1.

pH value

Tables 1, show all the pH values determined by pH meter (USP, 2014).

Bulk density and Tap density

The bulk and tap densities were obtained (Table 1) by dividing the weight of the sample in grams by the final volume in cm^3 of the sample contained in the cylinder (USP, 2014).

Loss on drying (LOD %) and Moisture contents

LOD % and moisture contents of the raw materials of antihypertensive herbal products were determined (Table 1) using LOD and Karl Fisher method (USP, 2014).

Gelatin Identification test

From Table 2, it was observed that that only product P16 failed gelatin test (USP, 2014).

Weight variation

With the help of electronic balance average weight of the tablets were determined (USP, 2014). Table 2 shows greater weight variation in products, P1, P2, P6, P12, P16, P17, P19 and P20.

Diameter and Thickness of tablets

The diameter and thicknesses of the tablets were determined by using Vernier caliper. Average diameter and thickness values were calculated (USP, 2014) as shown in Table 2.

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Table 3: Concentration (ppm) of various toxic heavy metals in raw material of local antihypertensive herbal drug products

Product code	Fe	Cd	Pb	Cr	Cu	Mn	Zn	Ni
P1	984 ± 9.12	17.50 ± 2.33	2.790 ± 0.0	1.743 ± 0.07	15.08 ± 0.91	43.96 ± 3.721	27.32 ± 0.82	0.5567 ± 0.13
P2	48.96 ± 4.11	40.24 ± 3.98	ND	2.593 ± 0.25	4.213 ± 0.07	19.12 ± 0.78	10.41 ± 0.50	19.60 ± 2.16
P3	314.8 ± 6.37	5.893 ± 1.05	2.010 ± 0.12	19.55 ± 1.70	0.5467 ± 0.07	ND	0.380 ± 0.11	2.513 ± 0.78
P4	1574 ± 5.36	32.42 ± 2.32	6.250 ± 0.20	ND	4.170 ± 0.13	20.09 ± 0.92	6.597 ± 0.80	3.083 ± 0.11
P5	836.60 ± 11.84	2.343 ± 0.78	7.063 ± 0.40	76.44 ± 0.35	7.767 ± 0.18	13.66 ± 1.51	14.98 ± 1.53	2.067 ± 0.17
P6	141.6 ± 1.24	5.370 ± 0.16	ND	5.573 ± 0.34	2.300 ± 0.25	12.68 ± 0.61	3.640 ± 0.38	8.450 ± 0.20
P7	48.19 ± 2.95	8.023 ± 1.01	0.550 ± 0.19	113.9 ± 4.42	1.447 ± 0.02	7.910 ± 0.74	5.487 ± 0.29	1.230 ± 0.09
P8	91.25 ± 1.41	8.200 ± 0.51	1.530 ± 0.18	118.8 ± 3.91	5.440 ± 0.14	14.80 ± 0.26	33.62 ± 2.29	22.85 ± 1.61
P9	1565 ± 62.36	3.003 ± 0.50	85.33 ± 7.52	96.25 ± 3.55	2.403 ± 0.27	22.73 ± 3.20	8.457 ± 0.58	2.267 ± 0.18
P10	36.45 ± 1.99	10.35 ± 0.52	ND	23.49 ± 1.25	29.55 ± 2.16	22.45 ± 1.13	81.98 ± 3.26	0.150 ± 0.03
P11	77.29 ± 1.07	4.860 ± 0.22	85.26 ± 4.13	ND	1.600 ± 0.21	16.43 ± 0.80	7.847 ± 0.63	9.043 ± 0.16
P12	763.3 ± 15.61	4.703 ± 0.51	ND	14.43 ± 0.68	4.763 ± 0.13	3.547 ± 0.17	79.61 ± 3.71	0.743 ± 0.16
P13	268.20 ± 20.43	5.613 ± 0.14	4.680 ± 0.17	0.563 ± 0.04	2.590 ± 0.16	12.62 ± 0.68	9.860 ± 0.90	7.577 ± 0.12
P14	14.62 ± 1.74	1.317 ± 0.25	0.353 ± 0.06	55.00 ± 3.06	22.71 ± 1.42	13.12 ± 1.70	31.75 ± 1.49	4.960 ± 0.23
P15	66.70 ± 12.91	8.947 ± 1.40	1.403 ± 0.25	3.167 ± 0.00	2.593 ± 0.19	4.677 ± 0.21	22.75 ± 1.42	0.89±0.01
P16	235.6 ± 12.76	8.357 ± 0.07	0.510 ± 0.19	0.583 ± 0.10	10.44 ± 0.60	8.767 ± 0.60	25.63 ± 0.57	5.090 ± 0.10
P17	1615 ± 86.85	1.353 ± 0.23	1.503 ± 0.15	0.533 ± 0.09	4.497 ± 0.16	17.30 ± 0.65	8.400 ± 0.12	52.13 ± 1.19
P18	569.1 ± 10.44	2.607 ± 0.20	8.153 ± 0.22	1.290 ± 0.23	2.603 ± 0.20	1.477 ± 0.11	14.63 ± 0.66	3.947 ± 0.12
P19	67.97 ± 0.80	12.10 ± 1.62	ND	1.377 ± 0.13	1.643 ± 0.02	20.15 ± 0.43	4.547 ± 0.08	8.143 ± 1.19
P20	45.50 ± 0.76	1.567 ± 0.15	0.510 ± 0.11	2.877 ± 0.17	1.540 ± 0.18	25.48 ± 2.14	6.313 ± 0.49	2.813 ± 0.22

ND=Not detected

Table 4: Concentration (ppm) of various toxic heavy metals in finished dosage form of local antihypertensive herbal drug products

Product Code	Fe	Cd	Pb	Cr	Cu	Mn	Zn	Ni
P1	985.81±5.44	17.33±4.03	1.76±0.05	1.44±0.25	14.88±1.42	30.96±0.56	26.31±1.27	0.59±0.28
P2	43.62±8.71	5.59±1.05	ND	15.88±3.96	0.38±0.19	10.33±0.08	0.34±0.10	2.53±0.12
P3	828.27±15.53	2.27±1.00	6.29±0.52	12.43±2.13	6.40±0.71	ND	13.01±0.58	1.12±0.13
P4	43.85±5.85	7.49±1.53	0.44±0.16	ND	1.01±0.03	6.57±0.84	4.82±1.66	1.20±0.66
P5	1597.20±43.33	2.12±0.09	61.32±8.18	96.91±4.47	2.27±0.44	23.39±3.81	7.45±0.82	2.43±0.26
P6	71.99±2.09	4.26±0.23	ND	10.2±6.25	1.62±0.23	15.42±1.13	6.51±1.02	7.37±0.26
P7	267.87±33.34	5.38±0.30	3.34±0.68	0.33±0.16	2.59±0.13	11.67±1.0	9.19±0.41	6.57±0.18
P8	66.36±22.82	8.38±2.10	1.00±0.02	3.14±0.01	1.86±0.10	5.17±0.68	20.78±1.22	22.76±0.21
P9	1648.06±157.7	1.15±0.40	1.19±0.14	0.32±0.07	5.09±0.44	17.10±1.25	7.93±0.10	45.46±4.13
P10	66.97±1.39	10.43±2.31	ND	1.16±0.09	1.33±0.40	18.84±0.21	3.88±0.43	7.47±1.28
P11	312.11±10.09	39.53±6.56	2.01±0.17	ND	4.48±0.24	19.12±2.44	10.41±0.83	14.93±4.57
P12	1568.23±5.49	32.31±4.01	ND	18.65±0.26	4.50±1.0	16.42±4.31	6.59±1.43	1.41±0.35
P13	134.63±5.52	5.17±0.06	5.31±0.23	5.06±1.47	1.96±1.28	12.01±1.01	2.64±0.54	6.78±1.33
P14	88.58±1.91	7.63±1.23	1.33±0.07	108.48±5.31	5.57±0.20	13.13±1.09	30.95±0.78	22.18±3.96
P15	31.78±0.10	11.34±0.90	16.59±0.98	21.82±3.58	28.22±2.56	20.78±1.73	80.31±6.67	0.13±0.04
P16	757.92±25.16	4.32±1.01	8.30±0.83	71.50±2.56	4.09±1.36	5.21±0.72	76.27±3.64	0.67±0.25
P17	11.28±1.46	1.39±0.44	0.36±0.07	54.0±5.02	21.04±0.86	12.12±2.41	27.42±5.01	5.09±0.35
P18	225.58±2.53	8.64±0.53	0.60±0.05	0.59±0.09	10.15±0.34	7.63±0.65	22.96±1.82	5.75±0.45
P19	565.80±12.64	1.94±0.25	ND	1.40±0.22	2.50±0.44	1.24±0.19	13.29±2.39	4.24±0.55
P20	43.16±3.37	1.16±0.13	0.47±0.12	2.84±0.25	1.39±0.19	21.81±2.37	6.31±1.26	2.41±0.47

ND= Not Detected

Table 5: Daily intake of metals (µg/day) according to daily dose of herbal antihypertensive products
ND= Not Detected

Product Code	Fe	Cd	Pb	Cr	Cu	Mn	Zn	Ni
P1	288.68±4.17	5.07±1.13	0.51±0.08	0.42±0.13	4.35±0.17	9.06±0.03	7.70±0.75	0.17±0.05
P 2	10.54±1.64	1.35±0.42	ND	3.83±0.06	0.09±0.03	2.16±0.39	0.083±0.47	0.61±0.42
P 3	173.47±7.6	0.47±0.06	1.31±0.08	14.97±1.91	1.34±0.08	ND	2.72±0.71	0.23±0.71
P 4	10.13±1.3	1.73±0.12	0.10±0.04	ND	0.23±0.02	1.51±0.73	1.11±0.50	0.27±0.02
P 5	256.03±53.7	0.34±0.10	10.03±0.98	15.86±1.11	0.37±0.12	3.82±0.41	1.22±1.16	0.39±1.73
P 6	25.28±5.38	1.49±0.41	ND	4.30±1.01	0.56±0.07	5.41±0.23	2.28±.61	2.59±0.75
P 7	96.29±5.26	1.93±0.10	1.20±0.19	0.11±0.72	0.93±0.03	4.19±1.8	3.30±0.68	2.36±0.50
P 8	7.023±1.95	0.88±0.09	0.10±0.08	0.33±0.04	0.19±0.03	0.54±0.20	2.20±1.16	1.48±0.21
P 9	202.25±1.76	0.14±0.16	0.14±0.01	0.03±0.06	0.62±0.09	2.09±0.75	0.97±0.11	5.57±0.21
P 10	13.40±1.43	2.08±0.21	ND	0.23±0.04	0.26±0.04	3.77±1.02	0.77±0.71	1.49±0.29
P 11	57.70±3.96	7.30±0.87	0.37±0.05	ND	0.82±0.27	3.53±1.83	1.92±0.90	2.76±0.44
P 12	562.74±3.66	11.59±0.72	ND	0.47±0.08	1.61±0.06	5.89±2.47	2.36±0.44	0.50±0.64
P 13	29.94±0.75	1.15±0.30	1.18±0.47	1.12±0.48	0.43±0.74	2.67±0.83	0.58±0.67	1.50±0.45
P 14	23.37±2.08	2.01±0.30	0.35±0.05	28.62±1.02	1.47±0.27	3.46±1.23	8.16±0.58	5.85±0.93
P 15	2.81±0.45	1.00±0.07	1.47±0.03	1.93±0.29	2.50±0.62	1.84±0.24	7.11±0.06	0.01±0.0
P 16	226.61±0.81	1.29±0.02	2.48±0.11	3.71±0.73	1.22±0.71	1.55±0.53	22.80±0.46	0.20±0.01
P 17	2.93±0.17	0.36±0.02	0.09±0.03	14.03±0.56	5.47±0.17	3.15±1.16	7.12±0.64	1.32±0.12
P 18	76.67±0.55	2.93±0.12	0.20±0.10	0.20±0.10	3.45±0.05	2.59±0.45	7.80±0.90	1.95±0.07
P 19	204.39±3.90	0.70±0.10	ND	0.50±0.10	0.90±0.13	0.44±0.13	4.80±0.52	1.53±0.14
P 20	13.62±0.62	0.36±0.07	0.15±0.05	0.89±0.42	0.43±0.16	6.88±0.94	1.99±0.58	0.76±0.07

Hardness of tablets

Products P1, P3, P8, and P11 failed the hardness test, specified in Pharmacopeia (USP, 2014).

Friability of tablets

Table 2 shows that friability of tablets of Products (P3, P9 and P11) failed the test (USP, 2014).

Evaluation of toxic heavy metals by flame atomic absorption spectrophotometer

In medicine, heavy metals poisoning can possibly include excessive amounts of iron, cadmium, lead, manganese, chromium, copper, zinc and nickel (Luo *et al*., 2011, Järup, 2003, Ernst, 2002). Iron is an essential element but once in excess, poseses a threat to cells and tissues, and therefore iron homeostasis has to be tightly controlled. (Papanikolaou and Pantopoulos, 2005). The recommended daily allowance of iron is 7-10 mg/day (Trumbo *et al*., 2001). All the tested products contained iron and the highest concentrations administered according to manufacturer dose were found in product P12 (562.74 µg/day) while lowest concentrations were found in product P15 (2.81 µg/day). Cadmium can cause severe complications and injuries to the tissues (Järup, 2003). According to Food and drug administration (FDA) and World Health Organization (WHO) the daily intake of Cadmium should not exceed 0.07 mg/day. Product P12 after administration provides (11.59 µg/day). High concentrations of lead is associated with status epileptics, infant fatal encephalopathy, congenital paralysis, sensori-neural deafness and growth retardation (Meeting and Organization, 2007). From Table 4 it can be seen product P5 (10.03 µg/day) provides highest concentration of lead. Exposure to high levels of Chromium can cause lungs cancer and demitits. The daily permissible intake of Chromium is 0.03 mg/day for adults (Health and Services, 1993). Among the herbal drugs under test the highest concentration of Chromium was provided by P14 (28.62 µg/day) after administration, while in product P9 it was found in permissible limits. However product P12 was found to have no traces of chromium. Copper is an essential element for human metabolism and in the synthesis of neurotransmitters (Health and Services, 1993). From Table 5 the daily consumption of copper from product P17 (5.47 µg/day) was the highest, while P2 (0.09 µg/day) was found the lowest among all the tested products. Product P1 after administration according to prescribed dose provides (9.06 µg/day) Manganese, Product P2 has no manganese contents while product P19 gives the lowest concentration (0.44 µg/day). Manganese is also an essential element and the daily requirements for children are about 1.2-1.5 mg/day, 2.3 mg/day for men and 1.8-2.0 mg/day for women (Normandin *et al*., 2004). The recommended daily allowance of Zinc for children is 4-5 mg/day while for adults it is 9-13 mg/day (Hotz and Brown, 2004). Except Product P16 (22.80 µg/day) all other tested samples show nearly optimum concentrations of Zinc. Least concentrations were found in product P2 (0.083 µg/day). As shown in Table 5 Product P14 provides highest concentrations of nickel (5.85 µg/day) while product P15 was found to have limited amounts of nickel. According to the agency of toxic substances and disease registry (ATSDR) exposure to Nickel is commonly associated with allergic reactions, blood, stomach, liver and kidney disorder (Anke *et al*., 1995).

Evaluation of allopathic drug contents using HPLC

Various allopathic drug contents of Ca^{+2} channel blockers (Amlodipine, Verapamil and Nifedipine) and a diuretic agent, Hydrochlorothiazide in combination with Amlodipine was detected and calculated for daily dose as recommended by the prescriber. From Table 6 it can be seen that raw material and the finished dosage form of the herbal products were found adulterated with allopathic contents. Higher concentration of adulterated allopathic contents of Amlpdopine administered according to manufacturer dose were found in P12 (20.30 mg/day), Verapamil in P2 (93.50 mg/day) and Nifedipine in P6 (38.65 mg/day). Products P4, P5 and P7 were found to have a combination of Amlopdipine and Hydrochlorothiazide and higher concentration were found in P5 (10.72 mg/day, 24.75 mg/day). In some of the procured herbal remedies under test the finished dosage form do not have any traces of allopathic contents (Product P1, Product P6) but the raw materials of these products were found to have adulteration, which indicates that these remedies might be contaminated to enhance their pharmacological and therapeutic effects by quacks, which may lead to severe toxic effects.

Table 6: Identified adulterated allopathic contents in raw and finished dosage form of antihypertensive herbal product

Product Code	Identified Allopathic compound in Raw/Finished dosage form	AML(Avg. mg/daily dose)	VER (Avg. mg/daily dose)	NFD (Avg. mg/daily dose)	AML+HCT (Avg. mg/daily dose)
P1	AML (R)	17.65±0.11	-	-	-
P2	VER (F)	-	93.50±0.40	-	-
P3	VER (F)	-	86.55±0.34	-	-
P4	AML+HCT (F)	-	-	-	(9.96±0.12)+(23.84±0.99)
P5	AML+HCT (F)	-	-	-	(10.72±0.20)+(24.75±0.27)
P6	NFD(R)	-	-	38.65±0.5	-
P7	AML+HCT (F)	-	-	-	11.02±0.78+22.20±0.79
P8	AML (F)	17.82±0.13	-	-	-
P9	ND	-	-	-	-
P10	ND	-	-	-	-
P11	NFD (F)	-		17.90±0.34	-
P12	AML (F)	20.30±0.85	-	-	-
P13	AML (F)	3.23±0.23		-	
P14	ND	-	-	-	-
P15	ND	-	-	-	-
P16	ND	-	-	-	-
P17	NID	-	-	-	-
P18	VER(F)	-	80.32±0.48	-	-
P19	ND	-	-	-	-
P20	ND	-	-	-	-

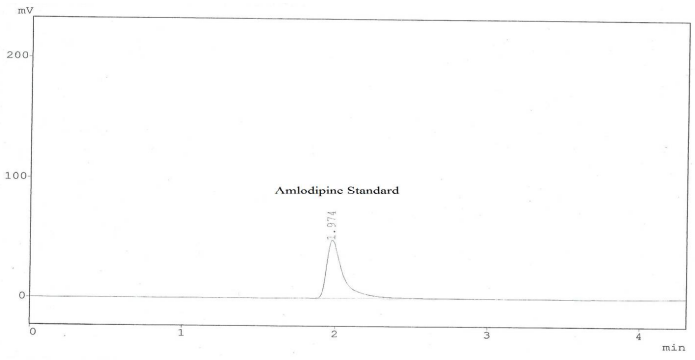

Figure 1: Chromatogram of Amlodipine Standard

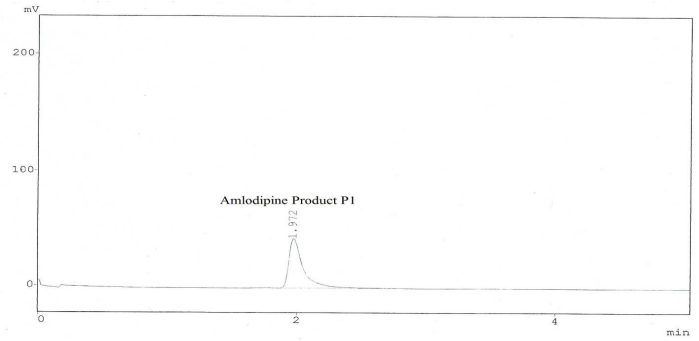

Figure 2: Chromatogram of Amlodipine identified in antihypertensive Product P1

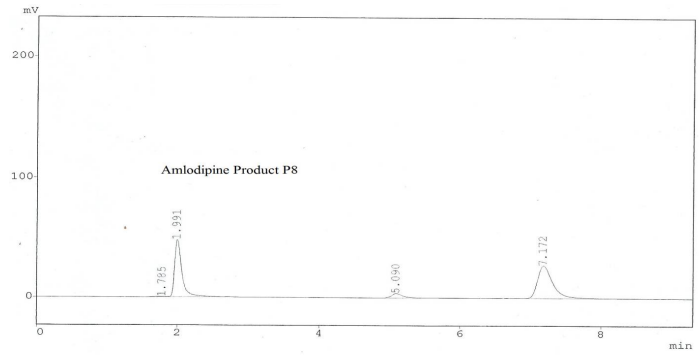

Figure 3: Chromatogram of Amlodipine identified in antihypertensive Product P8

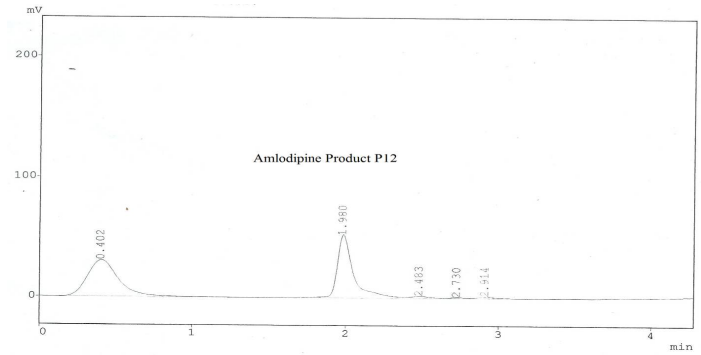

Figure 4: Chromatogram of Amlodipine identified in antihypertensive Product P12

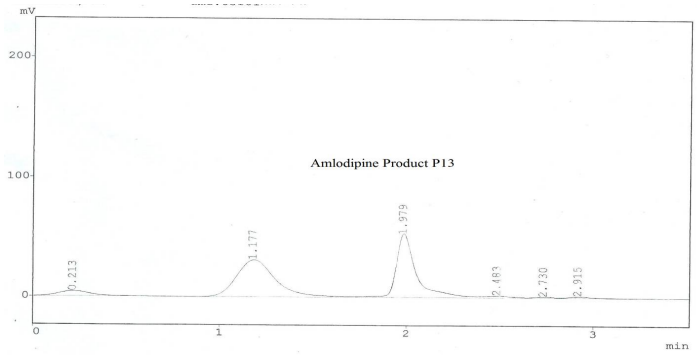

Figure 5: Chromatogram of Amlodipine identified in antihypertensive Product P13

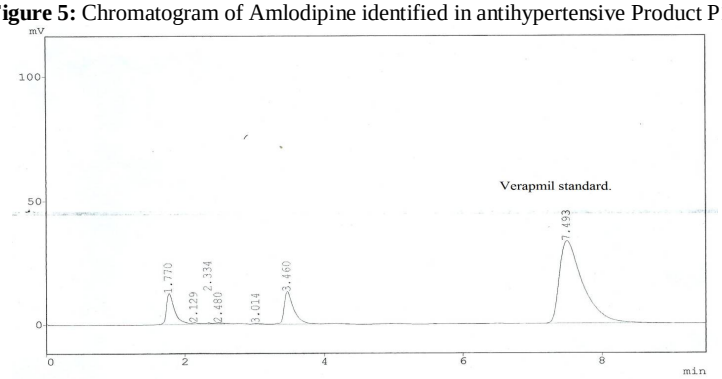

Figure 6: Chromatogram of Verapamil standard

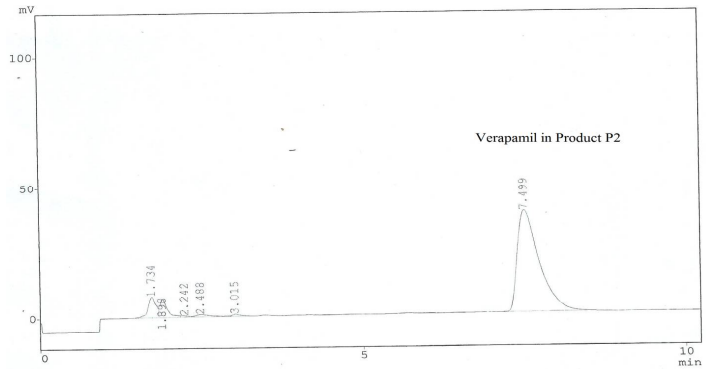

Figure 7: Chromatogram of Verapamil identified in antihypertensive Product P2

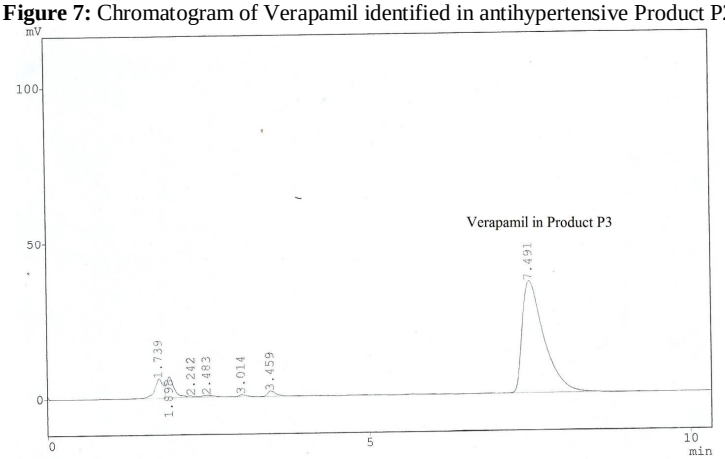

Figure 8: Chromatogram of Verapamil identified in antihypertensive Product P3

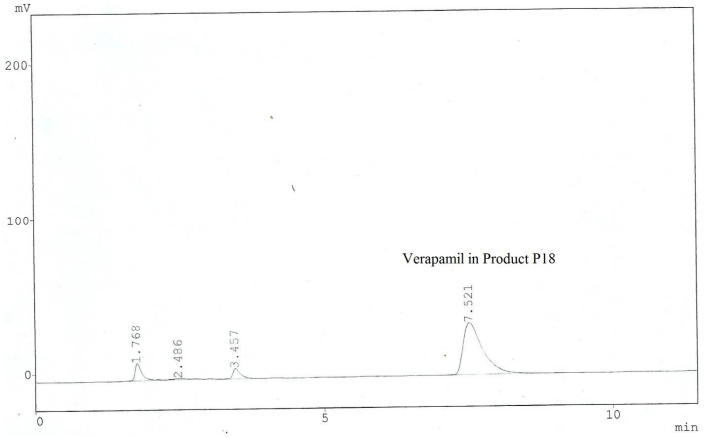

Figure 9: Chromatogram of Verapamil identified in antihypertensive Product P18

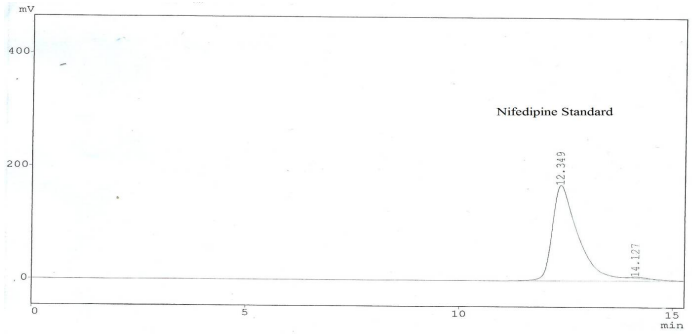

Figure 10: Chromatogram of Nifedipine Standard

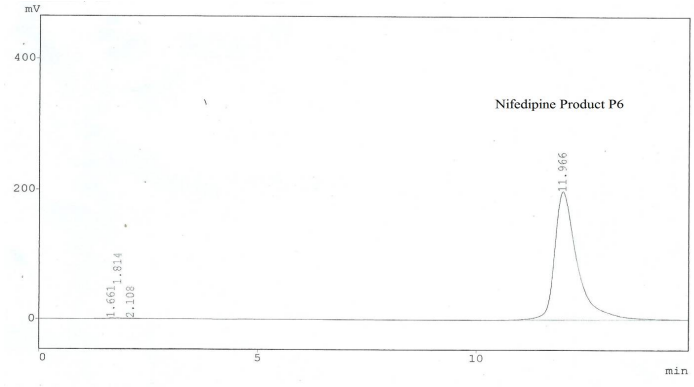

Figure 11: Chromatogram of Nifedipine identified in antihypertensive Product P6

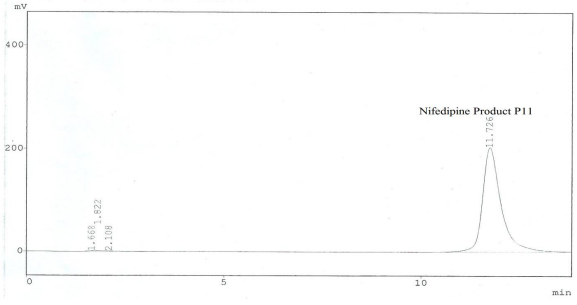

Figure 12: Chromatogram of Nifedipine identified in antihypertensive Product P11

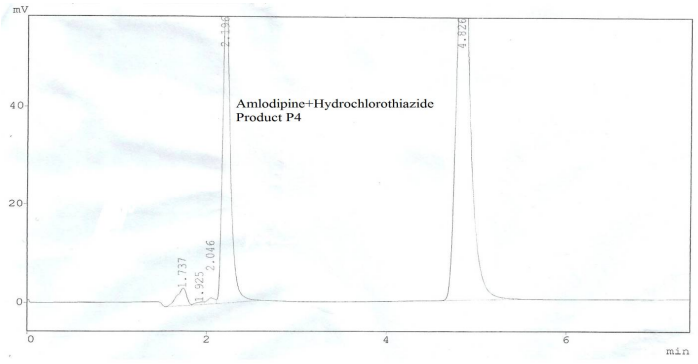

Figure 13: Chromatogram of Amlodipine+Hydrochlorothiazide identified in Product P4

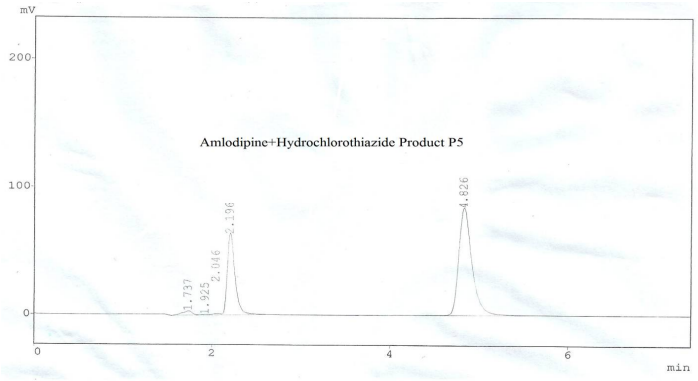

Figure 14: Chromatogram of Amlodipine+Hydrochlorothiazide identified in Product P5

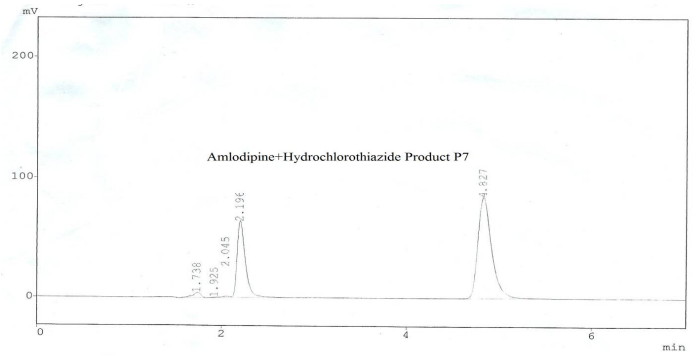

Figure 15: Chromatogram of Amlodipine+Hydrochlorothiazide identified in herbal Product P7

Conclusion

Societal laws allow for the marketing of herbal remedies without subjection or subjecting them to the same regulations required for the safety and efficacy of allopathic drug products. As the herbal remedies are often adulterated by quacks to enhance their pharmacological effects and this can be seen from the results, which clearly indicate that the pharmaceutical quality control parameters deviate the permissible pharmacopeial limits, heavy metals were quantified in toxic limits and adulterated allopathic drug contents were detected in raw materials and finished dosage forms of antihypertensive herbal products. Therefore, evidence of adulterant profile of the herbal products should be obtained both in raw and finished dosage form by well-designed analytical procedures. This will help to decrease the toxic effects of these remedies and increase the commercialization, internationalization and harmonization of antihypertensive herbal products.

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