Pak. J. Bot., 46(2): 645-658, 2014.

APPRAISAL OF MEDICINAL PLANTS USED IN ALTERNATIVE SYSTEMS OF MEDICINES FOR MICROBIAL CONTAMINATION, PHYSIOCHEMICAL PARAMETERS AND HEAVY METALS

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Abstract

The safety of herbal products has become a foremost apprehension in public health with their recognition and worldwide market growth and due in part to the widespread assumption that "natural" implies "harmless". The global market of medicinal plants has been growing at a rate of 7-10% annually; capitalizing on the growing awareness of herbal and aromatic plants globally. The present study was conducted to assess the physiochemical parameters, microbial contamination and presence of heavy metals. The 24 medicinal plants were collected from open market places of various cities of Pakistan and tested by employing WHO and AOAC guidelines. Medicinal plants were found polluted with wide variety of potentially pathogenic bacterias. Microbial count and levels of arsenic and mercury in some plants were found elevated. The percentage (%) of physiochemical parameters i.e., foreign organic matter, total ash, acid insoluble ash, alcohol soluble extract, water soluble extract and moisture count of these medicinal plants were found statistically noteworthy. The nonexistence of quality control values for medicinal plants has been one of the key lacunas. Quality assurance system and WHO's guidelines on good agricultural and collection practices be methodically enforced in the medicinal plants supply chain i.e., cultivation, collection and distribution, although it is tricky task.

Introduction

The safety of herbal products has become a foremost apprehension in public health with their recognition and worldwide market growth and due in part to the widespread assumption that "natural" implies "harmless". Therefore, phytotherapy has been incorporated into all alternative systems of medicine, often as the main source of healthcare in low or middle-income countries (Anon., 2007). The global market of medicinal plants has been growing at a rate of 7-10% annually, capitalizing on the growing awareness of herbal and aromatic plants globally and around 80% of developed are relying on herbal medicines (Dubey *et al.,* 2008, Shaikh *et al*., 2009, Shinwari & Qaisar, 2011). Uncultivated safe plants are essential assets with versatile uses and native people have ethnobotanical acquaintance of their usage (Debela Hunde Feyssa *et al.,* 2012). The local people of diverse regions of the world have years old information and traditional uses of many plants present in their regions have been accredited (Shinwari *et al.,* 2013; Nadeem *et al.,* 2013). It is reported that wild gathered food plants have been part of human diet since ancient times and it is argued that past societies made more use of the wild flora to overcome hunger than is done today (Dogan *et al.,* 2013). The nonexistence of quality control standards for medicinal plants has been one of the major lacunas in the wider acceptance of plant based drugs in various parts of the world. Despite these facts the World Health Organization (WHO) estimates that 65-80% of the world's health care services as 'traditional or complementary and alternative medicines (Jonas, 1997). Recently, Garrard and coworkers found wide-ranging label differences in the stated ingredients and recommended daily serving sizes across multiple brands for ten different herbs (Garrard *et al.,* 2003). However, this study was not an attempt to correlate label claims with the actual contents. As a result, the question concerning the precision in the stated composition of the products remained unreciprocated. Rise in the concurrent use of both herbal and conservative modalities, health care providers, in particular, have a crucial stake in knowing what their clients are consuming and in assessing the quality of these products (Corbin *et al.,* 2002). Health care providers who are currently hesitant to recommend or discuss botanical dietary supplements (BDS) use with their patients would likely be less restrained if they were confident about the product quality (Eisenberg, 1997; Smith *et al.,* 1999; Sleath *et al.,* 2001). These products have the potential of pollution with different microorganisms. This is due to raw materials contamination and insanitary production conditions. Herbal preparations used in different forms may carry a large number of microbes originating from soil usually adhering to leaves, stems, flowers, seed and root of the herbs (Adeleye *et al.,* 2005).

Plant samples in the market are stored under undesirable conditions over the years and often contain a mixture of other plant species thus adversely affecting their bio-efficacy (Khatoon *et al.,* 1993). The efficacy of many of drugs is fading because of the adulterated, dried raw materials profusely available in the indigenous market. It would be, therefore, advisable to treat plant drugs with non-toxic chemicals at various stages of storage and processing (Yadav *et al.,* 2008, Walter *et al*., 2011). The varied geographical locations, different varieties and vernacular names, adulteration or substitution encountered in the recent years, the quality control standards have become more relevant in the recent past (Blumenthal, 1997). The present study was aimed to evaluate the microbial contamination, presence of heavy metals and assessment of their physiochemical parameters of medicinal plants collected from various parts of the Pakistan by employing World Health Organization (WHO) and Association of Official Analytical Chemists (AOAC) guidelines (Anon., 1995; Limyati *et al.,* 1998).

Materials and Methods

Sample collection: Twenty four medicinal plants (about 1000 g) of seventeen different families were collected from various localities of the country i.e., Karachi, Lahore, Rawalpindi and the local market during April-June 2010 (Table 1). All plant samples were air dried

unrinsed, milled in a micro-hammer (without metal parts in it), and stored in clean paper bags. The plants were identified and were deposited at the Drugs Control and Traditional Medicines Division, National Institute of Health, Islamabad, Pakistan in its herbarium.

Physicochemical properties assessment and heavy metals presence in medicinal plants: The physicochemical determination of the medicinal plants moisture content (%), total Ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, foreign organic matter and commonly occurred heavy metals i.e., Arsenic, Mercury, Cadmium and Lead were carried out according to the methods by employing WHO and AOAC guidelines (Anon., 1995, Limyati *et al.,* 1998).

Microorganisms: The following strains of bacteria were used: *Escherichia coli, Salmonella Typhi, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans.* The medium used for the sub-culturing of the microorganisms was Macconkey's agar , Soybean casein digest agar and Sabouraud dextrose agar was sterilized by autoclave at 121ºC for 15 minutes. The final pH was 7.0±0.2. All the culture media were prepared and treated according to the manufacturer guidelines (Oxoid). The inoculums were prepared in Soybean Casein digest broth. All cultures media (soybean-casein digest broth, soybean casein digest agar medium, sabouraud dextrose broth & agar, Vogel- Johnson agar medium, manitol-salt agar medium, Macconkey's agar medium, selenitecystine medium, tetrathionate broth, brilliant green agar , bismuth sulfite agar , triple sugar-iron agar and sabouraud dextrose agar medium) from (Oxoid).

Preparation of samples for microbial contamination: A portion of each sample (10 g) was dispersed in fluid soybean-casein digest medium to make 100 ml (1:10 Dilution) in the aseptic conditions, clean rooms, areas and equipments (Limyati *et al.,* 1998).

Inoculation of microorganisms for recovery study: 1 ml of not less than 10^{-3} dilution of a 24 hrs broth culture of the indicator micro-organisms (*Escherichia coli, Salmonella Typhi, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans*) were incubated in soybean-casein digest broth and sabouraud dextrose broth), then incubated for 24- 48 hrs and up 96 hrs for *Candida albicans* and were evaluated for microbial growth in comparison with the colony morphology of positive blank (culture medium plus related microorganism). Doubtful results were confirmed by sub-culturing on selective media (Kudva *et al.,* 1998).

Bio-burden determination: The collected samples of herbal products were subjected to the following examinations: total aerobic viable count (TAVC) by pour plate and multiple tube methods and for presence or absence of *Escherichia coli, Salmonella Typhi, Staphylococcus aureus, Staphylococcus epidermidis* and *Candida albicans.* 10 g of each sample was suspended in appropriate medium. The total volume was adjusted up to 100 ml by adding soybean-casein digest broth for detection of bacteria and sabouraud dextrose broth for detection of molds and yeasts. Aerobic bacterial colony counts were made by the pour plate technique of 1ml of each dilution on soybean casein digest and Sabouraud dextrose agar up to the dilution not less than 10^{-3}. Plates were incubated in duplicate at 37°C for 48-72 hrs. After incubation, the number of colonies was recorded for each plate. Arithmetic mean counts were derived from each item having from 30 to 300 colonies per plate. On the other hand multiple-tube method based on USP-30 for detection of total aerobic count was carried out. Following the incubation period, by examining the tubes for growth, the most probable number of microorganisms per gram of solid dosage forms specimens was expressed by reference to related table in USP-30.

Media and isolation of pathogenic microorganisms: To determine the presence of *Staphylococcus epidermidis* and *Streptococcus aureus*, each sample diluted to 100 ml by adding soybean-casein digest broth and incubated at 35 ± 2°C for 24-48hrs. After growth, a portion of the medium was spread on the manitol-salt agar for *Staphylococcus epidermidis* and *Streptococcus aureus (orange colored colonies) and* the surface of Vogel-Johnson agar for detection of *Streptococcus aureus* (black shiney colonies). Add 10g of each sample in soybean-casein digest broth to make 100 ml for detecting *Escherichia coli and Salmonella* sp. streaked 1ml onto differential Mac-Conkey's agar plates which is selective medium for gram negative (pink colonies for *Salmonella* and *Escherichia coli.* 1ml aliquots was transferred into 9ml fluid selenite-cystine and fluid tetrationate (Selective for *Salmonella* sp.) growth of medium indicates presence of *Salmonella* sp., these cultures were incubated at 35 ± 2°C for 24-48hrs. Then these cultures were further sub-cultured on the surface of brilliant green agar (green colonies with metallic sheet) and bismuth sulfite agar media, (black colonies) indicates *Salmonella* sp. The butt-slant tube of triple sugar-iron agar medium was used for identification of gram-negative rods colonies, slant acidic and butt acid with gas for *Escherichia coli*, slant alkaline but acidic without gas for *Salmonella*. On the other hand 10 g of each sample were added to sabouraud dextrose broth to make 100 ml for detection of *Candida albicans*, incubated at 20-25°C for 7 days. The 1ml of incubated samples were examined and cultured in sabouraud dextrose agar plus chloramphenicol (SDA + C). In cases where microbial growth was observed, the colonies were identified by germ-tube test, the morphological characteristics of sporangia were examined microscopically.

Statistical analysis: Statistical analysis of data was performed by using the student's unpaired *t*-test and by analysis of variance (ANOVA). *p*-values less than 0.05 were considered significant.

Results and Discussion

A total of 24 medicinal plants were collected from four different places i.e., Karachi, Lahore, Rawalpindi and an Open Market. Physiochemical parameters i.e. Foreign Organic matter % age, total ash % age, acid insoluble ash % age, alcohol soluble extract %age, water soluble extract % age, moisture count % age and heavy metals contents of these medicinal plants were determined and analyzed

statistically. The name of medicinal plants, family, part used, place of collection results of the bacteriological analysis of the medicinal plants are shown in Table 1. The most frequently occurring member of the selected pathogenic bacteria screened were *Salmonella typhi* and *S. epidermidis.* They were isolated in 41(42.7%) and 26(26.04%) of the samples, respectively. *E. coli* occurred in 23 (23.9%) and *C. albicans* 26 (26.04%) and *S. aureus,* 9(9.3%) was least frequently present. The result presented in Table 2 are for foreign organic matter % age content of medicinal plants and were determined and expressed as Mean ± S.D. The value ranges from 0-39.3%. The values of foreign organic matter % age content of 14 (58.3%) of medicinal plants were found to be significant at *p*-value 0.05. The value of total ash% contents was determined and also expressed as Mean ± S.D. The value ranges from 0.4-26.9 and ash % age content values of *Acacia nilotica, Ferula assafoetida* and *Berberis aristata* were found to be significant at *p*-value 0.05 (Table 3). Acid insoluble ash % age contents ranges from 0-14.4%. Acid insoluble ash % age content values of *Embella ribes, Cassia absus, Carum copticum, Acorus calamus, Althea officinalis, Butea monosperma, Berberis aristata, Colchicum luteum* were found to be significant at *p*-value 0.05 (Table 4). Alcohol soluble extract % age content values ranges from 0-38.3% and alcohol soluble extract % age content of *Asparagh adscendens, Nigella sativa* were found to be significant at *p*-value 0.05 (Table 5). Water soluble extract % age content values ranges from 4.9%-83.73%. The values of Water Soluble Extract % age content of medicinal plants were evaluated at *p*-value 0.05 (Table 6). Moisture Count % age content ranges from 2.3%-17.2%. The values of Moisture Count % age content of medicinal plants were evaluated at *p*-value 0.05 (Table 7). Lead contents (ppm) of twenty four medicinal plants was determined and expressed as Mean± S.D. The range value is 0.0110 ppm-1.0600 ppm. The values of Lead contents (ppm) of 13 medicinal plants were found to be significant at *p*-value 0.05 (Table 8). Arsenic contents (ppm) of medicinal plants was also determined and value ranges from 0.0110 ppm-0.8299 ppm and values of Arsenic contents (ppm) of *Foeniculum vulgaare, Adiantum cappillus, Adiantum cappillus, Peucedanum graveolens, Brassica juncea, Viola odorata, Berberis aristata* were found to be significant at *p*-value 0.05 (Table 9). Cadmium contents (ppm) of all medicinal plants ranges value is 0.0006 ppm-0.0130 ppm. The values of Cadmium contents (ppm) of all medicinal plants were found to be significant at *p*-value 0.05 (Table 10). Mercury (Hg) contents (ppm) of medicinal plants were also determined and its value ranges from 0.0022 ppm-1.0446 ppm (Table 11). The values of Lead contents (ppm) of *Caccinia glauca, Anacyclus pyrethrum, Acorus calamus Peucedanum graveolens, Berberis aristata, Berberis aristata, Colchicum luteum* were found to be significant at *p*-value 0.05. Microbial count (MPN) by pour plate method, in 2 samples were high range between $10x10^5$ - $10x10^6$ cfu /gm, in 24 samples ranged between 0.5×10^2 - 11×10^2 cfu /g, 31 samples were less than 10cfu/g. Microbial count (MPN) by multiple tubes method in 2 samples were range between 1100 to > 1100 /gm, in 24 samples ranged between 93-240 /gm and in 31 samples less than 3 to 3 /gm.

Total no. of sample = 96, Contaminated samples: *S. typhi* 41=42.7%. *S. epidermidis* 26=27%, *E. coli* 23=23.9%, *C. albican* 26=27%.

Table 2. Foreign organic matter % of twenty four medicinal plants.

S. No.	RWP (%w/w)	KHI (%w/w)	LHR (%w/w)	Market (%w/w)	Range (%w/w)	Mean
1.	15	4.7	2.5	4	4-15	6.55 ± 5.7*
2.	10	0.3	0.4	20	0.3-2	7.68 ± 9.4*
3.	5.6	1.4	1.5	0.3	0.3-5.6	2.20 ± 2*
4.	51.3	7.84	12.6	37.6	7.8-51.3	27.33 ± 20.6
5.	0.02	6.31	0.2	1.6	0.02-6.3	2.03 ± 2.9*
6.	19	1.4	0.1	2	0.13-19	5.62 ± 8.9*
7.	31.2	4.5	4.6	23.8	4.5-31.2	16.02 ± 13.5*
8.	1.2	1.9	0.3	0.5	0.3-1-9	0.98 ± 0.7
9.	1.9	5.2	0.5	7	0.5-7	3.65 ± 2.9*
10.	1.7	1	5	8.3	1-8.3	4.0 ± 3*
11.	3.2	15	35	39	3.2-39	23.05 ± 16.8
12.	39.3	7.6	0.6	1.4	0.6-39-3	12.22 ± 18*
13.	0.72	0	0	0.1	0-0.72	0.21 ± 0.3*
14.	4	0.6	1.3	2.1	0.6-4	2.0 ± 1
15.	3.7	0.04	0.004	3.1	0.001-3.7	1.71 ± 2*
16.	0	0	0	32	0-32	8.0 ± 16.0*
17.	22.45	4.2	5.3	17.5	4.2-22.45	12.36 ± 9.0
18.	7.8	0.3	0.2	3.3	0.3-7.8	2.90 ± 3.5*
19.	17	14	7	3.3	3.3-17	10.32 ± 6.0
20.	1.8	1.4	0.6	0.5	0.5-1.8	1.07 ± 0.6
21.	5.7	1.6	5.9	5.5	1.6-5.9	4.67 ± 2.0
22.	0.4	0.3	0	0-3	0.3-0.4	0.25 ± 0.17
23.	11.4	26.6	10.3	10.6	10.3-26.6	14.73 ± 7.9
24.	0	0.01	0.01	0.3	0-0.3	0.08 ± 0.15*

*Shows values are significant at *p*-value 0.05

Table 1. List of medicinal plants, local name, family, parts used, place of collection and microbial contamination.

S. No.	Botanical name (Local name)	Family	Part used	Place of collection	*Escherichia coli*	*Salmonella Typhi*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
1.	*Acorus calamus* (Waji-l-Turkey)	Araceae	Rhyzome	a. RWP	-ve	-ve	-ve	-ve	- ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	+ve
				d. Market	+ve	+ve	+ve	-ve	+ve
2.	*Acacia nilotica* (Gumacacia)	Leguminosae	Gum	a. RWP	+ve	+ve	-ve	-ve	-ve
				b. KHI	+ve	+ve	-ve	+ve	-ve
				c. LHR	-ve	+ve	-ve	-ve	+ve
				d. Market	+ve	+ve	-ve	-ve	-ve
3.	*Adiantum cappillus-veneris* (Parsioshan)	Polypodiaceae	Aerial Parts	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	+ve	-ve	-ve	-ve
				c. LHR	-ve	+ve	-ve	-ve	-ve
				d. Market	+ve	+ve	-ve	-ve	+ve
4.	*Althea officinalis* (Tukhm-i-Khatmi)	Malvaceae	Seeds	a. RWP	-ve	-ve	-ve	+ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	+ve	+ve	-ve	-ve	+ve
5.	*Althea officinalis* (Gul - I - khatmi)	Malvaceae	Flower	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	+ve	+ve	-ve	-ve
				c. LHR	-ve	+ve	-ve	-ve	-ve
				d. Market	+ve	-ve	+ve	+ve	+ve
6.	*Anacyclus pyrethrum* (Akarkara)	Compositae	Roots	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	+ve
				d. Market	+ve	+ve	-ve	-ve	-ve
7.	*Asparagus adscendens* (Muscli safaid)	Liliaceae	Roots	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	ve
				c. LHR	-ve	-ve	-ve	-ve	+ve
				d. Market	-ve	+ve	-ve	+ve	+ve
8.	*Berberis aristata* (Zarishk)	Berberidaceae	Fruit	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. Market	+ve	+ve	+ve	-ve	+ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	-ve	-ve	-ve	-ve	-ve

Table 1. (Cont'd.)

S. No.	Botanical name (Local name)	Family	Part used	Place of collection	*Escherichia coli*	*Salmonella Typhi*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
9.	*Berberis aristata* (Rasaunt)	Berberidaceae	Extract	a. RWP	+ve	-ve	-ve	-ve	-ve
				b. Market	+ve	+ve	-ve	+ve	+ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	-ve	+ve	-ve	-ve	+ve
10.	*Brassica juncea* (Tukhm-i-Rai)	Cruciferae	Seed	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	+ve	+ve	+ve	-ve	-ve
11.	*Butea monosperma* (Gondpalas)	Berseraceae	Gum	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				d. Market	-ve	-ve	+ve	-ve	+ve
				c. LHR	-ve	-ve	+ve	-ve	+ve
12.	*Caccinia glauca* (Bargi-Gaozaban)	Boraginaceae	Leaf	a. RWP	-ve	-ve	-ve	-ve	+ve
				b. KHI	-ve	-ve	+ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	-ve	+ve	+ve	-ve	+ve
13.	*Cassia absus* (Chaksu)	Leguminosae	Seed	a. RWP	-ve	-ve	-ve	-ve	+ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	+ve	+ve	-ve	-ve	-ve
				d. Market	+ve	+ve	-ve	-ve	-ve
14.	*Carum copticum* (Ajwain desi)	Umbelliferae	Seeds	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	-ve	+ve	+ve	-ve	+ve
15.	*Colchicum luteum* (Soranjan)	Colchicaceae	Bulb	a. RWP	+ve	+ve	-ve	-ve	-ve
				b. Market	+ve	+ve	-ve	-ve	+ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	-ve	+ve	+ve	-ve	-ve
16.	*Curculigo orchiodes* (Musli siah)	Commelenaceae	Roots	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	+ve	+ve	+ve	-ve	-ve

Table 1. (Cont'd.)

S. No.	Botanical name (Local name)	Family	Part used	Place of collection	*Escherichia coli*	*Salmonella Typhi*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Candida albicans*
17.	*Embella ribes* (Baobarang)	Myrcinaceae	Fruit	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	+ve	+ve	-+ve	-ve
				c. LHR	-ve	+ve	-ve	-ve	-ve
				d. Market	-ve	+ve	-ve	-ve	+ve
18.	*Ferula assafoetida* (Heeng)	Umbelliferae	Resin	a. RWP	-ve	+ve	-ve	-ve	-ve
				b.KHI	-ve	-ve	+ve	-ve	-ve
				c. LHR	-ve	-ve	+ve	+ve	-ve
				d. Market	+ve	+ve	-ve	-ve	+ve
19.	*Foeniculum vulgaare* (Badyan)	Umbelliferae	Fruit	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	+ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	+ve	+ve	-ve	-ve	+ve
20.	*Fumaria parviflora* (Shahtra)	Fumeriaceae	Aerial parts	a. RWP	-ve	+ve	-ve	-ve	-ve
				b. KHI	-ve	+ve	-ve	-ve	-ve
				c. LHR	-ve	+ve	-ve	-ve	-ve
				d. Market	-ve	+ve	-ve	-ve	+ve
21.	*Nigella sativa* (Kalaongi)	Ranunculaceae	Seed	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. Market	-ve	+ve	-ve	-ve	-ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	-ve	-ve	-ve	-ve	-ve
22.	*Peucedanum graveolens* (Soya)	Umbelliferae	Seed	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. KHI	-ve	-ve	-ve	-ve	-ve
				c. LHR	-ve	-ve	-ve	-ve	-ve
				d. Market	+ve	+ve	-ve	-ve	+ve
23.	*Swertia chirata* (Charaita)	Gentianaceae	Aerial Parts	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. Market	+ve	-ve	+ve	-ve	+ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	-ve	-ve	-ve	-ve	-ve
24.	*Viola odorata* (Binafsha)	Violaceae	Aerial Parts	a. RWP	-ve	-ve	-ve	-ve	-ve
				b. Market	+ve	-ve	-ve	-ve	+ve
				c. KHI	-ve	-ve	-ve	-ve	-ve
				d. LHR	+ve	+ve	-ve	+ve	-ve

Table 3. Total ash % of twenty four medicinal plants.

S. No.	RWP (%w/w)	KHI (%w/w)	LHR (%w/w)	Market (%w/w)	Range (%w/w)	Mean
1.	8.2	10.7	7.6	8.9	7.6-10.7	8.85 ± 1.34
2.	1.7	2.9	5.8	2.2	1.7-5.8	3.15 ± 1.83
3.	3.4	3.3	2.6	3.3	2.6-3.4	3.15 ± 0.37
4.	26.9	20.3	22.4	22.5	20.3-26.9	23.02 ± 2.8
5.	5.1	4.9	5.7	5.9	4.9-5.9	5.40 ± 0.48
6.	5	3.3	5.4	5.7	3.3-5.7	4.90 ± 1.13
7.	8.5	10.4	8.7	8.5	8.5-10.4	9.02 ± 0.92
8.	8.5	4	4.4	4.8	4-8.5	5.43 ± 2.08
9.	5.3	6.9	4.8	5.6	4.8-6.9	5.65 ± 0.90
10.	4	4.4	4.8	3.6	3.6-4.8	4.20 ± 0.52
11.	18.7	14	16.6	14.6	14-18.7	15.98 ± 2.1
12.	8.4	7.5	7.1	7.6	7.1-8.4	7.65 ± 0.54
13.	2.7	0.4	0.3	1.7	0.3-2.7	1.28 ± 1.14*
14.	4.3	5.3	4.9	5.1	4.3-5.3	4.90 ± 0.43
15.	16.5	19.6	14.2	12.3	12.3-19.6	15.56 ± 3.1
16.	19.6	14.6	1.4	5	1.4-19.6	10.15 ± 8.4*
17.	9.34	7.3	7.8	11.8	7.3-11.8	11.56 ± 5.6
18.	5	6.3	7.2	6	5-7.2	6.13 ± 0.91
19.	16.2	9.6	12.7	4.8	4.8-16.2	10.83 ± 4.8
20.	10.8	10.2	10	6.4	6.4-10.8	9.35 ± 2.0
21.	3.1	2.9	3.4	7.1	2.9-7.1	4.13 ± 2.0
22.	4.7	5.3	4.9	2.8	2.8-5.3	4.43 ± 1.1
23.	11.4	26.6	10.6	10.3	10.3-26.6	14.73 ± 7.9*
24.	2.5	1.7	1.9	1.2	1.2-2.5	1.83 ± 0.54

*Shows values are significant at *p*-value 0.05

Table 4. Acid insoluble ash % age of twenty four medicinal plants.

S. No.	RWP (%w/w)	KHI (%w/)	LHR (%w/)	Market (%w/)	Range (%w/w)	Mean
1.	0.06	0.4	0.8	0.5	0.06-0.8	0.46 ± 0.30
2.	0.05	0.4	1.4	1.2	0.05-1.4	0.76 ± 0.64*
3.	0.03	0.01	0.037	0.7	0.01-0.7	0.19 ± 0.34*
4.	14.4	11.7	12.3	10.2	10.2-14.4	12.15 ± 1.74
5.	1.4	1.2	1.5	1.4	1.2-1.5	1.38 ± 0.13
6.	1.5	1.8	1	1.6	1-1.6	1.47 ± 0.34
7.	0.8	0.3	0.05	0.1	0.05-0.8	0.32 ± 0.34*
8.	0.2	0.8	1.8	0.7	0.2-1.8	0.87 ± 0.67
9.	0.01	1.1	0.6	0.4	0.01-1.1	0.53 ± 0.45*
10.	1.7	0.9	0.9	2.5	0.9-2.5	1.50 ± 0.77
11.	9	5	2.7	4.8	2.7-9	5.38 ± 2.63*
12.	2.5	2.2	3.7	3.8	2.2-3.8	3.05 ± 0.82
13.	0.2	0	0.1	0.09	0-0.2	0.10 ± 0.08
14.	2.1	2.8	1.9	2.5	1.9-2.8	2.32 ± 0.40*
15.	6.3	6.4	7	6	6-7	6.43 ± 0.42
16.	1.1	2.5	4.1	6.1	1.1-6.1	3.45 ± 2.15
17.	1.5	0.6	1.8	2.9	0.6-2.9	1.70 ± 0.95
18.	2.4	1.1	2	3.4	1.1-3.4	2.23 ± 0.95
19.	1.8	4.9	2.7	1.3	1.3-4.9	2.68 ± 1.59
20.	1.1	2.5	3.1	1.8	1.1-3.1	2.12 ± 0.87
21.	0.4	0.3	0.02	1	0.4-1	0.43 ± 0.41*
22.	0.4	0.4	1.2	1.6	0.4-1.6	0.90 ± 0.60
23.	13	14	10	11.8	10-14	12.20 ± 1.72
24.	0.02	0.1	0.05	0.8	0.02-0.8	0.24 ± 0.37*

Table 5. Alcohol soluble extract % age of twenty four medicinal plants.

S. No.	RWP (%w/w)	LHR (%w/w)	Market (%w/w)	Range (%w/w)	Mean
1.	10.7	11	8.3	8.3-11	9.97 ± 1.21
2.	20.3	7.8	13.1	7.8-20.3	12.47 ± 5.71
3.	6.5	6.1	6.9	6.1-6.9	6.42 ± 0.36
4.	2.8	4	3.1	2.8-4	3.20 ± 0.55
5.	1	2.3	1.3	1-2.3	1.72 ± 0.68
6.	2.7	3	2.4	1.7-3	2.45 ± 0.56
7.	1.2	1.1	7.9	1-7.9	2.80 ± 3.40*
8.	5	8.5	4.5	4.5-8.5	5.95 ± 1.78
9.	11.9	15	10	10-15	12.07 ± 2.11
10.	15.4	15	10.2	10.2-16.4	14.25 ± 2.76
11.	3.8	5.7	4.6	3.8-15.8	7.47 ± 5 .60
12.	5.7	10.7	5.5	5.5-10.7	7.95 ± 2.73
13.	0	0	0	0	0
14.	17.7	15.8	14.8	14.8-17.7	16.23 ± 1.23
15.	5.8	5.9	5.5	5.5-7	6.05 ± 0.66
16.	31.6	10.3	27.6	10.3-31.6	21.90 ± 9.58
17.	7.9	10	9	7.9-10	8.73 ± 0.98
18.	3.6	17	20.7	3.6-20.7	11.95 ± 8.20
19.	3.9	4	7.8	3.9-7.8	5.43 ± 1.86
20.	4.5	4.1	3.6	3.6-4.5	4.05 ± 0.37
21.	35	31.5	20	16-31.5	25.63 ± 9.07
22.	33.67	38.3	26	26-56.1	98.5 ± 112.1*
23.	8.8	13.7	12.2	8.8-13.7	11.0 ± 2.34
24.	1.8	0.1	2.1	0.1-2.1	1.24 ± 0.92

*Shows values are significant at *p*-value 0.05

Table 6. Water soluble extract % age of twenty four medicinal plants.

S. No.	RWP (%w/w)	KHI (%w/w)	LHR (%w/w)	Market (%w/w)	Range (%w/w)	Mean
1.	21.3	24.6	20.3	19.1	19.1-24.6	21.32 ± 2.4
2.	25.6	6.8	6.7	9.8	6.8-25.6	12.23 ± 9.0
3.	18.6	19.2	19.3	22.9	18.6-22.9	20.0 ± 1.96
4.	33.3	41	33.9	33.5	33.3-41	35.43 ± 3.7
5.	12.9	18.1	20.3	15.6	12.9-20.3	16.72 ± 3.2
6.	33.7	54.1	49.6	44	33.7-49.6	45.35 ± 8.0
7.	15.9	16.1	17.1	16.5	15.9-17.1	16.40 ± 0.5
8.	19.7	14.7	20.3	18.8	14.7-20.3	18.37 ± 2.5
9.	18.6	21.6	28.9	20.7	18.6-28.9	22.45 ± 4.5
10.	11.3	12.9	12.3	11	11-12.9	11.88 ± 0.9
11.	23.8	22.2	34.2	25.2	22.2-34.2	26.35 ± 5.4
12.	7.6	12.8	10	6.2	6.2-12.8	9.15 ± 2.90
13.	82.4	83.6	83.73	30	30-83.73	69.93 ± 26.63
14.	42	39	34	28	28-42	35.75 ± 6.13
15.	19.7	35.1	20.4	18.1	18.1-35.1	23.32 ± 7.91
16.	20	17.8	12.1	11.8	11.8-20	15.42 ± 9.11
17.	13.2	13	17.9	17.8	13-17.9	15.47 ± 2.74
18.	18.1	20	9.5	11.1	9.5-20	14.68 ± 5.15
19.	19.4	12.7	12.6	18.3	12.6-19.4	15.75 ± 3.61
20.	25.3	41	39.4	62.1	25.3-62.1	41.95 ± 15.17
21.	70	67.2	70.5	58.3	58.3-70.5	66.50 ± 5.66
22.	7.4	11.5	13.5	8.6	7.4-13.5	10.25 ± 2.77
23.	74	59	53	64	53-74	50.5 ± 31.0
24.	9.5	6.6	5.6	4.9	4.9-9.5	6.65 ± 2.02

Table 7. Moisture count % of twenty four medicinal plants.

S. No.	RWP (%w/w)	KHI (%w/w)	LHR (%w/w)	Market (%w/w)	Range (%w/w)	Mean
1.	6.7	7.8	6.7	2.3	2.3-7.8	5.87 ± 2.4
2.	9.8	9.9	8.7	9.7	8.7-9.9	9.52 ± 0.6
3.	10.3	10.2	10.07	9.12	9.12-10.3	9.92 ± 0.5
4.	9.96	9.23	9.81	10.9	9.23-10.9	9.97 ± 0.7
5.	8.08	10.2	9.9	8.29	8.08-10.2	9.12 ± 1.09
6.	8.9	9.5	9.8	9.1	8.9-9.8	9.33 ± 0.40
7.	7.62	8.93	8.22	10.04	7.62-10.04	8.70 ± 1.04
8.	5.9	10.8	10.5	6	5.9-10.8	8.30 ± 2.7
9.	11.1	11.82	12.51	17.2	11.1-17.2	13.16 ± 2.8
10.	9.1	8.2	8.6	8	9.1-8	8.48 ± 0.5
11.	9.1	9.3	9.6	10.9	9.1-10.9	9.73 ± 0.8
12.	8.8	12.8	7.8	11.3	7.8-12.8	10.18 ± 2.3
13.	12.2	12	12.4	8.27	8.27-12.4	11.22 ± 1.9
14.	12	14.6	8.1	15.4	8.1-15.4	12.53 ± 3.3
15.	8.1	7	8	8.5	7-8.5	7.90 ± 0.6
16.	6.5	8.6	7.9	14.62	6.5-14.62	9.41 ± 3.6
17.	9.6	9.8	9.7	9.5	9.5-9.8	9.65 ± 0.1
18.	6.4	6.5	5.9	7.1	5.9-7.1	6.48 ± 0.5
19.	9.1	8.3	8.6	11.3	8.3-11.3	9.33 ± 1.4
20.	9.7	10	9.2	7.6	7.6-10	9.12 ± 1.07
21.	8.9	7.4	7.3	10.02	7.3-10.02	8.41 ± 1.30
22.	6.2	6.2	6.1	9.6	6.1-9.6	7.02 ± 1.7
23.	8.1	7.6	12.3	8.7	7.6-12.3	9.18 ± 2.1
24.	8.2	9.4	8.5	7.5	7.5-9.4	8.40 ± 0.8

Table 8. Lead contents (in ppm) of twenty four medicinal plants.

S. No.	RWP	KHI	LHR	Market	Range	Mean
1.	0.1180	0.0176	0.1188	0.5441	0.0176-0.5441	0.2 ± 0.2*
2.	0.1420	0.1240	0.0777	0.3663	0.0777-0.3663	0.18 ± 0.1
3.	0.0220	0.4200	0.1701	0.6677	0.0220-0.6677	0.32 ± 0.3*
4.	0.1105	0.2124	0.1100	0.4499	0.1100-0.4499	0.22 ± 0.2
5.	0.1042	0.2316	0.2212	0.4548	0.1042-0.4548	0.25 ± 0.2
6.	0.1028	0.0344	0.0222	0.0689	0.0222-0.1028	0.06 ± 0.04
7.	0.1141	0.1321	0.1252	0.3390	0.1141-0.3390	0.18 ± 0.1
8.	0.1199	0.2218	0.0200	0.8813	0.0200-0.8813	0.31 ± 0.4*
9.	0.1108	0.0388	0.1322	0.3440	0.0388-0.3440	0.16 ± 0.1
10.	0.0182	0.0222	0.0206	0.1247	0.0182-0.1247	0.05 ± 0.05*
11.	0.1122	0.1042	0.1296	0.5399	0.1042-0.5399	0.22 ± 0.21*
12.	0.1013	0.0311	0.0116	0.0662	0.0116-0.1013	0.05 ± 0.04
13.	1.0348	0.0486	0.0216	0.0666	0.0216-1.0348	0.29 ± 0.5*
14.	0.1601	0.0212	0.0326	0.5542	0.0212-0.5542	0.19 ± 0.3*
15.	0.2229	0.1322	0.1226	1.0600	0.1226-1.0600	0.38 ± 0.5*
16.	0.1112	0.0470	0.0286	0.0508	0.0286-0.0508	0.06 ± 0.04
17.	0.2244	0.3332	0.1116	0.0442	0.0442-0.3332	0.18 ± 0.1
18.	0.1175	0.2242	0.0826	0.0554	0.0554-0.2242	0.12 ± 0.07
19.	0.0111	0.0621	0.0216	0.5120	0.0111-0.5120	0.15 ± 0.2*
20.	0.6183	0.0341	0.0986	0.1782	0.0341-0.1782	0.23 ± 0.3*
21.	0.0522	0.2406	0.1256	0.4200	0.0522-0.4200	0.21 ± 0.2*
22.	0.1133	0.0110	0.0860	0.3292	0.0110-0.3292	0.13 ± 0.1*
23.	0.2266	0.1111	0.0891	0.5040	0.0891-0.5040	0.23 ± 0.2*
24.	0.2420	0.2211	0.2777	0.3244	0.2211-0.3244	0.27 ± 0.1

*Shows values are significant at *p*-value 0.05

Table 9. Arsenic contents (in ppm) of twenty four medicinal plants.

S. No.	RWP	KHI	LHR	Market	Range	Mean
1.	0.1316	0.2460	0.2177	0.3041	0.0146-0.0441	0.22 ± 0.1
2.	0.2112	0.2210	0.1760	0.2650	0.0132-0.0660	0.22 ± 0.04*
3.	0.3660	0.2566	0.1717	0.4784	0.0259-0.0783	0.32 ± 0.1
4.	0.1662	0.1820	0.1888	0.2462	0.1662-0.2462	0.20 ± 0.03
5.	0.1224	0.1110	0.1216	0.2201	0.1110-0.2201	0.14 ± 0.05
6.	0.1248	0.1341	0.1220	0.4064	0.1220-0.4064	0.20 ± 0.1
7.	0.1131	0.2221	0.1242	0.2190	0.1131-0.2221	0.17 ± 0.1
8.	0.1014	0.1218	0.1216	0.3899	0.1014-0.3899	0.18 ± 0.1
9.	0.2132	0.1288	0.1116	0.4448	0.1116-0.4448	0.22 ± 0.2
10.	0.1182	0.1311	0.1206	0.3222	0.1182-0.3222	0.17 ± 0.10
11.	0.1092	0.1444	0.2228	0.4348	0.1092-0.4348	0.23 ± 0.2
12.	0.1017	0.1031	0.1016	0.5602	0.1016-0.5602	0.22 ± 0.2*
13.	0.1245	0.1048	0.10216	0.0666	0.0666-0.1245	0.10 ± 0.02
14.	0.0942	0.0110	0.0322	0.5542	0.0110-0.5542	0.17 ± 0.3*
15.	0.1141	0.1322	0.1226	0.3815	0.1141-0.3815	0.19 ± 0.1
16.	0.1441	0.2300	0.2206	0.5508	0.1441-0.5508	0.29 ± 0.2
17.	0.1132	0.1314	0.0116	0.3442	0.0116-0.3442	0.05 ± 0.1*
18.	0.1007	0.1022	0.1081	0.4055	0.1007-0.4055	0.18 ± 0.2*
19.	0.1200	0.1121	0.1259	0.3180	0.1121-0.3180	0.17 ± 0.10
20.	0.1281	0.2341	0.1026	0.6762	0.1026-0.6762	0.29 ± 0.3*
21.	0.0852	0.1406	0.0266	0.7140	0.0266-0.7140	0.24 ± 0.3*
22.	0.2066	0.3110	0.3160	0.8299	0.2066-0.8299	0.42 ± 0.3
23.	0.2019	0.4111	0.0993	0.6049	0.0993-0.6049	0.33 ± 0.2
24.	0.2004	0.1022	0.2777	0.4999	0.1022-0.4999	0.27 ± 0.2

*Shows values are significant at *p*-value 0.05

Table 10. Cadmium contents (in ppm) of twenty four medicinal plants.

S. No.	RWP	KHI	LHR	Market	Range	Mean
1.	0.0010	0.0006	0.0018	0.0041	0.0006-0.0041	0
2.	0.0130	0.0100	0.0017	0.0060	0.0017-0.0130	0.01 ± 0*
3.	0.0010	0.0024	0.0080	0.0071	0.0010-0.0080	0
4.	0.0018	0.0012	0.0018	0.0082	0.0018-0.0082	0
5.	0.0020	0.0011	0.0016	0.0040	0.0011-0.0040	0
6.	0.0040	0.0044	0.0022	0.0080	0.0022-0.0080	0
7.	0.0015	0.0021	0.0030	0.0036	0.0015-0.0036	0
8.	0.0040	0.0012	0.0024	0.0022	0.0012-0.0040	0
9.	0.0010	0.0080	0.0016	0.0040	0.0012-0.0040	0
10.	0.0040	0.0017	0.0006	0.0024	0.0006-0.0040	0
11.	0.0040	0.0016	0.0022	0.0032	0.0016-0.0032	0.01 ± 0.01*
12.	0.0030	0.0011	0.0016	0.0062	0.0016-0.0062	0
13.	0.0010	0.0006	0.0016	0.0066	0.0006-0.0066	0
14.	0.0020	0.0010	0.0020	0.0042	0.0010-0.0042	0
15.	0.0060	0.0022	0.0022	0.0044	0.0022-0.0060	0
16.	0.0020	0.0008	0.0010	0.0008	0.0008-0.0020	0
17.	0.0020	0.0014	0.0016	0.0042	0.0014-0.0042	0
18.	0.0012	0.0012	0.0016	0.0054	0.0012-0.0054	0
19.	0.0010	0.0021	0.0021	0.0018	0.0010-0.0021	0
20.	0.0020	0.0031	0.0026	0.0021	0.0021-0.0031	0
21.	0.0022	0.0008	0.0011	0.0026	0.0008-0.0031	0
22.	0.0021	0.0010	0.0016	0.0028	0.0010-0.0028	0
23.	0.0010	0.0011	0.0031	0.0043	0.0010-0.0043	0
24.	0.0020	0.0021	0.0027	0.0049	0.0020-0.0049	0

*Shows values are significant at *p*-value 0.05

Table 11. Mercury (Hg) contents (in ppm) of twenty four medicinal plants.

S. No.	RWP	KHI	LHR	Market	Range	Mean
1.	0.0382	0.0146	0.0188	0.0441	0.0146-0.0441	0.03 ± 0.01
2.	0.0132	0.0221	0.0176	0.0660	0.0132-0.0660	0.03 ± 0.02
3.	0.0366	0.0259	0.0783	0.0678	0.0259-0.0783	0.05 ± 0.02
4.	0.0086	0.0112	0.0188	0.0482	0.0086-0.0482	0.02 ± 0.02*
5.	0.0442	0.0311	0.0216	0.0542	0.0216-0.0542	0.04 ± 0.01
6.	0.0248	0.0344	0.0222	0.0689	0.0248-0.0689	0.04 ± 0.02
7.	0.0431	0.0321	0.0232	0.0396	0.0232-0.0431	0.03 ± 0.01
8.	0.0142	0.0212	0.0246	0.0842	0.0142-0.0842	0.02 ± 0.04*
9.	0.0042	0.0288	0.0316	1.0446	0.0042-1.0446	0.28 ± 0.51*
10.	0.0182	0.0011	0.0206	0.0242	0.0182-0.0242	0.02 ± 0.01
11.	0.0042	0.0411	0.0226	0.0342	0.0042-0.0441	0.03 ± 0.02
12.	0.0173	0.0311	0.0116	0.0602	0.0116-0.0602	0.03 ± 0.02
13.	0.0348	0.0486	0.0216	0.0666	0.0216-0.0666	0.04 ± 0.02
14.	0.0942	0.0110	0.0326	0.0542	0.0110-0.0542	0.05 ± 0.04
15.	0.0144	0.0322	0.0226	0.0864	0.0144-0.0864	0.04 ± 0.03
16.	0.0442	0.0378	0.0206	0.0508	0.0206-0.0508	0.04 ± 0.01
17.	1.0142	0.0311	0.0116	0.0442	0.0116-1.0142	0.28 ± 0.49*
18.	0.0075	0.0242	0.0816	0.0554	0.0075-0.0816	0.04 ± 0.03
19.	0.0220	0.0621	0.0216	0.0180	0.0180-0.0220	0.03 ± 0.02
20.	0.0281	0.0341	0.0286	0.0762	0.0281-0.0762	0.04 ± 0.02
21.	0.0052	0.0406	0.0256	0.0142	0.0052-0.0406	0.02 ± 0.02*
22.	0.0066	0.0110	0.0160	0.0292	0.0216-0.0292	0.02 ± 0.01
23.	0.0199	0.0111	0.0091	1.0043	0.0091-1.0043	0.26 ± 0.50*
24.	0.0042	0.0022	0.0277	0.0249	0.0042-0.0277	0.01 ± 0.01*

*Shows values are significant at *p*-value 0.05

The reports that some herbal products contain potentially harmful adulterants or having widely varying amounts of ingredients have heightened these concerns (Ko, 1998; Slifman *et al.,* 1998; Cui *et al.,* 1994; Anon., 1995, Hussain *et al*., 2011). The widespread public opinion is that being natural products, the herbal medicines are harmless, free from adverse effects and so even if the expected medical effect is not achieved, their consumption is not dangerous and WHO presumes that popularity of herbal medicines is connected with their ease access, therapeutic efficacy, relatively low cost and the assumption for absence of side toxic effects (Anon., 2002). The presence of large numbers of selected pathogenic bacteria in the analyzed samples of medicinal plants in this study may be due to process of harvesting, drying, storage, handling and the soil influence the which in turns affects the entire quality of the herbal preparation. Thus, manufacturers should ensure the highest possible level of hygiene during manufacturing as well as the lowest possible level of pathogenic organisms in their herbal products so as to maintain correct quality, safety and efficacy of the final herbal preparations. In the present study, the herbal medicinal plants contained high levels of bacteria, and the counts were beyond the European Pharmacopoeia stated limit, and also carried pathogenic Gram negative bacteria (such as *Salmonella* and *E. coli*) that are expected to be absent. Coliform such as *E. coli* are the most reliable indicators of faecal contamination, thus the test for their presence is an index of the degree of faecal contamination, which may indicate a possible presence of harmful disease-causing organisms (Anon., 1992). These bacteria constitute the intestinal flora of humans and other animals, and are therefore used as indicator organisms and as an index of possible contamination by human pathogens (Anon., 1992). The significance of faecal bacteria is that if these specific bacteria are present then other harmful microorganisms may also be present, such as *Salmonella* (Forest, 2004). Therefore, the high recovery rates of these suspected perilous bacteria from indigenous orally consumed herbal medications could be of clinical relevance.

The analytical results also showed foreign organic matter 0-51.3% and fourteen samples showed variation which was found to be significant. Total ash value of all samples varies from 1-2-26.6% and three samples had shown significant variation. The amounts of acid insoluble matter present in the plants were 0-14.4% and eight samples had also shown significant variation. The water-soluble extractive value was indicating the presence of sugar, acids and inorganic compounds. The water soluble extractive values in the samples were 4.9-83.73% and alcohol soluble extractive value was 0.1-56.1% with two samples had significant variations. The alcohol soluble extractive values indicated the presence of polar constituents like phenols, alkaloids, steroids, glycosides, flavonoids and secondary metabolites present in the plant sample. The results of the moisture

content showed that there was remarkable variation amongst the different medicinal plants samples. 72 (75%) of the samples have greater than 8% moisture contents. European Agency for the Evaluation of Medicinal products (1998) suggested that water content should be included in the list of comprehensive specifications for herbal medicinal products especially the powdered forms. The maximum moisture content limit of 8% /g of herbal preparations are satisfactory according to National Agency for Food and Drug Administration and Control (NAFDAC SOP, 2000). The bacterial counts observed in 75% medicinal plants with high moisture contents were high, suggesting that high moisture contents favored the growth of non-pathogenic as well as pathogenic bacteria in samples analyzed. High counts of harmful microorganisms such as *Salmonella* species may affect the human health and drug quality and therefore it becomes imperative to improve pl ant material quality and establishing better hygienic conditions during medicinal plants supply chain. Similarly, the low bacterial counts in the other samples could be attributed to very low moisture contents. A problem that now occurs is that traditional village-based activities have been expanded into the global marketplace and this may bring with it difficulties in controlling the quality, composition and purity of herbal supplies (Ernst, 2002).

All analyzed samples contained detectable levels of arsenic, cadmium, mercury and lead. Arsenic was found at levels up to 0.0132-0.8990 ppm, cadmium up to 0.0006-0.0082 ppm, mercury 0.0042-1.0446 ppm and lead up to 0.0116-1.0600. *Acasia nilotica* and *Fumaria paviliflora* purchased from Rawalpindi and open market were found to contain higher than the limit while three samples i.e., *Acocus calamus, Peucedanum graveolens* and *Berberis aristata* purchased from open market contains higher than limit prescribed by WHO. In all other samples the concentrations of As, Cd, Hg and Pb do not exceed the limits recommended for medicinal plants (Anon., 1999). The concentrations of arsenic, cadmium and lead found in this work were similar to the described for medicinal plants in Poland (Łozak *et al.,* 2002), Egypt (Abou-Arab *et al.,* 1999), Argenina (Gomez *et al.,* 2007), United States (Khan *et al.,* 2001). In Brazil, from the analyzed 10 types of medicinal plants some samples of *horse chestnut, centella asiatic* and G*inco biloba* had cadmium and lead concentrations higher than the permitted level (Caldas & Machado, 2004). In Italy, the concentrations of Pb measured in 79 samples of various herbal medicines were in the same order as in the present paper while for Cd concentrations up to 0.75 mg/kg were reported (De Pasquale *et al.,* 1993). It has also been reported that heavy metals, accumulated naturally in soil, surface water or through industrial and mining processes, pose a potential threat to various terrestrial and aquatic organisms (Greeger 1999**;** Larison *et al.,* 2000; Dwivedi & Dey 2002; Hsu *et al.,* 2006; Dhir *et al.,* 2008). Exposure to high metal concentrations impinges on the growth and development of plants (Rout & Das, 2003; Shanker *et al.,* 2005; Dhir *et al.,* 2009). Such growth effects result from alterations in physiological events such as photosynthesis, respiration, changes in lipid composition, enzyme activity, and distribution of macro and micronutrients at the cellular level (Sheoran *et al.,* 1990; Van Assche & Clijsters 1990; Rout & Das 2003; Shanker *et al.,* 2005, Sarwat *et al*., 2012).

Conclusion

It may be concluded from this study that most of the medicinal plants are contaminated with a wide variety of potentially pathogenic bacteria, some with higher levels of arsenic and mercury and it has become imperative that the quality assurance system should be thoroughly enforced in the medicinal plants supply chain i.e., cultivation, collection and distribution. WHO has developed a series of technical guidelines relating to the quality control of herbal medicines of which these WHO guidelines on good agricultural and collection practices (WHO, 2003) for medicinal plants are the latest. The guidelines provide a detailed description of the techniques and measures required for the appropriate cultivation and collection of medicinal plants and for the recording and documentation of necessary data and information during their processing. Despite such guidelines, there is still considerable disparity between knowledge and implementation. For example, it is a difficult task to train farmers and other relevant persons as producers, handlers and processors of medicinal plant materials. While pharmaceutical and other companies are striving to meet the requirements for the quality control of herbal medicines, they cannot force farmers, producers, handlers and processors to follow good agricultural and collection practices for medicinal plants. The training of farmers and other relevant persons is therefore one of many important measures to be taken to ensure that good agricultural and collection practices are adopted in order that medicinal plant materials of high quality are obtained. Quality control directly impacts the safety and efficacy of herbal medicinal products. Good agricultural and collection practices for medicinal plants is only the first step in quality assurance, on which the safety and efficacy of herbal medicinal products directly depend upon, and will also play an important role in the protection of natural resources of medicinal plants for sustainable use.

Acknowledgements

We are thankful to the staff of National Institute of Health, Islamabad, Pakistan and Wilson Pharmaceutical (Pvt), Islamabad, Pakistan for their sincere hard work and cooperation to materialize this study.

Competing interests: The author(s) declare that they have no competing interests.

References

Abou-Arab, A.A.K.., M.S. Kawther, E.l. Tantawy, M.E. Badeaa and R.I. Khayria. 1999. Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chemistry*, 67: 357-363.

Adeleye, I.A., G. Okogi and E.O. Ojo. 2005. Microbial contamination of herbal preparations in Lagos, Nigeria. *J. Health, Population and Nutrition*, 23(3): 296-297.

Anonymous. 1992. American Public Health Association (APHA). *Standard methods for the examination of water and wastewater,* 18th Ed., American Public Health Association, Washington D.C: 9-66.

Anonymous. 1995. AOAC. *Official methods of analysis of AOAC International,* 16th edition. AOAC International, Suite 400, 2200 Wilson Boulevard, Arlington, Virginia, USA.

Anonymous. 1995. Herbal roulette. Consumer Reports, 698-705.

Anonymous. 1999. World Health Organization: WHO. *Monographs on Selected Medicinal Plants.* Volume 1. Geneva: World Health Organization.

Anonymous. 2002. World Health Organization: WHO. *Monographs on Selected Medicinal Plants.* Volume 2. Geneva: World Health Organization.

Anonymous. 2003. WHO guidelines on good agricultural and field collection practices (GACP) for medicinal plants. Geneva, World Health Organization,

Anonymous. 2007. United States Pharmacopeia. *Microbial limits tests. In: United States Pharmacopeia*, 30th Revision, United States Pharmacopeia convention, Philadelphia. p. 83.

Anonymous. 2007. World Health Organization (WHO). *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residue.* Geneva: WHO.

Blumenthal, M. 1997. Industry alert: plantain adulterated with digitalis. *Herbalgram*, 40: 28-9 (letter).

Caldas, E.D. and L.L. Machado. 2004. Cadmium, mercury and lead in medicinal herbs in Brazil. *Food and Chemical Toxicology*, 42: 599-603.

Corbin Winslow, L. and H. Shapiro. 2002. Physicians want education about complementary and alternative medicine to enhance communication with their patients. *Arch. Intern. Med*., 162: 1176-81.

Cui, J., M. Garle, P. Eneroth and I. Björkhem. 1994. What do commercial ginseng preparations contain? *Lancet.,* 344:134 (letter).

De Pasquale, A., E. Paino, R. De Pasquale and M.P. Germano. 1993. Contamination by heavy metals in drugs from different commercial sources. *Pharmacological Research*, 27: 9-10.

Debela Hunde Feyssa1, Jesse T. Njoka, Zemede Asfaw and M.M. Nyangito. 2012. Uses and management of *Ximenia americana*, Olacaceae in semi-arid east Shewa, Ethiopia, *Pak. J. Bot.,* 44(4): 1177-1184.

Dhir, B., P. Sharmila and P. Saradhi. 2008. Photosynthetic performance of *Salvinia natans* exposed to chromium and zinc rich wastewater. *Braz. J. Plant Physiol.,* 20(1): 61-70.

Dhir, B., P. Sharmila, P. Saradhi and S.A. Nasim. 2009. Physiological and antioxidant responses of *Salvinia natans* exposed to Chromium-rich wastewater. *Ecotoxicol Environ. Saf.,* 72: 1790-1797.

Dogan, Y., U. Ilker and D. Nazmi. 2013. Wild edible plants sold in the local markets of Izmir, Turkey. *Pak. J. Bot*., 45(S1): 177-184.

Dubey, N.K., A. Kumar, S. Priyanka and S. Ravindra. 2008. Microbial contamination of raw materials: A major reason for the decline of India's share in the global herbal market. *Current Sci*., 95(6): 25-

Dubey, N.R., R. Kumar and P. Tripathi. 2004. Global promotion of herbal medicine: India's opportunity. *Current Sci.,* 86(1): 37-41.

Dwivedi, S.K. and S. Dey. 2002. Medicinal herbs: A potential source of toxic metal exposure for man and animals in India. *Arch. Environ. Health*, 57: 229-233.

Eisenberg, D.M. 1997. Advising patients who seek alternative medical therapies. *Ann Intern Med.,* 127: 61-9.

Ernst, E. 2002. Toxic heavy metals and undeclared drugs in Asian herbal medicines. *Trends Pharmacol Sci.,* 23: 136-9.

Forest, J. 2004. Fecal Coliforms. University of Iowa Hygienic Laboratory Manual. 36: 2-4.

Garrard, J., S. Harms, L.E. Eberly and A. Matiak. 2003. Variations in product choices of frequently purchased herbs: caveat emptor. *Arch. Intern. Med.,* 163: 2290-5.

Gomez, M..R., S. Cerruti, L.L. Sombra, M.M. Silva and L.D. Martinez. 2007. Determination of heavy metals for the quality control in Argentinean herbal medicines by ETAAS and ICP-OES. *Food and Chemical Toxicology*, 45: 1060-1064.

Greeger, M. 1999. Metal availability and bioconcentration in plants. In: *Heavy Metal Stress in Plants*. (Eds.): M.N.V. Prasad and J. Hagemeyer. Springer, New York, pp 1-29.

Hsu, M.J., K. Selvaraj and G. Agoramoorthy. 2006. Taiwan's industrial heavy metal pollution threatens terrestrial biota. *Environ. Pollut.,* 143: 327-334.

Hussain, J., F.U. Khan, R. Ullah, Z. Muhammad, N. Rehman, Z.K. Shinwari, I.U. Khan, M. Zohaib, I. Din and S. M. Hussain. 2011. Nutrient evaluation and elemental analysis of four selected medicinal plants of Khyber Pakhtoon Khwa, Pakistan. *Pak. J. Bot*., 43(1): 427-434.

Jonas, W.B. 1997. Researching alternative medicine. *Nat. Med.,* 3: 824-82.

Khan, I.A., J. Allgood, L.A. Walker, E.A. Abourashed, D. Schelenk and W.H. Benson. 2001. Determination of heavy metals and pesticides in ginseng products. *J. AOAC Int.,* 84: 936-939.

Khatoon, S., S. Mehrotra, U. Shome and B.N. Mehrotra. 1993. Analysis of commercial 'Ratanjot; by TLC fluorescence fingerprinting, *Int. J. Pharmacol.,* 31: 269-277.

Ko, R.J. 1998. Adulterants in Asian patent medicines. *N. Engl. J. Med.,* 339: 847.

Kudva, I.T., K. Blanch and C.J. Hovde. 1998. Analysis of E. coli O157: H7 survival in ovine or bovine manure and manure slurry. *Appl. Environ. Microbiol.,* 64: 3166-3174.

Larison, J.R., E. Likens, J.W. Fitzpatrick and J.C. Crock. 2000. Cadmium toxicity among wildlife in the Colorado rocky mountains. *Nature*, 406: 181-183.

Limyati, D.A. and B.L.L. Juniar. 1998. Jamu Gendong, a kind of traditional medicine in Indonesia: the microbial contamination of its raw materials and end product. *J. Ethnopharmacol.,* 63: 201-208.

Łozak, A., K. Sołtyk, P. Ostapczuk and Z. Fijałek. 2002. Determination of selected trace elements in herbs and their infusions. *Science of the Total Environment*, 289: 33-40.

Nadeem, M., Z.K. Shinwari and M. Qaisar. 2013. Screening of Folk remedies by genus Artemisia based on ethnomedicinal surveys and traditional knowledge of native communities of Pakistan. *Pak. J. Bot.,* 45(S1): 111-117.

National Agency for Food and Drug Administration and Control. Standard Operating Procedures (NAFDAC SOP), author Determination of moisture contents.Yaba, Lagos: Central Drugs and Vaccine Control Laboratory (CDVCL); 2000. pp. 1-2.

Rout, G.R. and P. Das. 2003. Effect of metal toxicity on plant growth and metabolism. *I. Zinc Agronomie*., 23: 3-11.

Sarwat, Z., K. Shinwari and N. Ahmad. 2012. Screening of potential medicinal plants from district Swat specific for controlling women diseases. *Pak. J. Bot.,* 44(4): 1193-1198.

Shaikh, H. Shahzad, Farnaz Malik, Henry James. Hamid Abdul. 2009. Trends in the use of complementary and alternative medicines: A population based survey. *Alter Comply Meds.* 15(5): 545-550.

Shanker, A.K., C. Cervantes, H. Loza-Tavera and S. Avudainayagam. 2005. Chromium toxicity in plants. *Environ. Int.,* 31: 739-753.

Sheoran, I., H. Singal and R. Singh. 1990. Effect of cadmium and nickel on photosynthesis and enzymes of the photosynthetic carbon reduction cycle in the pigeon pea (*Cajanus cajan*). *Photosynth Res.,* 23: 345-351.

Shinwari, Z.K. and M. Qaisar. 2011. Efforts on conservation and sustainable use of medicinal plants of Pakistan. *Pak. J. Bot*., 43 (Special Issue): 5-10.

Shinwari, Z.K., M. Saleema, R. Faisal, S. Huda and M. Asrar. 2013. Biological screening of indigenous knowledge based plants used in diarrheal treatment. *Pak. J. Bot*., 45(4): 1375-1382.

Sleath, B., R.H. Rubin, W. Campbell, L. Gwyther and T. Clark. 2001. Ethnicity and physician-older patient communication about alternative therapies. *J. Altern. Complement Med*., 7: 329-35.

Slifman, N.R., W.R. Obermeyer and B.K. Aloi. 1998. Contamination of botanical dietary supplements by Digitalis lanata. N. *Engl. J. Med.,* 339: 806-11.

Smith, M. and H.S. Boon. 1999. Counseling cancer patients about herbal medicine. *Patient Educ Couns*. 38(2): 109-20.

Van Assche, F. and H. Clijsters. 1990. Effect of metals on enzyme activity in plants. *Plant Cell Environ.,* 13: 195-206.

Walter, C., Z.K. Shinwari, I. Afzal and R.N. Malik. 2011. Antibacterial activity in herbal products used in Pakistan. *Pak. J. Bot*., 43(Special Issue): 155-162.

Yadav, N.P. and V.K. Dixit. 2008. Recent approaches in herbal drug standardization, *Int. J. Integrated Biology*, 2(3): 195.

(Received for publication 25 April 2012)