Assessment of heavy metal ions, essential metal ions, and antioxidant properties of the most common herbal drugs in Indian Ayurvedic hospital: For ensuring quality assurance of certain Ayurvedic drugs

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ABSTRACT

During the last few years, World Health Organisation (WHO) has emphasized for the quality control for herbal drugs. The present study was designed and executed to check the heavy metal ions content in the most commonly used Ayurvedic/herbal drugs in Indian Ayurvedic hospitals. Based upon the medicinal applications, we have examined 23 most common and important drug sample contained 7 vati, 5 guggulu, 7 churna, and 4 lauha samples. Essential metal ions, antioxidant and protein binding qualities of drug samples under study were analyzed. Metal ions (heavy metal and essential metal ions) were determined by using the Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The antioxidant potential was evaluated through spectrophotometric analysis by using 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating (MC), ferric reducing (FRAP), Scavenging activity of superoxide anion (SA) and nitric oxide (NO) assays. The observed total phenolic content of the samples varied from 14.91 to 208.34 mg gallic acid equivalent (GAE)/g dry weight (DW) of sample(s). The values of total flavanoids content were varied from 48.0 to 208.0 mg quercetin equivalent (QE)/g DW of sample(s). As, compared to control (ascorbic acid), herbal drug samples have shown excellent antioxidant activities over a range from 40.51 to 92.34%. The observed protein binding constants values were almost close to control (aspirin). It was noticed that maximum drugs have a good source of essential metal ions like Na, Mg, Ca, Fe, Cu, Zn etc. The observed concentration values for the heavy metal ions were within the prescribed limits of WHO and API. Only two drugs contained aluminium concentrations more than 50 mg/kg. These findings may validate and boost up the quality control process.

1. Introduction

Plants and their secondary molecules are the unique gift of God to humans. In 21st century, though people may not believe, but that is true, the most under-developed and developing countries are relying upon the traditional medicines (Jin et al., 2018; Kota et al., 2017; Newman and Cragg, 2016; Sridevi et al., 2018a). Secondary biomolecules of plants are considered as major therapeutic agents to cure the diverse class of disease (Apak et al., 2016; Chakraborty, 2018; Kumar et al., 2018a,b,c,d; Newman and Cragg, 2016). Despite of a glorious journey of synthetic Chemistry, Chemists are not quite successful in

synthesising medicines those were gifted by the nature or extracted from the plants, for example morphine, taxol, atropine, ephedrine etc. (Mulaudzi et al., 2017; Newman and Cragg, 2016). Most of the plant-derived drugs contained nutritional as well as medicinal values (Chakraborty, 2018; Mulaudzi et al., 2017; Newman and Cragg, 2016). The presence of multiclass secondary metabolites like polyphenols and flavonoids made them unique due to their medicinal, antioxidant and nutritional values (Ahmed et al., 2018; Bhadane and Patil, 2017; Chavan et al., 2018; Devi et al., 2016). These herbal products are very good alternatives of synthetic antioxidant or supplements (Chakraborty, 2018; Kumar et al., 2018c, 2018d; Newman and Cragg,

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2016). Presence of antioxidant in drugs makes it more important because such a drug may cure the specific disease and suppress the various redox reactions of free radicals (Chakraborty, 2018; Kumar et al., 2018c, 2018d; Pulido et al., 2000). Herbal drugs are a mixture of one or more plants or their different parts and these formulations are more vital w.r.t. medicinal, nutrient, and antioxidant values (Sridevi Vijayaraghavan et al., 2018b).

Recent years have seen spurt in use of herbal drugs both in developing and developed countries. Using herbal products including the herbal/traditional drugs is gaining popularity worldwide (Chakraborty, 2018). It is considered that India and China are the hubs of traditional medicines (Apak et al., 2016; Chakraborty, 2018). Indian Government is spending more budget to boost up the Indian Systems of Medicine. During the last decades the consumption of the herbal drugs has been growing in Western countries (Apak et al., 2016; Gülçin et al., 2004; Pulido et al., 2000). Economic point of view, herbal drug business is 150 million dollar (USD). India has improved its export business from USD 69 to USD 128 between the years 2005–06 to 2009–10, and it will be over 150 USD up to 2022 (Chakraborty, 2018). An increase in business may leads to the dilution in quality of herbal drugs. So, it is important to update quality control mechanism on a regular basis.

Most consumers have believed that the herbal drugs are less toxic with least side effects (Braca et al., 2002; Bhandari and Kawabata, 2004). But, due to industrialization and urbanization truth is totally different, hence natural origin of herbal drugs is not an insurance of their safety. Various cases have been noticed about the quality dilution of the traditional drugs where the limits of heavy metal and pesticides were over the prescribed limits of World Health Organisation (WHO) guidelines and The Ayurvedic Pharmacopeia of India (API) (Bressy et al., 2013; Fallah et al., 2011). Most cases were due to the poor good manufacturing practices (GMP), lack of standardization, adulteration, substitution, misidentification, inapt labelling and contamination (Gülçin et al., 2004; Pulido et al., 2000). To overcome this, most countries are trying to manufacture these drugs as per WHO and GMP guidelines (Sahoo et al., 2010).

Quality control is the major part of any pharmaceutical product, including the herbal products. In herbal industry, identification and standardization are the major part of quality control mechanism (Sahoo et al., 2010). The most common reason behind the poor quality of herbal drugs is the presence of pesticide residues, heavy metals, and microorganisms in the raw and consequently in the finished products (Bressy et al., 2013; Fallah et al., 2011; Gülçin et al., 2004; Pulido et al., 2000). Literature survey revealed that the heavy metal toxicity may cause gastrointestinal upsets, kidney damage, interstitial injuries, constipation, skin irritations, liver damage, rashes, diarrhoea, hepatotoxicity etc. (Bressy et al., 2013; Debbie et al., 2012; Fallah et al., 2011; Foguem and Foguem, 2014; Maiga et al., 2005; Mulaudzi et al., 2017; Obi et al., 2006). The main sources of the contaminations are poor agricultural practices (i.e. excess use of pesticides), air and soil pollution, poor manufacturing processes etc. (Apak et al., 2016; Mulaudzi et al., 2017; Okem et al., 2014).

In the present study, we designed and executed the current study to investigate the most commonly used 23 herbal drug samples. Drugs were selected based upon their frequency of prescription. The name of drugs and their medicinal applications are listed under Table 1. The objectives of present study were three fold i.e. (1) to check the quality (heavy metal ions) of drug samples, (2) nutritional (total phenolic, total flavanoids and antioxidant), and (3) medicinal qualities (antioxidant and protein binding) of the herbal drug samples. These drugs were supplied under the surveillance of government agency in the Indian Ayurvedic hospitals to treat various ailments. The present study may be a good concept to encourage the government agencies to check the quality of government manufacturing units. Recent case in India regarding the contamination of polio vaccines may be due to poor quality control mechanism (Internet link).

2. Experimental

2.1. Reagents and drugs collection

Analytical range (AR) grade chemicals and reagents were used and listed in supplementary section S1. Drug samples were collected from the store of the government Ayurvedic hospital. The government manufacturer had supplied these drugs. Drugs under investigation were coded (from A to W), and the name of drugs and their medicinal applications are listed under Table 1. Before a detailed study of the drug samples, we have checked basic quality control properties like packaging, labelling, expiry etc on the packets of drugs. Then drug samples were opened, reduced to the smallest size by using mixture grinder, dried and stored at room temperature (25 ± 2) °C.

2.2. Total metal analysis using ICP-OES

The drug samples were screened for the total metal ion analysis (i.e. essential and heavy metal ion analysis) by using Inductive Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The running conditions for the ICP-OES (Optima 8000; Perkin Elmer Inc., USA) instrument and microwave digestion based sample preparations were done as prescribed by the Okem et al. (2014). The operating wavelength (in nm) of each element was as: Na (589.592), Mg (285.213), Al (396.153), K (766.490), Ca (317.933), Cr (267.716), Mn (257.610), Fe (238.204), Co (228.616), Ni (231.604), Cu (327.393), Zn (206.200), Cd (228.802), As (193.696), Pb (220.353). The concentration of total mercury (Hg) was analyzed by employing atomic absorption spectroscopy (AAS) technique as prescribed by Fallah et al. (2011). All analyzes were performed in triplicate.

2.3. Preparation of extracts for antioxidant and protein binding activities

In the present study, hydro-alcoholic extract of samples was prepared. Briefly, 4 gm of powder was put into 100 ml distilled water. Mixture was shaken mechanically and the supernatant filtered after 24 h by using the Whatman filter paper No. 1. The supernatant was filtered and stored at 4–10 °C for biochemical analysis.

2.4. Antioxidant and protein binding qualities

Ultra violet (UV)-visible spectrophotometric methods were used to assay the antioxidant qualities. UV–visible spectrophotometer (Shimadzu-1800; range 190–1100 cm^{-1}) was used to carry out the biochemical tests. To warrant the results, various antioxidant assays were performed these assays were performed including the 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating (MC), ferric reducing (FRAP), Scavenging activity of superoxide anion (SA) and nitric oxide (NO) assays (Kumar et al., 2018c, 2018d; Kumar et al. 2017; Kumar et al. 2016a,b; Kumar et al. 2015b). Total phenolic content (TPC) and total flavonoids content (TFC) were analyzed by using Folin Ciocalteau reagent and $AlCl_3$ assays (Kumar et al., 2018c). The interactions of the BSA proteins with samples were checked in terms of binding constant (k) (Abdi et al., 2012; Kumar et al., 2017; 2016a; 2016b). The detailed protocols are mentioned in the Supplementary data under protocol section (S2−S5).

3. Results & discussions

3.1. Preliminary analysis of quality control and heavy metal content

Initial quality control properties like packaging, labelling, expiry etc. were performed and everything was found suitable as per API and WHO guidelines. No drug was found damaged, and all drugs were well packed to avoid any moisture and microbial load. After opening of individual drug no damage and contamination (foreign matter, moister

Table 1
List of various herbal drugs and their coding and traditional uses.

Drug Code	Name	Traditional Use	Ref.
A	Agnitundi Vati	Digestion, fever, liver, anaemia, semen defects and gynaecological problems and indigestion.	The Ayurvedic Pharmacopoeia of India (API)
B	Chandra Prava Vati	Urinary tract infection, constipation, bloating, abdominal colic pain, low back pain, cold, cough, rhinitis, bronchitis, asthma, eczema, dermatitis, pruritus, piles,	Internet sources 1: http://www.planetayurveda.com/
C	Kutajaghan Vati	Antidiarrheal, Antidysenteric, Antimicrobial, Anthelmintic, Astringent, Antiamoebic and Haemostatic.	Internet sources 2: https://www.ayurtimes.com/
D	Mahashanka Vati	Bloating of gas, Acidity, Indigestion, Diarrhoea, Peptic Ulcers, Bowel Syndrome and Ulcerative Colitis.	
E	Sanjevani Vati	Dyspepsia, Lack of digestion, Snake bite and Chronic fever.	
F	M- Sudarshan Ghan Vati	Lack of adequate sleep, hypertension and dizziness.	
G	Gokshuradi Guggul	Gout (raised uric acid), osteoarthritis, fistula and inflammatory conditions.	
H	Kaishore Guggul	Anti aging, skin health promoter, joint health, natural blood cleanser, etc.	
I	Kanchanar Guggul	Cervical lymphadentis, fibroid, cysts, tumor, tumor, ulcers, wounds distension, skin diseases etc.	
J	Punarnavadi Guggul	Gout, sciatica, low back ache, spondylosis, rheumatoid arthritis, and muscular and joint disorders.	
K	Yogaraj Guggul	Weight loss, piles, fistula and inflammatory conditions.	
L	Ajmodadi Choorna	Pain and inflammation of joints, osteoarthritis, back pain, and sciatica	
M	Aswagandha Choorna	Dizziness, lack of strength, indigestion, abdominal colic etc.	
N	Avipattikar Choorna	Hyperacidity, gastritis, burning ache due to excess acid, loss of appetite etc.	
O	Darshan Sanskar Choorna	It helps to strengthen teeth, improves shine and whiteness	
P	Sithophaladi Choorna	Respiratory, digestive, immune etc.	
Q	Talisadi Choorna	Respiratory, digestive, immune etc.	
R	Chandanadi Lauha	Chronic fever, cough, cold, gastritis, fatigue and weakness.	
S	Navavas Lauha	Chronic fever any weakness etc	
T	Shilajatyadi Lauha	Chronic respiratory diseases, tuberculosis, bronchitis, cough and cold.	
U	Saptamrit Lauha	Anorexia, vomiting, eye disorders, tiredness, fatigue, difficulty in urination, bloating, and inflammation.	
V	Sarphagandha Mishran	Hypertension, Anxiety and Depression, epilepsy and vertigo.	
W	Eladi Gutika	cough, cold, fever, hiccups, vomiting, dizziness, haematemesis (blood vomiting), abdominal pain.	

and microbial) was noticed with naked eyes. Although the information about packaging and expiry dates was fine, but it does not make sure the reliability and quality of the chemical composition of the drug.

The heavy metal ions content of herbal drugs under study was examined and reported in Table 2. The highest amount (i.e. 0.223 mg/kg)

Table 2
Quantitative analysis of heavy metals of the various herbal drugs. Values are expressed as mean ± SD (n = 3).

Drug Code	Heavy metal ion content mg/kg (mean ± SD)			
	Cd	Pb	Hg	As
A	0.111 ± 0.031	0.449 ± 0.031	BDL	BDL
B	BDL	0.388 ± 0.017	0.016 ± 0.005	0.011 ± 0.004
C	0.089 ± 0.017	BDL	0.022 ± 0.008	BDL
D	BDL	0.281 ± 0.015	BDL	BDL
E	0.136 ± 0.021	0.607 ± 0.044	0.016 ± 0.005	0.131 ± 0.011
F	BDL	0.312 ± 0.044	0.009 ± 0.003	BDL
G	BDL	BDL	BDL	0.006 ± 0.002
H	0.075 ± 0.012	0.388 ± 0.019	0.014 ± 0.005	BDL
I	0.126 ± 0.014	BDL	BDL	0.028 ± 0.005
J	BDL	0.378 ± 0.041	0.021 ± 0.007	0.019 ± 0.003
K	0.066 ± 0.008	BDL	0.009 ± 0.003	BDL
L	0.117 ± 0.008	1.116 ± 0.031	BDL	BDL
M	BDL	BDL	0.018 ± 0.004	0.136 ± 0.021
N	0.094 ± 0.008	0.731 ± 0.022	0.005 ± 0.002	BDL
O	BDL	BDL	BDL	BDL
P	0.111 ± 0.051	0.511 ± 0.074	BDL	0.089 ± 0.007
Q	0.101 ± 0.008	BDL	BDL	0.066 ± 0.005
R	BDL	0.587 ± 0.027	BDL	BDL
S	0.095 ± 0.005	BDL	0.013 ± 0.005	BDL
T	0.115 ± 0.011	0.648 ± 0.023	BDL	0.085 ± 0.004
U	BDL	BDL	0.009 ± 0.003	BDL
V	0.085 ± 0.005	1.216 ± 0.065	BDL	0.045 ± 0.003
W	0.223 ± 0.055	BDL	0.021 ± 0.003	BDL

Limits (mg/kg) set by API for: Lead = 10.0; Cadmium = 0.3; Mercury = 1.0; Arsenic = 3.0. BDL = below detection limit and not detected.

of cadmium (Cd) ion was noticed in eladi gutika which was far away from the prescribed limits of WHO (i.e. 0.3 mg/kg). Similarly, the highest amount of lead (Pb), mercury (Hg), and arsenic (As) ions was 1.216 mg/kg (found in sarphagandha mishran), 0.022 mg/kg (found in kutajaghan vati), and 0.136 mg/kg (found in aswagandha choorna) respectively (Table 2). The amount of heavy metal ions were within or below the prescribed limits of WHO (i.e. 10 mg/kg for Pb, 1 mg/kg for Hg, and 3.0 mg/kg for As). Overall, it was found that the concentrations of heavy metal ions were within the API and WHO limits (Table 2).

The presence of other toxic metal ion aluminium (Al) was found bit higher i.e. greater than 50 mg/kg. Only two drugs have shown higher value of Al metal ion i.e. mahashanka vati (70.106 ± 0.222 mg/kg) and punarnavadi guggul (95.027 ± 0.351 mg/kg) (Table 2). This may attributed to the various reasons including use of aluminium pot during the processes or manufacturing of drug. Other reason behind the higher value of Al may be the presence of higher amount of Al in the raw material which may grew in Al contaminated soils. Literature has indicated the variable (30–5000 mg/kg) intake limit of Al (Stahl et al., 2011). Excess use of Al may damage kidneys and causes Alzheimer's disease (Stahl et al., 2011). Due to adverse effects of Al, WHO have reduced the daily intake limit of Al from 7 mg/kg to 1 mg/kg (Stahl et al., 2011). Formulation, purification/processing or "Shodhana" of herbal drugs may alter the authentic quantity of the metal ions. Sometime, it may introduce the contamination of heavy metal ions (Mulaudzi et al., 2017; Stahl et al., 2011). So, quality control practices of finished products are important as that of raw materials of herbal products.

There are several reports of toxicity of heavy metals have been reported (Agency for Toxic Substances and Disease Registry, 2012; Mulaudzi et al., 2017; Stahl et al., 2011). The variation and toxicity of these metals may originate from the type of soil and environment. Sometime wrong agricultural practices (excess use of metalloids in the form of plant protector or plant growth regulators) are responsible for such toxicity (WHO, 2003, 2007; Mulaudzi et al., 2017; Stahl et al.,

2011). Another reason behind the poor quality and high toxicity of the herbal drugs may be the marketing of drugs with no authorization from authorities (Apak et al., 2016; Mulaudzi et al., 2017; Okem et al., 2014; WHO, 2003; WHO, 2007). Also, many people imagine that these drugs have low toxicity and may be used as a health supplement without any concerned of the doctor. Poor GMP's application is another reason behind the toxicity and low purity of herbal drugs. People envisage that these are the homemade drugs, so GMP concept is not applicable, hence no need of any kind of license (Okem et al., 2014; WHO, 2003; WHO, 2007). As like modern drug analysis, quality control procedures can be followed for both licensed and non-license herbal drugs. In order to develop the faith of common people (w. r. t. quality and efficacy) among the traditional drugs, there is a need to strengthen the market surveillance procedures and regulatory control system. Moreover, non-compliance of standard protocols (like API, BP, USP and IP) is the main reason for the failure of FDA approval for herbal drugs (Okem et al., 2014; WHO, 2003; WHO, 2007).

Current study may help quality control process w. r. t. analysis of heavy metal ions. Quality and purity of the product can influence both efficacy and safety of the product. So purity is prime most targets to achieve. It becomes more urgent when things are related to healthcare. In India, only 10% of the herbal drug formulation firms have in-house research and development facilities (Bressy et al., 2013; Maiga et al., 2005; Mulaudzi et al., 2017). So, quality control and safety is always a matter of concern with the products of such firms. Their various reports on the issues like heavy metals, pesticide residues, microbial load, adulteration, misbranded products, and non effective herbal has been published (Bressy et al., 2013; Debbie et al., 2012; Fallah et al., 2011; Foguem and Foguem, 2014; Maiga et al., 2005; Mulaudzi et al., 2017; Obi et al., 2006).

3.2. Essential metal ion content

Essential metal ions are considered as the basis of living things. These micro and macro ions have their own wide applications for the plant and animals (including the social animal). These metal ions could play the key role in many structural and functional mechanisms of the plants and the human body (Long et al., 2004). Minerals under different conditions and climate are the gift of nature. Herbal medicines are sourced to convey this gift to human through food and healthcare applications. All basic principles related to human therapy have been derived from herbs and aromatic plants (Mulaudzi et al., 2017; Okem et al., 2014). As per WHO reports, over three billion people are suffering worldwide from the deficiency of micronutrients (Manganese (> 10%), Iron (> 25%) and Zinc (> 45%)) deficiency) (Alloway, 2008; Kumar et al, 2015a; Kaur et al 2017; Long et. al., 2004). Due to natural sources of origin, herbal drugs may be the help to overcome such deficiencies.

In the current study macro (sodium (Na), magnesium (Mg), potassium (K) and calcium (Ca)) and micro (chromium (Cr), manganese (Mn), iron (Fe), copper (Cu) and zinc (Zn)) elements were quantified in twenty three herbal drugs. It was the first study where essential metal ions of herbal drugs were tested with the aim to check the nutritional value of herbal drugs in terms of the essential metal ions. The ICP-OES results have highlighted that all the herbal drugs contained good concentration of essential metal ions of Fe, Cu, Zn, Ca, Mg, and Na (Tables 3A and 3B). From Tables 3A and 3B, it was quite clear that herbal drugs under study are good source of essential metal ions like Fe and Zn.

Moreover, it is worthy to declare that the essential metal ions content of herbal drugs under study was within the WHO limits (Tables 3A and 3B). Most importantly, Cr, and Cu are within prescribed limits i.e. 2 and 150 mg/kg (Apak et al., 2016; Mulaudzi et al., 2017; Okem et al., 2014; WHO, 2003; WHO, 2007). It is well known that Cr is very useful to cure diabetes, but higher applications may cause cancer and respiratory problems (Agency for Toxic Substances and Disease Registry, 2012; Mulaudzi et al., 2017). The Cu is considered as a main

source of physiological processes, high intake of Cu may lead to gastrointestinal problems, liver and kidney damage, and even death (Agency for Toxic Substances and Disease Registry, 2004; Mulaudzi et al., 2017). Mn, Fe, Co and Zn are considered the basis of various biological functions. Mn has antioxidant properties and suppresses toxic oxygen forms. Co is a major part of vitamin B-12. Zn and Cu have anticancer properties and essential for proper cell and enzymatic reactions (Alloway, 2008; Long et al., 2004). Deficiencies of these elements may cause various diseases (Alloway, 2008; Long et al., 2004).

Now a day's concept of fortification has been introduced to overcome the deficiency of trace metal ions. The elemental composition of many aromatic plants is well known, hence the drugs from the aromatic plants may be a good alternate over synthetic fortified drugs. Sometime excess applications of these trace metal ions may cause adverse effects on the health of the users. So, it is very interesting to find the trace elements of polyherbal drugs.

3.3. In vitro antioxidant assays

Mono/polyherbal drugs contained diverse classes of chemical constituents having various medicinal values including the antioxidant activities (Jin et al., 2018; Kota et al., 2017; Sridevi et al., 2018a). To confirm the antioxidant activities of herbal drugs, multiple antioxidant assays namely 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating (MC), ferric reducing (FRAP), scavenging activity of superoxide anion (SA) and nitric oxide (NO) were performed along with the TPC and TFC studies. Table 4 has highlighted the significant (with bold letters) antioxidant properties of herbal drugs under study. As compared to control (ascorbic acid), drug sample including gokshuradi guggul, kaishore guggul, kanchanar guggul, yogaraj guggul, ajmodadi choorna, chandanadi lauha, navavas lauha, and saptamrit lauha have shown good DPPH scavenge free radicals activities (Table 4). Except, mahashanka vati, aswagandha choorna, darshan sanskar choorna, sithophaladi choorna, shilajatyadi lauha, and sarphagandha mishran, remaining all drugs have shown good FRAP scavenge free radicals activities (Table 4). Good MC, SA, and NO antioxidant activities have been shown by each and every herbal drug (Table 4). It is noticeable that few drugs have shown better antioxidant properties than control (Table 4). Table 4 has exhibited the drug samples including kaishore guggul, kanchanar guggul, darshan sanskar choorna, chandanadi lauha, and navavas lauha as an excellent source of antioxidant properties, even more than control(s).

Table 5 exhibited the results of TPC and TFC studies. TPC was calculated as gallic acid equivalents (GAE) (Y = 0.303X + 0.007), R^2 = 0.999) (Supplementary Data – S3). Similarly, TFC was calculated as quercetin equivalents (QE) (Y = 0.040X + 0.012), R^2 = 0.999) (Supplementary Data – S4). Overall, most drugs samples have shown good TPC and TFC values (i.e. ≥ 100 GAE or QRE in mg/g DW). The results showed that the TPC & TFC of drugs varied to a great extent. The observed TPC of the samples varied from 14.91 to 208.34 mg/g dry weight (DW) of sample. The TFC varied from 48.0 to 208.0 mg quercetin equivalent (QE)/g DW of sample (Table 5).

Plants are known for their multi-utility and secondary biomolecules of plants are unique gift of mature. Secondary biomolecules of plants are considered as major therapeutic agents to cure diverse class of disease (Braca et al., 2002; Bhandari and Kawabata, 2004). Designing and preparation of formulation of herbal drug on the basis of high phenolic and flavonoids content of the aromatic plant(s) is a demand of time (Apak et al., 2016; Pulido et al., 2000). High amount of phenolic and flavonoids content indicates the ability of plants to treat multiple diseases (Dehghan et al., 2016; Pulido et al., 2000). The herbal drugs with good antioxidant qualities may be a good alternate against synthetic antioxidants like butylated hydroxyl toluene, butylated hydroxyl anisole, and propyl gallate having adverse health effects (Braca et al., 2002; Bhandari and Kawabata, 2004).

Table 3A
Quantitative analysis of minerals (mg/kg) of the various herbal drugs. Values are expressed as mean $\pm$ SD (n = 3). *Here values in bold letters are showing the significant results noticed (Here η = conc. more than 50 ppm, = conc. more than 100 ppm).*

Drug Code	Na	Mg	Al	Si	K	Ca
A	**27.674 ± 0.151**	BDL	6.952 ± 0.077	**29.352 ± 0.183**	BDL	BDL
B	BDL	**13.382 ± 0.126**	4.366 ± 0.046	**84.294 ± 0.382$^{\eta}$**	BDL	**53.160 ± 0.255$^{\eta}$**
C	3.481 ± 0.067	1.462 ± 0.006	0.367 ± 0.011	0.811 ± 0.004	1.126 ± 0.021	**24.701 ± 0.204**
D	BDL	BDL	**70.106 ± 0.222$^{\eta}$**	2.127 ± 0.015	BDL	BDL
E	BDL	**13.382 ± 0.149**	4.366 ± 0.013	**84.294 ± 0.377$^{\eta}$**	BDL	**53.159 ± 0.333$^{\eta}$**
F	6.071 ± 0.022	BDL	2.058 ± 0.008	2.429 ± 0.022	BDL	BDL
G	**17.443 ± 0.101**	BDL	**15.502 ± 0.032**	10.546 ± 0.145	BDL	BDL
H	BDL	**29.82 ± 0.115**	3.359 ± 0.004	16.215 ± 0.158	BDL	**62.511 ± 0.351$^{\eta}$**
I	**20.559 ± 0.119**	**31.394 ± 0.151**	10.483 ± 0.088	3.905 ± 0.017	14.408 ± 0.123	**112.969 ± 0.504$^{\lambda}$**
J	BDL	BDL	**95.027 ± 0.351$^{\eta}$**	2.992 ± 0.011	BDL	BDL
K	BDL	7.815 ± 0.077	3.564 ± 0.066	1.312 ± 0.009	9.617 ± 0.098	BDL
L	BDL	**49.197 ± 0.192**	2.376 ± 0.046	**10.476 ± 0.107**	BDL	BDL
M	**20.128 ± 0.122**	BDL	**12.105 ± 0.102**	4.984 ± 0.034	BDL	BDL
N	BDL	**15.719 ± 0.166**	2.477 ± 0.084	4.187 ± 0.051	**64.408 ± 0.194$^{\eta}$**	**84.992 ± 0.431$^{\eta}$**
O	3.014 ± 0.081	1.249 ± 0.008	0.431 ± 0.006	0.595 ± 0.004	1.155 ± 0.009	19.565 ± 0.188
P	BDL	**10.581 ± 0.111**	1.789 ± 0.008	**64.841 ± 0.231$^{\eta}$**	**52.942 ± 0.222$^{\eta}$**	**51.933 ± 0.227$^{\eta}$**
Q	**19.557 ± 0.145**	BDL	5.229 ± 0.078	5.551 ± 0.022	BDL	BDL
R	BDL	**10.891 ± 0.107**	5.424 ± 0.101	3.889 ± 0.011	6.162 ± 0.102	BDL
S	**23.125 ± 0.161**	**35.634 ± 0.155**	**23.594 ± 0.151**	8.107 ± 0.088	BDL	BDL
T	**18.652 ± 0.135**	BDL	**14.009 ± 0.127**	1.704 ± 0.034	BDL	BDL
U	BDL	**38.874 ± 0.167**	2.718 ± 0.044	**16.615 ± 0.124**	BDL	**139.574 ± 0.415$^{\lambda}$**
V	**16.127 ± 0.111**	**15.308 ± 0.158**	**12.966 ± 0.133**	0.381 ± 0.006	**59.911 ± 0.277$^{\eta}$**	**64.247 ± 0.264$^{\eta}$**
W	BDL	BDL	**10.815 ± 0.118**	3.441 ± 0.011	BDL	BDL

BDL = below detection limit and not detected.

3.4. Protein binding assay

Basically, drug delivery is a complex mechanism. It has been assumed that drugs show their interactions to various proteins before reaching to target. Mode of interactions may define the toxicity, potency and efficacy of a drug. Here, interaction behaviour (in terms of binding constant) of various drugs was observed and compared with acetyl salicylic acid (control) (Table 5 and Supplementary Data – S5). The average values of protein binding constants are tabulated under Table 5. All these results suggest a weak interaction of various drugs with BSA protein, which is a good thing for the proper drug delivery (Kumar et al., 2018a,b,c,d; Kumar and Singh 2018). Generally, strong interactions of any medicine with proteins with binding constant (k) ranging from 10^6 -10^{11} M^{-1} is not a good thing for the proper drug delivery (Abdi et al., 2012; (Kumar et al., 2018a,b,c,d). Moreover, results mentioned under Tables 2–5 contained significant information. Results mentioned in bold letters are the significant results and may be considered for the demarcation of medicines with good mineral and nutritional values.

4. Conclusion

In present study, herbal drugs were evaluated for their quality control, nutritional and medicinal qualities. We found that drugs under studies were rich in nutritional values including TPC (from 14.91 to 208.34 mg (GAE)/g and TFC (from 48.0 to 208.0 mg (QE)/g). Compare

Table 3B
Quantitative analysis of minerals (mg/kg) of the various herbal drugs. Values are expressed as mean $\pm$ SD (n = 3). *Here values in bold letters are showing the significant results noticed (Here η = conc. more than 50 ppm, = conc. more than 100 ppm).*

Drug Code	Cr	Mn	Fe	Co	Ni	Cu	Zn
A	0.173 ± 0.016	5.776 ± 0.102	6.973 ± 0.031	0.008 ± 0.002	0.071 ± 0.008	1.516 ± 0.095	2.951 ± 0.024
B	0.136 ± 0.012	0.577 ± 0.022	6.861 ± 0.025	0.007 ± 0.002	0.046 ± 0.005	0.364 ± 0.045	0.475 ± 0.011
C	0.089 ± 0.009	0.031 ± 0.011	2.013 ± 0.078	0.005 ± 0.001	0.027 ± 0.002	0.253 ± 0.024	0.407 ± 0.010
D	0.799 ± 0.111	1.652 ± 0.075	**64.792 ± 0.388$^{\eta}$**	0.030 ± 0.005	0.351 ± 0.014	1.289 ± 0.074	3.036 ± 0.037
E	0.136 ± 0.018	0.577 ± 0.033	**6.861 ± 0.022**	0.007 ± 0.003	0.046 ± 0.005	0.364 ± 0.022	0.475 ± 0.018
F	0.098 ± 0.014	1.826 ± 0.041	2.903 ± 0.012	0.005 ± 0.003	0.045 ± 0.004	0.612 ± 0.027	2.507 ± 0.028
G	0.344 ± 0.075	0.033 ± 0.007	**12.979 ± 0.066**	0.015 ± 0.005	0.402 ± 0.025	0.905 ± 0.029	8.598 ± 0.088
H	0.146 ± 0.042	1.809 ± 0.021	**11.518 ± 0.054**	0.011 ± 0.004	0.218 ± 0.013	0.588 ± 0.024	1.149 ± 0.013
I	0.123 ± 0.032	0.924 ± 0.033	4.371 ± 0.029	0.005 ± 0.002	0.038 ± 0.007	0.289 ± 0.014	0.388 ± 0.010
J	0.174 ± 0.038	1.658 ± 0.017	**13.078 ± 0.077**	0.013 ± 0.004	0.059 ± 0.009	1.161 ± 0.049	1.465 ± 0.017
K	0.066 ± 0.005	0.096 ± 0.005	4.226 ± 0.035	0.004 ± 0.001	0.017 ± 0.003	0.135 ± 0.009	0.281 ± 0.005
L	0.117 ± 0.033	0.312 ± 0.044	**12.995 ± 0.065**	0.006 ± 0.002	0.072 ± 0.010	1.116 ± 0.022	1.607 ± 0.008
M	0.225 ± 0.088	0.449 ± 0.016	**14.595 ± 0.088**	0.011 ± 0.005	0.137 ± 0.014	0.767 ± 0.020	1.112 ± 0.014
N	0.117 ± 0.055	0.388 ± 0.019	8.158 ± 0.021	0.005 ± 0.002	0.038 ± 0.004	0.289 ± 0.017	0.388 ± 0.007
O	0.055 ± 0.010	0.024 ± 0.018	1.361 ± 0.016	0.005 ± 0.001	0.014 ± 0.002	0.253 ± 0.014	0.416 ± 0.009
P	0.125 ± 0.066	0.378 ± 0.055	4.012 ± 0.019	0.004 ± 0.001	0.041 ± 0.004	0.419 ± 0.021	0.521 ± 0.005
Q	0.173 ± 0.074	0.323 ± 0.051	7.548 ± 0.028	0.006 ± 0.003	0.052 ± 0.004	0.916 ± 0.033	0.749 ± 0.009
R	0.123 ± 0.021	2.117 ± 0.084	2.264 ± 0.021	0.004 ± 0.001	0.037 ± 0.003	0.587 ± 0.027	0.494 ± 0.004
S	0.198 ± 0.027	1.479 ± 0.065	**21.507 ± 0.039**	0.016 ± 0.004	0.103 ± 0.009	0.405 ± 0.022	0.507 ± 0.009
T	0.115 ± 0.009	5.435 ± 0.098	**14.928 ± 0.032**	0.017 ± 0.003	0.066 ± 0.008	0.648 ± 0.023	0.842 ± 0.011
U	0.158 ± 0.047	0.456 ± 0.022	**15.828 ± 0.021**	0.007 ± 0.002	0.059 ± 0.005	0.487 ± 0.018	0.439 ± 0.007
V	0.474 ± 0.097	7.864 ± 0.085	1.755 ± 0.009	0.024 ± 0.005	0.376 ± 0.044	1.216 ± 0.065	2.547 ± 0.012
W	0.223 ± 0.055	2.769 ± 0.029	**25.475 ± 0.087**	0.014 ± 0.004	0.082 ± 0.019	0.387 ± 0.041	0.966 ± 0.018

Table 4

In vitro antioxidant assays of the various herbal drugs, DPPH radical scavenging activity, Metal chelating scavenging activity, FRAP scavenging activity, Superoxide radical scavenging activity, and Nitric oxide radical scavenging activity. Values are expressed as mean ± SD (n = 3). *Here values in bold letters are showing the significant results noticed in various experiments of antioxidant assays.*

Drug Code	Antioxidant assays				
	DPPH	FRAP	NO	MC	SA
Control	96.07 ± 3.21 (Ascorbic acid)	93.45 ± 4.13 (Ferrous sulphate)	29.21 ± 1.58 (Ascorbic acid)	91.41 ± 3.08 (EDTA)	32.75 ± 2.15 (Ascorbic acid)
A	20.39 ± 1.21	**54.40 ± 2.36**	24.80 ± 2.19	63.86 ± 3.11	18.49 ± 1.02
B	25.44 ± 1.52	**62.63 ± 3.66**	24.71 ± 1.74	**76.11 ± 3.25**	**40.37 ± 2.21**
C	36.52 ± 1.98	**71.52 ± 4.10**	24.41 ± 1.34	**89.58 ± 4.19**	**40.98 ± 1.88**
D	12.85 ± 0.88	13.38 ± 0.75	24.61 ± 1.15	33.84 ± 1.78	**45.96 ± 2.22**
E	41.49 ± 2.11	**79.11 ± 3.52**	**36.31 ± 2.02**	**96.14 ± 4.12**	**75.36 ± 3.18**
F	33.85 ± 1.95	**69.73 ± 3.46**	26.35 ± 1.34	**86.64 ± 3.17**	**45.66 ± 2.19**
G	**61.96 ± 2.11**	**84.62 ± 3.61**	26.35 ± 2.21	**75.97 ± 2.12**	35.62 ± 2.03
H	**73.95 ± 3.07**	**84.45 ± 3.15**	**30.23 ± 2.18**	**89.25 ± 3.37**	**49.81 ± 3.15**
I	**84.51 ± 3.88**	**85.67 ± 3.11**	**39.72 ± 1.88**	**89.90 ± 4.01**	**42.71 ± 2.15**
J	33.93 ± 1.50	**65.44 ± 3.02**	27.32 ± 1.85	**81.85 ± 2.14**	16.11 ± 1.05
K	**58.04 ± 1.54**	**77.93 ± 2.83**	**51.35 ± 2.74**	**82.73 ± 3.54**	**41.50 ± 2.44**
L	**72.27 ± 2.74**	**86.38 ± 3.78**	**37.90 ± 1.81**	**90.40 ± 4.32**	18.49 ± 1.31
M	31.99 ± 1.11	40.45 ± 2.22	25.26 ± 1.94	**89.17 ± 3.54**	**47.69 ± 2.41**
N	45.45 ± 1.02	**79.22 ± 3.21**	**47.98 ± 2.16**	**89.40 ± 3.64**	33.20 ± 2.13
O	**64.42 ± 2.54**	**88.63 ± 3.96**	**39.51 ± 2.15**	**90.85 ± 3.33**	37.58 ± 2.88
P	37.47 ± 1.74	22.38 ± 2.15	23.79 ± 1.95	**88.59 ± 2.55**	**50.03 ± 2.98**
Q	36.99 ± 1.44	43.27 ± 2.44	23.79 ± 1.51	**90.25 ± 2.91**	**40.07 ± 2.31**
R	**51.91 ± 2.01**	**69.94 ± 3.02**	22.17 ± 1.21	**91.08 ± 4.03**	**45.66 ± 2.15**
S	**58.38 ± 2.33**	**81.45 ± 3.74**	29.03 ± 1.13	**91.05 ± 3.88**	**45.88 ± 2.78**
T	39.02 ± 1.22	47.65 ± 2.55	20.96 ± 0.99	**90.21 ± 3.77**	**49.96 ± 2.72**
U	**62.61 ± 2.66**	**84.32 ± 3.22**	25.80 ± 1.98	**90.95 ± 4.27**	**46.33 ± 2.56**
V	23.93 ± 1.05	45.44 ± 2.45	**47.32 ± 2.05**	61.85 ± 2.29	23.15 ± 2.01
W	33.88 ± 1.22	**65.41 ± 3.37**	27.32 ± 1.77	**79.85 ± 3.88**	19.91 ± 1.78

DPPH (2,2-diphenyl-1-picrylhydrazyl); MC (Metal chelating); FRAP (Ferric reducing); SA (Superoxide radical); NO (Nitric oxide).

Table 5

In vitro Total phenolic & flavonoids content, and BSA binding constants of various herbal drugs. Values are expressed as mean ± SD (n = 3). *Here values in bold letters are showing the significant results noticed in TPC/TFC and BSA assays.*

Drug Code	TPC (GAE in mg/g DW))	TFC (QRE in mg/g DW)	Protein Binding
Control	Gallic Acid (GAE)	Quercetin (QRE)	$11.31 \pm 0.05 \times 10^{-5}\,\mu M^{-1}$ (Aspirin)
A	**184.34 ± 3.08**	48.12 ± 2.09	$15.5 \pm 1.27 \times 10^{-5}\,\mu M^{-1}$
B	**168.34 ± 3.44**	**168.33 ± 4.12**	$14.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
C	62.05 ± 2.66	**128.22 ± 3.15**	$17.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
D	**201.48 ± 3.68**	48.11 ± 2.11	$11.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
E	**154.62 ± 2.98**	88.38 ± 1.89	$13.8 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
F	**124.21 ± 2.11**	96.12 ± 2.29	$11.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
G	**111.28 ± 2.19**	**231.11 ± 4.11**	$17.1 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
H	76.91 ± 1.88	**208.88 ± 3.85**	$14.2 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
I	14.91 ± 1.02	**218.12 ± 2.44**	$17.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
J	**130.62 ± 3.33**	**225.45 ± 2.02**	$14.5 \pm 1.25 \times 10^{-5}\,\mu M^{-1}$
K	83.77 ± 4.88	**208.66 ± 2.15**	$17.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
L	98.62 ± 3.55	**128.27 ± 2.88**	$13.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
M	**188.91 ± 3.23**	**128.81 ± 1.89**	$11.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
N	**163.77 ± 2.88**	88.77 ± 1.87	$10.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
O	68.91 ± 2.02	48.99 ± 1.54	$12.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
P	**208.34 ± 3.88**	48.17 ± 1.38	$16.2 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
Q	**195.77 ± 3.77**	**168.97 ± 4.15**	$13.6 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
R	**180.91 ± 3.89**	**208.39 ± 2.67**	$15.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
S	**166.05 ± 4.66**	**128.25 ± 2.55**	$17.7 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
T	**200.34 ± 3.12**	**168.84 ± 2.25**	$21.5 \pm 1.22 \times 10^{-5}\,\mu M^{-1}$
U	**155.77 ± 2.88**	**208.29 ± 3.77**	$17.1 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
V	78.45 ± 3.12	33.12 ± 1.65	$13.4 \pm 1.05 \times 10^{-5}\,\mu M^{-1}$
W	66.22 ± 2.55	44.34 ± 1.54	$12.7 \pm 1.25 \times 10^{-5}\,\mu M^{-1}$

to ascorbic acid, significant antioxidant qualities (40.51–92.34%) were noticed. ICP-OES study revealed that maximum drugs were good source of essential metal ions like Na, Mg, Ca, Fe, Cu, Zn etc. In terms of quality control evaluation, drugs under study have shown heavy metal ions concentration within the prescribed limits of WHO and API. In present study, the supplier of drugs was a government agency which is under regularity authorities and these drugs were prepared as per GMP and API guidelines. These findings may encourage the quality control mechanism of herbal drugs and build up the faith of users towards the herbal drugs. In concluding remarks, it is worthy and essential to check the quality and medicinal values of herbal drugs at regular intervals for the sake of safety and quality of Ayurvedic or herbal drugs. Present work may be a good concept to encourage the government agencies to check the quality and medicinal values of the herbal drugs manufactured by the private or government agencies.

Conflicts of interest

None declared.

Author contributions

Dr. Vijay Kumar designed the study, execute it and write the paper including the experimental work. Dr. Simranjeet Singh performed the experimental work (metal ion analysis) and edited the paper and refines it. Dr. Arjun Singh critically reviewed the manuscript.

Practical application

As per the WHO reports, 80% population is still depend on the folk drugs. Most consumers believe that these drugs are less toxic with least side effects, but, due to industrialization and urbanization truth is totally different. During last five years, various cases have been noticed about the quality dilution of the traditional/folk drugs where the limits of heavy metal and pesticides were over the prescribed limits of WHO and API. Hence, as per WHO norms, quality assessment of folk/herbal/traditional drugs is obligatory.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.01.056.

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