Determination of heavy metal contents by atomic absorption spectroscopy (AAS) in some medicinal plants from Pakistani and Malaysian origin

Sobia Akram[1]*, Rahila Najam[1], Ghazala H. Rizwani[2] and Syed Atif Abbas[3]
[1]Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan
[2]Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Pakistan
[3]School of Pharmacy, Taylor's University, Malaysia

Abstract: This study depicts a profile of existence of heavy metals (Cu, Ni, Zn, Cd, Hg, Mn, Fe, Na, Ca, and Mg) in some important herbal plants like (*H. Integrifolia, D. regia, R. communis, C. equisetifolia, N. oleander, T. populnea, M. elengi, H. schizopetalus, P. pterocarpum)* from Pakistan and an antidiabetic Malaysian herbal drug product containing (*Punica granatum L.* (Mast) Hook*, Momordica charantia L., Tamarindus indica L., Lawsonia inermis L.)* using atomic absorption spectrophotometer. Heavy metals in these herbal plants and Malaysian product were in the range of 0.02-0.10ppm of Cu, 0.00-0.02ppm of Ni, 0.02-0.29ppm of Zn, 0.00-0.04ppm of Cd, 0.00-1.33ppm of Hg, 0.00-0.54ppm of Mn, 0.22-3.16ppm of Fe, 0.00-9.17ppm of Na, 3.27-15.63ppm of Ca and 1.85-2.03ppm of Mg. All the metals under study were within the prescribed limits except mercury. Out of 10 medicinal plants/product under study 07 were beyond the limit of mercury permissible limits. Purpose of this study is to determine heavy metals contents in selected herbal plants and Malaysian product, also to highlight the health concerns related to the presence of toxic levels of heavy metals.

Keywords: Atomic Absorption Spectroscopy (AAS), pollutants, heavy metals, traditional medicines.

INTRODUCTION

Traditional medicines (TM) have been in used since ages. People around the globe are using TM because of their historical behavior and according to their cultural creeds. According to world health organization (WHO) about 70% of the world population is still depending on TM to fulfill their health needs (Uddin *et al*., 2012). As traditional medicines (TM) are from natural source, it is a misconception that TM is comparatively safer than synthetic drugs having chemicals. As researchers going advanced, it is found that plants not only contain toxic secondary metabolites, but they are also contaminated with environmental pollutants specially heavy metals, which on long term exposure can lead to serious health issues (Hina *et al.,* 2011).

Traditional medicines industry is very rapidly growing and it was estimated to be US$ 60 billion annually in 2008 (Uddin *et al*., 2012). In early 50s, 84% of the Pakistani population was depending on traditional and herbal medicines but nowadays this practice has been limited (Alam *et al*., 2011). In Malaysia, this industry is rapidly growing and people are using traditional medicines as their primary source of healthcare, this can be understood by this that in Malaysia, about US$ 500 million is spent annually on traditional medicines and natural source medicines as compared to about US$ 300 million on allopathic medicine (WHO, 2007). Heavy metals are natural components of the earth's crust. They cannot be destroyed and could enter human body via food, drinking, medicinal plants etc. Heavy metal refers to any elements that have a relatively high density and is toxic or poisonous at low concentrations and examples of heavy metals include mercury, cadmium, arsenic, chromium, thallium and lead. Heavy metals are also referred to as metals having atomic weight greater than sodium, and possess some level of toxicity (Adepoju-Bello *et al*., 2014). Heavy metals could be toxic and essential: Toxic heavy metals mainly Pb, Cd, Hg and As can cause metal poisoning to the patients. Some heavy metals are essential and required by the human body in trace amounts. However they become toxic when blood level increased. They may cause damage to vital organs of the body like heart, liver, kidneys and brain (Uddin *et al*., 2012).

Due to globally growing use of medicinal plants, regulatory authorities WHO and FDA are now more concern with the safety and efficacy of these traditional medicines (Helaluddin *et al.,* 2013). Monitoring of heavy metals is important due to two major reasons. The first reason is environmental pollution. Sources of this environmental pollution include industrial waste, traffic emissions and agricultural items, such as, organic mercury fungicides, insecticide. The second reason is that various traditional medicines, mainly of Asian origin, have been reported many times to contain toxic levels of heavy metals (Adepoju-Bello *et al*., 2014). FAO/WHO now

**Corresponding author:* e-mail: sobiya18@hotmail.com

strongly recommends heavy metal analysis in the TM (Hina *et al.*, 2011).

This article aims to provide the heavy metals content in 09 medicinal important plants from Pakistan and 01 combination herbal drug from Malaysia. Heavy metals were determined using atomic absorption spectroscopy (AAS).

MATERIALS AND METHODS

A total of 10 medicinal plants from Pakistan and Malaysian origin were analyzed using atomic absorption spectroscopy. Out of 10 nine were from Pakistan and was methanolic extracts of different parts of medicinal plants, one was obtained from Malaysia and it was a combination drug in capsule dosage form. Pharmacognostic features of medicinal herbs/products under study are mentioned in table 1.

Analytical grade nitric acid (HNO3) and 70% perchloric acid (HCLO4) supplied from fischer scientific were used as reagents for wet digestion of samples. Solutions were prepared using deionized water. All the glassware were thoroughly washed and rinsed before use.

Standard preparation

The standard solutions for all the heavy metals under study were prepared in three to five different concentrations to obtain a calibration curve by diluting stock standard solution of concentration 1000ppm.

Analysis method

Samples under study were first digested using wet digestion method. Briefly approx. 0.2gms of the sample were taken in 100ml volumetric flask and about 4 ml of HNO3 was added and solution was allowed to stand for few hours than it was carefully heated over water bath till red fumes coming from the flask completely ceased. Flask was allowed to cool at room temperature and than about 4 ml of perchloric acid was added and than flask was heated again over water bath to evaporate till a small portion which was than filter through whattman filter paper no.42 and made up the volume using distilled water till 100ml.

Instrumentation

Heavy metals determination in all the medicinal plants was done using atomic absorption spectroscopy (shimadzu). Standard operating parameters were set and given in table 3. The hollow cathode lamps for Cu, Cr, Cd, Co, Ni, Pb, Zn and Fe (shimadzu) were used as radiation source and fuel was air acetylene. All the samples and standard were run in duplicate.

RESULTS

Heavy metals concentrations in methanolic extracts of medicinal plants from Pakistan and Malaysia was determined using atomic absorption spectroscopy (AAS) and results obtained were tabulated in table 2. Hg content of herbal drugs is mentioned in fig. 1.

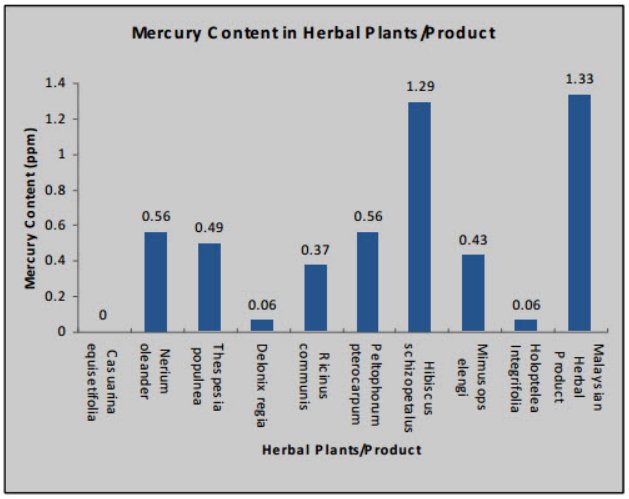

Fig. 1: Mercury content of herbal plants/products.

Sodium (Na)

Casuarina equisetifolia showed maximum concentration of sodium i.e. 9.18ppm and on the other hand minimum concentration was found in *M. elengi* i.e. 2.89ppm.Sodium (Na) content were also present in significant amounts like 8.07 ppm, 7.95ppm, 8.15ppm, 8.84ppm, 8.86ppm, 8.74ppm in *H. Integrifolia, R. communis, N. oleander, T. populnea, P. ptero-carpum D. regia* respectively. Medicinal herbal drug from Malaysia contains sodium content of 8.98 ppm.

Iron (Fe)

Malaysian herbal drug showed maximum content of Iron (Fe) i.e. 3.16ppm while *C. equisetifolia* showed minimum amount of iron content of 0.22ppm.

Magnesium (Mg)

T. populnea contains maximum magnesium content i.e. 2.03 ppm and minimum concentration was found in *N. oleander* i.e. 1.85 ppm.

Calcium (Ca)

Malaysian Herbal drug contains maximum concentration of calcium (Ca) i.e. 15.63ppm while minimum concentration was found in *D. regia* i.e. 3.27ppm. Calcium content was also present in significant amounts like 6.58ppm, 5.26ppm and 4.11ppm in *C. equisetifolia*, *H. Integrifolia*, *N. oleander* respectively.

Copper (Cu)

Highest concentration of Copper (Cu) was found in *T. populnea* which is 0.1ppm while least concentration of Cu was found in *C. equisetifolia* i.e. 0.02ppm.Other plants like *M. elengi* and Malaysian herbal drug product contains significant amounts of copper i.e. 0.08ppm, 0.05ppm respectively.

Table 1: Pharmacognostic features of tested medicinal herbs/product

S. No	Plant species	Family	Part used	Medicinal importance
1	*Casuarina equisetifolia*	Casuarinaceae	Fruit	Diarrhea, dysentery, beriberi
2	*Nerium oleander*	Apocynaceae	Flower	Cancer, asthma, muscular pain, menstural pain
3	*Thespesia populnea*	Malvaceae	Bark	Hepatoprotective, skin protective, dermatitis, wound healing.
4	*Delonix regia*	Fabaceae	Flower	Diarrhea,antiulcer
5	*Ricinus communis*	Euphorbiaceae	Leaves	Hepatoprotective, analgesic, laxative, emolient
6	*Peltophorum pterocarpum*	Fabaceae	Pod	Stomatitis, insomnia, constipation, dysentery, muscular pains, sores,skin disorders, analgesic
7	*Hibiscus schizopetalus*	Malvaceae	Flower	Hypolipidemic, antihyperglycemic, analgesic, antipyretic
8	*Mimusops elengi*	Sapotaceae	Leaves	antipyretic, analgesic, antidiabetic, hypoglycemic
9	*Holoptelea Integrifolia*	Ulmaceae	Flower	antidiabetic, antidiarrhoeal , anti-inflammatory
10	*Malaysian Herbal Product*	-	-	antidiabetic.

Table 2: Heavy metals contents of medicinal herbs/products (ppm) against permissible Limits.

	Na	Fe	Mg	Ca	Cu	Zn	Ni	Mn	Cd	Hg
Permissible limits	51340**	20	2000***	614**	10	50	1.5	200	0.3	0.1*
H. Integrifolia	8.07	1.40	2.01	5.26	0.07	0.20	0.02	0.00	0.04	0.06
D. regia	8.74	1.15	1.93	3.27	0.04	0.09	0.01	0.00	0.00	0.06
R. co mmunis	7.95	0.36	2.02	3.37	0.03	0.09	0.01	0.02	0.00	0.37
C. equisetifolia	9.18	0.22	1.97	6.58	0.02	0.03	0.02	0.00	0.01	0.00
N. oleander	8.15	0.77	1.85	4.11	0.04	0.12	0.00	0.00	0.00	0.56
T. populnea	8.84	1.44	2.03	3.77	0.10	0.16	0.03	0.01	0.01	0.49
M.elengi	2.89	0.87	2.00	5.08	0.08	0.20	0.00	0.13	0.00	0.43
H. schizopetalus	0.00	0.47	1.90	3.55	0.05	0.09	0.00	0.00	0.00	1.30
P. pterocarpum	8.86	0.78	1.95	3.94	0.04	0.15	0.02	0.01	0.01	0.56
Malaysian Product	8.98	3.16	2.02	15.63	0.05	0.29	0.00	0.54	0.01	1.33

Niaz *et al*., 2013, * Maobe *et al*., 2012, **Khan *et al*., 2012, *** Omokehide *et al*., 2013

Table 3: Working parameters of atomic absorption spectrophotometer

S. No	Element	Wavelength (nm)	Slit Width
1	Na	589	0.7
2	Fe	248.3	0.7
3	Mg	285.2	0.7
4	Ca	422.7	0.7
5	Cu	324.7	0.7
6	Zn	213.9	0.7
7	Ni	232	0.7
8	Mn	279.4	0.7
9	Cd	228.8	0.7
10	Hg	253.6	0.7

Zinc (Zn)

Maximum concentration of zinc was found in Malaysian herbal product which contains about 0.29ppm of zinc while minimum concentration was found in *C. equisetifolia* i.e. 0.03ppm.

Nickel (Ni)

Highest concentration of nickel (Ni) was found in *T. populnea* i.e. 0.03ppm while least concentration was found in *D. regia* and *R. communis* i.e. 0.01ppm. Ni was not found in medicinal plants like *N. oleander, M. elengi* and *H. schizopetalus.*

Manganese (Mn)

Malaysian herbal drug contains maximum concentration of manganese i.e. 0.54ppm while minimum concentration was found in *T. populnea* and *P. pterocarpum* i.e. 0.01ppm.

Cadmium (Cd)
Highest concentration of cadmium was found in *H. integrifolia* i.e. 0.04ppm while least concentration was found in *C. equisetifolia, T. populnea, P. pterocarpum* and Malaysian product i.e. 0.01ppm.

Mercury (Hg)
Maximum concentration of Mercury was found in Malaysian herbal drug i.e. 1.33ppm and Minimum concentration was found in *D. regia* and *H. integrifolia* i.e. 0.06ppm. Other significant mercuric content was found in *H. schizpetalus, N. oleander, P. pterocarpum, T. populnea, M. elengi* and *R. communis,* which are 1.30ppm, 0.56ppm, 0.56ppm, 0.49ppm, 0.43ppm and 0.37ppm respectively. Mercury was not detected in *C. equisetifolia*.

DISCUSSION

Casuarina equisetifolia L.
C. equisetifolia (Casuarinaceae) is beautiful tree with dropping branches, 10-50 m high. It is found in dry hill sides of open forests in India, Sri Lanka and Australia (Aher *et al.,* 2010). Part used in this study is fruit. Anticancer properties were reported in leaf extracts. Antidiarrheal activity of bark was also reported. Seeds are anthelmintic, antispasmodic and ant diabetic (El-Tantawy *et al.,* 2013). Aqueous extracts of this plant also showed antibacterial activity (Parekh and Chanda, 2007). Wood and bark extract also exhibit antihistaminic properties (Aher *et al.,* 2009). In this study, this medicinal plant contains maximum concentration of sodium (Na) i.e. 9.18ppm which is within the permissible limits. Humans and animals contain sodium in their extra cellular fluids. It plays a major role in depolarizing cell membrane and also to keep water equilibrium in ICF and ECF (Ştef *et al*., 2010). The institute of medicine's dietary reference intakes (DRIs) Canada recommends Canadian to limit their dietary Sodium intake from 1500mg to 2300mg per day. Authorities like WHO (World Health Organization) recommends limiting sodium to less than 2,000 mg a day and the AHA (American Heart Association) recommends limiting sodium to less than 1,500 mg a day (American Heart Association, 2013). Metals like Hg, Cu, Cd, Mn, Ni, Zn all were under the permissible limits as defined by WHO/FAO.

Nerium oleander L.
N. oleander (Apocynaceae) is evergreen flowering shrub. This ornamental plant frequently grown in parks and gardens (Altaee, 2011). Part used in this study is flower. It has been known to be a poisonous plant for animals and human beings either fresh or in dried form. Maximum toxic contents were present in roots and seeds. Suicidal use of this plant has also been reported (Altaee, 2011). Metals like Mn and Ni was not detected in this plant. Cd was found to be within the prescribed limits by WHO/FAO. Permissible limits set by WHO, China and Thailand for Cd was 0.3ppm. Mercury content was found to be 0.55ppm which is more than the prescribed limits set by WHO/FAO i.e. 0.1ppm. Inorganic and organic mercury can permanently damage the vital organs like brain, kidneys and growing fetus (Maobe *et al.,* 2012). Mercury exposure causes destruction of neurons in the visual cortex (Clarkson *et al.,* 2003). Children and adults exposed to mercury suffers from a disorder called acrodynia which includes symptoms like leg cramps, irritability, peeling of skin of hands, nose and sole of feet, excessive salivation, photophobia and sleeplessness. Maternal exposure during Pregnancy also causes impaired growth (TEACH Chemical Summary EPA, 2007).

Thespesia populnea L.
T. populnea (Malvaceae) is evergreen medium sized tree upto 10m long. Part used in this study is bark. *T. populnea* barks and fruits possess curative properties like it is used as astringent, anti-inflammatory, antidirrhoeal, antibacterial. They also reported for cutaneous treatments like scabies, psoriasis and ringworm (Phanse *et al.,* 2013) *T. populnea* is also reported to be useful in alzheimer's disease. Hypoglycemic effect also found in alcoholic bark extracts (Patil *et al*., 2012). All the metals under study was found within the prescribed limits set by WHO/FAO except mercury which was more than the prescribed limits for medicinal plant set by WHO/FAO. Mercury was found to be 0.49ppm.

Delonix regia L.
D. regia (Fabaceae) is a large tree with distinctive bright red flowers. Parts used in this study are Flower. It is a traditional antidiarrhoeal drug. *D. regia* flowers exhibit antibacterial, anti-inflammatory and analgesic activity. (Rajbhau *et al.,* 2011). Ethanolic extracts of *D.regia* flowers showed antidiarrhoeal activity in doses of 100, 250 and 500mg/kg (p.o), which is comparable to loperamide (1mg/kg) (Sarin and Bafna, 2012). Heavy metals like Cu were 0.04ppm which is within the permissible limits. WHO/FAO set for medicinal plants i.e. 10ppm. In China and Singapore medicinal plants should contain not more than 20ppm and 150ppm of Cu respectively. Increased level of copper can cause hair and skin discoloration, metallic mouth taste, nausea, dermatitis. Copper deficiency can cause anemia and congenital inabilities (Maobe *et al.,* 2012). Mn was not detected in D. regia. Mercury was within the prescribed limits as set by WHO/FAO.

Ricinus communis L.
R. communis (Euphorbiaceae) is a 4m high smooth, erect somewhat woody bush. Famously known as castor beans. Castor oil is cathartic and abortificient and acts as labor inducer. Ricinoleic acid has been used as contraceptive jellies in past times (Sadashiv, 2011) Apart from such deleterious effects, ethanolic extracts of *R. communis* acts

as a hepatoprotective agent in doses of 100mg/kg. (Padampriya *et al.,* 2012). Analgesic activity of *R. communis* was also reported in ethanolic extracts of root bark (Rajeshkumar *et al*., 2013). All the metals under study was detected and tabulated in table 2 were under permissible limits except mercury which is found to be 0.37ppm which is more than 0.1ppm.

Peltophorum pterocarpum (DC. Backer ex Heyne)

P. pterocarpum (Fabaceae) is 15-25m high ornamental tree grown round the World. Part used was pod. Different parts of the tree were used in different conditions like stomatitis, insomnia, skin problems, and constipation. Flowers of *P. pterocarpum* used for inducing sleep i.e. for the treatment of insomnia. Leaf decoction has been used for treating pains. Flowers are used as astringent and for the treatment of pain after birth and also for lotion for eye troubles (Jash *et al.,* 2014). It also possess antioxidant and anti-hemolytic activity (Khan *et al.,* 2013). All the metals under study was found within the permissible limits except mercury which was found to be 0.55ppm more than the allowable limit of 0.1ppm set by WHO/FAO.

Hibiscus schizopetalus (Mast) Hook

H. schizopetalus (Malvaceae) comprises of 275 species and out of all *H. schizopetalus* is the least studied specie of the genus. It is ornamental shrub grown in different parts if the World. It has been used by colombians to treat cough and cold (Zahid *et al.,* 2012). Flower and leaf extracts of H. schizopetalus possess significant analgesic and antipyretic effects (Zahid *et al.,* 2014). In this plant sodium and manganese was not detected. Out of 10 elements only mercury was found out of permissible limits set by WHO/FAO, it was about 1.29ppm, which is much higher than 0.1ppm set limit. Cd and Ni were not found. WHO/FAO defines Cd limit in edible plants of 0.21ppm, however for medicinal plants limits set by WHO, China, Canada was 0.3ppm (Maobe *et al.,* 2012). Cd fumes inhalation can cause acute pulmonary and kidney damage. It may cause acute and chronic poisoning causing adverse effects on Immune and vascular system (Maobe *et al*., 2012).

Mimusops elengi L.

M. elengi (Sapotaceae) is an evergreen tree found in different parts of the world. Part used in this study is leaves. Multiple activities have been reported of this plant. Bark of *H. schizopetalus* reported to have antioxidant, antiulcer, anti-inflammatory, analgesic activities. Boiled leaves are used to treat headaches. Leaf juice is used to treat sore eyes (*Kar et al.,* 2012). *M. elengi* also possesses diuretic and cognitive enhancing activity. Leaves of *M. elengi* possesses antipyretic and analgesic activity (Sakshi *et al.,* 2011). M. elengi reported to have hypoglycemic and hypolipidemic activity (Zahid, 2012). In this study Ni was not detected in *M. elengi* sample. WHO/FAO defines permissible limits for medicinal plants i.e. 1.5ppm. It is required by the body in very small quantity for insulin production in pancreas. Ni toxicity is not very common among humans because its absorption by the body is very low. Nickel itch, an allergic dermatitis is the most common condition, which could occur. It may affect lungs and nasal cavities by acting as a carcinogen. Ni exerts potent toxic effects on peripheral tissues and reproductive system (Maobe *et al.,* 2012). Other metals were within the limits except mercury which was found to be 0.43ppm which is higher than the limits of 0.1ppm.

Holoptelea integrifolia (Roxb) Planch

H. Integrifolia (Ulmaceae) is a large tree distributed throughout greater part of India. Part used in this study is flower. It is mainly used for gastric issues like inflammation, gastritis, colic, vomiting, and intestinal worms. It is also used for leprosy, diabetes, hemorrhoids. Bark and leaves are used as an astringent, carminative, laxative (Sharma and Singh, 2012).It is also used for hair loss treatment due to herpes Infection (Srivastava *et al.,* 2013). *H.integrifolia* also possess antitumour activity when given in doses of 250mg/kg and 500mg/kg.It increases the survival time in mice (Kumar *et al.,* 2012).All the metals under study was within the permissible limits including mercury.

Malaysian drug product

It is a combination marketed herbal drug product available in markets of Malaysia. It is used as an antidiabetic. It is a combination of *Punica granatum, Momordica charantia, Tamarindus indica, Lawsonia inermis*.

Punica granatum

It is used in the treatment of hematuria, hemorrhoids, dysentery and bronchitis. Roots and bark of *P. granatum* used as anthelmintic and astringent. Leaf paste is used in conjunctivitis (Rajan *et al.,* 2013). It is also used in the treatment of prostate cancer, arthrosclerosis, diabetes, male infertility, arthritis (Jurenka, 2008).

Momordica charantia L.

Commonly known as bitter melon. It lowers blood sugar level, prevents jaundice by strengthening liver functions. It is also used in treatment of piles and cholera (Kumar and Bhowmik, 2010).

Tamarindus indica L.

Exhibit broad spectrum antibiotic activity. It has potent activity against S. aureus, S. typhi, B. subtilus. Fruit of *T.indica* reported to be beneficial in abdominal pains (Bhadoriya *et al.,* 2011).

Lawsonia inermis L.

L. inermis possess different Pharmacological activities like antioxidant, antimalarial, hepatoprotective, antiinflammatory. Methanolic leaf extracts of L. inermis

used as hypoglycemic, while dried leaf extracts are used as ant carcinogen (Kamal and Jawaid, 2010).

CONCLUSION

All the metals under study in selected herbal plants and Malaysian product were under the prescribed limits except mercury. Mercury was found as a major pollutant and health concern in population using these herbal plants and product. Malaysian ant diabetic drug is a marketed product, which poses a threat to population using this product for diabetes as mercuric content was out of the permissible limit in Malaysian product. Proper guidance must be provided by the regulating authorities for the safety and efficacy of herbal plants during their growth and usage.

REFERENCES

Adepoju-Bello AA, Issa OA, Oguntibeju OO, Ayoola GA and Adejumo OO (2014). Analysis of some selected toxic metals in registered herbal products manufactured in Nigeria. *Afr. J. Biotech.*, **11**(26): 6918-6922.

Aher AN, Pal SC, Patil UK, Yadav SK and Bhattacharya S (2009). Evaluation of antihistaminic activity of *Casuarina equisetifolia* frost. (Casuarinaceae). *PhOL.,* **1**: 1144-1149.

Aher AN, Pal SC, Yadav SK, Patil UK, & Bhattacharya S (2010). Isolation and characterization of phytoconstituents from *Casuarina equisetifolia* (Casuarinaceae). *Asian J Chem.,* **22**(5): 3429-3434.

Alam N, Shinwari ZK, Ilyas M and Ullah Z (2011). Indigenous knowledge of medicinal plants of chahgharzai Valley, District Buner Pakistan. *Pak. J. Bot*., **43**(2): 773-780.

Altaee MF (2011). *In vivo* toxicity study of nerium oleander's leaves and flowers aqueous extracts in mice (Cytogenetic, Biochemical and hematological Study). *Baghdad Sci. Journal.,* **8**(1): 1111.

American Heart Association Meeting Report. March, 21 2013.

Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G and Jain AP (2011). *Tamarindus indica*: Extent of explored potential. *Pharmacogn. Rev*., **5**(9): 73-81.

Clarkson TW, Magos L and Myers GJ (2003). The toxicology of mercury current exposures and clinical manifestations. *N. Engl. J. Med.,* **349**: 1731-1737.

El-Tantawy WH, Mohamed SAH and Al Haleem ENA (2013). Evaluation of biochemical effects *Casuarina equisetifolia* extract on gentamicin-induced nephrotoxicity and oxidative stress in rats. Phytochemical analysis. *J. Clin. Biochem. Nutr*., **53**(3): 158-165.

Helaluddin ABM, Khalid RS, Khan UA and Abbas SA (2013). Review of heavy metals contamination in traditional medicinal products. *J. App. Pharm.,* **4**(2): 748-756.

Hina B, Rizwani GH and Naseem S (2011). Determination of toxic metals in some herbal drugs through atomic absorption spectroscopy. *Pak. J. Pharm. Sci*., **24**(3): 353-358.

Jash SK, Singh RK, Majhi S, Sarkar A and Gorai D (2014). Peltophorum Pterocarpum: Chemical and Pharmacological Aspects. *Int. J. of Pharm. Sci. Res.,* **5 (1)**: 26-36

Jurenka JS (2008). Therapeutic Applications of Pomegranate (*Punica granatum* L.): A Review. *Altern. Med. Rev.,* **13**(2): 128-144

Kamal M and Jawaid T (2010). Pharmacological activities of *Lawsonia inermis* Linn: A review. *Int. J. Biomed. Res.,* **1**(2): 62-68.

Kar B, Kumar RS, Karmakar I, Dola N, Bala A, Mazumder UK and Hadar PK (2012). Antioxidant and *in vitro* anti-inflammatory activities of *Mimusops elengi* leaves. *Asian Pac. J. Trop. Biomed.,* **2**(2): S976-S980

Khan H, Khan MA, Tariq SA, Saeed M, Muhammad N, Gul F and Inayat H (2012). Analysis of metals contents of samples Gloriosa superba (Colchicacae) collected from two different locations of Pakistan. *Trop. J. Pharm. Res*., **11**(4): 631-636

Khan M, Rizwani GH, Shareef H, Cavar S and Zia-Ul-Haq M (2013). Assessment of total phenolic content and antioxidant potential of methanol extract of *Peltophorum pterocarpum* (DC.) Backer ex K. Heyne. *Pak. J. Pharm. Sci*., **26**(5): 967-972.

Kumar D, Kumar K, Gupta J, Bishnoi N and Kumar S. (2012). A mini review on chemistry and biology of *Holoptelea integrifolia* Roxb. Planch (Ulmaceae). *Asian Pac. J. Trop. Biomed.,* **2**(2): S1200-S1205.

Kumar KS and Bhowmik D (2010).Traditional medicinal uses and therapeutic benefits of *Momordica charantia* Linn. *Int. J. Pharm. Sci. Rev. Res.,* **4**(3): 23-28

Maobe MA, Gatebe E, Gitu L and Rotich H. (2012). Profile of heavy metals in selected medicinal plants used for the treatment of Diabetes, Malaria and pneumonia in Kisii region, Southwest Kenya. *Global J. Pharmacol.,* **6**(3): 245-251.

Niaz A, Ullah N, Rehman A, Ahmad I, Ikhlaq M and Rehman HU (2013). Pollution based study of heavy metals in some selected medicinal plants by dry digestion method. *Int. J. Pharm. Sci. Res.,* **4**(2): 17-24.

Omokehide A, Lajide L, Hammed O and Babatunde O (2013). Trace elements and major Minerals evaluation in *Fluerya aestuans* Linn. *Int.J Pharm. Sci.*, **3**(5): 328-332.

Padmapriya B, Leema MCE, Prasanna kumar A, MH Muhammad Ilyas MH and Rajeswari T (2012). Antihepatotoxicity of *Ricinus communis* (L.) against Ketoconazole induced hepatic damage. *Adv. Biol. Res.,* **6**(1): 30-36.

Parekh J and Chanda SV (2007). *In vitro* antimicrobial activity and phytochemical analysis of some Indian Medicinal Plants. *Turk. J. Biol.,* **31**: 53-58.

Patil PS, Argade PD, Ghule RS, Venkatnarayanan R and Shinde PR (2012). Protective effects of thespesia populnea (L.) Sol ex. Correa in inflammatory, nociceptive and arthritic conditions on experimental animals. *Br. J. Pharm. Res.,* **2**(4): 215-227.

Phanse MA, Patil MJ and Abbulu K (2013). Review on Pharmacological studies of *Thespesia populnea* Linn. *Int. J. Pharm. Pharm. Sci.*, **5**(3): 1-5

Rajan S, Ravi J, Suresh A, & Guru S (2013). Hidden Secrets of '*Punica Granatum*' use and its effects on oral health: A Short Review. *J. Orofac. Res.*, **3**(1): 38-41

Rajeshkumar D, Nagachaitanya V, Manasa G, Usharani A and Nagaraju K (2013). Pharmacological evaluation of analgisic activity of aqueous extract of *Ricinus communis* root bark. *Int. J. Toxicol. Pharmacol. Res.*, **5**(4): 94-95.

Sadashiv PS (2011). Acute toxicity study for *Ricinus communis. Scholars Research Library Der Pharmacia Lettre.*, **3**(5): 132-137.

Sakshi S, Vineet G, Rajiv G and Shubhini AS (2011). Analgesic and antioyretic activity of *Mimusops elengi* L. (Bakul) leaves. *PhOL.,* **3**: 1-6.

Sarin RV and Bafna PA (2012). Herbal antidiarrhoeals: A Review. *Int. J. Res. Pharm. Biomed. Sci.*, **3**(2): 637-649

Sharma J and Singh V (2012). *Holoptelea integrifolia*: An Overview. *Eur. J. Appl. Sci.,* **4**(1): 42-46.

Srivastava J, Prasad SK, Dwivedi KN and Pandey HP (2013). *Holoptelea integrifolia* Planch: A potential Ayurvedic medicinal plant. *Int. J. Pharm. Sci. Rev. Res*., **21**(2): 281-286.

Ştef DS, Gergen I, Hărmănescu M, Pop C, Drugă M, Bujancă G and Popa M (2010). Determination of the macro elements content of some medicinal herbs. *Scientific Papers, Animal Science and Biotechnologies,* **43**(1): 122-126

TEACH Chemical Summary (2007). US EPA, Toxicity and Exposure Assessment for Children's Health. *Environmental Protection Agency.*10/29/2007

Uddin AH, Khalid RS and Abbas SA (2012). Determination of heavy metal concentration of different traditional medicine formulations available at the east coast region of Malaysia. *Afr. J. Pharm. Pharmacol.,* **6**(20): 1487-1491.

WHO (2007). Exposure to Mercury: A Major Public Health Concern WHO Traditional Medicine Strategy 2002-2005- *World Health Organization* WHO/EDM/ TRM/2002.1.

Zahid H (2012) Hypoglycemic and hypolipidemic effects of *Mimusops elengi* Linn extracts on normoglycaemic and alloxan-induced diabetic rats. *Int. J. Pharm. Biol. Arch.,* **3**(1): 56-62.

Zahid H, Rizwani GH, Shareef H, Ahmed M and Hina B (2012). Analgesic and antipyretic activities of Hibiscus schizopetalus (Mast) Hook. *Int. J Pharm. Pharm. Sci.*, **4**(3): 218-221.

Zahid H, Rizwani GH, Shareef H, Khursheed R, Huma A, and Hasan SM. (2014). Antihyperglycemic and hypolipidemic effects of *Hibiscus schizopetalus* (Mast) hook in alloxan-induced diabetic rats. *Pak. J. Pharm. Sci.,* **27**(1): 83-89.