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Phytochemical, elemental and functional group analyses of herbal material and extracts of *Cassia sieberiana* used in herbal drug formulation

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Cassia sieberiana is used traditionally for the treatment of several ailments. The lack of knowledge in the levels of essential mineral contents and heavy metal constituents of C. sieberiana normally collected from various sites for preparation of herbal products can pose serious health risks to consumers. The elemental contents (Ca, Mg, K, Na, Cl, Hg, Pb, As, Ni, Cd, Cu, Fe, Mn, Zn, N, S and C) of a mixture of the stem and root barks (CSR) of Cassia sieberiana collected from Agomeda in the Eastern Region of Ghana and its extracts (absolute ethanol (CSE 1), 70% ethanol (CSE 2)) were investigated in this study. For the purposes of identification and characterization of CSR, CSE 1 and CSE 2, FT-IR and phytochemical analyses were conducted. The quantity of metals in all test samples were within the acceptable WHO permissible limits except for Cl, Fe, Mn, Pb and Cd contents in CSR. It is mostly the extract of CSR which is consumed traditionally. The quantity of powdered CRS consumed during treatment of ailment may be too low to cause Cl, Fe, Mn, Pb and Cd toxicity. FTIR studies showed similar functional groups in CSR, CSE 1 and CSE 2. Preliminary phytochemical screening showed the presence of reducing sugar, saponins, polyphenols, anthracenosides and triterpenes in CSR, CSE 1 and CSE 2. The elements found in Cassia sieberiana and extracts are vital for human health. Their reported contents indicate CSE 1 and CSE 2 may be suitable for use in drug formulation.

Key words: *Cassia sieberiana, herbal medicine, macronutrients, trace elements, heavy metals, functional groups, WHO/FAO*.

INTRODUCTION

Increase in global consumption of herbal medicine is not only due its ready availability, affordability and accessibility (Hussin, 2001)' but also, the perception that, herbal medicines are safer as compared to orthodox medicines

(Inamdar et al., 2008; Jayaraj, 2010). This is evident in the global market where herbal medicine was valued at USD 71.19 billion in 2016 (Hexa Research, 2017) and this is expected to increase to USD 117.02 billion by 2024 (Report Buyer, 2018). This high demand, together with herbal medicine being cited as a possible source of heavy metal toxicity to animals and man have necessitated the need in ensuring that, plant medicines are safe prior to their use (Alwakeel, 2008; Dwivedi and Dey, 2002; Rahimi et al., 2012; Saeed et al., 2011; Saper et al., 2004). The World Health Organization (WHO) recommends that, herbal medicines should be checked for the presence of heavy metals. Generally, factors which can cause the presence of heavy metals and other essential macronutrients in herbal medicines include; the geochemical soil characteristics, geography, use of contaminants such as pesticides, heavy metal poisoning from water bodies, microbes, adulteration, chemical toxins, contaminants in air, soil and water (industrial waste, mining and fertilizers). Contamination may also occur during cultivation, harvesting, collection, cleaning, drying processing, transportation and storage of plant material and this can significantly affect the quality and properties of resulting formulations (Saad et al., 2006; Tong et al., 2000). Basically, most medicinal plants are also eaten as food hence, investigating their nutritional benefits can help appreciate the worth of these plants (Pandey et al., 2006). WHO has also affirmed the importance and the need in determining the mineral nutrients as far as standardization of plant medicine is concerned (WHO, 2002). *Cassia sieberiana* of family Fabaceae is a shrub native to Africa. It grows mostly in savanna and woody grasslands. It has numerous ethnomedicinal and ethnobotanical properties such as its use in the treatment of dysentery, diarrhoea, etc. In Ghana, the Centre for Plant Medicine Research prescribes capsules formulated from the mixture of the stem and root bark of the plant to treat dysmenorrhea and pain associated with gastric ulcer. Several works have reported on the biological activities as well as phytochemical constituents of *Cassia sieberiana* (Archer et al., 2019) with little report on its essential minerals and heavy metal concentrations from various collection sites.

Agomeda is a village in the Shai Osuduko district in the Greater Accra region of Ghana. This district is found in the southeastern part of the region and has a 1,102 km total land area, characterized by undulating land below 70 m and a few secluded hills. Generally, this district records very low rainfall (762.5 - 1220 mm of annual mean rainfall). The vegetation is savanna interspersed with short trees and shrubs. Some light forest with tall trees is also found along the foothills of the Akuapem ridge, especially around Dodowa, Ayikuma and Agomeda. Agomeda abounds in various species of medicinal plants and is one of the principal areas where individuals and some institutions such as the Centre for Plant Medicine Research do plant collection for the production of essential herbal medicines (Dangme West District Assembly, 2013). This study aims at investigating the presence and contents of some essential minerals and heavy metal contaminants in a mixture of the root and stem bark of *Cassia sieberiana* as well as the contents in its extracts (absolute ethanol extract (CSE 1) and 70% ethanol (CSE 2)) as compared to the established WHO standard limits. Also, for the purpose of standardization, the FT-IR and phytochemical screening of CSR, CSE 1 CSE 2 was investigated.

MATERIALS AND METHODS

Collection and identification of plant

The stem and root bark of *Cassia siebieriana* were collected from Agomeda, (6°07'20.3''N, 014'36.2W) in the Eastern region of Ghana in March, 2020. Some of the plant specimen collected were pressed and processed following standard practices (Martin, 1995) and voucher specimen (CPMR 4978) have been deposited at the Centre for Plant Medicine Research (CPMR) herbarium. Plant identification was achieved via comparison of voucher specimen collected with already identified specimen at CPMR herbarium. Nomenclature and classification of the species of plants follows The Plant List database (http://www.theplantlist.org; accessed on 18/11/2020).

Extraction of powdered root and stem barks of *Cassia sieberiana*

An amount of 1.5 kg of a mixture of coarse powdered root and stem bark of *C. sieberiana* was cold macerated with 3.5 L of absolute ethanol for 75 h and filtered. Another 1 L of absolute ethanol was added to the residue and left for 16 h and then filtered. Both filtrates were mixed and evaporated at 40°C under reduced pressure using rotary evaporator to remove all the ethanol. The obtained paste was then lyophilized and stored in cellophane bag at room temperature until required for analyses (Donkor et al., 2014). The same process was repeated using 70% ethanol as extraction medium.

Preliminary phytochemical screening of CSR, CSE 1 and CSE 2

CSR, CSE 1 and CSE 2 were screened for the presence or absence of saponins by foam or froth test (Arthur, 1954; Yadav and Agarwala, 2011), polyuronides by Acetone test (Ciulei, 1982), polyphenols by Lignans test (Ciulei, 1982), reducing sugars by Fehling's test (Daniels et al., 1960; De et al., 2010), alkaloids by Mayer's test (Surmaghi et al., 1992), flavonoids by Shibata test (Khadraoui et al., 2015; Tahara et al., 1987), anthracenosides by Borntrager's test (Trease and Evans, 2002), cyanogenic glycosides by Guignard's test (Francisco and Pinotti, 2000; Harborne, 1972), triterpenes and phytosterols by Leibermann Burchard's test (Cook,

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1961; Kumar et al., 2014).

Determination of FT-IR of CRS, CSE 1 and CSE 2

The equipment was first scanned using the reference film (FT-IR Spectroscopic MIR polystyrene reference film) before the IRs of the samples were investigated. Twenty milligram (20 mg) of CSR was triturated with 200 mg spectroscopic grade KBr using agate mortar and pestle. The obtained mixture was then spread uniformly into a suitable dye. It was then compressed into a transparent disc. The obtained disc was placed in the sample compartment of the PerkinElmer Fourier Transform Infrared Spectrophotometer (L1600301 spectrum Two Lila) to obtain the IR spectrum at 400-4000 cm^{-1}. This was repeated for CSE 1 and CSE 2 (Fosu et al., 2016).

Elemental analysis of CSR, CSE 1 and CSE 2

Determination of Na and K contents in CSR, CSE 1 and CSE 2

An amount of 1 g of CSR was weighed into crucibles and placed in a muffle furnace at a temperature of 500°C for 3 h. After cooling an ash solution of the sample was then prepared by adding 10 ml of 1:2 solution of HNO_3 and H_2O and placed on a hot plate. At the first sign of boiling, it was removed and filtered into a 100 ml volumetric flask and made up to volume. This was repeated for CSE 1 and CSE 2. An atomic absorption spectrophotometer AAS (AAS, Agilent Technology 200 series 240 FS AA, USA) was used to determine the presence and amounts of Sodium (Na) and Potassium (K) at wavelengths 589 and 766 nm, respectively in each digest.

Determination of Pb, As, Hg, Ni, Cd, Ca, Fe, Zn, Mn, Mg, Cu, N and C contents in CSR, CSE 1 and CSE 2

An amount of 0.5 g of CSR was weighed into a microwave vessel and 10 ml of concentrated nitric acid was added to the sample. The vessel was then closed and placed into a microwave digester (Preekem Microwave Digestions System Model KJ-100, China) at set temperature, pressure and time regime. After completion of wet digestion, the sample was removed and ultra-pure water was added to make up a volume of 30 ml. It was then filtered into a receiving flask and made up to a volume of 100 ml. This was repeated for CSE 1 and CSE 2. The presence and concentration of mercury (Hg), nickel (Ni), lead (Pb), arsenic (Ar), cadmium (Cd), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) in each digest were determined using the atomic spectrophotometer at wavelengths Hg, 253.7 nm; Ni, 341.5 nm; Pb, 283.3 nm; Ar, 193.7 nm; Cd, 228.9 nm; Cu, 324.8 nm; Mn, 279.5 nm; Fe, 248.3 nm; and Zn, 213.9 nm.

The nitrogen (N) and carbon (C) contents in CSR, CSE 1 and CSE 2 were also investigated using the modified Kjeldahl (Motsara and Roy, 2008) and Walkley-Black wet oxidation (Nelson and Sommers, 1982) methods, respectively.

Determination of total sulphur (S) contents in CSR, CSE 1 and CSE 2

A volume of 5 mL aliquot of the filtrate from the wet digested plant material was measured into a 25 ml volumetric flask. A volume of 10 mL of sodium acetate-acetic acid buffer was added to maintain the pH at about 4.8. A volume of 1 mL of acacia gum mucilage and 1 g of $BaCl_2$ crystals were added and shaken well. The volume was made up to 25 mL with distilled water. The S content was determined using AAS at 440 nm (FAO, 2008).

Determination of chlorine contents in CSR, CSE 1 and CSE 2

CSR was ground into fine powder and an amount of 250 mg of the powder was weighed into a 90 ml glass vial. A volume of 50 mL deionized water was added and the vial capped. The vial was then placed on a reciprocating shaker and shaken at 250 rpm for 30 min. min. The content was filtered through Whatman No. 42 filter into a plastic vial for Cl analysis (Taylor and Francis, 1998). The Cl in the filtrate was analyzed using the argentometric method/Mohr's titration where an amount of 5 g of potassium chromate (5%) was added to 25 mL aliquot of the filtrate and then titrated with 0.0141 N silver nitrate to a brick red end point. This was repeated for CSE 1 and CSE 2.

Quality control and quality assurance

All experiments were performed in triplicates including reagent blanks. Deionized water was used throughout the study. All glassware were cleaned with 20% HNO_3. All reagents were of analytical grade. Additionally, a certified reference material (IACE-7) has been analysed (n=3) in conjunction with the samples to verify the accuracy and precision of the analytical procedure for total metal concentration. The recovery values were provided by IAEA-7. Moreover, 10 procedure blanks were tested during the total metal determinations and metal concentrations were below detected limits for all studied metals.

Statistical analysis

Data was analyzed with Excel and Graph Pad Prism for windows version 5 (Graph Pad Software Inc., San Diego, CA, USA) using one-way ANOVA. A p value < 0.05 was considered significant. All measurements were done in triplicates and results stated as mean ± standard deviation.

RESULTS AND DISCUSSION

Phytochemical screening of CSR, CSE 1 and CSE 2

A preliminary phytochemical analysis of CSR, CSE 1 and CSE 2 is shown in Table 1. They all contain saponins, reducing sugars, polyphenols, anthracenosides and triterpenes. The presence of these phytochemicals may be responsible for the biological activity of *C. sieberiana* and supports its folkloric use.

The presence of some phytochemical constituents in plants serves as a protective agent against toxic metal toxicity when ingested by humans. For instance, consumption of food and medicinal plants rich in polyphenols may lead to the prevention of some diseases caused by toxic metals (Brzóska et al., 2016). Polyphenols are able to control the level of toxic metals in the body through complexation. The numerous hydroxyl groups found in polyphenols form stable complexes with these metals (Borowska and Brzóska, 2016). This prevents their absorption from the gastrointestinal tract but enhances their excretion from the body via urination (Borowska et al., 2018). It has also been established that Saponins are able to remove heavy metals from aqueous solutions via biochemical reaction. Saponins may remove

Table 1. Phytoconstituents present in CSR, CSE 1 and CSE 2.

Phytoconstituents	CSR	CSE 1	CSE 2
Reducing Sugars	+	+	+
Saponins	+	+	+
Polyuronides	-	-	-
Polyphenols	+	+	+
Alkaloids	-	-	-
Flavonoids	-	-	-
Anthracenosides	+	+	+
Flavonoids	-	-	-
Triterpenes	+	+	+
Phytosteroles	-	-	-
Cyanogenic glycosides	-	-	-

(-) Absent; (+) Present. CSR: Mixture of stem and root bark, CSE 1: absolute ethanol extract of CSR, CSE: 70% ethanol extract of CSR.

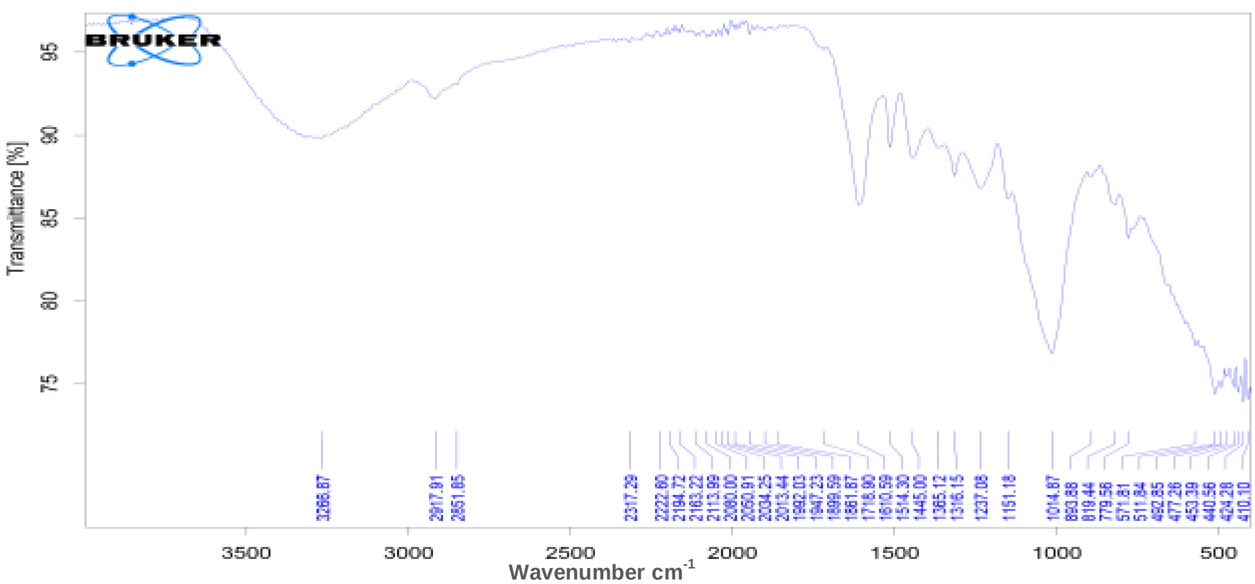

Figure 1. Results of FT-IR analysis of CSR.

heavy metals by biochemically forming coordinated complex with the saponins as the natural bio-ligand and the heavy metal at the central part of the complex.

However, this process is affected by pH, setting time and concentrations of heavy metal and saponins (Abed El Aziz et al., 2017).

The presence of metals in the soil is known to affect the presence and content of phytochemical constituents of their cultivated plants. The presence of Cd significantly affects the content of reducing sugar in plants by causing a reduction in the uptake of water for photosynthesis. This affects plant growth due to neutered sugar metabolism. This can be prevented by the addition of Ca which will ensure water uptake so as to revive the metabolism of sugar (Devi et al., 2007; Prado et al., 2010).

FT-IR analyses of CSR, CSE 1 and CSE 2

The surface functional groups present in CSR, CSE 1 and CSE 2 were identified using FT-IR spectra, as illustrated in Figures 1 to 3 with spectral peaks simplified in Tables 2 to 4. The spectra showed peak in the range of 400 to 4000 cm^{-1}. FT-IR of CSR, CSE 1 and CSE 2 indicates the presence of aromatic rings, alkenes, amines, nitro compounds, alkanes, alcohols and phenols. Detection of hydroxyl groups is an indication of the presence of flavonoids, phenolic and alcoholic compounds (Pawar and Kamble, 2017; Sim et al., 2004)

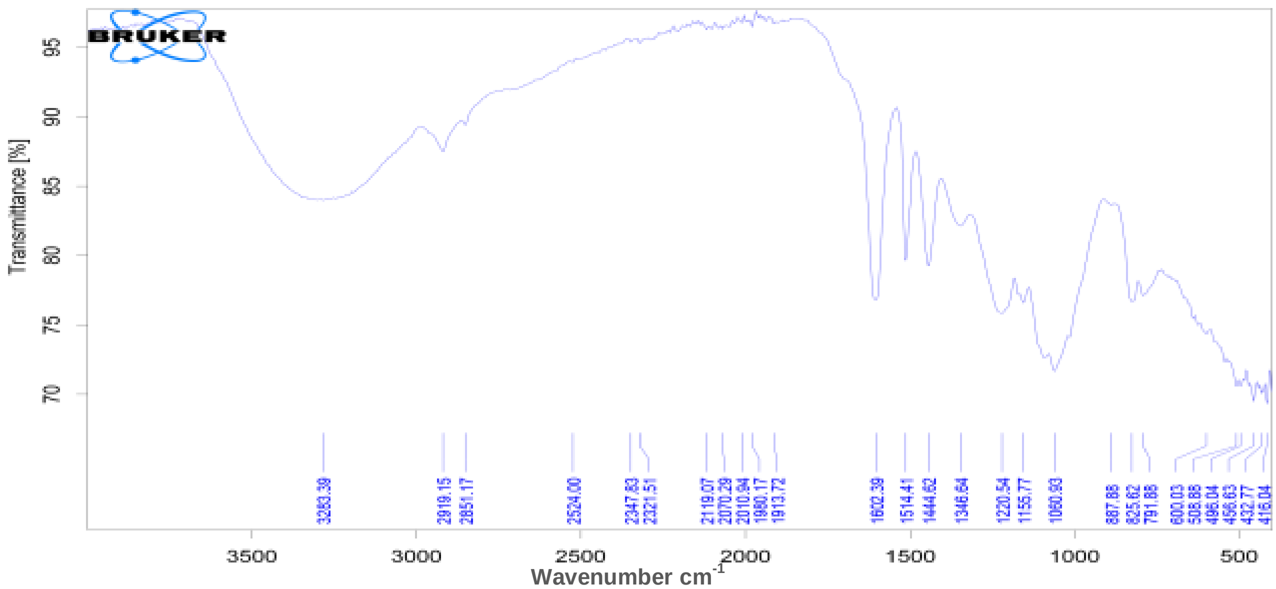

Figure 2. Results of FT-IR analysis of CSE 2.

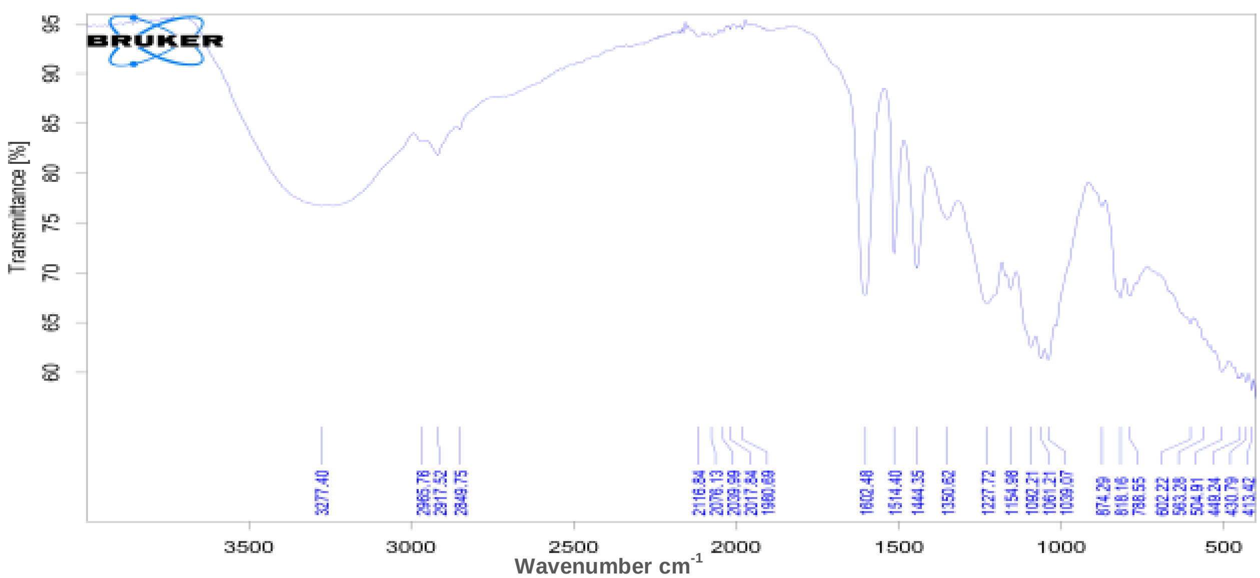

Figure 3. Results of FT-IR analysis of CSE 1.

which have all been previously detected in the various extracts of the plant (Archer et al., 2019) .

From the results of the various extraction methods, it can be concluded that constituents in all three samples were similar. By visual recognition, there is no significant difference in the characteristic absorption bands but the intensity of certain wavelengths differs from each other especially near the fingerprint region (1700-1400 cm^{-1}) for CSR compared to CSE 1 and CSE 2. These slight peak changes observed may be attributed to the different methods of extraction.

Essential minerals and heavy metals contents of CSR, CSE 1 and CSE 2

Macro minerals are essential minerals required in larger amounts in diet and are present in higher levels in the body. The presence of macronutrients in medicinal plants is advantageous because, they provide the body with essential minerals to help in its proper function. Among the investigated macronutrients (Ca, Mg, Na, K, Cl and S), Cl recorded the highest level with S being the least shown in Table 5.

Table 2. FT-IR peak values of CSR.

No.	Peak (wave number cm^{-1})	Bond	Functional group assignment	Group frequency
1	779.56	C=C Bend	Alkene	1000-650
2	819.44	C=C Bend	Alkene	1000-650
3	893.88	C=C Bend	Alkene	1000-650
4	1014.87	C-N Stretch	Amine	1400-1000
5	1237.08	C-N Stretch	Amine	1250-1020
6	1316.15	O-H bend	Phenol	1390-1310
7	1445.00	–	Unknown	-
8	1514.30	N-O stretch	Nitro compound	1550-1500
9	1610.59	N=H bend	Amine	1650-1580
10	1718.90	C-H Bend	Aromatic compound	2000-1650
11	1861.87	C-H Bend	Aromatic compound	2000-1650
12	2851.85	C-H Stretch	Alkane	3000-2840
12	2917.91	C-H Stretch	Alkane	3000-2840
13	3266.87	O-H Stretch	Alcohol, Phenol	3550-3200

Table 3. FT-IR peak values of CSE 2.

No.	Peak (wave number cm^{-1})	Bond	Functional group assignment	Group frequency
1	791.88	C=C	Alkene	1000-650
2	825.62	C=C	Alkene	1000-650
3	1060.93	C-N Stretch	Amine	1250-1020
4	1155.77	C-O Stretch	Tertiary alcohol	1205-1124
5	1220.54	C-N stretch	Amine	1250-1020
6	1346.64	O-H Bend	Phenol	1390-1310
7	1444.62	-	Unknown	-
8	1514.41	N-O stretch	Nitro compound	1550-1500
9	1602.39	N-H bend	Amine	1650-1580
10	1913.72	C-H Bend	Aromatic compound	2000-1650
11	1980.17	C-H Bend	Aromatic compound	2000-1650
12	2851.17	C-H Stretch	Alkane	3000-2840
13	2919.15	C-H Stretch	Alkane	3000-2840
14	3283.39	O-H Stretch	Alcohol, Phenol	3350-3200

Calcium is essential for the normal development and maintenance of healthy bones, blood and teeth (Charles, 1992). It helps in blood clotting, oocyte activation, nerve transmission and cardiac function (Julian et al., 2017). Calcium is also essential throughout life for the maintenance of bone density so as to prevent rickets in children and osteoporosis in adults (IOM Institute of Medicine, 2010). From Table 5, the contents of calcium were 0.01% for both CSE 1 and CSE 2 while CSR was 1.12% which was the highest amongst the tested samples. However, the content of Ca in them are below 36.61% which is the WHO/FAO permissible limit of Ca content in medicinal plants (Teerthe and Kerur, 2019; WHO, 2003). Statistical analysis of the results shows no significant difference in Ca contents in CSE 1 and CSE 2 but a significant difference ($p < 0.05$) between CSR and the two extracts.

Magnesium recorded contents in the range of 0.005 to 0.10% in the CSR, CSE 1 and CSE 2 (Table 5). A comparative study statistical analysis shows a significant difference ($p < 0.05$) between CSR and the two extracts but no significant difference in Mg contents in CSE 1 and CSE 2. Mg is needed for the structural development of bones via its role in the active transport of potassium and calcium ions (Famewo et al., 2018). Mg is also used for the synthesis of the antioxidant glutathione and nucleic acid. It is also involved in various physiological pathways such as glycolysis, energy production and oxidative phosphorylation. In addition, magnesium acts as cofactor in over 300 enzyme systems which regulates several

Table 4. FT-IR peak values of CSE 1.

No.	Peak (wave number cm^{-1})	Bond	Functional group assignment	Group frequency
1	788.55	C=C bend	Alkene	1000-650
2	818.16	C=C bend	Alkene	1000-650
3	1039.07	C-N stretch	Amine	1250-1020
4	1061.21	C-N Stretch	Amine	1250-1020
5	1092.21	C-N Stretch	Amine	1250-1020
6	1154.98	C-O Stretch	Tertiary alcohol	1205-1124
7	1227.72	C-N Stretch	Amine	1250-1020
8	1350.62	O-H bend	Phenol	1390-1310
9	1444.35	-	Unknown	-
10	1514.40	N-O Stretch	Nitro Compound	1550-1500
11	1602.48	N-H Bend	Amine	1650-1580
12	1980.69	C-H Bend	Aromatic compound	2000-1650
13	2849.75	C-H Stretch	Alkane	3000-2840
14	2917.52	C-H Stretch	Alkane	3000-2840
15	3277.40	O-H Stretch	Alcohol, Phenol	3550-3200

Table 5. Macro minerals contents in CSR, CSE 1 and CSE 2 (n=3).

Macro mineral	Macro nutrient content (%)		
	CSR (n=3)	CSE 1 (n=3)	CSE 2 (n=3)
Ca	1.12 ± 0.00	0.01 ± 0.003	0.01 ± 0.001
Mg	0.10 ± 0.20	0.005 ± 0.00	0.01 ± 0.00
K	0.53 ± 0.20	0.02 ± 0.002	0.07 ± 0.00
Na	0.24 ± 0.10	0.02 ± 0.00	0.03 ± 0.01
Cl	7.49 ± 0.10	0.71 ± 0.03	0.80 ± 0.07
S	0.15 ± 0.0	0.0034 ± 0.001	0.01 ± 0.001

Ca: Calcium; Mg: Magnesium; K: Potassium; Na: Sodium; Cl: Chlorine; S: Sulphur

biochemical reactions in the body (Famewo et al., 2018; IOM Institue of medicine, 1997). The results obtained shows that, the content of Mg in plant material and extracts are below 35% which is the WHO/FAO permissible limit of Mg content in medicinal plants (Teerthe and Kerur, 2019; WHO, 2003).

From Table 5, CSR recorded the highest sodium content of 0.24%. A statistical analysis showed significant difference ($p < 0.05$) in the Na levels between CSR and the extracts (CSE 1 and CSE 2). Na is a cation needed to maintain serum osmolality as well as extracellular fluid volume in the body. This essential role helps to keep a balanced fluid and electrolyte level, maintains the cell's membrane bound potential, ensures active transport of molecules across cell membranes and transmits nerve impulses in the body (Pohl et al., 2013). This recorded low contents of sodium in CSR, CSE 1 and CSE 2 is of immense advantage. This is because excessive consumption of sodium in humans can lead to increased risk of arterial hypertension which could result to stroke, heart diseases and kidney disease (Pohl et al., 2013).

The obtained result of potassium content for CSR, CSE 1 and CSE 2 (Table 5) were 0.53, 0.02 and 0.07%, respectively. A statistical comparison showed a significant difference ($p < 0.05$) in the K contents with CSR recording the highest level of 0.53%. There have been no reported WHO/FAO permissible limits for potassium in medicinal plants (Turkson et al., 2020). The presence of this macro mineral nutrient which is a predominant cation in the intracellular fluid of the body plays an essential role in protein synthesis and the normal function of the cell. An example is, neurotransmission and digestion (Pohl et al., 2013). It also functions as an electrolyte, helps to maintain the pH inside the cell and proper functioning of the nerve and muscle contraction (Young, 2012).

The concentration of Cl in CSR, CSE 1 and CSE 2 were 7.49, 0.71 and 0.80%, respectively. Statistical analysis also shows significant difference ($p < 0.0001$) in their respective Cl contents. The values of Cl obtained for CSR was higher than 1.0% which is above the WHO/FAO permissible limit for chlorine in medicinal

Table 6. Micro mineral contents in blend of powdered plant parts and extracts.

Micro minerals	Micro mineral content (mg/kg)		
	CSR (n=3)	CSE 1 (n=3)	CSE 2 (n=3)
Cu	9.80 ± 0.20	0.35 ± 0.02	0.8 ± 0.01
Fe	208.2 ± 0.09	12.23 ± 0.02	20.38 ± 0.01
Mn	217.8 ± 0.60	2.97 ± 0.01	5.97 ± 0.05
Zn	29.6 ± 0.00	0.81 ± 0.001	2.50 ± 0.01

Cu: Copper; Fe: Iron; Mn: Manganese; Zn: Zinc.

plants which is <1.0% (WHO, 2003). Nonetheless, the amount of CSR consumed as powder may be too small to cause Cl toxicity. However, the Cl content in CSE 1 and CSE 2 falls within the WHO/FAO permissible limit. Cl is an important essential mineral which works with potassium and sodium to maintain the levels of body fluid balance. Also, Cl is needed by the cells in the stomach lining to produce HCl, an important component of digestive juices. Though chlorine helps in the proper digestion of food and the absorption of other relevant elements needed for survival, continuous consumption of medicinal plants with excessive levels could lead to increase in blood pressure and water retention in the body (Kaur et al., 2012).

The result in Table 5 shows that, CSR recorded the highest amount of sulphur, 0.15%. The content of sulphur in plant material and extracts are below 1.0% which is the WHO/FAO permissible limit of S content in medicinal plants (Teerthe and Kerur, 2019; WHO, 2003). A statistical analysis also showed a significant difference ($p < 0.05$) between the S contents in the two extracts (CSE 1 and CSE 2) and CSR. Sulphur is an essential component of methionine and cysteine which are used in the formation of most protein molecules of the body (Hewlings and Kalman, 2019). Sulphur has demonstrated antioxidant effect via the reduction in the reactive oxygen and nitrogen species production (Beilke et al., 1987). Other functions of sulphur include: metal transport, protein synthesis and stabilization, repair and methylation of DNA, metabolism of lipids, functionality of enzyme, free radical scavenging, detoxification of xenobiotics, control of gene expression, protection of tissue integrity and repair of extracellular matrix constituents (Palego et al., 2015).

The presence of these macro nutrients in CSR, CSE 1 and CSE 2 with their contents within the WHO permissible limits makes CSR collected from Agomeda and its extract (CSE 1 and CSE 2) not only ideal for the treatment of dysmenorrhea and pain associated with peptic ulcer as currently used by CPMR but also a good source of these macro nutrients for its consumers.

Micro minerals

Micro minerals or trace elements are those essential minerals required in smaller amounts in the diet and are also present at low levels in the body. Among the analyzed trace elements, Mn recorded the highest amount while Cu recorded the least amount in CSR.

Iron, which is one of the abundant minerals recorded in this study is an essential part of myoglobin in the muscle (Wessling-Resnick et al., 2014), haemoglobin in the red blood cells (Famewo et al., 2018) and enzymes involved in redox reaction and cytochromes (Abbaspour et al., 2014). Fe also plays an essential role in normal cell function, metabolism, development of connective tissues and hormones (Murray-Kolbe and Beard, 2010). Iron deficiency would not only lead to anaemia but also would increase one's susceptibility to infections (Karyadi et al., 2000). Unsafe levels of Fe could lead to overdose which is associated with liver damage, diarrhoea, dizziness, joint pain, nausea and vomiting (Martin and Griswold, 2009; Obi et al., 2006). CSR recorded the highest content of Fe (208 mg/kg) while CSE 1 recorded the least (12.23 mg/kg) which is below the WHO maximum permissible limit (15 mg/kg) (World Health Organisation, 2007b, a). Thus the contents of Fe in CSR and CSE 1 (Table 6) were above the WHO maximum permissible limit. For instance, traditionally, CSR is mostly prepared as infusions and decoctions in treating ailments (Lim, 2012). Hardly is the raw plant consumed. However, most formulations from CSR are in small doses. Example, CPMR's formulation from CSR contains approximately 320 mg of CSR powder which is taken when necessary (prn) with maximum daily dose of 960 mg (in 3 divided dose) and maximum dose of 2.88 g per treatment course. Also, formulation from CSE 1 also contains approximately 28 mg of CSE 1 per dose which is also taken prn with maximum daily dose of 84 mg and maximum of 252 mg per treatment course (Archer et al., 2020). This means that, the amount of CSR and CSE 1 consumed is too small to cause Fe toxicity. Statistical analysis showed a significant difference ($p < 0.05$) in Fe contents in CSR, CSE 1 and CSE 2. This could be due to the employed method of extraction and the different solvents used.

Copper is one of the cofactors for super oxide dismutase in the body. It is needed for the manufacture of collagen, important for building strong tissues, nerve function, bone growth, helps in the utilization of sugar and maintain blood volume in the body. Copper is also involved in generation of energy from carbohydrates and helps in the incorporation of iron into the red blood cells for the prevention of anaemia (IOM Institue of Medicine, 2001). It is likely that, the presence of copper together with the other detected minerals could enhance the immunity of consumers of *Cassia sieberiana* collected from Agomeda and its formulations from the extracts. Cu deficiency is known to cause the Menkes' kinky hair syndrome. From Table 6, CSR recorded the highest Cu content (9.8 mg/kg), while CSE 1 recorded the least, 0.35 mg/kg. Cu contents in all tested samples however, are below the WHO permissible limit of 10 mg/ kg (World Health Organisation, 2002). This result shows a significant difference ($p < 0.05$) in the levels of Cu in CSR, CSE 1 and CSE 2. This shows that, CS collected from Agomeda and its extracts pose no danger with respect to Cu toxicity.

The presence of manganese (Mn) in the plant material and its extracts is advantageous as Mn does not only help the body to form blood clotting factors, connective tissues and sex hormones (Young, 2012) but also plays an essential role in bone formation, metabolism of carbohydrates, fats, amino acids and cholesterol (IOM Institue of Medicine, 2001). It plays a significant role in the regulation of blood sugar, absorption of calcium and also forms a component of the antioxidant enzyme which is required to fight free radicals (Young, 2012). CSR recorded the highest content of Mn (217.8 mg/kg), which is a little higher than 200 mg/kg, the reported WHO permissible limit of Mn in medicinal plants (World Health Organisation, 1998). Nonetheless, the amount of CSR consumed istoo small to cause Mn toxicity. However, CSE 2 and CSE 1 recorded 5.97 and 2.97 mg/kg content of Mn, respectively which are all below the permissible limit. This shows that, upon extraction using these solvents, some amounts of Mn content are lost which could be as a result of the inability of these solvents to completely extract Mn from the plant material. The obtained results also showed a significant difference ($p<0.05$) in the recorded Mn contents in CSR, CSE 1 and CSE 2.

The extracts and plant material have been shown to be rich in zinc (Table 6). The concentration of Zn in CSR, CSE 1 and CSE 2 were 29.6, 0.81 and 2.50 mg/kg, respectively which also demonstrated a significant difference ($p < 0.05$) in Zn contents among all test samples. The values obtained are below 50 mg/kg which is the FAO/WHO permissible limit for Zn in medicinal plants (World Health Organisation, 2002, 2006). This essential trace element is required in various functions such as catalytic activity of enzymes, cell metabolism, cell division, DNA synthesis, protein synthesis and wound healing. Zinc also plays an important role in individual's immune system and also helps to protect cells against free radicals. Zinc deficiency causes susceptibility to infections as a result of impaired immunity (Muthuraj et al., 2010; Ramakrishnan et al., 2008).

Toxic heavy metals

Toxic metals are dense metalloids or metals which are known for their potential toxicity (Pourret and Hursthouse, 2019), particularly in environmental context (Srivastava and Goyal, 2010; Zhang et al., 2019). Mercury, lead, arsenic, nickel and cadmium are part of the 10 listed toxic metals of major public health concern (FAO/WHO, 1984). Trace amounts of some of these elements are essential for the biological and physiological functions of the human body (Korfali et al., 2013a; 2013b), however, its prolong exposure may lead to toxicity and adverse health effects. This may be the result of bioaccumulation of it causing distortion in the functions of the vital organs in the body such as the kidneys, liver and brain (Çelik and Oehlenschläger, 2007; Ray and Ray, 2009).

The results obtained shows that, the highest level of mercury content was recorded by CSR, 0.009 mg/kg shown in Table 7. A statistical comparison of Hg contents shows no significant difference ($p >0.005$) of Hg contents in CSR, CSE 1 and CSE 2. These values are below 0.5 mg/kg, the permissible limit as proposed by WHO (World Health Organisation, 2007a). Organic, metallic and inorganic mercury poisoning is associated with damage to developing foetus, brain and kidney. The damage to brain function results in tremors, irritability, shyness, memory problems and changes to vision and hearing. The exposure to methyl mercury is worse off for young children than adults. This is because, methyl mercury easily passes into their brain where it interferes with their normal growth and development (Vaikosen and Alade, 2011).

Lead (Pb) poisoning occurs when its plasma concentration reaches 100 to 140 µg/l (Goldfrank et al., 1998). This can lead to drastic reduction in the cognitive function of children, spontaneous abortion, impaired neurodevelopment and low birth weight of foetus as a result of high Pb level during pregnancy (Canfield et al., 2003; Salawu et al., 2009). Also, prolonged exposure could lead to a reduction in the renal clearance and the performance of nervous function (Salawu et al., 2009).The concentration of Pb in CSR, CSE 1 and CSE 2 was found to be 52, 5.73 and 4.72 mg/kg, respectively. The lowest concentration as recorded by CSE 2 could be due to the presence of relatively higher volume of water present in the extraction procedure; water has been found to poorly extract lead making it a safer alternative in medicinal plant preparation as compared to absolute ethanol extraction (Adie and Adekunle, 2017). According to WHO, the maximum permissible limit for Pb is 10 mg/kg (World Health Organisation, 2006). This implies that consumption of herbal medicines prepared form either absolute or 70% ethanol extract of CS from Agomeda would not cause Pb toxicity. There was also a significant difference ($p < 0.0001$) in the amount of Pb found in CSR, CSE 1 and CSE 2. Due to the increasing Pb toxicity in herbal medicines, it would be more

Table 7. Toxic heavy metal contents in CSR, CSE 1 and CSE 2.

Toxic heavy metals	Toxic heavy metal contents (mg/kg)		
	CSR (n=3)	CSE 1 (n=3)	CSE 2 (n=3)
Hg	0.009 ± 0.01	0.0006 ± 0.00	0.0007 ± 0.004
Pb	52.0 ± 0.02	5.73 ± 0.02	4.72 ± 0.01
As	1.2 ± 0.29	0.05 ± 0.00	0.08 ± 0.00
Ni	0.2 ± 0.08	0.03 ± 0.00	0.08 ± 0.01
Cd	1.2 ± 0.09	0.12 ± 0.0	0.2 ± 0.01

Hg: Mercury; Pb: Lead; As: Arsenic; Ni: Nickel; Cd: Cadmium.

advantageous to use hydroethanolic extract of medicinal plants in formulation.

Arsenic (As) exposure could result in chronic adverse health effect such as cancer in diverse body organs especially of the lungs and skin as well as acute adverse health effects, example, capricious appetite coupled with weight loss and cramping of the abdomen (Franzblau and Lilis, 1989; Ng et al., 2003). From Table 7, CSR showed the highest As content with concentration of 1.2 mg/kg. CSE 2 was found to contain 0.08 mg/kg of As while CSE 1 was 0.05 mg/kg. The concentrations recorded fall below the maximum permissible limit for As which is 10 mg/kg (World Health Organisation, 2007a). Also, a comparison of the contents only showed a significant difference ($p < 0.05$) in Pb contents in CSR and the extracts (CSE 1 and CSE 2).

The highest content of nickel which was 0.2 mg/kg was found in CSR while the lowest amount, thus 0.034 mg/kg was found in CSE 1. However, a comparative study showed no significant difference ($p > 0.05$) between the Ni contents in CSR, CSE 1 and CSE 2. These values obtained are below the WHO permissible limits for medicinal plants, 1.5 mg/kg (World Health Organisation, 2002) and also lower than the reported FAO/WHO permissible limit of 1.63 mg/kg for edible plants (FAO/WHO, 1984). Nickle has been identified as a carcinogen (Maobe et al., 2012) but it also has some health benefits. In small amounts such as the recommended daily intake of < 1 mg/ kg (McGrath, 1990), Ni is needed by the body for the production of insulin and its deficiency is known to cause hepatic disorders (Pendias, 1992). The exposure to toxic levels of Ni is the occurrence of nickel itch which is a form of allergic dermatitis (Maobe et al., 2012).

The exposure to toxic levels of cadmium (Cd) from sources such as medicinal plants could lead to the increased risk of cancer, destruction of the red blood cells and high blood pressure (Afkhami et al., 2006). Cd toxicity in the body can also be caused by the replacement of ions in the body's metallo-enzyme by Cd^{2+}. This could be due to the competition for binding site and their chemical similarities (Duruibe et al., 2007). The results from the experiment shown in Table 7 showed Cd concentrations for CSR, CSE 1 and CSE 2. The Cd content in CSR is higher than the WHO permissible limit, 0.3 mg/kg (World Health Organisation, 2006), however, the amount of CSR consumed may be too little to cause Cd toxicity. Statistical comparison of the contents showed a significant difference ($p < 0.05$) in Cd contents found in CSR and extracts (CSE 1 and CSE 2).

Carbon and nitrogen

The mixed stem and root barks together with its extracts has demonstrated to be rich in nitrogen which is needed for the growth and repair of worn out tissues, synthesis of amino acids which intends makes protein in our body and for the synthesis of nucleic acid (DNA and RNA) (Rosca and Duca, 2009). From Table 8, CSR demonstrated the highest amount of nitrogen content, thus 0.95% with CSE 1 showing the least N content, 0.45%. A statistical analysis showed a significant difference ($p < 0.0001$) in N contents in the tested samples. Currently, there are no reported WHO/FAO permissible limits for nitrogen contents in medicinal plants.

Carbon is one of the elements which form the building blocks in all living things due to its ability to form stable bonds with itself and many other elements. Carbon is the backbone of all organic compounds and as such carbon cycle is an important biological process. It is the main component of carbohydrate, proteins, nucleic acid, fats, muscle tissues and almost everything in the body. Energy is released upon breaking of carbon molecules which drives all cellular processes (Anon, 2021). The highest C content of 49.58% was found in CSE 1. However, all the values recorded are below the WHO/FAO permissible range for medicinal plants 65% (WHO, 2003) shown in Table 8. Statistical analysis shows a significant difference ($p < 0.05$) in the contents of C in CSR, CSE 1 and CSE 2.

Conclusion

Agomeda is one of the key areas where some major institutions do plant material collection for the mass production of herbal medicines. Hence, the study gives a new perspective to the presence of essential elements

Table 8. Carbon and nitrogen contents in blend of powdered plant parts and extracts.

Other elements	Other minerals (%)		
	CSR (n=3)	CSE 1 (n=3)	CSE 2 (n=3)
N	0.95 ± 0.20	0.25 ± 0.08	0.45 ± 0.05
C	47.22 ± 0.10	49.58 ± 0.06	48.96 ± 0.10

N: Nitrogen; C: Carbon.

and heavy metals in CS. The results show that, except for Cl, Fe, Pb and Cd in CSR, all essential elements and heavy metal contents in CSR, CSE 1 and CSE 2 and its extracts were within the permissible limits sets for metals in edible plants and the permissible limits set for medicinal plants. It was generally observed that, the elemental contents in the plant material were higher than the extracts. These results indicate that, it is safer to use extracts of *CS* sourced from Agomeda, rather than consuming the raw plant material. High contents of Pb and Cd in CSR should be of concern when consumed in very high amounts. Quality control practices for screening herbal medicines must be in place to protect consumers from toxicity. Preliminary phytochemical screening and FT-IR of CSR, CSE 1 and CSE 2 showed the presence of similar constituents and functional groups, respectively. This study shows that, the safe contents of these elements in CSE 1 and CSE 2 coupled with the fact that they contain similar compounds as CSR makes them suitable for use in drug formulation. Further studies should be conducted to identify the source of these identified heavy metal contaminants of *Cassia sieberiana* in Agomeda. Also, such studies should be done on *CS* and other medicinal plants collected on all principal areas of Ghana. This will help address public health concerns with regards to safety of use herbs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbaspour N, Hurrell R, Kelishadi R (2014). Review on iron and its importance for human health. Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences 19(2):164.

Abed El Aziz M, Ashour A, Madbouly H, Melad AS, El Kerikshi K (2017). Investigations on green preparation of heavy metal saponin complexes. Journal of Water and Environmental Nanotechnology 2(2):103-111.

Adie GU, Adekunle A (2017). Evaluation of Potentially Toxic Metal Contamination of Local Medicinal Plants and Extracts Sold in Ibadan, Nigeria. Journal of Health and Pollution 7(14):23-29. https://doi.org/10.5696/2156-9614-7.14.23

Afkhami A, Madrakian T, Siampour H (2006). Flame atomic absorption spectrometric determination of trace quantities of cadmium in water samples after cloud point extraction in Triton X-114 without added chelating agents. Journal of Hazardous Materials 138(2):269-272. https://doi.org/10.1016/j.jhazmat.2006.03.073

Alwakeel SS (2008). Microbial and heavy metals contamination of herbal medicines. Research Journal of Microbiology 3(12):683-691.

Anon (2021). Significance of Carbon. https://bio.libretexts.org/@go/page/6260 [Accessed July 23, 2021].

Archer MA, Agyei A, Mintah S, Adjei P, Kumadoh D, Asiedu-Larbi J (2019). Medicinal uses of Cassia sieberiana; a review. International Journal of Sciences: Basic and Applied Research 48:161-180.

Archer MA, Kumadoh D, Yeboah GN, Kyene MO, Kumatia EK, Antwi S, Appiah AA (2020). Formulation and evaluation of capsules containing extracts of Cassia sieberiana for improved therapeutic outcome. Scientific African 10:e00609. https://doi.org/https://doi.org/10.1016/j.sciaf.2020.e00609

Arthur HR (1954). A phytochemical survey of some plants of North Borneo. Journal of Pharmacy and Pharmacology 6(1):66-72.

Beilke MA, Collins-Lech C, Sohnle PG (1987). Effects of dimethyl sulfoxide on the oxidative function of human neutrophils. The Journal of Laboratory and Clinical Medicine 110(1):91-96.

Borowska S, Brzóska MM (2016). Chokeberries (Aronia melanocarpa) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Comprehensive Reviews in Food Science and Food Safety 15(6):982-1017. https://doi.org/10.1111/1541-4337.12221

Borowska S, Brzoska MM, Tomczyk M (2018). Complexation of bioelements and toxic metals by polyphenolic compounds–implications for health. Current Drug Targets 19(14):1612-1638. https://doi.org/10.2174/1389450119666180403101555

Canfield RL, Henderson Jr CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP (2003). Intellectual impairment in children with blood lead concentrations below 10 µg per deciliter. New England Journal of Medicine 348(16):1517-1526.

Çelik U, Oehlenschläger J (2007). High contents of cadmium, lead, zinc and copper in popular fishery products sold in Turkish supermarkets. Food Control 18(3):258-261. https://doi.org/10.1016/j.foodcont.2005.10.004

Charles P (1992). Calcium absorption and calcium bioavailability. Journal of Internal Medicine 231(2):161-168. https://doi.org/10.1111/j.1365-2796.1992.tb00519.x

Ciulei I (1982). Methodology for analysis of vegetable drugs. practical manual on the industrial utilisation of medicinal and aromatic plants. Romania: Center Building, pp. 67-81

Cook RP (1961). Reactions of steroids with acetic anhydride and sulphuric acid (the Liebermann-Burchard test). Analyst 86(1023):373-381.

Dangme West District Assembly (2013). Medium Term Development Plan (MTDP) 2010 -2013.

Daniels R, Rush CC, Bauer L (1960). The Fehling and Benedict tests. Journal of Chemical Education 37(4):205.

De S, Dey YN, Ghosh AK (2010). Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of Amorphaphallus paeoniifolius (Araceae). International Journal of Pharma and Bio Sciences 1(5):150-157.

Devi R, Munjral N, Gupta AK, Kaur N (2007). Cadmium induced changes in carbohydrate status and enzymes of carbohydrate metabolism, glycolysis and pentose phosphate pathway in pea. Environmental and Experimental Botany 61(2):167-174. https://doi.org/10.1016/j.envexpbot.2007.05.006

Donkor K, Okine LNK, Abotsi WKM, Woode E (2014). Acute and subchronic toxicity studies of aqueous extract of root bark of *Cassia sieberiana* DC in rodents. Journal of Applied Pharmaceutical Science 4(04):084-089

Duruibe JO, Ogwuegbu MOC, Egwurugwu JN (2007). Heavy metal pollution and human biotoxic effects. International Journal of Physical Sciences 2(5):112-118.

Dwivedi SK, Dey S (2002). Medicinal herbs: a potential source of toxic metal exposure for man and animals in India. Archives of Environmental Health: An International Journal 57(3):229-231. https://doi.org/10.1080/00039890209602941

Famewo EB, Clarke AM, Afolayan AJ (2018). Evaluation of important mineral nutrients and vitamins in poly herbal medicines used forthe treatment of tuberculosis in the Eastern Cape Province, South Africa. International Journal of Phytomedicine 10(1):16-22.

FAO/WHO (1984). Contaminants.: Vol. XVII (In Codex and Alimentarius (ed.); 1st ed.). FAO/WHO, codex Alimentarius Commision.

Food and Agriculture Organization (FAO) (2008). Guide to laboratory establishment for plant nutrient analysis. Viale delle Terme di Caracalla.

Fosu MA, Ofori-Kwakye K, Kuntworbe N, Bonsu MA (2016). Investigation of blends of cashew and xanthan gums as a potential carrier for colonic delivery of Ibuprofen. International Journal of PharmTech Research 9(7):369-380.

Francisco IA, Pinotti MHP (2000). Cyanogenic glycosides in plants. Brazilian Archives of Biology and Technology 43(5):487-492.

Franzblau A, Lilis R (1989). Acute arsenic intoxication from environmental arsenic exposure. Archives of Environmental Health: An International Journal 44(6):385-390. https://doi.org/10.1080/00039896.1989.9935912

Goldfrank LR, Flomenbaum NE, Hoffman RS, Howland MA, Lewin NA, Weisman RS (1998). Goldfrank's toxicologic emergencies. 6th ed. Appleton & Lange: Stanford (CT) 1227-309

Harborne JB (1972). Phytochemical ecology. Phytochemical Ecology. Academic Press, London and New York, USA P 272.

Hewlings S, Kalman D (2019). Sulfur in human health. EC Nutrition 14:785-791.

Hexa Research (2017). Herbal Medicine Market Size and Forecast and Trend Analysis pp. 2014-2024.

Hussin AH (2001). Adverse effects of herbs and drug-herbal interactions. Malaysian Journal of Pharmacy 1(2):39-44.

Inamdar N, Edalat S, Kotwal VB, Pawar S (2008). Herbal drugs in milieu of modern drugs. International Journal of Green Pharmacy 2(1).

IOM Institue of Medicine (1997). Food and Nutrition Board. Dietary Reference intakes for calcium, phosphorus, magnesium, vitamin D and fluoride. Washington DC: National Academy Press.

IOM Institue of Medicine (2001). Food and Nutrition Board: Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. The National Academies Washington.

IOM Institute of Medicine (2010). Food and Nutrient Board. Committee to Review Intakes for vitamin D and Calcium. Dietary reference intakes for calcium and vitamin D. Washington D.C: National Academy Press, (Ross A).

Jayaraj P (2010). Regulation of traditional and complementary medicinal products in Malaysia. International Journal of Green Pharmacy 4(1).

Julian AV, Umagat MR, Reyes RG (2017). Mineral Composition, Growth Performance and Yield of Pleurotus ostreatus on Rice Straw-Based Substrate Enriched with Natural Calcium Sources. Euro-Mediterranean Conference for Environmental Integration pp 1573-1575.

Karyadi E, Schultink W, Nelwan RHH, Gross R, Amin Z, Dolmans WMV, Van Der Meer JWM, Hautvast JGAJ, West CE (2000). Poor micronutrient status of active pulmonary tuberculosis patients in Indonesia. The Journal of Nutrition 130(12):2953-2958. https://doi.org/10.1093/jn/130.12.2953

Kaur R, Kumar A, Kaur N, Mohanty BP, Oswal M, Singh KP, Behera BR, Singh G, Puri R, Sharma S (2012). Investigation of Major and Trace Elements in Some Medicinal Plants Using PIXE. International Journal of PIXE 22(01n02):113-119. https://doi.org/10.1142/S0129083512400177

Khadraoui A, Hachama K, Khodja M, Khelifa A, Mehdaoui R, Harti H, Abid S, Najda BA, Chahboun N (2015). Extraction study and the antibacterial activity of phenol and flavonoid pp 2501-2508.

Korfali SI, Mroueh M, Al-Zein M, Salem R (2013). Metal concentration in commonly used medicinal herbs and infusion by Lebanese population: health impact. Journal of Food Research 2(2):70.

Korfali SI, Hawi T, Mroueh M (2013). Evaluation of heavy metals content in dietary supplements in Lebanon. Chemistry Central Journal 7(1):10.

Kumar RS, Balasubramanian P, Govindaraj P, Krishnaveni T (2014). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. roots (Coriander). Journal of Pharmacognosy and Phytochemistry 2(6):74-78.

Lim TK (2012). Garcinia macrophylla. In Edible Medicinal And Non-Medicinal Plants. Springer pp. 71-75.

Brzóska M, Borowska S, Tomczyk M (2016). Antioxidants as a potential preventive and therapeutic strategy for cadmium. Current Drug Targets 17(12):1350-1384.

Maobe MAG, Gatebe E, Gitu L, 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 Journal of Pharmacology 6(3):245-251. 10.5829/idosi.gjp.2012.6.3.65128

Martin G (1995). Ethnobotany-A manual of methods. London: Chapman et Hall.

Martin S, Griswold W (2009). Human health effects of heavy metals. Environmental Science and Technology Briefs for Citizens 15:1-6.

McGrath SP (1990). Chromium and nickel. Heavy Metals in Soils pp. 125-150.

Motsara MR, Roy RN (2008). Guide to laboratory establishment for plant nutrient analysis (Vol. 19). Food and agriculture organization of the United Nations Rome.

Murray-Kolbe LE, Beard JI (2010). Encyclopedia of Dietary Supplements L, and N. Y. I. Healthcare 2:432-438.

Muthuraj M, Kamatchiyammal S, Usharani B, Manupriya S, Ayyappan ARN, Divyalakshmi K. (2010). Serum zinc, calcium and albumin levels in pulmonary tuberculosis patients co-Infected with HIV. Global Journal of Biotechnology and Biochemistry 5(1):27-35.

Nelson DW, Sommers LE (1982). Methods of soil analysis. Part 2, pp. 539-579.

Ng JC, Wang J, Shraim A (2003). A global health problem caused by arsenic from natural sources. Chemosphere 52(9):1353-1359.

Obi E, Akunyili DN, Ekpo B, Orisakwe OE (2006). Heavy metal hazards of Nigerian herbal remedies. Science of the Total Environment 369(1-3):35-41.

Palego L, Betti L, Giannaccini G (2015). Sulfur metabolism and sulfur-containing amino acids: I-molecular effectors pp. 1-8.

Pandey M, Abidi AB, Singh S, Singh RP (2006). Nutritional evaluation of leafy vegetable paratha. Journal of Human Ecology 19(2):155-156.

Pawar S, Kamble V (2017). Phytochemical screening, elemental and functional group analysis of Vitex negundo L. leaves. International Journal of Pharmacy and Pharmaceutical Sciences 9(6):226-230.

Pendias H (1992). Trace elements in soils and plants.

Pohl HR, Wheeler JS, Murray HE (2013). Sodium and potassium in health and disease. Interrelations between Essential Metal Ions and Human Diseases pp. 29-47.

Pourret O, Hursthouse A (2019). It's time to replace the term "heavy metals" with "potentially toxic elements" when reporting environmental research. International Journal of Environmental Research and Public Health 16(22):4446.

Prado C, Rodríguez-Montelongo L, González JA, Pagano EA, Hilal M, Prado FE (2010). Uptake of chromium by Salvinia minima: effect on plant growth, leaf respiration and carbohydrate metabolism. Journal of Hazardous Materials 177(1-3):546-553.

Rahimi M, Farhadi R, Balashahri MS (2012). Effects of heavy metals on the medicinal plant. International Journal of Agronomy and Plant Production 3(4):154-158.

Ramakrishnan K, Shenbagarathai R, Kavitha K, Uma A, Balasubramaniam R, Thirumalaikolundu SP (2008). Serum zinc and albumin levels in pulmonary tuberculosis patients with and without HIV. Japanese Journal of Infectious Disease 61(3):202-204.

Ray SA, Ray MK (2009). Bioremediation of heavy metal toxicity-with special reference to chromium. Al Ameen Journal of Medical

Sciences 2(2):57-63.
Report Buyer (2018). Report buyer, https://www.reportbuyer.com. Accessed on 2/3/20 at 13:22 GMT.
Rosca V, Duca M (2009). MT d. Groot and MTM Koper. Chemical Reviews 109:2209-2244. Available at: 10.1021/cr8003696
Saad B, Azaizeh H, Abu-Hijleh G, Said O (2006). Safety of traditional Arab herbal medicine. Evidence-Based Complementary and Alternative Medicine 3(4):433-439.
Saeed M, Muhammad N, Khan H (2011). Assessment of heavy metal content of branded Pakistani herbal products. Tropical Journal of Pharmaceutical Research 10(4):499-506.
Salawu EO, Adeleke AA, Oyewo OO, Ashamu EA, Ishola OO, Afolabi AO, Adesanya TA (2009). Prevention of renal toxicity from lead exposure by oral administration of Lycopersicon esculentum. Journal of Toxicology and Environmental Health Sciences 1(2):22-27.
Saper RB, Kales SN, Paquin J, Burns MJ, Eisenberg DM, Davis RB, Phillips RS (2004). Heavy metal content of ayurvedic herbal medicine products. Jama 292(23):2868-2873.
Sim CO, Hamdan MR, Ismail Z, Ahmad MN (2004). Assessment of herbal medicines by chemometrics–assisted interpretation of FTIR spectra. J Analytica Chimica Acta 1:14.
Srivastava S, Goyal P (2010). Novel biomaterials: decontamination of toxic metals from wastewater. Springer Science & Business Media.
Surmaghi MHS, Amin YAGH, Mahmoodi Z (1992). Survey of iranian plants for saponins alkaloids flavonoids and tannins. IV. DARU Journal of Pharmaceutical Sciences 2(2-3):1-11.
Tahara S, Hashidoka Y, Mizutani J (1987). Flavonoids as medicines. Agricultural and Biological Chemistry 51:1039-1045.
Taylor and Francis (1998). Handbook of Reference Methods for Plant Analysis. Soil and Plant Analysis Council Inc. CRC Press.
Teerthe S, Kerur B (2019). Study of multielemental concentrations and Nano-micro structural morphology in myrtaceae family medicinal plants by field Emission Scanning electron microscope-energy dispersive X-ray spectroscopy method. Asian Journal of Pharmaceutical and Clinical Research 12(2):432-435.
Tong S, Schirnding YE, Prapamontol T (2000). Environmental lead exposure: a public health problem of global dimensions. Bulletin of the World Health Organization 78:1068-1077.
Trease GE, Evans WC (2002). Pharmacognosy. Saunders," Elsevier, Amsterdam, The Netherlands 36:51.
Turkson BK, Mensah MLK, Sam GH, Mensah AY, Amponsah IK, Ekuadzi E, Komlaga G, Achaab E (2020). Evaluation of the Microbial Load and Heavy Metal Content of Two Polyherbal Antimalarial Products on the Ghanaian Market. Evidence-Based Complementary and Alternative Medicine.
Vaikosen EN, Alade GO (2011). Evaluation of pharmacognostical parameters and heavy metals in some locally manufactured herbal drugs. Journal of Chemical and Pharmaceutical Research 3(2):88-97.
Wessling-Resnick M, Ross AC, Caballero B, Cousins RJ, Tucker KL, Ziegler RG (2014), Modern Nutrition in Health and Disease. Baltimore MD: Lippincott Williams & Wilkins.
World Health Organisation (WHO) (2003). Traditional Medicine. 56th World Health Assembly Provisional Agenda item 14.10: A56/19. Report by the Secretariate.
World Health Organisation (WHO) (1998). Quality control methods for medicinal plant materials. World Health Organization.
World Health Organisation (WHO) (2002). Quality Control Methods for Medicinal Plants materials. Delhi, AITBS, Publisher.
World Health Organisation (WHO) (2006). WHO Guidelines for Assessing Quality of Herbal Medicines with reference to contaminants and residues. In World Health Organisation, Geneva, Geneva, Switzerland.
World Health Organisation (WHO) (2007a). Monographs on selected medicinal plants. In World Health Organisation, (Vols. 1-3).
World Health Organisation (WHO) (2007b). WHO Guidelines for assessing quality of herbal medicines with reference to contaminants and residues.
Yadav RNS, Agarwala M (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology 3(12):10-14.
Young DB (2012). Role of potassium in preventive cardiovascular medicine. Springer Science & Business Media.
Zhang H, Walker TR, Davis E, Ma G (2019). Ecological risk assessment of metals in small craft harbour sediments in Nova Scotia, Canada. Marine Pollution Bulletin 146:466-475.