

Analysis of metals and persistent organic pollutants in ethyl acetate extract of *Peltophorum africanum*

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Received 24 November 2018; Revised 09 October 2019

This study was aimed to analyze the metals and persistent organic pollutants (POPs) accumulated in the ethyl acetate extract (EAE) of *Peltophorum africanum* a medicinal plant commonly used in South Africa. Metal analysis revealed the presence of aluminum (Al) [17.2%], chlorine (Cl) [2.7%], sodium (Na) [5.7%], nitrogen (N) [1.3%], sulphur (S) [3.0], carbon (C) [6.5%], oxygen (O) [6.5%], titanium (Ti) [6.1%], silicon (Si) [17.2%], gold (Au) [15.9%], copper (Cu) [2.9%], zinc (Zn) [3.0%], and potassium (K) [4.3%]. The functional group of phosphorus-oxy (P=O); halogenated compounds (C-F, C-Cl), thiols and thio-substituted (C-S/C-I, S-S/CICN) organic pollutants with the frequency wavelength range of 420.23–1287.62 cm⁻¹ were identified. Colchicine, n-hexadecanoic acid, lanosta, nitroanthraquinone, stigmaterol, octamethylcyclotetrasiloxane, and ferrocene were also detected with percentage quantity of 0.4, 6.4, 2.9, 0.2, 1.6, 0.6 and 0.1% respectively. Some of the metals and POPs identified from the EAE of *Peltophorum africanum* in this study have been linked or associated with various human health risks.

Keywords: Chemicals, Environmental impact, Human health, Medicinal plant, Metal contamination, Pollutants.

IPC Code; Int. cl. (2015.01)-A61K 36/00

Introduction

Metals are common environmental contaminants, often as a result of human activities and mostly arise together with persistent organic pollutants (POPs). Pollutants can lead to different effects on plant metabolism and the detoxification system¹. POPs are compounds which are resistant to environmental degradation (photolytic, biological, and chemical), such as per- and polyfluoroalkyl substances (PFASs) which persist for a long period of time in the environment, and consequently bio-magnify and bio-accumulate via plants and animals². Perfluoroalkyl sulfonic acids (C₄, C₆–C₈, C₁₀-PFSA) and carboxylic acids (C₄–C₁₄-PFCA) have been found in some baking and sandwich papers³ with plants analyses revealing a high variability of PFAS concentrations in vegetative compartments⁴. Cadmium (Cd), mercury (Hg), lead (Pb), arsenic (As), benzene, fluoride, dioxin and pesticides have been among the toxic heavy metals and pollutants listed as a major public health concern by

World Health Organisation⁵. People staying in the proximity of a polluted area by heavy metals are prone to renal disease, hypertension, diabetes, and stroke⁶. Toxic metals react with various proteins in the body that may modify their functions and reaction rates⁷. Human exposure to mercury is mainly from fish, dental fillings and cosmetics^{8,9}. A study conducted in India revealed that metal concentration in the Bhopal wetland lower lake was very high and the fishes were severely contaminated with Pb and chromium (Cr); hence not fit for human consumption¹⁰. Risk assessment analysis reveals that metal pollution generally poses medium to high risk at different sites due to anthropogenic activities including industrial, domestic, and agricultural¹¹. Arsenic is used in the manufacture of insecticides, herbicides and pesticides; thus, contributes to the groundwater pollution which is dangerous to plants, animals and human health globally⁷. It has been reported that the levels of As, Cd, and Pb were significantly increased in the mother with diabetes and their newborns¹². One of the risk factors of developing diabetes is exposure to high levels of Hg and Cd. This may result in the malfunction of insulin-producing cells in the pancreas^{13,14}. To address such

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diseases in developing nations, phyto-remedial strategies are used. Herbal medicine is known to have numerous vital and dietetic elements, is being used worldwide since ancient times as a readily available source for management of various diseases and to reinforce the human body's immune system. However, their surplus or shortage may perturb regular biochemical activities of the human body, with the contamination of medicinal plants by POPs and metals via aquaporins, further exacerbating contaminant ingestion^{15,16}.

Peltophorum africanum, known as African Wattle or Weeping Wattle is a medicinal plant used traditionally in the management of wound infection, toothache, intestinal parasites and gastric problems¹⁷. Plants and vegetables are expected to be safe because of their existence in nature; meanwhile, studies have reported the presence of high level of toxic organic chemical and metal contaminants sourced from transportation, industrial discharge, energy production, and agriculture. Risks, such as poisoning with lethal effects on the nervous system, intestine, stomach, blood, and liver, have been reported^{18,19}. Aquaporins (AQPs) are water channels made mainly of proteins that enable the transport of liquid and tiny molecules through organic membranes. In plants, aquaporin is confined in the endoplasmic reticulum, vacuoles, plastids, plasma sheath, and transports various metals, biological substrates and POPs apart from the influx of water into expanding cells and maintenance of normal cytosolic osmolality^{2,20,21}. These contaminations occur at different phases of the plant growth consequently of contributions from fertilizers, soil, water, atmospheric dust, rainfall, and plant protective agents¹. Multivariate techniques such as cluster analysis (CA), principal component analysis (PCA) and factor analysis (FA) are used to determine the similarity in elemental composition, the proximity of groups of elements, and the mode of absorption of nutrients by plants respectively. FA has revealed that metal contamination of plant could be based mainly on the active uptake of nutrients from the soil, passive absorption, root pressure, and gaseous absorption from atmospheric driven transpiration pull²². In the study conducted by Kumar *et al.*^{23,24}, the concentration of most metal (e.g., Cd, Cr, Cu, Ni, Si etc) contaminants identified in different parts of the plants ranges from 0.0008 to 13,800.00 µg/g. There is no known study on the analyses of metals and organic pollutants in EAE of *Peltophorum africanum*. The

study, therefore, sought to investigate its metal and chemical pollutants together with the review of related literature on the assessment of the associated human health risk. Scanning Electron Microscope with Energy Disperse X-ray Spectroscopy (SEM-EDS), Fourier-Transform Infrared Spectroscopy (FT-IR), Gas Chromatography and Mass Spectroscopy (GC-MS) were used for the identification of metals, functional groups, and volatile compounds respectively.

Materials and Methods

Sample collection and preparation

The plant was collected in Limpopo Province and identified in collaboration with a Botanist at the University of Venda, South Africa. Voucher specimen BP01 was deposited at the herbarium of the University. Stem bark was used in this study because of the antimicrobial and cytotoxicity activity recorded in our previous study¹⁷. The sample was sun-dried (two weeks), grounded to powder (100 g) and subjected to cold extraction by percolation using 900 mL of 99% ethyl acetate (EA). The sample was exhaustively extracted, filtered and concentrated to dryness using evaporator (Steroglass, Italy) at 70 °C. The working stock was prepared by purifying in DMSO and kept at 4 °C. Potassium bromide, pyridine, and empty poly-L-lysine-coated glass coverslip were used as process control blanks for FT-IR, GC-MS and SEM-EDS analyses respectively. The experiments were conducted in duplicate and the instruments used calibrated. Prior to use, the SEM magnification settings were calibrated for range accuracy as well as the EDS energy level by checking the copper L α and aluminum K α .

Fourier-Transform infrared spectroscopy analysis

The functional group of toxic organic chemicals in the extract was characterized using infrared spectra fingerprint. The dried crude ethyl acetate extract (EAE) was pulverized into a powder with potassium bromide (KBr) in ratio 1:100, and subjected to FT-IR analysis (Perkin Elmer System, 2000, England). The frequency wavelength (cm⁻¹) was interpreted using the interpretation of infrared spectra, a practical approach in the encyclopedia of analytical chemistry²⁵.

Gas chromatography and mass spectrophotometer analysis

The GC-MS analysis was done using Agilent 6890 Series and Restek 12723-127 together with Agilent 5973 detector software. EAE was purified in 100% DMSO and diluted in sterile water to make up a

concentration of 100 mg/mL. The EAE was fractionated by means of toluene/ethanol (T:Et; 90:5) and benzene/ ethanol/ ammonium hydroxide (B:Et:A; 90:10:1) solvent systems on thin-layer chromatography (TLC) and column chromatography. The samples were dissolved in pyridine and derivatized with *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) before injection onto a GC column. The oven was programmed at 150, 180, and 325 °C for 1, 4 and 20 minutes, respectively before Ramp1 (50 °C/min) and Ramp2 (10 °C/min). Pure helium was the carrier gas used at a flow rate of 1 mL/min and injection volume of 1 µL (Mode ratio- 15:1). Injection temperature and MS transfer were set at 280 °C with acquisition mode scanning mass range of 40 to 550 m/z (Energy: 70 eV)²⁶.

Scanning electron microscope and energy-dispersive X-ray spectroscopy analysis

EAE was analyzed using Scanning Electron Microscope (SEM; JSM-6390LV, Jeol, Japan) and Energy Dispersive X-ray Spectroscopy (SEM-EDXS; Thermo super dry II Xray detector, Jeol, Japan). A 10 mg/mL of EAE diluted in sterile water was prepared in 96-well plates and incubated at 27 °C for 3 days after which it was harvested using a sterile scraper and washed with phosphate-buffered saline (PBS). The sample fixed in 2.5% gluteraldehyde after 24 hours of incubation at 37 °C was prepared on poly-L-lysine-coated glass coverslip (sticky). The post fixation was carried out with 1% Osmium tetroxide (OsO₄) and dehydrated (30, 50, 70, 85 and 95% ethanol) cocktail mounted onto stubs and coated using IB3 Ion Coater (EIKO, Japan). The sample was micro-analyzed and the representative spectra obtained²⁷.

Results and Discussion

Functional group of compounds

Table 1 presented the compounds in the EAE of *P. africanum* considered to be POPs and can endanger human health. Phosphorus-oxy compound (P=O), for example, phosphorus oxychloride may cause respiratory irritation. This is toxic when ingested or inhaled with skin and tissue irritation. It causes weakness, abdominal pain, nausea, vomiting, nephritis, injuries of the mucous membranes of the mouth and gastrointestinal tract and may be lethal^{28,29}. Aromatic ether oxy compound (Φ-O-H) such as furan is toxic and carcinogenic in humans with respiratory tract irritation^{30,31}. Halogenated compounds (C-F, C-Cl) are used in agrochemicals and pharmaceuticals (organofluorines), including drugs such as ciprofloxacin (Cipro), fluoxetine (Prozac), 5-post-fixation (Paxil), fluconazole, and mefloquine³². Halogenated compounds constitute one of the largest groups of environmental pollutants, such as trichloroethylene, dichloromethane, dichlorodiphenyltrichloroethane (pesticides) and dioxins³³. Excessive consumption of fluoride has been linked with the risk of developing dental and skeletal fluorosis; while the inhalation of chlorine at a concentration up to 500 parts per million for less than 60 min is lethal^{5,34}.

Thiols and thio-substituted compound/ halogenated compound of disulfides (C-S stretch)/ aliphatic iodo compound C-I stretch or aryl disulfides (S-S stretch)/ ClCN/ triatomic inorganic molecule are organosulphur compound found in space, volcanoes, and oceans. It occurs in all existing creatures as essential amino acids such as methionine, and cysteine, which are

Table 1 — Functional group of the organic pollutant from ethyl acetate extract of *Peltophorum africanum*

Origin/functional group	Frequency wavelength (cm ⁻¹)	Assignment	Compound	Hazard/Toxicity
P=O	1287.62	Phosphate	Phosphorus-oxy compound	Very toxic (T ⁺), Harmful (Xn), Corrosive (C), LD ₅₀ (median dose at 50%) 36 mg/kg (oral, in rat) e.g., phosphorus oxychloride ^{26,27}
Φ-O-H	1235.96	Aromatic ethers	Ether and oxy compound	Carcinogenic in humans e.g., Furan ^{28,29}
C-F	1142.04	Aliphatic fluoro compound C-F stretch	Halogenated compound	Development of dental and skeletal fluorosis ^{5,30,32}
C-Cl	723.92	Aliphatic chloro compound (organohalogen)	Halogenated compound	C-Cl in the extract can be carcinogenic and genotoxic in both man and animal ³⁰⁻³²
C-S/C-I	598.75	Disulfides (C-S stretch)/Aliphatic iodo compound C-I stretch	Thiols and thio-substituted compound/Halogenated compound	Toxic to organs like kidneys, respiratory system, liver, central nervous system, and spleen ³³
S-S/ClCN	420.23	Aryl disulfides (S-S stretch)/ triatomic inorganic molecule	Thiols and thio-substituted compound/ linear molecule	Potent hepatotoxic and hemolytic agents ³³

constituents of proteins, vitamins, hormones, and enzymes. Organosulphur compounds are found in such foods as garlic, onion, coffee and other sulfur compounds such as penicillin, cephalosporin, and sulfanilamide, which are essential antibiotics. Meanwhile, some of the organosulphur compounds such as thiophenol is an irritant and toxic when ingested and the target organs are kidneys, respiratory system, liver, central nervous system, and spleen³⁵.

Volatile compounds

Mass spectroscopy (MS) identifies compounds through electric charge, accelerating over a magnetic field, and detecting the compounds after breaking down into charged fragments³⁶. Hexadecanoic acid also known as palmitic acid with 6.4 % total quantity in the fraction as shown in Table 2, is common saturated fatty acids found in plants and animals (palm oil, olive oil, and body lipids) with nutritional and medicinal advantages³⁷. Meanwhile it is associated with schizophrenia, a disorder or disease characterized by hallucinations and other cognitive disorder³⁸. Nitroanthraquinone (0.166%) and its similar derivatives have been suspected to be a carcinogen, capable of causing interference to the genomic constituents and cellular metabolic processes, with acute neurotoxic effects^{39,40}. Colchicine's (0.4058%) side effects depend on its dosage and the human lethal dose (LD) of 6–7 mg has been reported. It has a resilient irritation effect on the gastrointestinal (GI) tract and can obstruct the blood cells, causing neutropenia deficiency and aplastic anaemia. It can also result in respiratory centre paralysis^{41,42}.

According to a study conducted in China in 1992, 4 mg/kg of Lanosta-8, 24-dien-3-ol, acetate(3 beta)-

(2.8456%) induced abortion after thirteen (13) weeks of conception⁴³. Ferrocene (0.1162 %) can moderately poison humans with vomiting and diarrhoea, shock, GI haemorrhage, coma, seizures and hepatotoxicity being the primary clinical outcomes associated with its ingestion⁴⁴. It has also been reported to be toxic to the lungs and kidney after a repeated or prolonged inhalation or usage⁴⁵. Moreover, cyclotetrasiloxane, octamethyl-(0.6284%) is suspected of reproductive toxicity; hence detrimental to fertility or the unborn child⁴⁶. Inhalation of octamethylcyclotetrasiloxane induces CYP2B1/2 protein which results in hepatomegaly⁴⁷. Stigmasterol (1.5827%) has been associated with a disorder or disease called sitosterolemia or phytosterolemia, a condition with lipid or sterols metabolic disorder that results in the increase of fatty substances in the human tissues and blood^{48,49}.

Metal composition

Aluminum (Al), chlorine (Cl), sodium (Na), nitrogen (N), sulphur (S), carbon (C), oxygen (O), titanium (Ti), silicon (Si), gold (Au), copper (Cu), zinc (Zn), and potassium (K) with percentage quantity of 17.2, 2.7, 5.7, 1.3, 3.0, 6.5, 6.5, 6.1, 17.2, 15.9, 2.9, 3.0, and 4.3% were identified in the EAE respectively as represented in Fig. 1. Forte *et al.*⁵⁰ and Feng *et al.*⁵¹ reported that aluminium (Al), Titanium (Ti), Copper (Cu), and Zinc (Zn) have been implicated in diabetes. Al and Si are the most abundant in this study with the highest total quantity of 17.2% in the EAE. This is high compared to the study conducted by Kumar *et al.*²² with the concentration range of 0.05-12.78% for both elements, meanwhile, the concentration of elemental composition, especially of

Table 2 —Volatile organic pollutant from fractions of the ethyl acetate extract

Solvent system	Retention Time (min)	Compounds	Quality march	Total percentage	Hazard/Toxicity
TEt	9.2047	n-Hexadecanoic acid	99	6.4	Linked with Schizophrenia ³⁶
BEtA	14.7153	1-(2-Naphthylloxy)-4-nitroanthraquinone	46	0.166	Carcinogen ^{37,38}
BEtA/TEt	17.0248	Colchicine, N-desacetyl-N-TFA-/ Colchicine, N-(trifluoroacetyl)methyl- N-deacetyl-	50	0.4058	Overdose results in death from respiratory failure. May cause genetic defects (Germ cell mutagenicity) ^{39,40}
BEtA	17.6471	Lanosta-8, 24-dien-3-ol, acetate (3 beta)-	43	2.8456	Results in abortion ⁴¹
BEtA	19.0008	Ferrocene, [benzoyl(phenylmethyl)amino]-	32	0.1162	Hematochezia, lethargy, shock, acidosis, and coagulopathy. Necrosis to the GI tract. Decrease in total blood volume, hypotension, CNS depression, and hepatotoxicity have been demonstrated ^{42,43}
BEtA	19.3985	3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-	43	0.6284	Results in the fall of blood pressure and cardiac output. Induces CYP2B1/2 protein and causes liver enlargement ^{44,45}
BEtA	19.4691	Stigmasteroltrimethylsilyl ether	95	1.5827	Associated with Sitosterolemia ^{46,47}

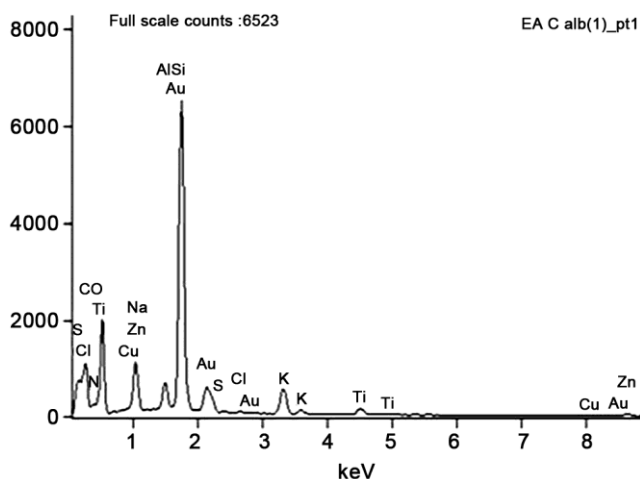


Fig. 1 — SEM-EDS micrograph of metal analysis in ethyl acetate extract of *Peltophorum africanum*

Saccharum bengalense Retz was similar to EAE, except for high C (20.01%) and O (31.51%) recorded in that study. The toxicity of aluminium results in lung and bone disease, neurotoxicity effects, impaired iron absorption, and Alzheimer⁵². Titanium and its derivatives used in food colourings such as candy, gum and toothpaste have been associated with lung and respiratory disorder as well as degenerative brain and as possibly carcinogenic to humans^{53,54}. High accumulation of metals in vegetables has been associated with atmospheric depositions¹⁹. Metals with high transfer factor (HTF) can easily gain access to comestible part of a plant which results in high accumulation of metals, especially in food crops⁵⁵. Although lack of some of the essential minerals in the human body such as Zn, K, and Fe have been associated with a high chance of developing chronic kidney disease (CKD)⁵⁶.

Conclusion

This study revealed that there is accumulation of elemental (Al, Cl, Na, N, S, C, O, Ti, Si, Au, Cu, Zn, K) and chemical pollutant (Phosphorus-oxy, Colchicine, Ferrocene, and Lanosta) in stem bark of *P. africanum*, a medicinal plant locally used in Africa. The findings showed that metal and persistent organic pollutant in the soil is a serious challenge. This will, therefore, necessitate the protective propagation and cultivation of the plant used for medicinal and consumption as plants could be contaminated by gaseous absorption from the atmosphere, active uptake of nutrients from the soil, passive absorption, and root pressure through plant aquaporin. The chemical contaminants may result in disorder or debilitating

diseases after extensive accumulative effects in the human body. Hexadecanoic acid, Nitroanthraquinone, Colchicine, Lanosta-8, 24-dien-3-ol, acetate(3 beta)-, and Ferrocene have been reported to result in schizophrenia disorder, neurotoxic effects, aplastic anaemia, induced abortion and hepatotoxicity respectively. Meanwhile, the total percentage of these chemical pollutants is within the range of 0.1–6.4%. Moreover, most known proteins contain metal such as Na^{2+} , K^{+} , Mg^{2+} that helps in protein structure stabilization and enzyme catalysis which are important in the activation of many fundamental life processes.

Conflict of interest

The authors declare no conflict of interest. The funding body had no role in the design of the study; the collection, analyses, and interpretation of data; nor in the writing of the manuscript, and the decision to publish the results.

Acknowledgement

We thank the Cape Peninsula University of Technology, University of Fort Hare and National Research Foundation (CSUR2008052900010) of South Africa for funding.

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