Simultaneous Analysis of Eleven Heavy Metals in Extracts of *Sonneratia caseolaris* (L.) Engl. by ICP-MS

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ABSTRACT

Sonneratia caseolaris (L.) Engl. has been used in traditional medicine to treat various diseases. The high concentrations of heavy metals in edible parts of *S. caseolaris* can lead to poisoning. Therefore, the aim of this study was to determine the concentrations of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in various parts of *S. caseolaris* (breathing roots, calyces, fruits, leaves, seeds, and stamens) and their extracts by using inductively coupled plasma-mass spectrometry (ICP-MS). The concentrations of As, Cd, and Hg found in the raw plant samples and their extracts were below the permissible limits. However, the contamination of Al, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in some parts of plant samples and their extracts were rather high. It is concluded that quality control of the raw material must be carried out before further utilization of *S. caseolaris*.

Keywords: *Sonneratia caseolaris*, Heavy Metals, Extracts, ICP-MS

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INTRODUCTION

An exotic mangrove plant *Sonneratia caseolaris* (L.) Engl. (family Sonneratiaceae) is commonly known as lamphu in Thailand. This species can also be found in Sri Lanka, Sumatra, Java, Borneo, Celebes, Philippines, Moluccas, Timor, New Guinea, Solomon Islands, and New Hebrides [1]. The sour taste of its young fruits can be used for making healthy fruit juice, vinegar, and curry. This plant is a folk remedy for cough, angina, diabetes, diarrhoea, dysentery, hematuria, haemorrhage, and eradication of gastrointestinal parasites [2]. Previous studies revealed that triterpenoids, flavonoids, nor-lignans, and 6*H*-benzo[*b,d*]pyran-6-one derivatives were found in the fruits of *S. caseolaris* [3]. Furthermore, (-)-(*R*)-nyasol, (-)-(*R*)-4'-*O*-methylnyasol and maslinic acid isolated from the fruits showed moderate cytotoxic activity against a rat glioma C-6 cell line using the MTT assay method [3]. Two flavonoids (luteolin and luteolin 7-*O*-*β*-glucoside) from its leaves exhibited antioxidant activity [4]. The analysis of gas chromatography-time of flight-mass spectrometry (GC–TOFMS) chromatograms of bark and wood of *S. caseolaris* indicated the presence of organic compounds, such as alkanes, alkenes, aromatic compounds, alcohols, phenols, carboxylic acids, amides and amines [5]. In our previous work, aqueous and methanolic crude extracts from calyces, seeds, and stamens of *S. caseolaris* composed of gallic acid, luteolin-7-*O*-glucoside, and luteolin showed antioxidant activity, with TEAC assay [6-8]. Moreover, the extracts of this plant containing polyphenols, flavonoids, triterpenoids, and sterols exhibited antioxidant, anti-inflammatory, and hepatoprotective activities [9]. At present flavonoids are plant secondary metabolites, which are important determinants in quality of various parts of *S. caseolaris*.

Concentrations of heavy metals such as aluminium (Al), arsenic (As), cadmium(Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn) have been reported in mangrove tissues [10,11]. Mangrove plants have been used in traditional medicine for the treatment of human diseases. Edible parts of mangrove plants containing toxic heavy metals exceed permissible limits may cause human health hazards such as renal failure, liver damage, and symptoms of chronic toxicity [11,12]. The bioaccumulation of heavy metals in mangroves depends on many factors, including species, tissues, life stage, season, salinity, toxic waste, sediment, dispersed oil, and pesticides [10]. According to the stipulation of the World Health Organization (WHO), toxic heavy metals must definitely be controlled in herbal plants in order to assure the safety [13]. The aim of this study was to find out the concentrations of eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in various parts of *S. caseolaris* such as breathing roots (pneumatophores), calyces, fruits, leaves, seeds, and stamens as well as their extracts by using inductively coupled plasma-mass spectrometry (ICP-MS). These extracts were prepared by Soxhlet extraction and simple solvent extraction.

MATERAILS AND METHODS

Materials

The solvents used for extraction were methanol and distilled water. Analytical reagent grade methanol was obtained from Merck (lot K40351807 934, Darmstat, Germany). The ultrapure water ASTM type I, 18.2 MΩ x cm used for determination of heavy

metals was generated by a TKA GenPure ultra pure water machine (TKA Wasseraufbereitungssysteme GmbH, Germany). Nitric acid for sample digestion was of analytical reagent grade (lot K40352656 935, Merck, Darmstat, Germany). An ICP multi element standard solution XIII (Lot HC813513, Agilent, USA) was prepared immediately in 5% v/v nitric acid solution prior to use. All glassware containers used for metal analysis were soaked overnight in 20% v/v nitric acid solution and rinsed several times with ultrapure water to eliminate metal contamination.

Plant Material

S. caseolaris (L.) Engl. were collected from the mangrove forest close to Mae Klong river in Amphur Mueng, Samut Songkhram province, Thailand, in October 2008. The voucher specimen was deposited in the Faculty of Pharmacy, Silpakorn University, Thailand.

Plant Treatment

This study presented a comparative analysis of eleven heavy metals in breathing roots, calyces, fruits, leaves, seeds, and stamens of *S. caseolaris* (L.) Engl. All plant samples were washed with distilled water and rinsed several times with ultrapure water. Each part of plants was dried in hot air oven at 60°C. The dried samples were then grounded separately with an IKA MF-10 Microfine Grinding Mill (Werke GmbH & Co. KG, Germany), equipped with a 0.5 mm hole size sieve and stainless steel grinder features interchangeable grinding heads. The powdered samples were stored in plastic bags inside a desiccator to protect them from humidity before extraction and acid digestion.

Plant Preparation

Soxhlet Extraction

Each ground sample (50 g) and 200 ml of 75% methanol (MeOH) was refluxed for 3 days using Soxhlet extraction glassware system (250 ml siphon system, Pyrex). Each extracted solution was filtered and dried on a rotary evaporator (Buchi R205, Germany) to give the solid mass of MeOH Soxhlet extract (MSE). The extracts were kept in a desiccator at room temperature.

Simple Solvent Extraction

For preparation of MeOH extracts (ME), the ground samples (25 g) were separately extracted with 100 ml of 95% MeOH at room temperature for 3 days. The obtained MeOH solution was dried at 50°C under reduced pressure in a rotary evaporator. For preparation of water extracts (WE), the ground samples (25 g) were separately extracted with 100 ml of hot distilled water at 95 °C for 3 hrs. The water soluble part was collected and filtered through a Whatman No.1 filter paper. Each filtrate was dried using spray dryer (Minispray Dryer, Büchi 190, Switzerland) with 130°C of an inlet air temperature and 80°C of an outlet air temperature. All extracts were kept in a desiccator prior to further assays.

Sample Digestion

The ground samples and extracts were separately digested with 60% v/v nitric acid solution on a hot plate at 110-130 °C until the digested solutions were clear. The digested solutions were then diluted with ultrapure water to the final volume before analysis by ICP-MS. Each sample was digested in duplicate.

Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

Eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) in digested samples were analyzed using ICP-MS spectrometer (Model 7500 ce, Agilent). The instrument was operated in helium collision mode using RF power 1,500 w, Ar gas flow rate 0.9 l/min, and nebulizer pump speed 0.1 rps.

Validation Method

For the quantitative analysis of extracts, calibration curves were built at six different concentrations. Standard solutions were prepared in 5% v/v nitric acid solution (the same concentration of nitric acid present in sample solutions) by diluting an ICP multi element standard solution XIII containing eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn). The absence of polyatomic interferences was checked when required, by measuring several isotopes of the heavy metals and checking the isotopic ratio.

Several criteria such as range of linearity, limits of detection (LODs), limits of quantification (LOQs), specificity, precision under repeatability conditions and within-laboratory reproducibility (intermediate precision) were evaluated. The procedure and calculation were modified according to the European Standard for the analyses of heavy metals [14,15]. During the validation, an internal quality control procedure was adopted by testing the blank level of the instrument reading.

RESULTS AND DISCUSSION

Data Validation: For validation of the method, there were no certified reference materials for the different kinds of extracts from various parts of *S. caseolaris*. Therefore, recovery studies for eleven heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) were performed in extracts in order to validate the proposed method. The recoveries ranged from 98.99% to 107.58% in all heavy metals. The obtained recoveries confirmed that no significant metal losses occurred during the extraction. For analytical parameters, all the measurements were carried out using the full quantitative mode analysis. The correlation coefficients (r^2) for all the calibration curves were at least 0.996, indicating good linear relationships throughout the concentration range studied. The LODs and LOQs were calculated as three and ten times of the standard deviation (SD) of ten measurements, respectively. The blanks were prepared in the same way as the samples, but without adding extracts. The LODs (μg/L) for all the studied metals were 2.64 (Al), 4.29 (As), 1.74 (Cd), 0.93 (Cr), 2.55 (Cu), 4.95 (Fe), 3.15 (Hg), 1.23 (Mn), 1.50 (Ni), 4.89 (Pb), and 2.43 (Zn). The LOQs (μg/L) were 8.80 (Al), 14.31 (As), 5.80 (Cd), 3.10 (Cr), 8.10 (Cu), 16.50 (Fe), 10.50 (Hg), 4.08 (Mn), 4.99 (Ni), 16.30 (Pb), and 8.10 (Zn). The intra-day and inter-day repeatability showed

the good precision. The precision expressed as relative standard deviations (RSD), was found to be 0.85% to 1.79% for intra-day analysis (n = 10) and 0.99-3.35% for inter-day analysis (n = 10). Therefore, this analysis method has been applied to the determination of eleven heavy metals in various parts of *S. caseolaris* (breathing roots, calyces, fruits, leaves, seeds, and stamens) and their extracts.

Application of the method to various parts of *S. caseolaris* and their extracts: To cope with the increasing demand of *S. caseolaris* extracts, the quality and safety assessment became absolutely essential to human health. In Thai Herbal Pharmacopoeia, the permissible limits of heavy metals in plant raw materials have been fixed only for As (4 mg/Kg), Cd (0.3 mg/kg), and Pb (10 mg/Kg) in agreement with the WHO permissible limits for Cd and Pb, based on ADI (acceptable daily intake) [13,16]. Under the regulation of the Prevention of Food Adulteration Act, 1986 in Thailand, the permissible limits for Cu, Hg and Zn have been set at 20, 0.02 and 100 mg/Kg, respectively [17]. A comparison of the results obtained in this study (Table 1) with permissible limits set by Thai Herbal Pharmacopoeia and the Prevention of Food Adulteration Act revealed that the concentrations of As, Cd, Hg and Zn in all parts of *S. caseolaris* and their extracts including MSE, ME, and WE were lower than the permissible limits (As 4 mg/Kg, Cd 0.3 mg/Kg, Hg 0.02 mg/Kg, and Zn 100 mg/Kg). The results revealed that plant samples and their extracts were safe from As, Cd, Hg, and Zn.

However, the concentrations of Cu and Pb in all parts of raw plant samples were lower than the permissible limits (Cu 20 mg/Kg and Pb 10 mg/Kg) while those concentrations in some extracts especially MSE of fruits (Cu 27.180 mg/Kg and Pb 10.210 mg/Kg), WE of calyces (Cu 20.413 mg/Kg), WE of leaves (Cu 29.950 mg/Kg), and WE of seeds (Cu 21.272 mg/Kg) were higher than the permissible limits as shown in Table 1. These results suggest that these plant extracts were similarly vulnerable to Cu and Pb. The efficiency of methanol and water in extracting Cu and Pb from raw plant materials varied depending on the particular metals. It is concluded that WE from calyces, leaves, and seeds were the high-risk sources of Cu contamination.

In case of other heavy metals including Al, Cr, Fe, Mn, and Ni, the permissible limits of these heavy metals were not set by Thai Herbal Pharmacopoeia and the Prevention of Food Adulteration Act in Thailand. As shown in Table 1, the highest concentrations of Al, Cr, Fe, Mn, and Ni in raw plant samples were found in leaves (Al 34.083 mg/kg and Ni 3.481 mg/kg) and breathing roots (Cr 0.243 mg/Kg, Fe 39. 848 mg/Kg, and Mn 48.209 mg/Kg). The highest concentrations of Al, Cr, Fe, Mn, and Ni in plant extracts were found in MSE of leaves (Al 58.397 mg/Kg and Mn 70.040 mg/Kg), WE of leaves (Cr 0.815 mg/Kg), and ME of breathing roots (Fe 45.755 mg/Kg and Ni 11.025 mg/Kg) as shown in Table 1. The above results showed that the highest concentrations of Al, Cr, Fe, Mn, and Ni in plant extracts were higher than the highest concentrations of those metals in raw plant samples. It is important to attend the considerable amounts of Al, Cr, Fe, Mn, and Ni in raw plant samples especially leaves and breathing roots as well as their extracts.

Table 1. The concentrations (mg/Kg) of 11 heavy metals in various parts of *S. caseolaris* (breathing roots, calyces, fruits, leaves, seeds, and stamens) and their extracts (MSE, ME, and WE).

Samples	The concentrations (mg/Kg) of 11 heavy metals										
	As	Cd	Cu	Hg	Pb	Zn	Al	Cr	Fe	Mn	Ni
Parts of plants											
Breathing roots	n.d.	n.d.	0.499	n.d.	n.d.	0.293	7.891	0.243	39.848	48.209	2.683
Calyces	0.002	n.d.	0.658	n.d.	n.d.	0.102	4.752	0.020	5.140	13.056	0.295
Fruits	0.003	n.d.	0.702	n.d.	n.d.	0.101	3.435	0.031	12.831	21.757	0.267
Leaves	0.003	n.d.	1.816	n.d.	n.d.	n.d.	34.083	0.091	26.390	34.644	3.481
Seeds	n.d.	n.d.	1.233	n.d.	n.d.	0.133	12.077	0.050	18.391	44.961	1.763
Stamens	0.001	n.d.	0.019	n.d.	n.d.	0.019	3.266	0.065	16.596	34.651	0.988
MSE											
Breathing roots	0.515	n.d.	1.356	n.d.	0.435	n.d.	11.895	n.d.	34.356	41.435	6.536
Calyces	n.d.	n.d.	17.747	n.d.	n.d.	4.743	5.644	n.d.	9.827	37.362	0.950
Fruits	n.d.	0.095	27.180	0.004	10.210	11.080	9.852	0.710	11.314	10.676	2.142
Leaves	0.429	n.d.	2.785	n.d.	0.206	n.d.	58.397	n.d.	25.138	70.040	7.935
Seeds	n.d.	n.d.	12.472	n.d.	n.d.	9.903	14.302	n.d.	19.278	63.085	3.126
Stamens	0.204	n.d.	13.124	n.d.	0.506	n.d.	4.868	0.131	26.376	31.600	4.695
ME											
Breathing roots	0.501	n.d.	1.758	n.d.	1.552	n.d.	12.940	0.132	45.755	37.254	11.025
Calyces	0.009	n.d.	10.063	n.d.	n.d.	n.d.	13.155	0.039	9.672	30.738	1.654
Fruits	0.020	n.d.	6.937	n.d.	0.792	0.005	7.346	n.d.	33.711	39.795	6.493
Leaves	0.121	n.d.	12.419	n.d.	0.312	n.d.	38.399	0.387	24.919	57.949	5.651
Seeds	0.011	n.d.	11.152	n.d.	0.459	0.005	13.619	n.d.	17.285	50.107	8.178
Stamens	0.221	n.d.	5.604	n.d.	0.625	n.d.	4.116	0.634	16.260	48.728	9.571
WE											
Breathing roots	n.d.	n.d.	18.723	n.d.	n.d.	20.226	15.743	n.d.	39.232	49.260	2.788
Calyces	n.d.	n.d.	20.413	n.d.	n.d.	22.007	15.995	n.d.	23.562	29.584	2.500
Fruits	0.148	n.d.	13.822	n.d.	n.d.	5.777	20.851	n.d.	16.100	11.042	2.184
Leaves	n.d.	n.d.	29.950	n.d.	1.165	n.d.	30.384	0.815	39.403	44.162	7.687
Seeds	n.d.	n.d.	21.272	n.d.	n.d.	7.815	11.597	n.d.	14.071	46.666	3.056
Stamens	n.d.	n.d.	14.300	n.d.	n.d.	n.d.	11.100	n.d.	10.210	50.700	3.100
Permissible limits	4	0.3	20	0.02	10	100	-	-	-	-	-

n.d.: not determined; %SD: 0.001-0.410

CONCLUSION

After validation step, the selected criteria of performance (linearity, LODs, LOQs, specificity, repeatability, and reproducibility) demonstrated that the nitric acid digestion followed by ICP-MS analysis could be used in the laboratory for determination of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn in *S. caseolaris* plant samples and their extracts. The obtained results indicated that there is no danger in consuming As, Cd, and Hg contaminated in raw plant samples of *S. caseolaris* and their extracts. Furthermore, it is safe to consume Cu and Pb contaminated in only raw plant samples. However, since the concentrations of Cu and Pb in MSE and WE of some *S. caseolaris* parts including fruits, calyces, leaves, and seeds were higher than the permissible limits, it is vulnerable to intake these extracts. For the contamination of Al, Cr, Fe, Mn, and Ni, the extracts of breathing roots and leaves contained high levels of these heavy metals. It is concluded that some of *S. caseolaris* extracts in the study were contained high levels of essential elements (Al, Cu, Fe, and Mn) and non-essential elements (Cr, Pb, and Ni), so they were not safe for human consumptions. However, the safe way to prevent high intake of heavy metals through *S. caseolaris* and their extracts is quality control of the raw plants before their further extraction.

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