CHEMISTRY

FUNDAMENTALS AND APPLICATIONS

Editors
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UNIVERSITI MALAYSIA SARAWAK
UNIMAS

Chemistry: Fundamentals and Applications

Compilations of Papers Presented at
2nd Junior Chemist Colloquium (2nd JCC)
at Universiti Malaysia Sarawak

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Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

Chemistry: Fundamentals and Applications
Editors Zainab Ngaini, Devagi Kanakaraju, Kamarul' Ain Mustafa.
ISBN 978-967-5418-02-0

Printed by SSR Technology

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Contents

Preface

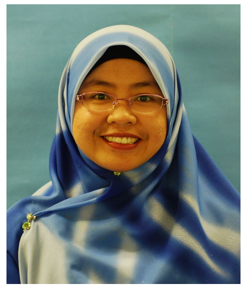
This proceeding, *Chemistry: Fundamentals and Applications* is the ultimate outcome of the 2nd Junior Chemist Colloquium or JCC. JCC was organized with the aim to provide a platform for graduate students of Department of Chemistry, Unimas to share their research findings internally, and to nurture a good research culture among them. The success of the 1st JCC 2008 has lead to the setting up of 2nd JCC 2009, where this time, the invitation for participation was extended to graduate students in chemistry related field in other public universities in Malaysia.

The responses were quite encouraging. A total of 54 postgraduate participants from six public universities including Unimas attended this colloquium, presenting papers on several topics in the field of chemistry including Natural Product Chemistry, Environmental Chemistry, Analytical Chemistry, Synthetic Chemistry and Physical Chemistry. The sessions were very informative and educative. The energetic debate and discussion during the sessions was an eye opener for the postgraduate students especially the new ones who come with limited knowledge and experience. This was further enhanced with the presence of some distinguished lecturers from participating universities giving plenary talks, joining in asking questions, providing suggestions and alternatives out of some tricky situation.

All of the selected papers from the sessions were compiled and edited and are included in this proceeding. The proceeding is to be disseminated among the attendees and participants. Gratefulness is therefore directed to fellow editors who work diligently in the editorial process. We also want to thank all those involved in the success of organizing this JCC.

Editors
Zainab Ngaini
Devagi Kanakaraju
Kamarul' Ain Mustafa

Message From The Chairman of the Organizing Committee 2nd JCC 2009



Once again the Department of Chemistry, Universiti Malaysia Sarawak (UNIMAS) is hosting the annual Junior Chemist Colloquium (JCC).

I would like to take this opportunity to thank all the advisors and committee members of 2nd JCC 2009, Faculty of Resource Science and Technology, Centre for Technology Transfer and Consultation of UNIMAS, all plenary speakers and participants for making 2nd JCC 2009 happen. Indeed it is my hope that this proceeding would benefit all the attendees and participants.

Thank you

Rafeah Wahi
Chairman of Organizing Committee

Study of Chemical Constituents from *Wedelia Biflora* and *Wedelia Trilobata*

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ABSTRACT

A study was carried out to isolate, characterize and elucidate the chemical constituents from *W. biflora* and *W. trilobata* for the modern treatment regarding its value in the traditional medicine. *W. biflora* and *W. trilobata* was collected in disturbed environments in area of Kota Samarahan. The dried sample of whole plants was extracted with cyclohexane and followed by 95% ethanol. The ethanol crudes from both species were fractionated using column chromatography and two pure components were isolated from each species. Based on NMR and FT-IR analysis, the the component was expected to be triterpenes derivatives.

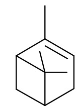
INTRODUCTION

Wedelia is a member of the family Compositae, known as the aster, daisy or sunflower family and well-known for its medicinal properties. They are one of the genera commonly called "creeping-oxeyes" and "trailing daisy", although some people mistakenly call it "Singapore Daisy".[1] It is a vigorous, creeping, herbaceous groundcover native to tropical America. It will thrive in sun or shade, but full sun produces the best flowering.[2] *Wedelia* is named in honored of George Wolfgang Wedel), Professor of Botany at Jena, Germany. *Wedelia* has about 70 species of tropical and subtropical regions[1] and 2 species found in Malaysia; W. *biflora* and *W. trilobata.*

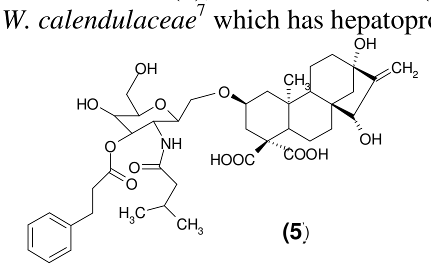
W. biflora, known as *saruni* or *sunai laut,* is a large native yellow-flowered herb grows to height of 1.5 m. In Malaysia, a paste of the leaves is used to heal sores, wounds, insect bites, soothe swelling and inflamed parts.[3]. The leaves are soaked in coconut oil and used to massage sprained or bruised limbs, and a decoction of the leaves is used for bacillary dysentery, infective hepatitis, hemorrhoids and infected bladder.[4] The leaves and stems are used for treating appendicitis and eczyma and the stems for pimples.[4]

The essential oil of the leaves contains (-)-α-pinene (**1**) as the major component.[4]. Four compounds, veratrylidenehydrazide, 3,3'-di-O-methylquercetin (**2**), 2,7-dihydroxy-3(3'-methoxy-4'-hydroxy)-5-methoxyisoflavone (**3**) and 3,7'-di-O-methylquercetin (**4**) were isolated from the dichloromethane extract of dried leaves of *W. biflora*. Compound 4 possessed antifungal and boll weevil antifeedant activity, whereas 2 showed antifeedant activity and 3 showed antifungal activities.[5]

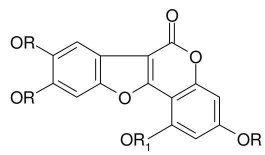
It would be interesting to learn whether a more intensive study on this species would disclose any molecules of pharmacological interest such as wedeloside, a kaurene aminoglycoside (**5**) from *W. asperima*, which has antitumor activity[6] and wedelolactone (**6**), norwedelolactone (**7**), norwedelic acid (**8**) and tri-O-methylwedelolactone (**9**) from *W. calendulaceae*[7] which has hepatoprotective activity.[8]



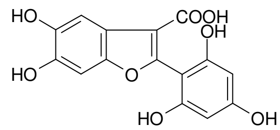
(**1**)



(**5**)



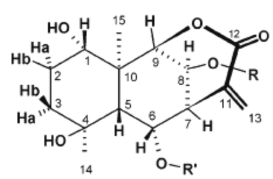
(**6**) R_1 = Me, R = H
(**8**) R_1 = R = H
(**9**) R_1 = R = Me

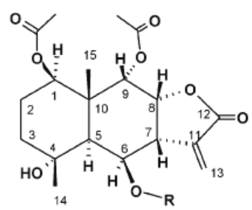


(**7**)

W. trilobata is a mat forming perennial herbs with yellow-orange flowers that grows up to 10 inch tall. This species has been used in traditional medicine: The crushed leaves were used as poultice and its tea was believed to alleviate symptoms of fever and colds [9] and ear infection[10] and whole plants is used to treat menstrual pain, unspecified female complaints and amenorrhea.[11] *W. trilobata* contain the diterpene (kaurenic acid), eudesmonolide lactones and luteolin[11,12] Kaurenoic acid has antibacterial, larvicidal and tripanocidal activity; it is also a potent stimulator of uterine contractions,[12] whereas luteolin exerts antitumoural, mutagenic and antioxidant effects, has depressant action on smooth muscles and a stimulant action on isolated guinea pig heart.[12]

Two new sesquiterpene lactones, wedelolides A (**10**) and wedelolides B (**11**), were isolated by bioassay-guided fractionation from the leaves of *W. trilobata,*[13] together with known trilobolide-6-O-isobutyrate (**12**) and trilobolide-6-O-methacrylate[14] (**13**).(Wedelolides A and B are a new antimalarial products constitute a new type of sesquiterpene δ-lactone; the (9R)-eudesman-9,12-olides framework.





(**10**) R = tigloyl, R' = isobutyroyl (**12**) R = isobutyroyl
(**11**) R = tigloyl, R' = methacryloyl (**13**) R = methacryloyl

MATERIALS AND METHODS

General: R spectra were obtained on Perkin-Elmer Model FT-IR Spectrum GX. ^{1}H and ^{13}C NMR spectra were recorded on 500 Mhz spectrometers in $CDCl_3$. Chemical shift were referenced to residual $CHCl_3$ $_{\delta H}$ 7.26 and $CDCl_3$ $_{\delta C}$ 77.0. DEPT-135 spectra was recorded on 125 and 500 MHz with a 2.0 s relaxation delay and 135 ° selection angles in $CDCl_3$, and chemical shift were referenced based on ^{13}C chemical shifts. HMQC and HMBC spectra were recorded on 500 and 125 MHz spectrometers in $CDCl_3$ with one dimension of the 2D map representing ^{13}C chemical shifts and the other representing ^{1}H chemical shifts.

Material: The plant samples of W. biflora and W. trilobata were harvested in Kota Samarahan in August, 2008 and identified by Mr. Qammil Muzzammil Abdullah, Department of Plant Science and Environmental Ecology, Universiti Malaysia Sarawak. The plant material was air-dried for 2 weeks and blended using blender (Panasonic PB-325, mill cutter).

Isolation of Pure Components

The dried plants of *W. biflora* (494 g) was extracted with cyclohexane and followed by 95% EtOH to yield 16 g of crude cyclohexane and 25 g of crude EtOH. 20 g of crude EtOH were subjected to chromatography on a silica gel column with a mixture of C_6H_{14}-CH_2Cl_2-EtOAc-MeOH and separated into 30 fractions. Fraction 4 was further purified by TLC ($CHCl_3$/EtOAc, 3:1) to afford compound **A** (21 mg). Fraction 5 and 6 were combined and purified by TLC ($CHCl_3$/EtOAc, 3:1) followed by further chromatography on a silica gel column with a mixture of CH_2Cl_2-$CHCl_3$-EtOAc and separated into 11 fractions. Fraction 3 was further purified by TLC ($CHCl_3$/EtOAc, 3:1) to afford compound **B** (40 mg).

The extraction method of *W. trilobata* is similar to *W. biflora* and yield 6 g of crude cyclohexane and 14 g of crude ethanol. 10 g of crude ethanol were subjected to chromatography on a silica gel column with a mixture of C_6H_{14}-CH_2Cl_2-EtOAc-MeOH and separated into 31 fractions. Fraction 8 were further purified by TLC ($CHCl_3$/EtOAc, 3:1) followed by further chromatography on a silica gel column with a mixture of CH_2Cl_2-$CHCl_3$-EtOAc and separated into 11 fractions; fraction 1 and 2 was further purified by TLC ($CHCl_3$/EtOAc, 3:1) to afford compound **C** (10 mg) and compound **D** (20 mg).

RESULTS AND DISCUSSION

The EtOH extracts of *W. biflora* and *W. trilobata* was successively extracted after extraction with cyclohexane. The fractionation of both EtOH extracts using silica gel column chromatography, followed by purification on TLC and further chromatography on a silica gel column, afforded four isolated compounds, compound A and B from *W. biflora* and compound C and D from *W. trilobata.* Compound A, B, C and D were suspected to be triterpenes derivatives and its chemical structure is still in the progress of characterization and elucidation.

The 1D and 2D NMR spectra of A were illustrated in Figure 1 – Figure 3. The IR spectrum was illustrated in Figure 4.Compound A was isolated as a colorless amorphous solid. The ^{13}C NMR and DEPT-135 spectra of A (Figure 2 –Figure 3) indicated 36 carbon atoms.

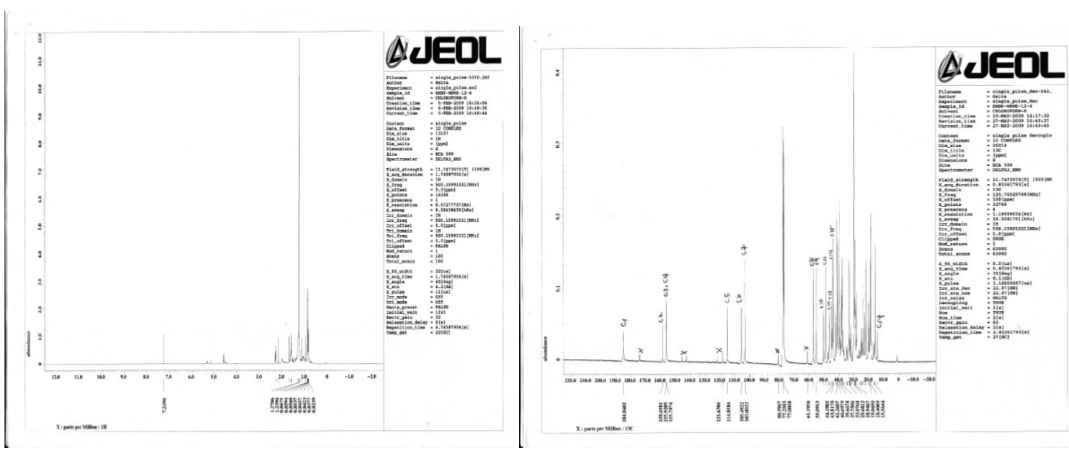


Figure 1: ^1H and ^{13}C NMR spectra of compound A

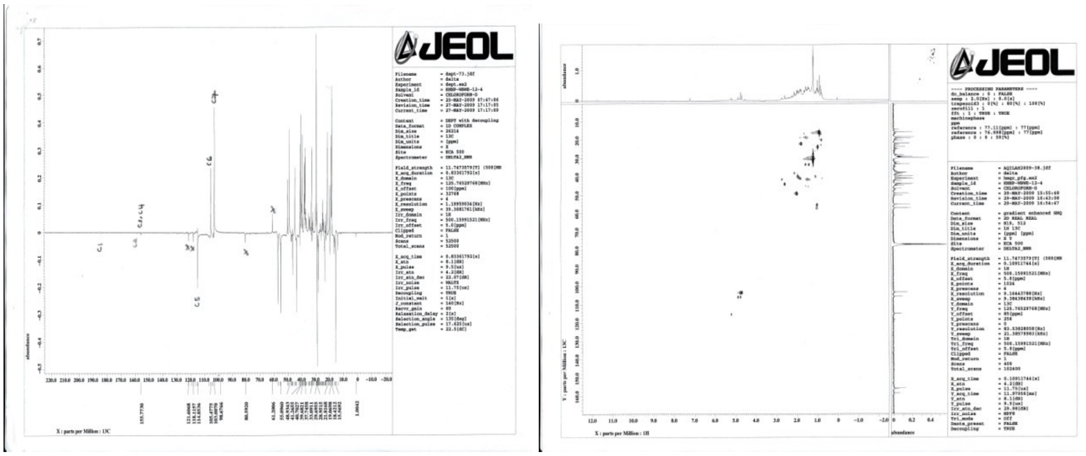


Figure 2: DEPT and HMQC NMR spectra of compound A

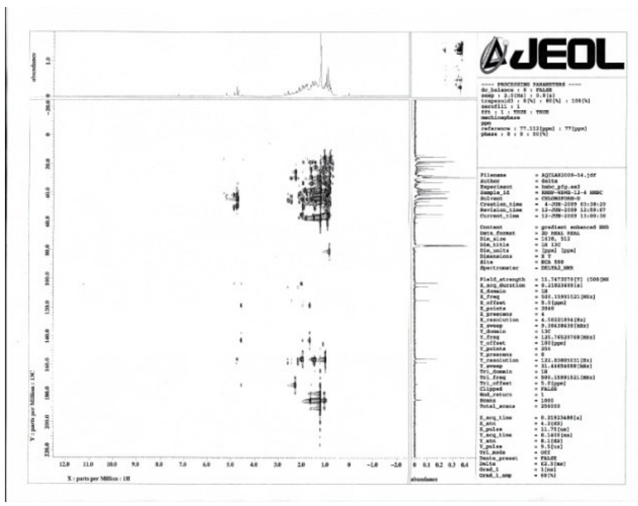


Figure 3: **HMBC NMR spectra of compound A**

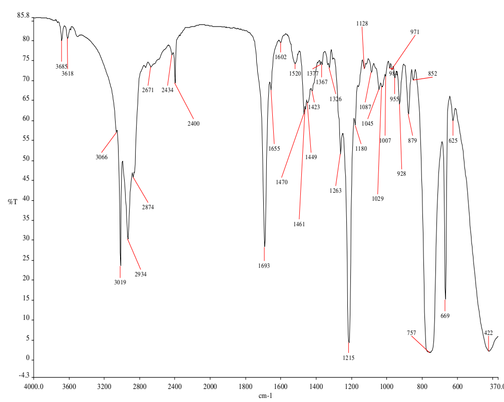


Figure 4: IR Spectra for Compound A

Compound B was isolated as a colorless crystalline powder. The 1D and 2D NMR spectrum of B were illustrated in Figure 5-7. The ^{13}C NMR and DEPT-135 spectra of B (Figure 5 –Figure 6) indicated 41 carbon atoms. The IR spectrum was illustrated in Figure 8.

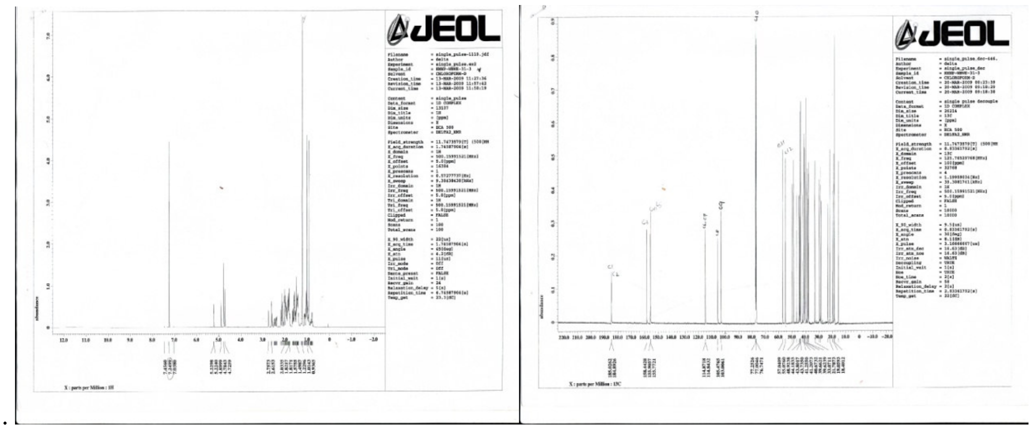


Figure 5: [1]H and [13]C Spectra of Compound B

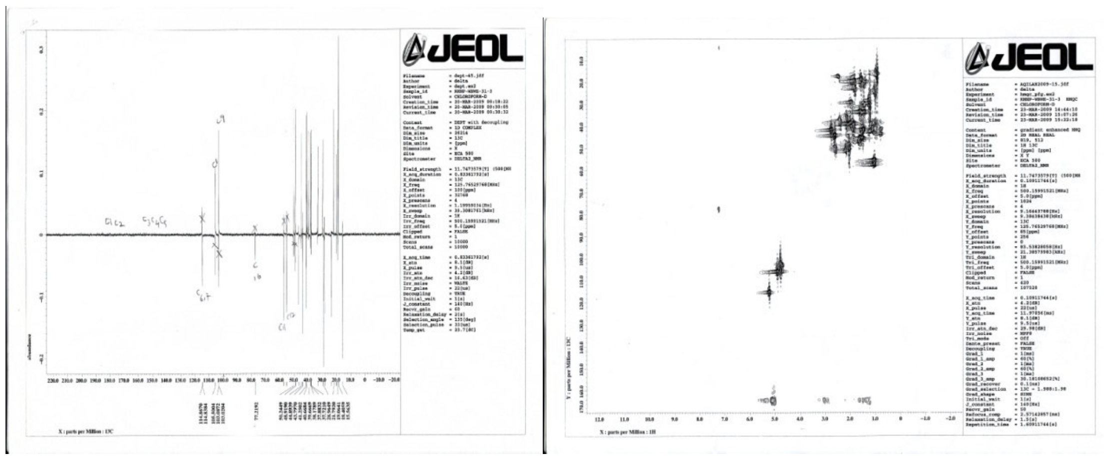


Figure 6: DEPT and HMQC NMR Spectra for Compound B

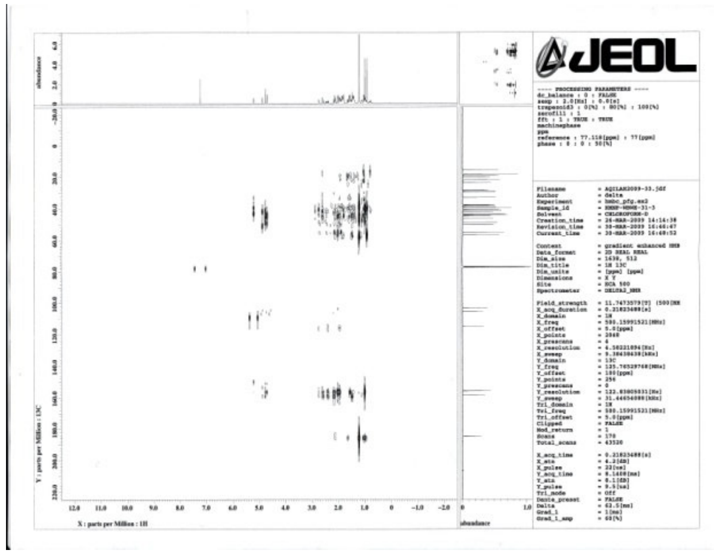


Figure 7: HMBC Spectra for Compound B

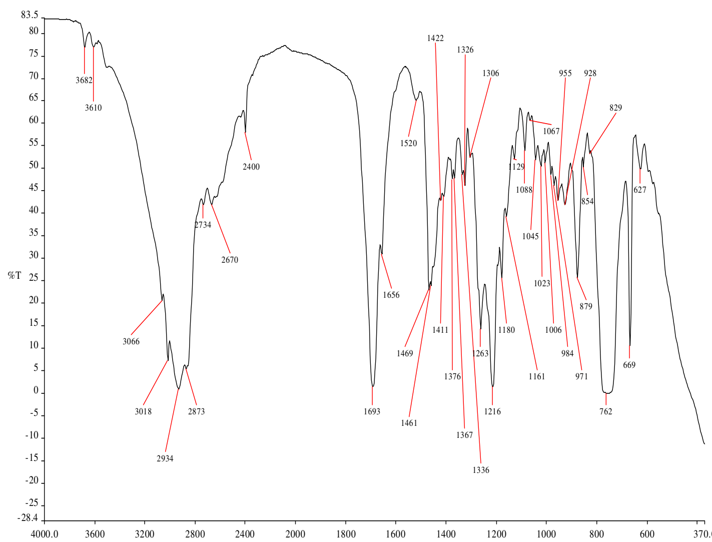


Figure 8: IR Spectra for Compound B

Compound C was isolated as a colorless amorphous solid. The 1D and 2D NMR spectrum of C were illustrated in Figure 9-10. The ^{13}C NMR and DEPT-135 spectra of C (Figure 9 –Figure 10) indicated 37 carbon atoms. The IR spectrum was illustrated in Figure 11.

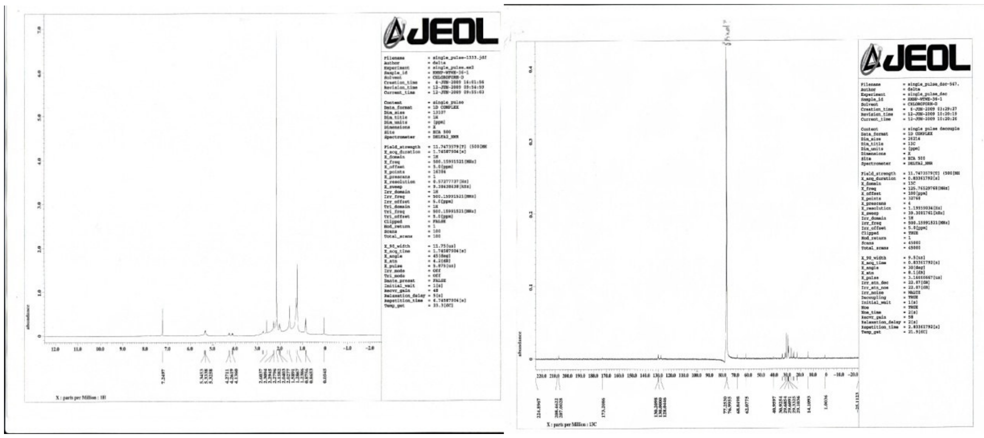


Figure 9: [1]H and C[13]C spectra for compound C

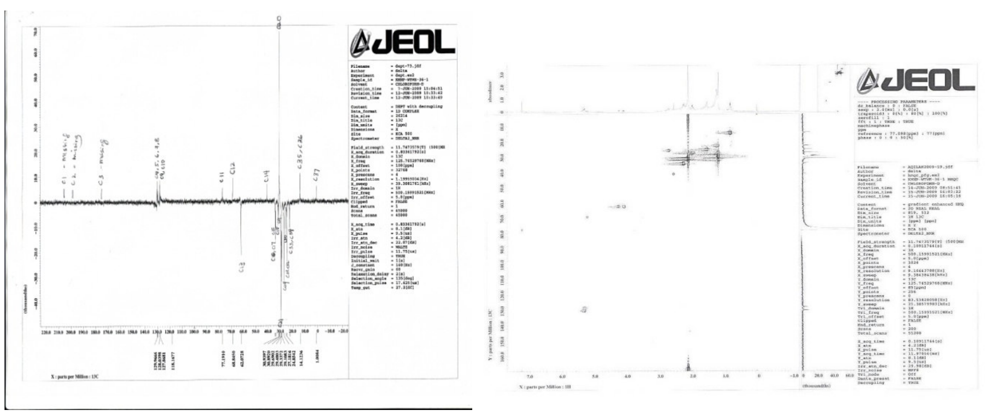


Figure 10: DEPT and HMQC Spectra for Compound C

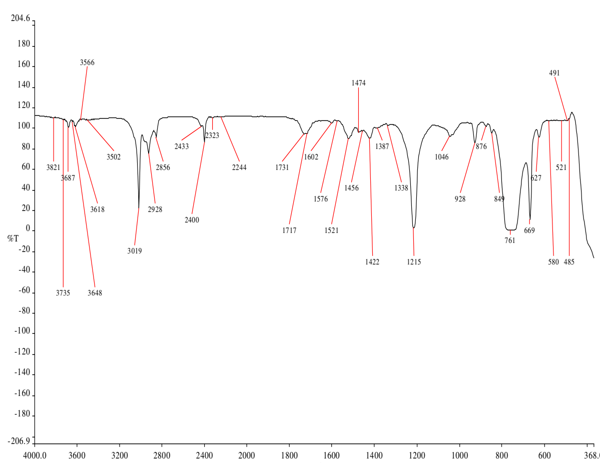


Figure 11: **IR Spectra for Compound C**

Compound D was isolated as a colorless amorphous solid. Figure 12 and Figure 13 showed the [1]H NMR and IR spectra for D.

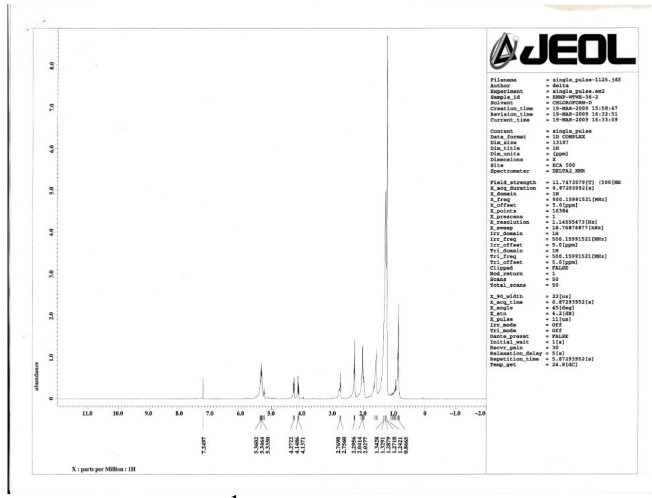


Figure 12: ^{1}H Spectra for Compound D

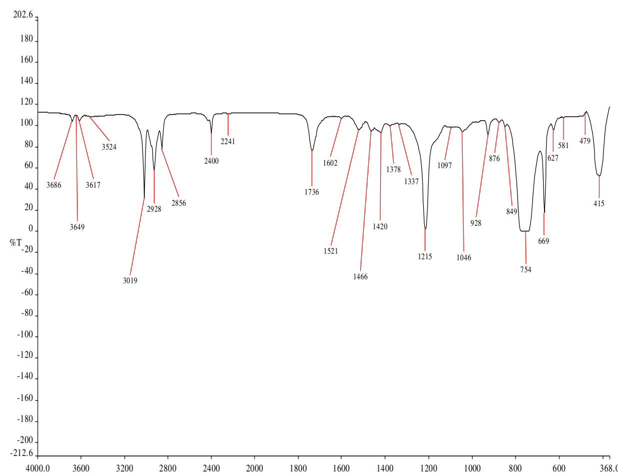


Figure 13: IR Spectra for Compound D

CONCLUSION

Interpretation of ^{1}H, ^{13}C, DEPT-135, HMBC and HMQC of NMR spectra with additional of IR spectra for compound A, B, C and D led to the unambiguous elucidation of the structure. ^{1}H and ^{13}C-NMR spectra of A resembled very closely to B and ^{1}H NMR and IR spectra of C resembled to D, so both were suspected to be its own triterpenes derivatives; just differing in the number of carbon atoms.

ACKNOWLEDGEMENTS

Many thanks to Mr. Qammil Muzzammil Abdullah for species identification, Mdm. Nurhayati for recording the ^{1}H, ^{13}C, DEPT and 2D NMR spectra.

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Phytoremediation of Soil Contaminated by Heavy Metals

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ABSTRACT

Phytoremediation includes all biological, chemical and physical processes using plants including rhizosphere for *in situ* or *ex situ* removal, transfer, stabilization or destruction of contaminants in soils, sludge, sediments and water. Plants mineralized some toxic organic compounds and accumulate heavy metals and other inorganic compounds from soil into aboveground shoots. Four native plant species, namely *Asystasia coromandeliana*, *Phyllanthus amarus*, *Kaempheria rotunda* and *Jathropha podagrica* were chosen to evaluate their abilities to accumulate heavy metals from contaminated soils. *K. rotunda* and *J. podagrica* showed potential as accumulator for heavy metals from contaminated soil. This study also revealed that *A. coromandeliana* and *P. amarus* did not survive in heavily contaminated soils with heavy metals.

Keywords: Phytoremediation, heavy metals, *Asystasia coromandeliana*, *Phyllanthus amarus*, *Kaempheria rotunda*, *Jathropha podagrica*

INTRODUCTION

Phytoremediation is a technique that uses plant to remediate contaminated soil and water.[1] The process includes all biological, chemical and physical processes using plants, including rhizosphere for *in situ* or *ex situ* removal, transfer, stabilization or destruction of contaminants in soils, sludge, sediments, other solids or groundwater. Plants mineralize toxic organic compounds and accumulate heavy metals and other inorganic compounds from soil into aboveground shoots.[1] Phytoremediation mechanisms include: phytodegradation the uptake, metabolism and breakdown of organic contaminants and herbicides within the plant to simpler molecules or degradation of contaminants in the soil, sediments, sludge, groundwater by enzymes produced and released by the plant; phytovolatilization the uptake of organic and inorganic contaminants by plant from soil, water or a mixed soil and water matrix, converts it to volatile form and release it to the atmosphere, usually through leaf stomata and rhizodegradation takes place at the intersection of bioremediation and phytoremediation.[2-5]

Phytoextraction is the choice for phytoremediation of metals and metalloids where the contaminants will be removed from the sensitive matrix without destroying it. In this process, the removal of contaminants is achieved through the root network and the accumulation potential into the plant biomass. The biomass is then harvested to complete the extraction of contaminants from the environment.[6] The uptake of metals by plants is regulated by specific abilities of the plant and soil factors such as pH, water regime, organic matter content, cation exchange capacity, nutrient balance, clay content, concentration of other trace metals and climatic conditions. Mechanisms of uptake differ among plants and types of metal. As per their ability to absorb, accumulate and tolerate metal within their tissues, plants exhibit three major responses and can be classified into three categories, which are hyperaccumulators indicators and excluders.[7,8] Plants with extreme levels of metal tolerance are called hyperaccumulators.[9] Indicators regulate metal uptake so that the internal concentration reflects the external levels, while excluders maintain low and constant metal concentration in their shoots.[10]

Plants used for phytoextraction should be fast growing, deep and wide-spreading root system, easily propagated, large biomass and accumulate large amount of the target metal.[11, 12] Salt *et al.* [13] reported that *Brassica juncea* (Indian mustard) could efficiently accumulate Pd, Zn, Cd, Ni, Cr and Cr (IV) from soils or water in both roots and stems. Additionally, study done by Kumar et al.[14] showed that six Brassica species, *B. nigra*, *B. oleracea*, *B. campestris*, *B. carinata*, *B. juncea* and *B. napus* have the ability to accumulate heavy metals. They found that Cr has the highest phytoextraction coefficient, followed by Cd, Ni, Zn and Cu. *G. Americana* also shows adaptive capabilities to adverse conditions such as eroded soil, flooding[15], low pH and low fertility. Another study reported that the seedlings of *G. americana* shows a great uptake of Cr^{3+} from the nutrient solution, immobilizing and storing this metal in the root system in high concentration, with a very little translocation to aerial parts. Hence, this species can be considered as phytoremediators tree in Cr^{3+} contaminated watershed.[16]

Arsenic hyperaccumulation by terrestrial plants is a rare phenomenon. The first known As hyperaccumulator *Pteris vittata* L., also known as Chinese brake fern was reported by Komar *et al.*[17] and Ma et al.[18] Since then, several other fern species including *Pityrogramma calomelanos*, *Pteris cretica*, *Pteris longifolia* and *Pteris umbrosa* have been reported to accumulate As.[19-21] Ma *et al.*[20] reported *P. vittata* can accumulate 12-64 mg As/kg in its fronds from uncontaminated soils containing 0.5-7.5 mg As/kg, and up to 22,630 mg As/kg from a soil amended with 1500 mg As/kg.

Cd concentration in the shoot of rainbow pink grown in a contaminated site in northern Taiwan for five weeks increased from 1.56 (under controlled condition) to 115 mg/kg and the total Cd uptake was about 100g/ha/yr.[22] Additionally, the accumulated Cd concentration can reach the threshold (100 mg Cd/kg) of a hyperaccumulator of Cd.[23] High concentration of Pb reduced the growth, biomass and total chlorophyll content of vetiver grass. However, vetiver grass accumulated from 250 to 750 mg Pb/kg in its shoots when the grass was grown in Pb-contaminated sandy loam soil.[24]

Over 400 plant species of hyperaccumulators from all over the world can accumulate high concentrations of metals at contaminated sites.[23] However, many of them have a low growth rate and very low biomass, hence; they need much time to remove contaminants from soils. Researchers found out two strategies to increase the phytoextraction of metals with higher biomass or by transgenic plants, which involving chelating agents and genetic engineering. Synthesized chelating agents such as ethylenediaminetetraacetic acid (EDTA), diethylenediaminepentaacetic acid (DTPA), hydroxyethylenediaminetetraacetic acid (HEDTA), trans-1,2-cyclohexylenediaminetetraacetic acid (CDTA) and ethylenebis (oxyethylenetrinitrilo) tetraacetic acid (EGTA) were applied to metal-contaminated soil to increase the mobility and bioavailability of the metal in the soils and also to increase the amount of heavy metals accumulated in the upper parts of plants.[25-27] Results from these research revealed that adding synthetic chelating agents can increase both the solubility of metal in soil solution and the concentration of metal in the shoots of plants. However, in soils contaminated with multiple metals, the application of synthetic chelating agents can reduce both the biomass of the plant and the total amount of metal removed because the high concentrations of other metals in the soil solution are toxic to the plant. [28]

The goal of this project is to evaluate management strategies using phytoremediation for land contaminated by heavy metals. Selected native plant species had been investigated for their potential as hyperaccumulator for heavy metals.

MATERIALS AND METHODS

Experimental Set Up

Experiments were conducted using soil spiked with 50 ppm salt solutions of Cd, Cr, Ni, Pb, Zn, and As. The solutions were uniformly mixed with air-dried soil and placed in polyethylene bags (1 kg). The soil pH was 5.32. Soil pH was measured in a distilled water using solid to liquid ratio 1:2.5. Soil moisture content was 18.19 ± 0.73 and organic matter was 13.48 ± 0.08 %. To prevent from leaching and soil loss, the polyethylene bags were placed inside another polyethylene bag without holes. Three replicates and a control were used and the polyethylene bags were placed in greenhouse shaded with transparent polythene sheet. Plants were grown under natural conditions and no artificial fertilizers or soil amendments were added to the soil during the course of the experiment. After one week of equilibration, seedlings of *Asystasia coromandeliana*, *Kaempferia rotunda*, *Phyllanthus amarus* and *Jatropha podagrica* were planted in each pot. About 5 mmol/kg soil of EDTA was applied to each pot except control, a week after plant transplanting.

Soil Analysis

Soil analysis was performed using method described by Binning and Baird.[29] Soil was air-dried and sieved through 2 mm sieve before analysis. About 2 g of soil sample was added to 20 mL of aqua-regia (1cHNO_3: 3cHCl). The samples were allowed to stand overnight and then heated until near dryness. After cooling, 20 mL of 5 M HNO_3 was added and the samples were allowed to stand overnight. Samples were filtered through Whatman No. 41 filter paper into 100 mL volumetric flask. The solution was made up to mark with 0.5 M HNO_3. Metal analysis and quantification was conducted using inductively coupled plasma-mass spectrometry (ICP-MS).

Plant Analysis

Plants were harvested 14, 60 and 90 days after transplanting. Plants were washed with distilled water to remove any dust deposits and surface soil, and oven-dried at 60°C for 72 h. Dry weights of plants was determined and the samples were ground. Analysis of plant tissue was done according to the method described by Soon.[30] Grounded plant tissue was ashed in the muffle furnace for 16 h at 480°C. After cooling, 10 drops of ultrapure water was added followed by 2 mL of 50% (v/v) HNO_3. Then, the sample was evaporated to dryness on a hot plate. After cooling, the ash was dissolved in 2 mL 20% (v/v) HNO_3 by heating on a hot plate at approximately 100°C. Sample was filtered through Whatman No. 42 paper to a 10mL volumetric flask. Metal contents in plants were determined using ICP-MS.

Instrumental Analysis

ICP-MS operating conditions were the following: carrier gas flow, 0.60 L/min; Plasma gas flow, 1.5L/min; coolant gas flow, 7L/min; radio frequency (RF), 1200 W.

Statistical Analysis

Statistical analyses were conducted using Microsoft Excel 2007 where data were presented as the mean value of triplicates and standard deviations were calculated.

RESULTS AND DISCUSSION

Plant Growth and Survival in Heavy Metals

All plant species showed signs of stunted growth with toxicity symptoms such as chlorosis and necrosis as well as loss of leaves for the first and second weeks of the experiment. *K. rotunda* and *J. podagrica* were adapting and growing while *A.coromandeliana* and *P. amarus* showed continuous loss of leaves and died after two weeks of the experiment. Similar toxicity symptoms were also observed by other researchers.[12, 31] Stunting could be due to specific toxicity of the metal to the plant metabolism, or antagonism with other nutrients in plants, which is commonly observed in a wide range of plant metal induced soils.[32]

Heavy Metals Accumulation in Plants

Figure 1 shows the mean metals accumulation in *K. rotunda* at different sampling times. The highest mean concentration of metals accumulate by *K. rotunda* was Cd (134.2 mg/kg), followed by Ni (106.6 mg/kg), Zn (93.1 mg/kg), Pb (32.2 mg/kg), Cr (20.4 mg/kg) and As (0.5 mg/kg). Metal accumulation in *J. podagrica* (Figure 2) was found lower than *K. rotunda* with metal concentration varied between 6.5 and 39.3 mg/kg for Zn, between 6.5 and 30.9 mg/kg for Ni, between 2.5 and 28.0 mg/kg for Cd, between 1.9 and 14.0 mg/kg for Cr, between 2.3 and 9.2 mg/kg for Pb and between 0.1 and 0.5 mg/kg for As. Uptake of As in both control and EDTA-treated plants of *K. rotunda* and *J. podagrica* was low and insufficient to consider for phytoextraction, thus it will not be discussed in further detail.

Chromium

Cr accumulation increased through time in *K. rotunda* with highest accumulation was found at day 90 (20.4 mg/kg), while highest Cr accumulation in *J. podagrica* was found at day 60 with concentration 14.0 mg/kg and decreased on day 90. Research done by Sampanpanish *et al*.[31] on Cr removal using weed species in Thailand showed similar trend and it was suggested that the decreased in Cr concentration may because the plant had grown well and resulted high biomass on day 90. However, Cr accumulation in *K. rotunda* and *J. podagrica* can be considered as low. A plant can behave as Cr accumulator if it can accumulate more than 1000 mg/kg in its tissue.[33] Research done by Turgut *et al*.[34] showed no statistical different in Cr uptake when using 0.1 g/kg of EDTA but when using 0.3 g/kg of EDTA, high accumulation of Cr in dwarf sunspot sunflower was found. Our study showed 2-3 times higher accumulation of Cr in *K. rotunda* as well as *J. podagrica* planted in soil amended with 0.5 mmol/kg of EDTA (~2.0 g/kg) compared to control.

Nickel

Both *K. rotunda* and *J. podagrica* showed highest Ni accumulation on day 60 of the experiment with concentration of 106.6 mg/kg and 30.9 mg/kg, respectively. Similar Ni concentration (30.9 mg/kg) was also found in the shoot of *Baccharis sorothroides* Gray.[35] EDTA could increase Ni in plant tissue more than double the Ni concentration obtained in control soil.[34]

Zinc

Zinc accumulation in control treatment of *K. rotunda* was increased through time with highest accumulation of 93.1 mg Zn/kg (Figure 1). No significant difference was observed in Zn concentration when planted in soil treated with EDTA in days 14, 30 and 90. Zn uptake capacities in *J. podagrica* peaked on day 60 and decreased on day 90. Furthermore, it is showed that Zn uptake in *J. podagrica* were higher in EDTA-treated plant compared to control. Plant tissue concentration on day 60 and 90 were 23.4 mg/kg in control and 39.3 mg/kg in EDTA-treated and 6.5 mg/kg in control and 8.1 mg/kg in EDTA-treated, respectively. The uptake of Zn in *T. caerulescens* was thought to be facilitated by specific Zn^{2+} transporters.[36] Hence, this study also suggests that the uptake of Zn in *J. podagrica* showed the same mechanism.

Cadmium

Both *K. rotunda* and *J. podagrica* showed highest Cd accumulation (134.2 mg/kg and 28.0 mg/kg) on day 60 with accumulation more than double Cd uptake on day 14 and day 90 (Figure 1 and Figure 2). The Cd uptake capacities in EDTA-treated *J. podagrica* peaked on day 14 and decreased on days 60 and 90. As observed in Figure 1 and Figure 2, Cd accumulation in both control plants was higher compared to EDTA-treated plants. Previous study also reported that application of EDTA reduced Cd uptake by plants.[38]

Lead

Pb accumulation in *K. rotunda* was peaked on day 60 with concentration of 32.2 mg/kg in control and 23.1 mg/kg in EDTA-treated plant and decreased on day 90. In contrast, *J. podagrica* accumulated highest concentration of Pb (9.2 mg/kg) on day 14. Pb hyperaccumulator plants are those that can accumulate more than 1000 mg/kg Pb in their tissues.[39] Hence, both *K. rotunda* and *J. podagrica* are not Pb accumulator, since Pb accumulation were not more than 40 mg/kg in both plants.

Low accumulation in both plants may due to low solubility of Pb in soils, and the ready precipitation of Pb by sulfate and phosphate at the root system that minimize the plant uptake.[23] Adding EDTA could increase Pb concentration in plants.[40] Nevertheless, this study showed that Pb accumulation was higher in control plants. It is suggested that adding EDTA may increase metal mobility in soil but not increasing the Pb accumulation in both *K. rotunda* and *J. podagrica*.

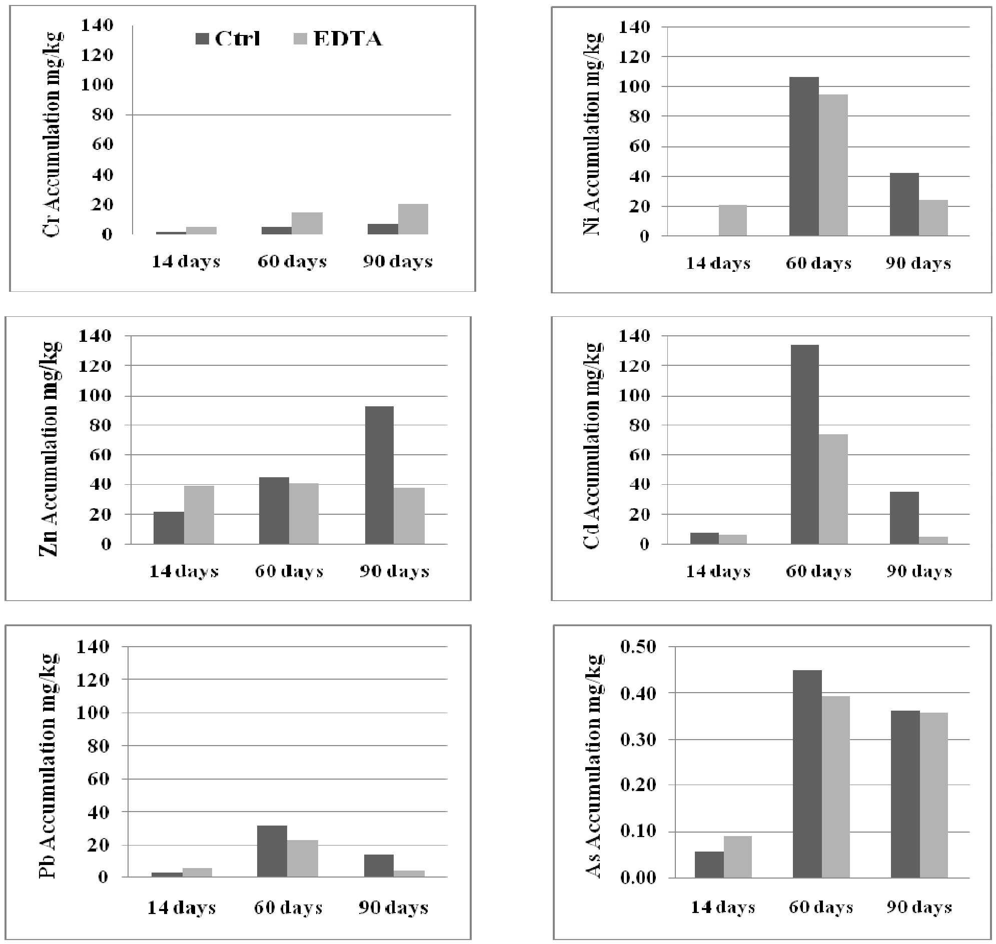

Figure 1: Cr, Ni, Zn, As, Cd and Pb accumulation in *K. rotunda*

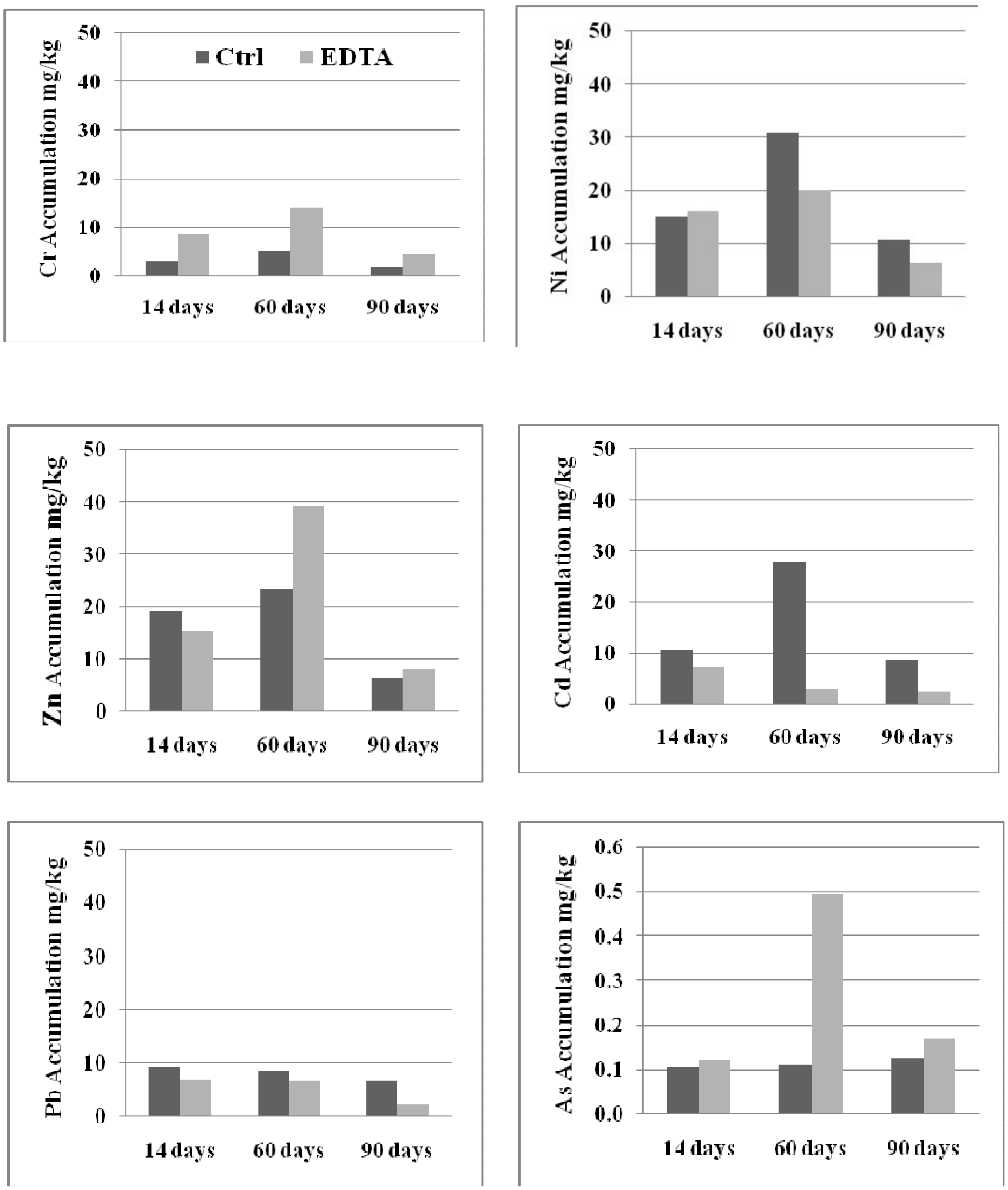

Figure 2: Cr, Ni, Zn, As, Cd and Pb accumulation in *J. podagrica*

CONCLUSION

This study showed that *K. rotunda* and *J. podagrica* have the ability to accumulate metals into their tissues. *K. rotunda* uptake selectivity was Cd>Ni>Zn>Pb>Cr>>As while uptake selectivity for *J. podagrica* was Zn>Ni>Cd>Cr>Pb>>As. *K. rotunda* have the potential to act as hyperaccumulator compared to *J. podagrica*, especially in accumulating Cd and Ni where the highest concentration found in its tissues were 134.2 mg/kg in control and 73.8 in EDTA-treated and 106.6 in control and 94.6 in EDTA-treated, respectively. It is also reported that EDTA amendment could reduced Ni and Cd uptake in plant but increased Cr and Zn uptake in plant. This study also revealed that *A. coromandeliana* and *P. amarus* did not survive in heavily contaminated soils with heavy metals.

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