Research Article

Effect of lead (Pb) on phytochemical variability of *Jatropha curcas* (L.): a versatile perennial of Euphorbiaceae family

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Received 08 August 2018; Accepted 30 August 2018; Published online 12 September 2018

Abstract

Jatropha curcas is a perennial plant belonging to the Euphorbiaceae family and it is widely used for phytoremediation techniques and biodiesel production. Jatropha curcas is also reported as a medicinal herb. The present study was performed to evaluate the effect of lead on phytochemical properties of the plant. Seedlings of J. curcas were subjected to different concentrations of Pb. The plant materials were prepared for detecting metal accumulation, antioxidant activity and active phytochemical compounds. The content of Pb in the seedlings was measured after one week of the application using atomic absorption spectrophotometer. The accumulation of Pb was significantly increased with increasing of the metal in the growth media. The antioxidant activity was measured using 1,1,-diphenyl-2-picryl-hydrazil (DPPH) assay. It was found to be increased in the plants treated with the highest Pb concentration. The GC-MS analysis revealed presence of different compounds depending on Pb treatment. Some identified compounds were found to have a biological activity as antioxidants such as Hexadecanoic acid, methyl ester; 9,12-Octadecadienoic acid (Z,Z)-,1,3,5,7-Tetroxane; p-Dioxane-2,3-diol and Heptadien-3-yne. The results indicated the ability of the plant to tolerate Pb stress and to induce its antioxidant defense system. Phytochemical screening was found to be valuable to release any risks related to plants of metal affinity to avoid possible adverse effects when used as medicine or for any other purposes.

Key words: Pollution; Heavy metals; *Jatropha curcas*; Lead; Phytoremediation; phytochemicals; GC-MS, Bioactive compounds; antioxidants

Introduction

Heavy metal pollution is presently a worldwide challenge frightening the healthy development of plants and other organisms of the environment. Metals induce modifications at the sub-organizational level in plants. These changes are exhibited by visible symptoms of metal toxicity as a result of a direct interaction of the toxic metals with structural components. At consequence, alteration of phytochemical properties is pronounced in response to metal stress (Solanki and

Dhankhar, 2011; Bielen et al., 2013). *Jatropha curcas* is a versatile plant with several potential uses in phytoremediation, biofuel production and medicinal purposes. Virtually, all parts of the plant have benefits in medicinal use and have been investigated in numerous studies (Openshaw, 2000; Mangkoedihardjo, 2008; Prasad, 2012; Dixit et al., 2015; Bauddh et al., 2017).

Plants have a rich diversity of phytochemical compounds such as phenolics, alkaloids, terpenoids, tannins, lignins and flavonoids. Most of these compounds are proven to have biological activities, *e.g.*, antibacterial, anti-inflammatory, and antioxidant (Brantner, et al., 1993; Chawech et al., 2015; Rathee et al., 2016; Al-Marzoqi et al., 2016).

Drugs of herbal origin have a great contribute to human health. Accumulation of heavy metals in these herbs is a pronounced challenge of quality control in the herbal drugs. Therefore, phytochemical screening can release any risks related to specific herb to avoid possible adverse effects when used as medicine or for any other purposes (Barthwal et al., 2008; George and Josekumar, 2016; Zoufan et al., 2017).

The present study was assumed to identify the phytochemical profile of *J. curcas* which have affinity to heavy metals. Pb is a toxic metal released by traffic emission and industrial sources such as mining. *J. curcas* is reported to survive lead-polluted soils even in high concentrations of Pb. In addition, it could accumulate higher metal concentration within its tissue. Consequently, it is widely used for efficient phytoremediation procedure because it is non-edible and could grow abundantly in a large scale.

The present study was a part of research project which had been designed to study the phytoremediation efficiency of *J. curcas*. Here, the effect of Pb on the phytochemical properties of Pb-stressed *J. curcas* was studied.

Materials and Methods

Growth conditions and Pb application

Seeds of *J. curcas* were collected from the arid zone around the campus of Aswan University, Aswan city, Egypt (23°59'56"N, 32°51'36"E). The seeds were surface sterilized for 5 min with 0.4% sodium hypochlorite, followed by ethyl alcohol for 3 min and then properly rinsed with distilled water. The seeds were germinated and after two months, homogenous seedlings were transferred to tanks of hydroponics supplied by nutrient solution. Tanks were divided into six groups. The first group was served as a control (T0). The other tanks were assigned as T1, T2, T3, T4 and T5 and they received 0.5, 5, 10, 20 and 40 mg/L Pb, respectively.

Digestion of plant material for metal accumulation analysis

Dried powdered plant material was digested by mixing with a mixture of 4 ml HNO₃ (69%) and 2 ml perchloric acid (70%). The mixture was gently boiled at 125 °C for 2 h and then the temperature elevated to 200 °C for 2 h more. The extracts were filtered and the filtrates were adjusted to 50 ml distilled water (Hseu , 2004). Standard stock of Pb (1000 ppm) was used to prepare diluted series of Pb. Lead in the plant material was measured using atomic absorption spectrophotometer (SHIMADZU AA6800). The results were reported as mg/kg dry weight.

Plant extraction and material preparation for GC-MS analysis

After one week of metal application, the seedlings were removed from hydroponics. They were rinsed with EDTA to remove any metal residue. The seedlings were air-dried. The dried samples

were ground to a fine powder and prepared for further extraction process. For each sample, dried plant material (100 mg) was dissolved in 4 mL methanol (100%) and vortexed for 30 s. The mixture was placed in a water bath for 1 h at 40 °C. Then, the mixture was centrifuged for 5 min at 800 rpm. The supernatant was used for phytochemical screening.

The chemical composition of the extracts was performed using Aligent GC mass spectrometer and the method was modified from Easwaran and Ramani (2014). The initial temperature of column oven was held at 120 °C. Then, it was increased by 5 °C/min to 200 °C with holding 2 min and finally increased to 280 °C (10 °C /min). Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The temperature of injector and detector was kept at 250 °C. The solvent delay was 2 min and diluted samples of 1 ml were injected automatically using Autosampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 40e550 in full scan mode. The ion source and transfer line temperatures were set at 200 and 250 °C, respectively. The compounds were identified by comparison of their retention times and mass spectra with those of NIST 11 Mass Spectral (2011) database.

Measuring antioxidant activity of plant extracts

Antioxidant activity was measured by free radical scavenging assay. It was evaluated by 1,1,diphenyl-2-picryl-hydrazil (DPPH) assay. From each plant extract, 1 ml was placed in a test tube. Then, 2 ml DPPH (1 mM) was added and incubated in dark condition at room temperature for 30 min., the absorbance of the mixture was read at 520 nm. Three ml of DPPH was taken as control. The percentage of radical scavenging activity of the plant extracts was calculated according to Patel et al. (2010).

Statistical analysis

All data were presented in the replicates' means \pm standard deviation. One-way analysis of variance (ANOVA; from Minitab version 12.21) was used to test the significant difference (at p ≤ 0.05) in the effect of Pb accumulation and antioxidant properties of the samples.

Results and discussions

Accumulation of Pb in the plant tissue

The property of plants to accumulate heavy metals trigger their use for phytoremediation purposes (Raskin et al., 1997; Glick, 2003). However, the plants vary in their ability to accumulate different metals. Lead has low mobility and efficiency of phytoremediation of plants depends on the ability to accumulate Pb in their tissues (K1sa et al., 2017). Here, the ability of *J. curcas* to extract and accumulate Pb inside its tissue was calculated by measuring the content of Pb inside the plant.

The content of lead was evaluated in the seedlings of J curcas subjected to different concentrations of Pb. In each treatment, the whole seedling was dried, digested and Pb was evaluated as described in the material section. The concentration of Pb was increased in the plants with increasing Pb in the medium. The highest content of Pb was about 400 mg/kg dry weight observed in plants treated with 40 mg/L (T5) (Fig. 1).

The current results indicated the ability of *J. curcas* to accumulate Pb within a narrow range of time scale. Similar results were obtained from previous studies of the ability of plants to

accumulate metals within hours or days of metal application. However, several toxicity symptoms were observed in response to metal application (Shu et al., 2014; Al-Rubaie and Al-Kubaisi, 2015). In the current study, there were no symptoms of toxicity observed on the plants even after they were treated with the highest concentration of Pb.

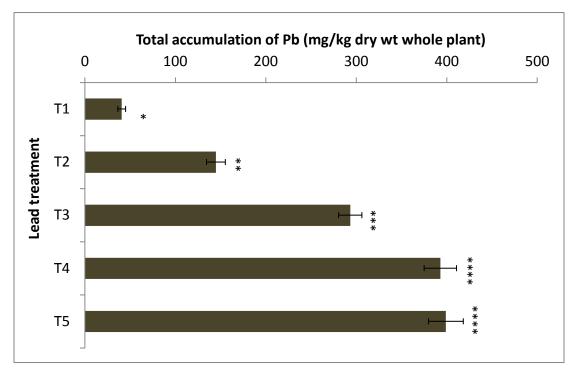


Figure 1. The content of Pb in *Jatropha curcas* seedlings subjected to different concentrations of Pb. Values are the mean \pm SD. Different symbols on bars indicate significant differences at P ≤ 0.05 (Duncan test)

Antioxidant activity of Pb treated plants

The antioxidant activity is one of the biological activities which are possessed by a plant. Metal stress induced the antioxidant defense system in many plants (Vijayalakshmi et al., 2010; Kandziora-Ciupa, 2016)

The antioxidant activity using DPPH assay was measured for methanolic extracts of dried seedlings subjected to different concentrations of Pb. The IC₅₀ values were calculated as mentioned in the materials. The results of antioxidant activity are shown in (Fig. 2). The lowest IC₅₀ value indicated highest antioxidant activity. As Pb accumulated inside *J. curcas* tissue the antioxidant activity was increased. The highest antioxidant activity was recorded for plants in treatment T5 of IC₅₀ value (454±25 µg/mL). The values of antioxidant activity in the different treatments were significantly different at $p \le 0.05$.

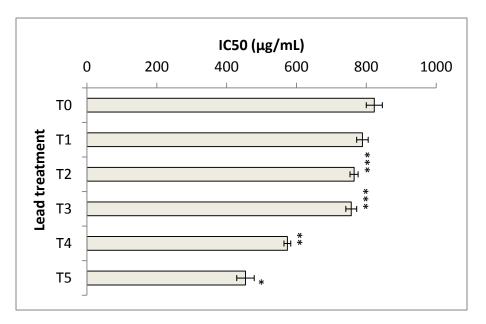


Figure 2. The antioxidant activity measured by inhibitory concentration (IC₅₀) of methanolic extract of seedlings subjected to different concentrations of Pb. Values are the mean \pm SD. Different symbols on bars indicate significant differences at P ≤0.05 (Duncan test).

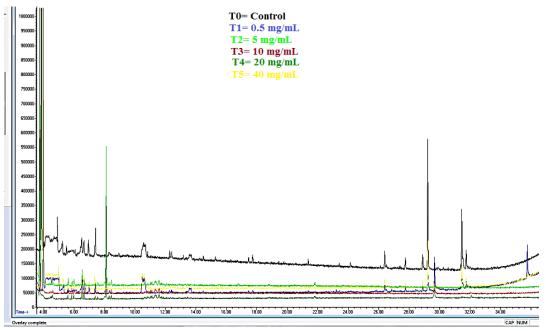


Figure 3. Overlapped GC-MS chromatograms of the methanolic extracts of seedlings of *Jatropha curcas* treated with different concentration of Pb.

GC-MS analysis of Pb treated plants

The details of qualitative and quantitative phytochemical of the methanolic extracts of seedlings of *J. curcas* treated with different concentrations of Pb were investigated using gas

chromatography coupled with mass spectrometry (GC-MS) analysis (Fig.3). The details of names of compounds, retention time (RT) and peak area percentages (area %) are depicted in tables 1, 2, 3, 4 and 5 for control (T0), T1, T2, T3, T4 and T5, respectively. The analysis revealed presence of different chemical constituents due to Pb application.

The GC-MS analysis performed in the methanolic extract of *J. curcas* seedlings in control treatment (T0) showed the presence of 29 compounds (Table 1). The major compounds that were identified in the extract based on peak area percentage include Benzene, 2-benzyloxy-1-methoxy-4(2nitroethenyl) (25.09%); p-Dioxane-2,3-diol (24.78%); Hexadecanoic acid (8.64%); Acetophenone (4.59); Octadecenoic acid (4.3%); Decane (2.66).

There were 31 compounds identified in the methanolic extract of *J. curcas* seedlings treated with 0.5 mg/L Pb (T1) (Table 2). The major compounds that were identified in the extract include Methanamine, N-methoxy- (18.36%); p-Dioxane-2,3-diol (10.88%); -Heptadien-3-yne (17.06%); Decane (9.07%); Hexadecanoic acid (7.34%); Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate (4.06%).

Table (3) shows the phytochemical analysis of the methanolic extract of *J. curcas* seedlings treated with 5 mg/L (T2). Different compound groups were investigated in this treatment and the major ones are Tetraethyl silicate (39.9%); Ethanone, 2-bromo-1-phenyl- (29.28%); Nonen-3-ol (7.98%); Acetophenone (5.42%); Thiazole, 5-methyl- (4.3%).

Tetraethyl silicate was the major compound of area percentage (40.84%) in the methanolic extract of *J. curcas* seedlings treated with 10 mg/L (T3). The other major compounds were Benzoic acid, 2-(2-chlorophenoxy)eyl ester (11.29%); Methoxymethyl isothiocyanate (8.77%); Methyl-isoxazol-5(4H)-one (4.39%) (Table 4).

The major compounds in the methanolic extract of *J. curcas* seedlings treated with 20 mg/L (T4) were Phenacyl thiocyanate (51.93%); Tetraethyl silicate (29.19%); Thiocyanic acid (5.48%); Thiocyanic acid (5.48%) (Table 5).

There are 25 compounds identified in the methanolic extract of *J. curcas* seedlings treated with 40 mg/L (T5). The major compounds are Methanamine, N-hydroxy-N-methyl-(29.53%); 1,5-Heptadien-3-yne (25.48%); Decane (7.48%); Hexadecanoic acid (7.26%); 2,3-Dihydrothiophene 1,1-dioxide (3.39%); 9-Octadecenoic acid (2.25%) (Table 6).

Many secondary metabolites and have wide range of effect on the biological activities (Nicolson and Subin, 2017).

Most of identified compounds in the methanolic extracts of *J curcas* were previously confirmed to be bioactive compounds such as Hexadecanoic acid, methyl ester which was found to have antioxidant, hypocholesterolenic, antiandrogenic, nematicide activities (Lalitharani et al., 2009; Easwaran and Ramani, 2014); 9,12-Octadecadienoic acid (Z,Z)-, to have anti-cancer activity (Yu et al., 2005). 13-Octadecenoic acid, methyl ester has anti-inflammatory, antileukotriene and cancer preventive activities (Krishnamoorthy and Subramaniam, 2014); Octadecanoic acid, methyl ester is found to be antimicrobial agent (Belakhdar et al., 2015); 1,3,5,7-Tetroxane is antimalarial, antipyretic or antiinflammatory agents. p-Dioxane-2,3-diol is of anticancer, pancreaprotective and antiasthmatic activity (Nicolson and Subin, 2017).

With increasing Pb concentration in T2, T3 and T4, the major compound was Tetraethyl silicate. It is an alkoxide addied to natural colorants to improve their stability. It was proven to improve/ thermal, photo, and pH stabilities of color red-beet-pigment obtained from *Beta vulgaris*. In addition, it sustained the antioxidant activity of the pigment (Molina et al., 2014).

Several medicinal plants growing near the industrial areas and they have the ability to accumulate high amounts of heavy metals (Zoufan et al., 2017). Elevated levels of heavy metals

lead to toxic effects and growth inhibition in plants. In addition, sensitive plants react to heavy metal stress by a decrease in biological properties where bioactive phytochemicals were reduced due to phytochemical-metal complex formation (Hussain et al., 2011). On the other hand, some plants show a great adaptability to rapid changes in the environment through modification of their metabolism. Hence, various bioactive compounds are stress derived. These compound integrate in the defense system of the plant to tolerate stress conditions (Vijayalakshmi et al., 2010). In the present study, bioactive compounds were accumulated due to Pb stress and elevated the antioxidant activity of the plants. In addition, identifying of phytochemicals ameliorates our understanding of the tolerance mechanism.

| No. | Compound | RT | Area% |
|-----|--|--------|-------|
| 1 | p-Dioxane-2,3-diol | 3.15 | 24.78 |
| 2 | Benzene, 2-benzyloxy-1-methoxy-4 (2nitroethenyl)- | 3.842 | 25.09 |
| 3 | Benzenamine, N-ethyl-N-nitroso | 4.02 | 0.7 |
| 4 | Methyl cis-3-chloropropenoate | 4.117 | 1.02 |
| 5 | Acetophenone | 4.157 | 4.59 |
| 6 | Benzamide, N-[6-(2-furyl)-2-oxo-2H -pyran-3-yl]- | 4.672 | 1.72 |
| 7 | Benzene, nitroso | 4.895 | 2.04 |
| 8 | N-Aziridyl]propane-2-thiol | 5.17 | 1.03 |
| 9 | Tetramethyl silicate | 5.221 | 1.49 |
| 10 | Benzenamine, N-ethyl-N-nitroso- | 6.423 | 0.4 |
| 11 | Benzamide, N-[6-(2-furyl)-2-oxo-]-2H pyran-3-yl | 6.452 | 0.21 |
| 12 | Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate | 6.497 | 1.5 |
| 13 | Ethyl(dimethyl)ethoxysilae | 6.641 | 1.47 |
| 14 | Methylbenzyl alcohol, tert-butyldimethylsilyl ether | 6.938 | 1.46 |
| 15 | Benzyl alcohol, benzyldimethylsily ether | 7.39 | 1.78 |
| 16 | Benzenemethanol, .alpha.,.alphadimethyl- | 10.52 | 2.02 |
| 17 | Decane | 10.572 | 2.66 |
| 18 | Indolinol, 1-benzoyl- | 10.68 | 1.69 |
| 19 | N-Benzoylglycine ethyl ester | 12.282 | 0.47 |
| 20 | Carbonic acid, isohexyl methyl ester | 12.42 | 0.49 |
| 21 | Benzamide, N-[6-(2-furyl)-2-oxo-2H -pyran-3-yl]- | 13.175 | 1.42 |
| 22 | Dimethylamphetamine | 17.73 | 0.44 |
| 23 | Benzenemethanol, .alpha.,.alphadimethyl- 1,2 | 21.369 | 0.34 |
| 24 | Methyl tetradecanoate | 26.387 | 1.22 |
| 25 | Pentadecanoic acid, methyl ester | 27.743 | 0.83 |
| 26 | Hexadecanoic acid, methyl ester | 29.214 | 8.64 |
| 27 | Octadecenoic acid, methyl ester, (E)- | 31.457 | 4.3 |
| | Benzene, (1-methylethyl 5-Isoxazolecarboxylic acid, 4,5- | | |
| 28 | dihydro-3-phenyl- | 31.52 | 1.4 |
| 29 | Methyl stearate | 31.743 | 1.35 |

Table 1. GC-MS analysis of the methanolic extract of seedlings of *Jatropha curcas* in control (T0) treatment showing the details of phytochemicals

Table 2. GC-MS analysis of the methanolic extract of seedlings of *Jatropha curcas* treated with 0.5 mg/L Pb (T1) treatment showing the details of phytochemicals

| 1 Hydroxyurea, N,N',O-trimethyl N-(2- 3.196 0.52 2 (Methylthio)-1-butene 3.247 0.92 3 p-Dioxane-2,3-diol 3.299 10.88 4 Methanamine, N-methoxy- 3.327 18.36 5 -Heptadien-3-yne 3.968 17.06 6 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 4.163 2.07 7 Thiazole, tetrahydro- 4.191 1.62 8 1,3-Dioxolan-4-on-5-acetic acid, trichloromethyl- 4.329 0.66 9 Dihydrothiophene 1,1-dioxide 4.403 1.29 10 Pentaethylene glycol 4.449 1.18 11 2,3-Dihydrothiophene 1,1-dioxide 4.603 0.32 12 Tetramethyl silicate 5.318 1.08 Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 < | No. | Compound | RT | Area% |
|--|-----|---|--------|-------|
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| 9 Dihydrothiophene 1,1-dioxide 4.403 1.29 10 Pentaethylene glycol 4.449 1.18 11 2,3-Dihydrothiophene 1,1-dioxide 4.603 0.32 12 Tetramethyl silicate 5.318 1.08 Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic | 7 | Thiazole, tetrahydro- | 4.191 | 1.62 |
| 10 Pentaethylene glycol 4.449 1.18 11 2,3-Dihydrothiophene 1,1-dioxide 4.603 0.32 12 Tetramethyl silicate 5.318 1.08 Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 0 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid | 8 | 1,3-Dioxolan-4-on-5-acetic acid, trichloromethyl- | 4.329 | 0.66 |
| 11 2,3-Dihydrothiophene 1,1-dioxide 4.603 0.32 12 Tetramethyl silicate 5.318 1.08 Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl est | 9 | Dihydrothiophene 1,1-dioxide | 4.403 | 1.29 |
| 12 Tetramethyl silicate 5.318 1.08 Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester | 10 | Pentaethylene glycol | 4.449 | 1.18 |
| Propanedioic acid, (benzoylhydrazino)hydroxy-, 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 25 cis-13-Octadecenoic acid, methyl ester 31.742 0.87 28 28 20.87 28 28 27.59 3 | 11 | 2,3-Dihydrothiophene 1,1-dioxide | 4.603 | 0.32 |
| 13 dimethyl ester 5.582 0.29 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 0.45 1 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 <td>12</td> <td></td> <td>5.318</td> <td>1.08</td> | 12 | | 5.318 | 1.08 |
| 14 Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate 6.56 4.06 15 -Propanamine, N-methyl-N-nitro- 6.703 2.21 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 <td< td=""><td></td><td></td><td></td><td></td></td<> | | | | |
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| 16 Benzaldehyde, O-ethyloxime 6.995 1.25 17 Ethanone, 1-phenyl-, oxime 7.384 0.76 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 14 | | | |
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| 18 Decane 10.468 9.07 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 27.749 0.45 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 16 | Benzaldehyde, O-ethyloxime | 6.995 | 1.25 |
| 19 Methyl tetradecanoate 26.387 0.94 Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) 27.749 0.45 20 ester 27.749 0.45 21 Pentanone, 1-phenyl 28.887 0.64 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 17 | Ethanone, 1-phenyl-, oxime | 7.384 | 0.76 |
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| 21Pentanone, 1-phenyl28.8870.6422Hexadecanoic acid, methyl ester29.2137.3423n-Hexadecanoic acid29.6653.8924Acetophenone29.7860.5225cis-13-Octadecenoic acid, methyl ester31.4562.7826Benzamide, N-hydroxy-31.5250.8527Heptadecanoic acid, 16-methyl-, methyl ester31.7420.8728Diisooctyl phthalate35.7593.6829Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]-35.8340.5430Tetrasiloxane, decamethyl-37.2760.29 | | Thiophene-2-carboxylic acid, (2-oxo-2-phenylethyl) | | |
| 22 Hexadecanoic acid, methyl ester 29.213 7.34 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 20 | ester | 27.749 | 0.45 |
| 23 n-Hexadecanoic acid 29.665 3.89 24 Acetophenone 29.786 0.52 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 21 | Pentanone, 1-phenyl | 28.887 | 0.64 |
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| 25 cis-13-Octadecenoic acid, methyl ester 31.456 2.78 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 23 | n-Hexadecanoic acid | 29.665 | 3.89 |
| 26 Benzamide, N-hydroxy- 31.525 0.85 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 24 | Acetophenone | 29.786 | 0.52 |
| 27 Heptadecanoic acid, 16-methyl-, methyl ester 31.742 0.87 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 25 | cis-13-Octadecenoic acid, methyl ester | 31.456 | 2.78 |
| 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 26 | Benzamide, N-hydroxy- | 31.525 | 0.85 |
| 28 Diisooctyl phthalate 35.759 3.68 29 Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- 35.834 0.54 30 Tetrasiloxane, decamethyl- 37.276 0.29 | 27 | Heptadecanoic acid, 16-methyl-, methyl ester | 31.742 | 0.87 |
| 30Tetrasiloxane, decamethyl-37.2760.29 | 28 | | 35.759 | 3.68 |
| | 29 | Benzamide, N-[6-(2-furyl)-2-oxo-2H pyran-3-yl]- | 35.834 | 0.54 |
| 31 Methyltris(trimethylsiloxy)silane 37.705 0.79 | 30 | Tetrasiloxane, decamethyl- | 37.276 | 0.29 |
| | 31 | Methyltris(trimethylsiloxy)silane | 37.705 | 0.79 |

Table 3. GC-MS analysis of the methanolic extract of seedlings of *Jatropha curcas* treated with 5 mg/L Pb (T2) treatment showing the details of bioactive compounds

| No. | Compound | RT | Area% |
|-----|--------------------------------------|--------|-------|
| 1 | 2,2-Diethoxyacetophenone | 3.253 | 2.18 |
| 2 | Nonen-3-ol | 3.396 | 7.98 |
| 3 | Ethanone, 2-bromo-1-phenyl- | 3.785 | 29.28 |
| 4 | 2-Propanone, 1-(ethylthio | 4.609 | 1.15 |
| 5 | Acetophenone | 5.61 | 1.87 |
| 6 | 1,2-Propanedione, 1-phenyl-, 2-oxime | 5.868 | 1.07 |
| 7 | 8-Benzoyloctanoic acid | 5.988 | 2.26 |
| 8 | Thiazole, 5-methyl- | 6.531 | 4.3 |
| 9 | 5-Nitro-m-xylene | 6.663 | 2.52 |
| 10 | Tetraethyl silicate | 8.111 | 39.9 |
| 11 | alphaNitroacetophenone | 8.448 | 2.06 |
| 12 | Acetophenone | 11.304 | 5.42 |

Table 4. GC-MS analysis of the methanolic extract of seedlings of *Jatropha curcas* treated with 10 mg/L Pb (T3) treatment showing the details of phytochemicals

| No. | Compound | RT | Area% |
|-----|---|--------|-------|
| 1 | Methoxymethyl isothiocyanate | 3.373 | 8.77 |
| 2 | Ethanone, 2-bromo-1-phenyl- | 3.762 | 27.06 |
| 3 | Benzene, 1,2,4-trimethyl- | 5.604 | 1.87 |
| 4 | Silane, triethoxymethyl- | 5.856 | 1.54 |
| 5 | 2,3-Dimethylamphetamine | 5.982 | 1.32 |
| 6 | Methyl-isoxazol-5(4H)-one | 6.526 | 4.39 |
| 7 | Ethanone, 1-phenyl-, oxime | 6.657 | 1.24 |
| 8 | Tetraethyl silicate | 8.105 | 40.84 |
| 9 | 2,3-Heptadien-5-yne, 2,4-dimethyl- | 8.448 | 1.48 |
| 10 | cis-2,3-Epoxyoctane | 11.561 | 0.19 |
| 11 | Benzoic acid, 2-(2-chlorophenoxy)e yl ester | 29.654 | 11.29 |

| Table 5. GC-MS analysis of leaf methanolic extract of seedlings of Jatropha curcas treated with |
|---|
| 20 mg/L Pb (T4) treatment showing the details of phytochemicals |

| No. | Compound | RT | Area% |
|-----|--|--------|-------|
| 1 | Thiocyanic acid, methyl ester | 3.333 | 5.48 |
| 2 | Phenacyl thiocyanate | 3.722 | 51.93 |
| 3 | 6-Benzoylhexanoic acid | 5.582 | 1 |
| 4 | Silane, triethoxymethyl- | 5.839 | 1.24 |
| 5 | 2,3-Dimethylamphetamine | 5.965 | 1.31 |
| 6 | Thiazole, 5-methyl- | 6.509 | 3.03 |
| 7 | Benzoic acid trimethylsilyl | 6.64 | 2.01 |
| 8 | Tetraethyl silicate | 8.099 | 29.19 |
| 9 | Octane, 2-methyl- | 11.555 | 0.1 |
| 10 | 1,3-Dioxolane, 2-phenyl-2-(phenylm ethyl)- | 29.654 | 4.69 |

Table 6. GC-MS analysis of the methanolic extract of seedlings of Jatropha curcas treated with40 mg/L Pb (T5) treatment showing the details of phytochemicals

| No. | Compound | RT | Area% |
|-----|--|--------|-------|
| 1 | Butane, 2,2'-thiobis- | 3.161 | 0.41 |
| 2 | 2-Aminoethylethyl sulfide | 3.207 | 0.9 |
| 3 | Methanamine, N-hydroxy-N-methyl- | 3.247 | 29.53 |
| 4 | 1,5-Heptadien-3-yne | 3.922 | 25.48 |
| 5 | Thiazole, tetrahydro- | 4.111 | 1.04 |
| 6 | Propanoic acid, 2-methyl-, 2-ethyl -3-hydroxyhexyl ester | 4.134 | 0.7 |
| 7 | Propyl nitrite | 4.151 | 1.42 |
| 8 | 2,3-Dihydrothiophene 1,1-dioxide | 4.237 | 3.39 |
| 9 | Pentaethylene glycol | 4.306 | 1.03 |
| 10 | Methyl trans-3-chloropropenoate | 4.569 | 2.86 |
| 11 | Benzeneethanol, .betamethylalphaphenyl- | 4.958 | 2.07 |
| 12 | Tetramethyl silicate | 5.284 | 1.39 |
| 13 | 4-Methoxy-2-phenyl-butyraldehyde | 5.559 | 0.43 |
| 14 | 5-Isoxazolecarboxylic acid, 4,5-di hydro-3-phenyl- | 6.474 | 1.11 |
| 15 | Isobutyl 2,5,8,11-tetraoxatridecan -13-yl carbonate | 6.543 | 2.53 |
| 16 | 1,1-Difluoro-2-methyl-2-vinyl-cyclopropane | 6.686 | 1.65 |
| 17 | 3-(3-Pyridyl)propenoic acid | 6.978 | 1.19 |
| 18 | Benzyl alcohol, bromomethyldimethy | 7.367 | 1.45 |
| 19 | Acetophenone | 8.305 | 1.8 |
| 20 | Decane | 10.457 | 7.48 |
| 21 | Methyl tetradecanoate | 26.387 | 0.67 |
| 22 | Hexadecanoic acid, methyl ester | 29.213 | 7.26 |
| 23 | 9-Octadecenoic acid, methyl ester, (E)- | 31.456 | 2.25 |
| 24 | Methyl stearate | 31.743 | 0.98 |
| 25 | 4-Cyanobenzophenone | 35.754 | 0.42 |

Conclusions

J. curcas is a multiple purpose plant with potential for biodiesel production and medicinal uses. The plant has a long history of usage in treatments of a wide range of ailments in many countries. The present study proved the ability of the plant to accumulate Pb from hydponics within limited range of time scale. High affinity of the plant to this particular heavy metal and its use as traditional medicine shed light on cautions to be taken for dual properties of traditional uses and applications. Further studies are required to control these controverting properties. The therapeutic utilization may be ideal when the phytochemical investigation is screened or the active compounds are used in purified form.

Acknowledgments

This current study is a part of the research project concerning plant-based bioremediation. MD appreciates TEMPUS for his JM scholarship to do part of this study at BOKU, Vienne, Austria.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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