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Original Article

Acute toxicity, secondary metabolites, and antioxidant activity of *Macaranga tanarius* from post-coal mining and non-mining areas in East Kalimantan, Indonesia

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Abstract

Coal plays a crucial role in Indonesia's foreign exchange and East Kalimantan's revenue sharing, yet its environmental impacts, including soil acidification, raises concerns. Reclamation measures involve revegetation with pioneer plants such as *Macaranga sp*., known for their medicinal properties. However, the pharmacological properties of these plants are influenced by secondary metabolites, which depend on soil parameters such as pH and nutrient levels. The aim of this study was to evaluate the acute toxicity, secondary metabolites, and antioxidant activities of *Macaranga tanarius* leaf extracts from post-coal mining area (MTPCMA) and non-mining area (MTNMA) alongside soil parameters. Acute toxicity of *M. tanarius* leaf extracts and soils were assessed using the brine shrimp lethality test (BSLT). Phytochemical screening was done using thin-layer chromatography (TLC), determining total phenolic (TPC) and flavonoid content (TFC). The DPPH radical scavenging assay was used to assess the antioxidant activity. A comparative analysis between MTPCMA and MTNMA was conducted using Student t-test. The data showed no significant difference in toxicity between MTPCMA and MTNMA leaf extracts (LC_{50} of 100–1000 µg/mL) (p=0.062), and soils from both areas were non-toxic (LC_{50} of >1000 µg/mL). Although heavy metal concentrations were higher in PCMA than in NMA soil (p<0.001), secondary metabolite compounds and TFC in both extracts were not significantly different (p=0.076). Both extracts contained flavonoids and polyphenols with antioxidant activity and terpenoids without antioxidant activities. The DPPH radical scavenging test suggested insignificant antioxidant activity between MTPCMA and MTNMA extracts (p=0.237). In conclusion, non-toxic soils in post-mining land and insignificant differences between MTPCMA and MTNMA extracts suggest good soil nutrient availability, highlighting the success of land recovery after 10 years of revegetation with *M. tanarius*.

Keywords: Acute toxicity, antioxidant, coal mining, revegetation, *Macaranga tanarius*, secondary metabolites

Introduction

East Kalimantan is the second-largest coal producer in Indonesia [1]. Exploitation begins with stripping the soil and vegetation covering the coal area. However, during the mining process, the

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topsoil is not reinstated to its original place; instead, it is piled up for several years. Later, upon completion, the soil is either restored to its initial state or relocated, causing soil heterogeneity due to the amalgamation of excavated soil and leftover mining materials [2]. This condition might cause alterations in soil nutrients and pH, reduce soil fertility, and promote toxicity to the affected environment [3,4], making coal mining as a significant ecosystem deterioration by humans.

To overcome this problem, various reclamation measures to restore damaged land have been conducted [5,6]. This included revegetation with pioneer plants such as *Macaranga sp*. [7], which often abundantly grows on post-coal mining landscapes. Ethnobotanically, *Macaranga sp*. has been traditionally used for medicinal purposes to reduce fever and diabetes, attributed to its antioxidant properties [8]. However, the quantity and quality of plants' secondary metabolites are highly influenced by soil pH and nutrients [9-11], which in turn, impact their biological activities. Therefore, evaluating the secondary metabolite profile and antioxidant activity of *Macaranga sp*. such as *M. tanarius* growing on post-mining lands, along with several soil parameters such as pH and nutrients, may help evaluate the success of post-mining land recovery.

According to the Food and Drug Monitoring Agency, plants used for traditional medicines should meet safety standards [12]. Plant parts used as raw materials for medicines require toxicity tests, including acute, sub-chronic, and chronic toxicity tests *in vivo* [12]. Nevertheless, to minimize the use of experimental animals in toxicity assays, acute toxicity screening using the brine shrimp lethality test (BSLT) has been considered a recommended alternative [13]. This study aimed to evaluate and compare the toxicity status, secondary metabolites, and antioxidant activity of *M. tanarius* growing in post-mining and non-mining areas, along with several soil parameters such as pH and nutrients. This study offers a revegetation model with substantial economic value through medicinal plant cultivation for the pharmaceutical industry, emphasizing medicinal plant cultivation in humid tropical forests.

Methods

Extract preparation

The leaves of healthy *M. tanarius* from the post-coal mining area in Sangata (MTPCMA) and *M. tanarius* from the non-mining area in Kutai Kartanegara (MTNMA), East Kalimantan, Indonesia, were collected (1 kg each). After being cleaned and washed thoroughly, the samples were dried in a drying cabinet at a temperature of 50°C before being ground into powder. Extraction was performed by the maceration method at a ratio of 1:5 (v/v), in which 300 g of simplicial was soaked in 1500 mL of ethanol for three days with occasional stirring. The concoction was then filtered to obtain macerate and lees. The macerate was collected and preserved in a place protected from light, whereas the lees fraction underwent the same maceration procedure until the mixture became colorless. The obtained macerate was then evaporated in a rotary evaporator at a temperature of 50°C. The yielded thick solution was subsequently transferred to a suitable container and placed in an oven at a temperature of 50°C to obtain a concentrated extract. The extracts were then stored at 4°C until use.

Toxicological evaluation using the brine shrimp lethality test (BSLT)

The toxicity of the extracts and soil from post-coal mining and non-mining areas was assayed using the BSLT. The eggs of *Artemia salina* (brine shrimp) were hatched in a container containing sterile artificial seawater with constant aeration for 48 hours. Ten active larvae were collected using a capillary pipette and transferred into different tubes containing 4.5 mL of saltwater and 0.5 mL of each sample solution (extracts and soil). Sterile salt water with larvae without the addition of the extract or soil was also prepared as a control. After 24 hours of incubation at room temperature, the number of living larvae was recorded, and the percentage of larvae mortality was calculated. The toxicity was assessed by determining the lethal concentrations 50 (LC_{50}) value using probit analysis in linear regression [14,15,16]. The toxicity levels were classified as very toxic (LC_{50} of ≤30 µg/mL), moderately toxic (LC_{50} of ≥30–1000 µg/mL), and low toxic (LC_{50} of >1000 µg/mL) [17]. All tests were performed in triplicate.

Soil nutrient and pH analysis

Soil samples from post-coal mining and non-mining lands were collected for nutrients, heavy metals, and pH analysis. Among the soil nutrients tested were organic carbon and nitrogen using the Kjeldahl method [18], phosphorus using the Olsen or Bray method [19], and potassium using the Morgan method [20]. In addition to soils, the levels of heavy metals were also assessed in MTPCMA and MTNMA leaf extracts [20]. Soil pH was measured using a pH meter. Soil nutrient and pH analyses were conducted in the Analyses Laboratory, Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, Indonesia.

Phytochemical screening

Phytochemical analysis, both qualitatively using thin layer chromatography (TLC) and quantitatively in the form of total phenolic content (TPC) and total flavonoid content (TFC), was carried out on *M. Tanarius* leaf extracts. Qualitative screening included alkaloids (Dragendorff), flavonoids ($AlCl_3$ 10%), polyphenols (FeCl3), and triterpenoids/steroids (Liebermann/Burchard) tests.

Thin layer chromatography (TLC)

Identification using TLC was performed by applying 10 µL of extracts onto the TLC plates and allowing them to dry at room temperature. Once dried, the plates were placed in a chromatography chamber containing eluents specific to the target compounds: (a) ethyl acetate: methanol: water (ratio of 6:4:2 v/v) for alkaloids; (b) butanol: acetic acid: water (BAW) (3:1:1 v/v) for flavonoids; (c) chloroform: ethyl acetate: formic acid (0.5:9:0.5 v/v) for polyphenols; and (d) n-hexane: ethyl acetate (4:1 v/v) for terpenoids/steroids assays. The plates were then sprayed with the respective reagents to visualize the spots, followed by spraying with 2,2-diphenyl-1-picrylrazyl (DPPH) to determine antioxidant activity, characterized by the presence of a pale-yellow spot with a purple background on the plate [21].

Total phenolic content (TPC)

TPC was assessed using a previously described method [22]. Briefly, a total of 0.2 mL of *M. Tanarius* leaf extract with a concentration of 30 mg/mL (prepared by dissolving 0.3 g extract in 10 mL of absolute ethanol), 15.8 mL of distilled water, and 1 mL of Folin-Ciocalteu 50% (v/v) were mixed and homogenized in a flask. After eight min of incubation, the concoction was added with 3 mL of Na_2CO_3 5% (w/v), homogenized, and incubated for two hours at room temperature, protected from light. The absorbance was measured using a UV-Vis spectrophotometer BioSpectrometer® from Eppendorf at 725 nm. The results are expressed as mg gallic acid/g extract (mg GAE/g). The test was performed in triplicate.

Total flavonoid content (TFC)

A total of 10 mL of the extract with a concentration of 0.1 mg/mL, 0.7 mL of distilled water, and 0.1 mL of 5% $NaNO_2$ were mixed and incubated for five min. Then, 0.1 mL of 10% $AlCl_3$ was added and left to stand for six min before the addition of 0.5 mL of 1M NaOH. After ten min of incubation, the solution's absorbance was measured at a wavelength of 510 nm using a UV-Vis spectrophotometer BioSpectrometer® from Eppendorf, and TFC was expressed as milligrams equivalent of catechins per gram of dry extract (mg QE/g). A 95% ethanol (1 mL) was used as a blank, and the assay was performed in triplicate [23].

DPPH radical scavenging assay

DPPH solution (1 mL) was gently added into a test tube containing the extracted sample (2 mL) of various concentrations: 2, 4, 8, 16, and 32 µg/mL. A DPPH solution (2 mL) and ethanol (0.5 mL) were prepared as a blank, and a concentration routine of 2, 4, 8, 16, 32 µg/mL was used as the positive control. After 15 min of incubation at 37°C, the absorbance was measured at a wavelength of 517 nm using a UV-Vis spectrophotometer, and the results were expressed as the effective concentration 50 (EC_{50}) value [8,24,25].

Statistical analysis

The LC_{50} of the toxicity test and EC_{50} of antioxidant activity were determined using linear regression between the extract concentrations (X-axis) and the percent of inhibition (Y-axis)

using probit analysis. A parametric Student t-test was used to identify the significance of differences between the extracts from post-coal mining and non-mining areas on all the parameters tested, and a *p*-value of ≤0.05 was considered statistically significant. All statistical analyses were performed using SPSS software (IBM, New York, USA).

Results

Toxicity test

The results of the toxicity test on the extracts and soil are presented in **Table 1**. The extract of MTNMA had a higher LC_{50} value (491.867±50.940 µg/mL) than that of MTPCMA (317.598±44.884 µg/mL), suggesting that *M. Tanarius* leaves growing in the post-coal mining area had slightly higher toxicity than those growing in the non-mining region. However, the result of the Student t-test analysis suggested no significant difference in the level of toxicity between the two extracts (p=0.062), in which both of them showed moderate toxicity (LC_{50} of ≥30–1000 µg/mL).

On the contrary, there was a statistically higher LC_{50} value for NMA soil (mean ± SEM: 51179.19±5224.52 µg/mL) compared to that of PCMA soil (20971.26±4109.40 µg/mL) (p=0.010); however, both soils did not show toxicity towards brine shrimp since the LC_{50} values were greater than 1000 µg/mL.

Table 1. LC_{50} values of *M. tanarius* leaf extracts and soils against *A. salina* larvae after 24 hours of exposure

Samples	LC_{50} (µg/mL)			Mean ± standard error	*p*-value
	I	II	III		
Extracts					
Non-mining area	449.06	593.34	433.21	491.87±50.94	0.062
Post-coal mining area	372.65	351.48	228.66	317.60±44.88	
Soil					
Non-mining area	41503.04	52601.73	59432.82	51179.19±5224.52	0.010*
Post-coal mining area	14740.41	19445.14	28728.23	20971.26±4109.40	

*Statistically significant at p=0.05

Nutrient status of soils and extracts

Nutrient status and pH of soils and extracts are summarized in **Table 2**. The pH, organic carbon, and available phosphorus were found to be significantly higher in the soil of post-coal mining area compared to that of non-mining area (p<0.05); however, their concentrations in the leaf extracts of both areas were not significantly different (p>0.05). Similarly, the concentrations of heavy metals such as Pb, Cd, As, and Hg in the soil of post-coal mining area were significantly higher (p<0.05) than those in non-mining area; in contrast, the concentrations of these metals in post-coal mining area leaves were lower than those in non-mining area. The concentrations of potential phosphorus and potassium in either soil or leaves from both lands, on the other hand, were similar.

Phytochemical analysis using TLC

The results of qualitative phytochemical screening of *M. tanarius* leaf extracts are presented in **Figure 1**. The TLC test revealed the presence of flavonoids (yellow spot after being sprayed with 10% $AlCl_3$) and polyphenols (black spot after being sprayed with $FeCl_3$) with antioxidant activity (yellow spot with a purple background after being sprayed with DPPH) (**Figure 1B-C**), as well as the presence of terpenoids (purple spot) without antioxidant potential (**Figure 1D**) in both MTPCMA and MTNMA leaf extracts. However, alkaloids were absent in the extracts from both areas (**Figure 1A**).

Total phenolic content (TPC) and total flavonoid content (TFC)

The results of TPC and TFC evaluation of *M. tanarius* leaf extracts are presented in **Table 3**. The leaves obtained from the post-coal mining area exhibited significantly higher compared to those from the non-mining area (p<0.001). On the contrary, there was no significant difference between the extracts from both lands in terms of TFC (p=0.076).

Table 2. Comparison of nutrients, pH, and heavy metals in soil and *M. tanarius* leaf extracts from post-coal mining and non-mining areas

Nutrients	Soil			Leaf extracts		
	Mean ± standard error		*p*-value	Mean ± standard error		*p*-value
	Non-mining area	Post-coal mining area		Non-mining area	Post-coal mining area	
pH						
H_2O	4.38±0.11	5.68±0.03	0.008*			
KCl	4.26±0.07	5.48±0.01	0.004*			
Organic C (%)	1.05±0.03	3.07±0.03	<0.001*	29.61±10.89	36.045±1.23	0.617
Total N (%)	0.19±0.05	0.25±0.01	0.325	2.81±0.11	2.825±0.06	0.947
Available P(Bray I) (ppm P_2O_5)	<0.08±0.00	12.02±0.89	0.006*			
Cation-exchange capacity (Cmol/kg)	5.96±0.28	10.69±0.02	0.004*			
Exchangeable Mg (Cmol Mg/kg)	0.08±0.06	5.0±0.15	0.001*			
Exchangeable Ca (Cmol Ca/kg)	2.58±0.06	4.50±0.02	0.001*			
Exchangeable K (Cmol K/kg)	0.46±0.01	0.20±0.01	0.002*			
Exchangeable Na (Cmol Na/kg)	0.05±0.01	0.11±0.00	0.027*			
Exchangeable Al (Cmol Al/kg)	1.06±0.05	0.05±0.05	0.005*			
Exchangeable H (Cmol H/kg)	0.19±0.09	0.37±0.01	0.199			
Potential						
P (mgP_2O_5/100g)	18.10±13.55	14.59±0.04	0.820	0.16±0.01	0.15±0.00	0.095
K (mgK_2O/100g)	26.15±3.72	28.36±15.15	0.900	0.66±0.01	0.91±0.02	0.01*
Water content (% w/b)	0.81±0.05	1.76±0.01	0.004*			
Total Mg (%)	0.01±0.00	0.14±0.01	0.001*	0.40±0.01	0.37±0.01	0.198
Total Ca (%)	0.03±0.00	0.08±0.00	<0.001*	1.73±0.02	0.89±0.06	0.007*
Total Na (%)	2.47±1.86	15.47±1.02	0.026*	0.02±0.00	0.02±0.00	1
Total S (%)	0.02±0.00	0.03±0.00	<0.001*	0.12±0.01	0.22±0.00	0.024*
Total Fe (ppm)	6,155.0±52.00	17,977.00±11.00	<0.001*	106.19±0.62	262.68±36.44	0.05
Total Mn (ppm)	19.6±0.90	169.36±3.74	<0.001*	284.05±26.38	67.11±4.41	0.015*
Total Cu (ppm)	3.52±0.45	17.79±1.13	0.007*	59.83±10.91	11.57±0.48	0.048*
Total Zn (ppm)	13.55±2.27	49.78±0.90	0.005*	66.43±3.09	70.61±4.39	0.518
Total B (ppm)	3.90±0.02	9.26±0.01	<0.001*	7.00±0.88	9.27±0.66	0.175
Rough silicate (% SiO_2)	88.89±0.13	80.89±0.22	0.001*	2.15±0.11	3.03±0.01	0.017*
Pb (ppm)	5.88±0.11	10.90±0.14	0.001*	1.18±0.47	0.53±0.04	0.302
Cd (ppm)	0.02±0.00	0.08±0.01	0.014*	0.03±0.00	0.01±0.00	<0.001*
Cr (ppb)	16.00±0.02	23.66±0.62	0.007*			
As (ppm)	1.80±0.07	7.43±0.23	0.002*	0.13±0.01	0.26±0.01	0.008*
Hg (ppm)	0.01±0.00	0.03±0.00	<0.001*	0.02±0.00	0.01±0.00	<0.001*
Co (ppm)	0.62±0.01	10.48±0.31	<0.001*			
Ash (%)				7.48±0.01	7.33±0.06	0.133
Total starch (%)				1.18±0.00	0.91±0.04	0.021*
Total glucose (%)				1.54±0.01	2.26±0.04	0.004*

*Statistically significant at p=0.05
**Statistically significant at p=0.001

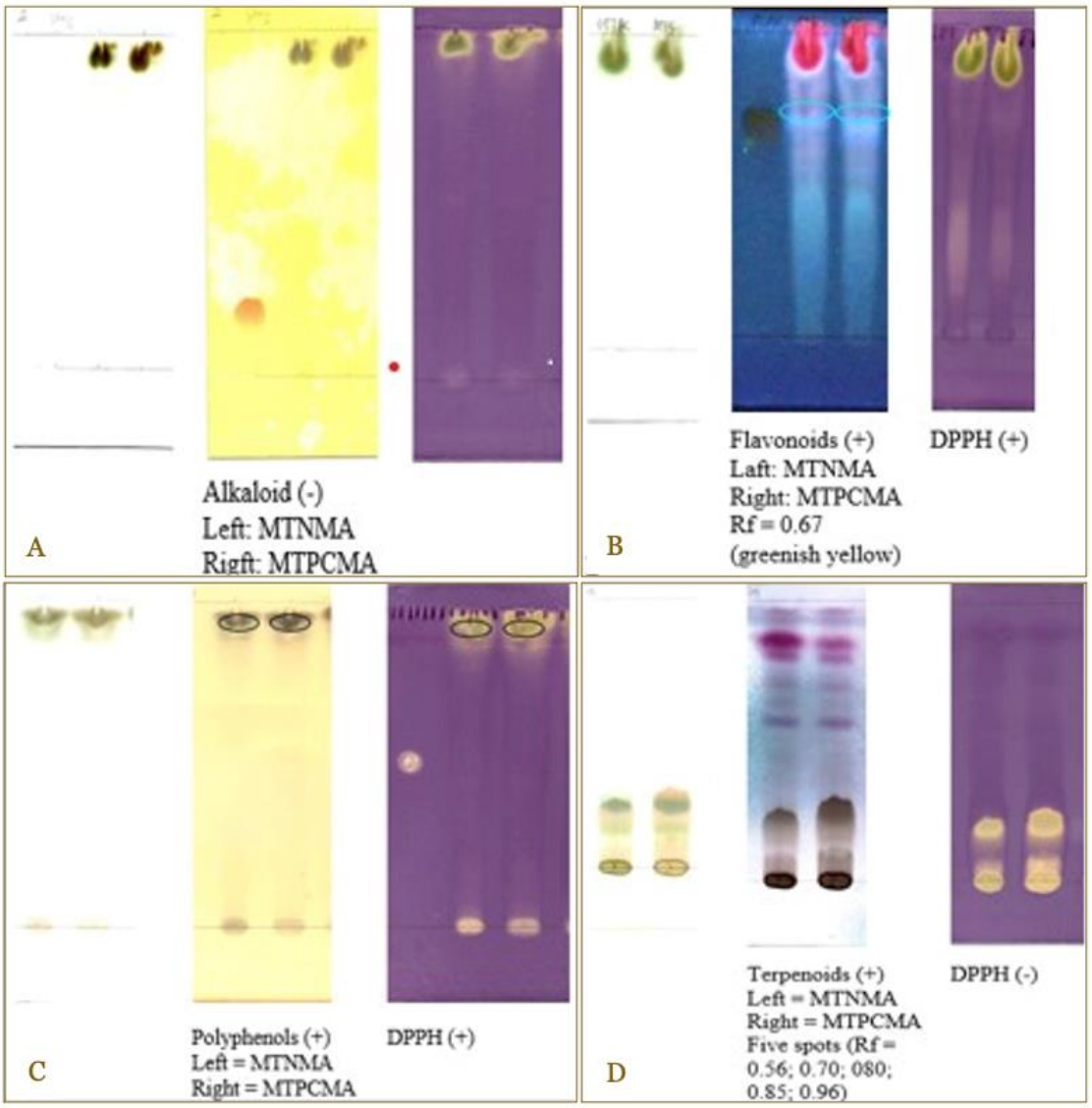

Figure 1. The results of the TLC assay of *M. tanarius* leaf extract: (A) alkaloids (-); (B) flavonoids (+), antioxidant (+); (C) polyphenols (+), antioxidant (+); (D) terpenoids (+), antioxidant (-). Each figure (A-D): Left: before being sprayed with reagent; middle: after being sprayed with reagent; right: after being sprayed with DPPH.

Table 3. Total phenolic content (TPC) and total flavonoid content (TFC) of *M. tanarius* ethanol leaf extract from post-mining (PCMA) and non-mining area (NMA)

Phytochemical constituents	I	II	III	Mean ± standard error	*p*-value
Total phenolic content (TPC) (mg GAE/g)					
Non-mining area	1541.00	1557.67	1544.33	1547.67±5.09	<0.001**
Post-mining area	2271.00	2277.67	2291.00	2279.89±5.88	
Total flavonoid content (TFC) (mg QE/g)					
Non-mining area	283.14	286.29	280.29	283.24±1.73	0.076
Post-mining area	291.14	288.86	286.00	288.67±1.49	

*Statistically significant at p=0.05
**Statistically significant at p=0.001

DPPH radical scavenging activity

The antioxidant activities of *M. tanarius* leaf extracts are illustrated in **Figure 2** and **Table 4**. The MTNMA extract exhibited a higher DPPH radical scavenging percentage, as well as a lower EC_{50} value compared to that of MTPCMA. However, based on the t-test analysis, the EC_{50} scores did not differ significantly between the extracts from both

areas (p>0.05), justifying the same antioxidant potential of *M. tanarius* leaves growing in revegetated post-coal mining and non-mining lands.

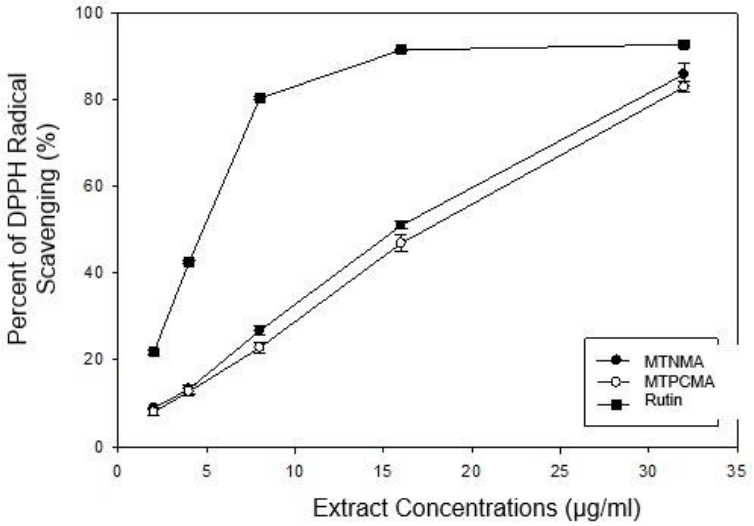

Figure 2. Percentage of DPPH radical scavenging of *M. tanarius* leaf extracts from post-mining (MTPCMA) and non-mining areas (MTNMA).

Table 4. EC_{50} value of *M. tanarius* leaf extracts against DPPH free radical

Extracts	EC_{50} (µg/mL) Mean ± standard error	p-value
M. tanarius from non-mining area (MTNMA)	17.38±0.53	0.237
M. tanarius from post-coal mining area (MTPCMA)	18.48±0.49	
Concentration routine	7.64±0.09	

Discussion

This study evaluated and compared the toxicity effect, secondary metabolites, and antioxidant activity of *M. tanarius* ethanol leaf extracts from revegetated post-coal mining and non-mining areas in East Kalimantan, Indonesia. We also analyzed soil toxicity and nutrient status from both areas to evaluate the effect of *M. tanarius* revegetation on post-coal mining land recovery. We found that MTPCMA and MTNMA soils did not exhibit toxicity during the BSLT test. On the contrary, the leaves were found to be toxic against brine shrimp, but the level of toxicity between both leaves was not significantly different (**Table 1**). This similarity might be attributed to the lower concentrations of heavy metals (Pb, Cd, As, Hg) in MTPCMA leaves as compared to those in MTNMA. Despite significantly higher heavy metal levels being observed in MTPCMA soil (**Table 2**), it is hypothesized that secondary metabolites contained in MTPCMA may act to eliminate absorbed toxins or chemicals through the roots, ensuring their survival [10]. On the other hand, the toxic effects of *M. tanarius* leaves have provided insights that the plant might serve as an anti-cancer candidate, as previous studies reported that various *Macaranga* genera, including *M. hosei*, *M. tanarius*, and *M. gigantea*, showed cytotoxic effects against cancer cell lines [26,27].

Qualitative phytochemical screening of MTPCMA and MTNMA revealed the presence of flavonoids and polyphenols with antioxidant activity, as well as the presence of terpenoids without antioxidant potential (**Table 3**). This is in line with the findings of previous studies, which reported that some species of the *Macaranga* genus, such as *M. hosei*, contain flavonoids [26], whereas *M. bancana* contains polyphenols and terpenoids [28]. Furthermore, the quantitative analysis suggested that MTPCMA had significantly higher TPC compared to MTNMA, highlighting higher polyphenol levels in MTPCMA than in MTNMA. On the other hand, TFC in both leaf extracts was not significantly different, indicating good soil nutrient availability in both areas. Additionally, the antioxidant potentials of both extracts, assessed using DPPH radical scavenging, were not significantly different (**Table 4**), suggesting a similar quality of

secondary metabolites of *M. tanarius* growing in post-coal mining and non-mining lands. These findings justify that revegetation with *M. tanarius* has contributed to the successful recovery of the post-coal mining area.

Assessment of plant pharmacological effects and toxicity to determine the recovery status of revegetated post-mining lands should not be limited to a single plant species; it necessitates involving multiple plant species from the same family [29]. Additionally, the use of plants with similar characteristics, such as those with the same height, diameter, and age, is crucial as these factors affect the quantity and quality of active ingredients, which in turn influence their pharmacological activities and toxicity effects [10]. Indifferent toxicity statuses between plants growing in post-mining and non-mining areas are indicative of the successful recovery of post-mining land through the revegetation process.

Typically, it takes approximately three to eight years for revegetation to recover post-mining lands and restore their nutrient levels, pH, and other essential elements to normal values [28]. In this study, according to post-mining land managers in Sangata, revegetation has been ongoing for more than 10 years. Soil nutrients and heavy metal contents such as Fe, Cu, Mn, Pb, Cd, As, and Hg were found to be higher in this area compared to those in non-mining regions. This condition was presumably associated with the open-pit mining system employed in Indonesia, which involves clearing the vegetation (land cleaning), removing layers of soil to access ore deposits, collecting the mining seeds, refilling and compacting the mining hole with overburden and mining waste material (tailings), and covering the area with topsoil previously set aside for future revegetation [30]. This waste soil resulting from the mining ore extraction process contains a significant number of heavy metals. However, interestingly, the heavy metals contained in MTPCMA leaves were lower than those of MTNMA, and the toxicity status, secondary metabolites, and antioxidant activity of the leaves from both areas were not significantly different. This suggests that the recovery process of post-mining land with *M. tanarius* revegetation for more than 10 years has been successful.

Conclusion

This study conducted a comparison between *M. tanarius* leaf extracts from post-coal mining (MTPCMA) and non-mining (MTNMA) areas to evaluate the success of the revegetation process on post-mining land recovery. Despite significantly higher heavy metal contents being observed in MTPCMA soil, there were no significant differences between MTPCMA and MTNMA leaf extracts in terms of toxicity status, secondary metabolites (TFC), and DPPH radical scavenging activities, suggesting good soil nutrient availability in both areas. This highlights a successful post-coal mining land recovery upon ten-year revegetation with *M. tanarius*.

Moving forward, it is essential to acknowledge this study's limitations. Focusing solely on *M. tanarius* may limit the findings' comprehensiveness, neglecting the long-term effects of its revegetation on soil quality and ecosystem dynamics. Additionally, the sample size may hinder the result's generalizability. To advance understanding, future research should consider recommendations. Including a broader range of plant species would enhance evaluation. Long-term monitoring studies are necessary to assess sustainability. Moreover, larger sample sizes and replication would improve reliability. Investigating underlying mechanisms, such as interactions between plant species and soil microorganisms, would provide valuable insights. Addressing these recommendations can enhance post-coal mining land recovery's effectiveness and sustainability.

Ethics approval

The Scientific and Ethical Review Committee of Universitas Mulawarman, Indonesia (approval number 116/KEPK-FK-VII/2022) and the Analyses Laboratory Department of Agronomy and Horticulture, Faculty of Agriculture, Institut Pertanian Bogor, Indonesia (approval number 399/08/DL/23), approved the study protocol.

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Competing interests

The authors declare that there is no conflict of interest

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Underlying data

Derived data supporting the findings of this study are available from the corresponding author on request.

How to cite

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References

1. Kementerian ESDM. Provinsi dengan Jumlah Cadangan Batubara Terbesar 2020. Available from: https://www.esdm.go.id/. Accessed: 11 November 2023.
2. Oktavia R. Identifikasi jenis tumbuhan dan kondisi tanah revegetasi lahan bekas tambang batubara. Bionatural. 2019;6(1):67-69.
3. Feng Y, Wang J, Bai Z, Reading L. Effects of surface coal mining and land reclamation on soil properties: A review. Earth-Sci Rev 2019;191:12-25.
4. Ma K, Zhang Y, Ruan M, *et al*. Land subsidence in a coal mining area reduced soil fertility and led to soil degradation in arid and semi-arid regions. Int J Environ Res Public Health 2019;16(20):3929.
5. Agus F, Soelaeman Y, Anda M. Petunjuk teknis rehabilitasi lahan bekas tambang untuk pertanian. Badan Penelitian dan Pengembangan Pertanian, Jakarta; 2019.
6. Hendrychová M, Svobodova K, Kabrna M. Mine reclamation planning and management: Integrating natural habitats into post-mining land use. Resour Policy 2020;69:101882.
7. Setiawan KA, Sutedjo S, Matius P, Diana R. Komposisi jenis tumbuhan bawah di lahan revegetasi pasca tambang batubara. ULIN: Jurnal Hutan Tropis 2018;1(2).
8. Fikriah I, Marliana E, Ismail S. Aktivitas antioksidan dan hepatoprotektor batang macaranga tanarius secara *in vitro*. Jurnal Riset Teknologi Industri 2016;6(12):20-28.
9. Fatimah S, Handarto BM. Pengaruh komposisi media tanam terhadap pertumbuhan dan hasil tanaman sambiloto (Andrographis paniculata, Nees). Jurnal Embryo 2008;5(2):133-148.
10. Syahadat RM, Aziz SA. Pengaruh komposisi media dan fertigasi pupuk organik terhadap kandungan bioaktif daun tanaman kemuning (Murraya paniculata (L.) Jack) di pembibitan. Bul Littro 2012;23(2):142-147.
11. Ekawati I, Wati HD. Pengaruh media tanam terhadap respon pertumbuhan dan produksi genotipe *Moringa oleifera* (L). Cemara 2019;17(1):8-13.
12. Badan Pengawas Obat dan Makanan RI. Peraturan kepala badan pengawas obat dan makanan nomor 12 Tahun 2014 tentang persyaratan mutu obat tradisional. Jakarta: BPOM RI; 2014.
13. Kosala K, Ismail S, Fikriah I, Gunawan Y. *In vitro*, tracheospasmolytic effect and acute toxicity screening of Coptosapelta flavescens Korth root's water extract. International Journal of Research in Pharmaceutical Sciences. 2020;11(SPL4):949-54.
14. Saragih G, Tamrin T, Marpongahtun M, *et al*. Phytochemical screening and toxicity of ethanolic extract of mangrove (*Rhizophora mucronata*) leaves from Langsa, Aceh Timur. Rasayan J Chem 2020;13(1):476-480.
15. Karchesy YM, Kelsey RG, Constantine G, Karchesy JJ. Biological screening of selected Pacific Northwest forest plants using the brine shrimp (Artemia salina) toxicity bioassay. Springerplus 2016;5:1-9.

16. Musdalipah M, Tee SA, Karmilah K, *et al*. Total phenolic and flavonoid content, antioxidant, and toxicity test with BSLT of meistera chinensis fruit fraction from Southeast Sulawesi. Borneo J Pharm 2021;4(1):6-15.
17. Meyer B, Ferrigni N, Putnam J, *et al*. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med 1982;45(5):31-34.
18. Yusmayani M. Analisis kadar nitrogen pada pupuk urea, pupuk cair dan pupuk kompos dengan metode kjeldahl. Amina 2019;1(1):28-34.
19. Umaternate GR, Abidjulu J, Wuntu AD. Uji metode Olsen dan Bray dalam menganalisis kandungan fosfat tersedia pada tanah sawah di Desa Konarom Barat Kecamatan Dumoga Utara. Jurnal MIPA 2014;3(1):6-10.
20. Eviati, Sulaeman, Herawaty L, *et al*. Analisis kimia tanah, tanaman, air, dan pupuk. 3 ed. Bogor: Balai Penelitian Tanah; 2023.
21. Apridamayanti P, Fajriaty I, Hatita E. Antioxidant activity and analgesic assessment of Lansium domesticum stem bark infusion. Nusantara Bioscie 2018;10(2):71-5.
22. Mu'nisa A, Wresdiyati T, Kusumorini N, Manalu W. Aktivitas antioksidan ekstrak daun cengkeh. Jurnal Veteriner 2012;13(3):272-277.
23. Zou Y, Lu Y, Wei D. Antioxidant activity of a flavonoid-rich extract of Hypericum perforatum L. *in vitro*. J Agric Food Chem 2004;52(16):5032-5039.
24. Rachmawaty R, Mu'nisa A, Hasri H, *et al*. Analysis of phenolic content and antioxidant activity of cocoa pod husk (*theobroma cacao* l.). J Phys: Conf Ser 2019;1317:012087.
25. Qarani W, Husna F, Yulia W, *et al*. Antioxidant and antiaging activities of Cinnamomum burmannii, *Michelia champaca* and their combinations. Narra J 2023;3(2):e111.
26. Marliana E, Astuti W, Kosala K, *et al*. Chemical composition and anticancer activity of Macaranga hosei leaves. Asian J Chem 2018;30(4):795-798.
27. Arung ET, Amirta R, Zhu Q, et al. Effect of wood, bark and leaf extracts of Macaranga trees on cytotoxic activity in some cancer and normal cell lines. J Indian Acad Wood Sci 2018;15:115-119.
28. Putri R, Sy RH, Teruna HY. Phytochemical screening and toxicity test from extracts of mahang (*Macaranga bancana*) leaves. Photon: J Sain Kesehat 2019;9(2):230-234.
29. Budiana IGE, Jumani J, Biantary MP. Evaluation of soil revegetation success rate ex-pit coal mine in Kitadin site Embalut Kutai in East Kalimantan. Agrifor 2017;16(2):195-208.
30. Oktorina S. Kebijakan reklamasi dan revegetasi lahan bekas tambang: Studi kasus tambang batubara Indonesia. Al-Ard: Jurnal Teknik Lingkungan. 2018;4(1):16-20.