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Biological potential of eight medicinal plants collected in the restored landscape after mining in South Kalimantan

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Abstract

Land restoration is expected to enhance the supply of valuable ecosystem resources such as herbaceous bushes and weeds. This study aimed to determine the primary phytochemical constituents and bioactivities of methanol extracts from eight bushes and weeds collected from a restored post-mining landscape in South Kalimantan, Indonesia. Qualitative phytochemical analysis showed the presence of phenolic compounds, flavonoids, tannins, alkaloids, steroids, terpenoids and saponins in the methanol extracts of herbaceous plants. Their antioxidant activity was measured by using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay. Their superoxide dismutase (SOD) activity was also measured. In addition, selected plant extracts were screened against the common human pathogens *Staphylococcus aureus* and *Candida albicans*. Phytochemical analysis showed that the methanol extracts contained all the bioactive compounds examined in this study except the one from *Lycopodium cernuum,* which lacked flavonoids and alkaloids. Further investigation revealed that all methanol extracts except the one from *L. cernuum* had promising antioxidant potential. The methanol extracts from *Chromolaena odorata* (stem)*, Trema micrantha, Melastoma malabathricum* (flower and leaf) and *Thypa angustifolia* exhibited effective antibacterial activity. In addition, the methanol extracts from *M. malabathricum* (flower and leaf), *T. micrantha, Scleria sumatrensis* and *Breynia cernua* (leaf) exhibited effective antifungal activity. *M. malabathricum* (flower and leaf) has the greatest potential as a herbaceous plant since its methanol extract exhibits the most potent antioxidant, antibacterial and antifungal activities.

Article Highlights

- Land restoration after the post-mining period provided useful medicinal plants with antioxidant and antimicrobial properties in vitro.
- The methanol extract of *M. malabathricum* (flower and leaf) showed the most promising antioxidant and antimicrobial action.
- The discovery of promising antioxidant and antifungal activities of *S. sumatrensis* is highlighted as the novelty of this study.

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1 Introduction

Land restoration is one crucial aspect after mining has ceased. The ex-mining land should be transformed into safe, stable, and non-polluting landforms and provide habitats and ecosystem services and/or support the economic activities of the new land users, including local communities [1, 2]. Land restoration can also enhance the supply of valuable ecosystem resources. For example, medicinal plants from bushes and weeds can grow rapidly and easily dominate re-vegetated areas [3]. Some are traditionally used to treat infectious or non-infectious diseases due to their various activities, such as antihypertensive, antimalarial, anti-inflammatory, anticancer, antioxidant, antibacterial and antifungal [4–7].

In vitro studies have also proven that they contain active compounds that benefit human health. For example, the largest weed from the genus *Eucalyptus* has been reported to have antimicrobial activity against many bacteria, such as *Escherichia coli*, *Enterobacter faecalis* and *Staphylococcus aureus* [8, 9]. The weed *Mimosa pudica* also exhibits antimalarial and insecticidal activities [10]. Other examples include *Hyssopus officinalis* and *Peganum hamala* (wild rue), which are reported to have antioxidant activity [11–13]. Many studies have also demonstrated that the shrub *Breynia cernua* has the potential as an anticancer candidate [14, 15]. Moreover, another wild plant, *Physalis angulata* Linn. (ciplukan), has been reported to have antifibrosis activity [16–18].

As a country with immense biodiversity, Indonesia has an abundance of medicinal plants. Studies have emphasised the importance of biodiversity to human health, with one of its most apparent advantages being the pharmaceuticals derived from the natural world [19–21]. More than 50% of commercially available pharmaceuticals are based on plant bioactive compounds [22, 23]. Examples of drugs originating from biological sources include vinblastine to fight Hodgkin's lymphoma, quinidine to treat cardiac arrhythmias, vincristine to treat acute childhood leukaemias, D-tubocurarine to help induce deep muscle relaxation without general anaesthetics, digoxin to treat heart disease and even aspirin [24].

In this study, we focused on the phytochemicals of several common bushes and weeds from restored post-mining landscapes. We specifically investigated the antioxidant, antibacterial and antifungal activities of their extracts. These extracts were studied as candidates for adjuvant therapy, particularly as standardised herbal medicines. Herbal medicines have some advantages under particular conditions. Pure drugs derived from a plant have extremely high production costs [25, 26], especially their compound isolation steps, such as extraction, fractionation and purification, which require expensive organic solvents and a large pharmaceutical industry. In contrast, herbaceous plants grow quickly with very low production costs. At a comparable dose, a pure drug rarely has a similar degree of activity to the unpurified extract due to synergy and positive interactions between the components in the extract [26–30]. Additionally, various plants contain substances that inhibit multidrug resistance [24, 31]. Moreover, plant extracts are rich in complex compounds that can increase their bioavailability and pharmacokinetic and pharmacodynamic properties [32–35].

Redox homeostasis has a critical role in disease prevention. Oxidative stress contributes to the pathogenesis of many diseases, such as neurodegeneration, cardiovascular diseases, immune disorders, cancers and diabetes [36, 37]. Recent studies have revealed that phytochemicals have potent antioxidant activity, of which polyphenols and carotenoids are two prominent groups [38, 39]. In addition, antibiotic resistance is a serious problem in treating infectious diseases [40, 41]. This emerging problem is caused by pathogenic bacteria developing antibiotic resistance via a broad range of mechanisms of action, such as biofilm formation, drug target modification, enzymatic inactivation, an increase in efflux pump and changing cell permeability. Consequently, new antibiotic substances, especially with novel modes of action, are needed to overcome this problem [42, 43].

Exploring plant-derived antibiotics has gained attention because of their advantages: lack of side effects, low cost and wide availability [44]. Based on their structure, phytochemicals with antibacterial activity are grouped into polyphenols, terpenoids, alkaloids and organosulfur. The compounds investigated to date have shown antibacterial activity with various modes of action [45].

Nature in Indonesia can provide plant resources with potential therapeutic activities, even from restored post-mining areas. For example, there are eight bushes and weeds that grow dominantly in the restored post-mining landscape in South Kalimantan: *B. cernua*, *Chromolaena odorata*, *Dicranopteris linearis, Lycopodium cernuum, Melastoma malabathricum*, *Thypa angustifolia, Trema micrantha* and *Scleria sumatrensis* [46]. Some studies have identified the chemical components of these plants and their bioactivities. Extracts from the aerial part of *B. cernua* contained thioinosinic monophosphate as their main component and showed antioxidant and antiparkinsonism activities [47]. Essential oil from the root of *C.*

odorata containing α-pinene (42.2%) and β-pinene (10.6%) exhibited antibacterial activity against *Bacillus cereus* and antifungal activity against *Aspergillus niger* [48]. The leaf extract of *D. linearis* contained α-tocopherol and showed biofilm inhibition activity against *S. aureus* [49]. Compounds isolated from *M. malabathricum,* such as α-amyrin, betulinic acid, quercetin and quercitrin, have been reported to have anti-inflammatory activity [50]. Typhaneoside and isorhamnetin-3-O-neohesperidoside isolated from *T. angustifolia* exhibit antioxidant activities [51]. Therefore, this study aimed to determine the phytochemical composition and antioxidant, antibacterial and antifungal activities of methanol extracts of eight bushes and weeds collected from a restored post-mining landscape in South Kalimantan, Indonesia.

2 Material and methods

2.1 Materials

Methanol extracts of selected weeds and bushes: *B. cernua* (leaf), *C. odorata* (stem), *D. linearis* (aerial parts), *L. cernuum* (aerial parts), *M. malabathricum* (flower & leaf), *M. malabathricum* (stem), *T. angustifolia* (aerial parts), *T. micrantha* (aerial parts), and S. *sumatrenis* (aerial parts) were assayed for their antioxidant activities and six of them were assayed for their antimicrobial and antifungal activities.

2.2 Sampling

The sampling of plant species was carried out by a qualitative method (floristic survey) and a quantitative method (vegetation analysis). Sampling was carried out in the reclamation area of the Kusan-Girimulya site, Borneo Indobara company, located in Angsana District, Tanah Bumbu Regency, South Kalimantan Province, Indonesia. The coordinates of the samplinga area are 3°36′06.3″S, 115°38′20.8″E. A floristic survey was conducted by exploring the study area representatively. A plant taxonomist, Reza Abdul Kodir recorded and identified the species directly in the field. Each plant species was recorded in the species list sheet and documented for further identification purposes. Plant species that could be identified were directly written in scientific names, while those that could not be identified were codified for further identification using the literature [46]. The plant material's taxonomic identification was validated by the Plant Taxonomy Laboratory of the Faculty of Mathematics and Natural Sciences of Universitas Padjadjaran. The Laboratory prepared and preserved voucher specimens.

2.3 Plant extraction

Two days after collecting the plants, the samples were sent to the laboratory and immediately processed for extraction. Methanol extraction was performed as follows, 200 g of the plant's part was washed and chopped, then the samples were oven dried at 50 °C for 48 h. The dried samples were macerated in 100 mL of 70% methanol for 24 h at room temperature. Finally, the solvent was removed by oven drying (70 °C, 4 h) [52] and the methanol extract was stored in the refrigerator. For antibacterial and antifungal activity studies, each extract was dissolved in DMSO to get stock a solution 50,000 mg/L and was stored in refrigerator.

2.4 Phytochemical analysis

Qualitative phytochemical analysis was carried out to investigate the presence or absence of phenolic compounds, flavonoids, tannins, alkaloids, steroids, terpenoids and saponins from crude extracts of plants.

2.4.1 Phenolic compounds

One mL of the extract solution was added with 2 drops of solution FeCl3 5%. Samples containing phenolics are indicated with the formation of a strong green or blue color [53, 54].

2.4.2 Flavonoids test

Five hundred mg of extract was solubilized with 10 mL of methanol. Next, the sample extract was added with 2 mL of concentrated HCl (37%) and shaken vigorously. After that, 0.2 g of Mg powder was added and shaken vigorously again. A color change to orange indicated positive samples containing flavonoids (assay A), as previously described [55]. Positive samples containing flavonoids using H_2SO_4 2N reagent were marked by a very striking yellow, red, or brown color change (assay B), as previously described [56]. After that the plant extract was dripped with a 10% NaOH solution. If the color of plant extracts changes from red to brown, the sample is declared positive for phenol group flavonoids [57].

2.4.3 Tannins

One mL of extract was added with 2–3 drops of 1% FeCl3. Sample contains tannins when the color changes blackish green [53].

2.4.4 Alkaloids test

Five hundred mg of extract was solubilized with 10 mL of 10% ammonia:chloroform solution (25%:75% v/v). The samples were then transferred to tubes A and B. Dragendorff's reagent and Wagner's reagent were added to each tube. The sample in tube A and B is positive for alkaloids if there is a reddish precipitate and a brown precipitate respectively [58].

2.4.5 Saponins test

Five hundred mg of extract was solubilized with 10 mL of 10% ammonia: chloroform solution (25%:75% v/v). Then the sample was shaken vigorously and then 2N HCl was added. Positive samples contain saponins if there is foam with a lot of intensity and consistency for 10 min [58].

2.4.6 Terpenoids and steroids test

Five hundred mg of extract was solubilized with 10 mL of ethanol. After that, the extraction was carried out again with chloroform: water (1:1). Two drops of chloroform extract were dropped on a drip plate and left to dry. Then, one drop of concentrated sulfuric acid and anhydrous acetic acid was added. Positive samples contain triterpenoids if they experience a red or brown color change and are positive for steroids if they experience a blue, purple, or green color change [58].

2.5 Antioxidant activity assay

The antioxidant activity of the extracts was measured by using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and the SOD (superoxide dismutase) assay.

2.5.1 Free-radical scavenging assay

DPPH free radical scavenging assay was carried out for screening the antioxidant activity of plant extracts in vitro. 4×10^{-4} mol/L DPPH solution was prepared and put into a vial and closed tightly. The vial was covered with aluminum foil to protect it from light. Samples varied in the range of 5–1,500 mg/L were assayed for antioxidant activity. In each test tube, 1 mL of DPPH solution was added to each test tube and then left for 30 min. Then, it was measured using a UV–Vis spectrophotometer at 517 nm wavelength (Genesys 10S UV–Vis**)**. The ability to scavenge DPPH free radicals (inhibition) was calculated using the following equation:

$$\%h = Ab - As\ Ab\text{x } 100\%$$

where % *h* is % inhibition (free radical inhibition); *Ab*, Absorbance blank; *As,* sample absorbance. The value of 50% free radical inhibition concentration (IC50) was calculated using the regression equation $y = ax + b$.

IC50 of each extract was measured in triplicates [52].

2.5.2 Superoxide dismutase (SOD) activity assay

A xanthine-xanthine oxidase system capable of producing superoxide radicals (O_2) was used to measure SOD activity. Samples varied in the range of 25–4,000 mg/L were assayed for SOD activity. The percent inhibition of nitro blue tetrazolium (NBT) reduction to form blue formazan was measured at 550 nm. SOD activity was obtained from IC50 data that defined as a 50% inhibition of NBT reduction. A negative control experiment for DPPH and SOD assay was conducted using DMSO as an extract solvent. IC50 of each extract was measured from triplicate assay [52, 59, 60].

2.6 Antibacterial and antifungal activity

The antibacterial and antifungal activities of the extract were assayed against *Staphylococcus aureus* (ATCC 25923) and *Candida albicans* (ATCC14053), respectively. Bacterial and fungal isolates were obtained from the Advanced Biomedical Laboratory, Faculty of Medicine, Universitas Padjadjaran. *S. aureus* was grown on Luria–Bertani (LB; 1% tryptone, 0.5% yeast extract, and 1% NaCl) medium (Himedia) at 37 °C, and *C. albicans* inoculate, in Potato Dextrose Broth (PDB) at 37 °C. The Minimum inhibitory concentration (MIC) by The Clinical and Laboratory Standards Institute (CLSI) was conducted using the broth microdilution method [61]. Extracts varied in the range of 30–200,000 mg/L were placed immediately in a microtiter plate containing Mueller Hinton broth. The bacterial inoculum was applied to get a final concentration of 5×10^5 CFU/mL in each well, then incubated for 24 h at 37 °C. The MIC was considered the lowest extract concentration that inhibits bacterial and fungal growth. The minimum bactericidal concentration (MBC) and the minimum fungicidal concentration (MFC) were considered the lowest concentration of the extract resulting in no growth of bacteria and fungi in agar plates after overnight incubation at 37 °C. A negative control experiment for antibacterial and antifungal assay was conducted using DMSO as an extract solvent. MIC, MBC and MFC of each extract were measured from triplicate assays.

2.7 Data analysis

Data from antioxidant, antibacterial and antifungal activity assays are presented as mean values with standard deviation from triplicate experiments. Data processing and statistical analysis was performed using Microsoft Office Excel 2021.

Table 1 Identification of plant species in the reclamation area

No.	Plant species	Local name	References
1	*Breynia cernua*	Katuk Hitam	[124]
2	*Chromolaena odorata*	Kirinyuh	[124]
3	*Dicranopteris linearis*	Paku andam	[125]
4	*Lycopodium cernuum*	Paku kawat	[124]
5	*Melastoma malabathricum*	Senduduk	[124]
6	*Scleria sumatrensis*	Kerisan	[124]
7	*Trema micrantha*	Guacimilla	[126]
8	*Typha angustifolia*	Lembang	[127]

3 Results and discussion

3.1 Floristic survey and vegetation analysis

A floristic survey and vegetation analysis of the restored post-mining land observed growing herbaceous plant species. Among them, eight species predominated [46]. The local names of these plants are listed in Table 1. The species belong to various genera. *B. cernua*, *C. odorata*, *M. malabathricum* and *T. micrantha* are shrubs. *D. linearis* and *L. cernuum* are ferns. *S. sumatrensis* and *T. angustifolia* are grasses. The people of Indonesia already know these plants and have used them widely as traditional medicines; some of them are even included in the World Health Organisation catalogue as traditional medicines [62].

3.2 Phytochemical analysis

Active compounds often used as drugs, such as flavonoids, tannins, alkaloids, steroids, terpenoids and saponins, are secondary metabolites of plants [63]. The active compounds can be found in various plant parts, such as the stems, leaves and flowers. Phenolic and flavonoid compounds are reported to have anti-inflammatory and antibacterial activities. In addition, these two groups of compounds protect the skin from UV rays, improve the immune system and have cardioprotective effects [64]. Phenolic compounds are also associated with antioxidant activity. The antioxidant activities of natural compounds are promising in inhibiting carcinogenesis and metastasis mechanisms and are, therefore, extensively studied in chemopreventive strategies [65]. The flavonoids and other phenolic compounds function at the molecular level to mitigate oxidative stress caused by reactive oxygen species [66]. These compounds are also reported to be important in controlling diseases, including cancer [67].

The results of the qualitative phytochemical analyses of each plant are provided in Table 2. All of the plant extracts were found to contain phenolic compounds. In addition, the results of three test methods (A, B, and C) showed that all extracts except the one for *L. cernuum* contained flavonoids. Moreover, all extracts except the one for *D. linearis* contained tannins. Furthermore, all extracts except the one for *L. cernuum* contained alkaloids. Finally, the extracts of almost all species contained terpenoids and steroids. However, the extracts of only five species contained saponins.

Phytochemical analysis of *B. cernua* showed that this plant contained phenolic compounds, flavonoids, tannins, alkaloids, steroids, terpenoids and saponins, consistent with the previous studies that reported that *B. cernua* contained alkaloids, triterpenoids, saponins, flavonoids, steroids, tannins, glycosides and hydrocarbons [68, 69]. *C. odorata* contained all the phytochemicals except terpenoids and saponins. A previous study reported that the *C. odorata* stem contained alkaloids, flavonoids, saponins, tannins, steroids, terpenoids and cardiac glycosides but not phlobatannins [70]. *D linearis* contained all the phytochemicals except flavonoid B and tannins. A previous study that examined the methanol extract of *D. linearis* reported that it contained flavonoids, saponins, tannins, phenolic compounds, and

Table 2 Qualitative phytochemical analysis of methanol extracts from 8 plant species. Flavonoid A: HCl-Mg test, flavonoid B: sulphuric acid test, flavonoid C: ethyl acetate-ammonia test

No.	Sample	Part	Phenolics	Flavonoids			Tannins	Alkaloids	Steroids	Terpenoids	Saponins
				A	B	C					
1	*Breynia cernua*	Leaf	+	+	+	+	+	+	+	+	+
2	*Chromolaena odorata*	Stem	+	+	+	+	+	+	+	–	–
3	*Dicranopteris linearis*	Aerial parts	+	+	–	+	–	+	+	+	+
4	*Melastoma malabathricum*	Stem	+	+	+	+	+	+	–	+	+
5	*Melastoma malabathricum*	Flower + leaf	+	+	+	+	+	+	+	+	+
6	*Lycopodium cernuum*	Aerial parts	+	–	–	–	+	–	+	+	+
7	*Thypa angustifolia*	Aerial parts	+	+	+	+	+	+	+	+	–
8	*Trema micrantha*	Aerial parts	+	+	+	+	+	+	+	+	+
9	*Scleria sumatrensis*	Aerial parts	+	+	+	+	+	+	+	+	–

B. cernua (leaf), *C. odorata* (stem), *D. linearis* (aerial parts), *M. malabathricum* (stem), *M. malabathricum* (flower & leaf), *L. cernuum* (aerial parts), *T. angustifolia* (aerial parts), *T. micrantha* (aerial parts) and S. *sumatrenis* (aerial parts)

steroids but not alkaloids [71]. The *M. malabathricum* stem contained all the phytochemicals except steroids, while the *M. malabathricum* flower and leaf contained all the phytochemicals. A previous study reported that the methanol extract of *M. malabathricum* leaves or flowers contained all the phytochemicals except saponins, while that of the *M. malabathricum* stem contained all the phytochemicals except saponins, alkaloids and terpenoids [72]. *L. cernuum* contained almost all of the phytochemicals except flavonoids and alkaloids. A previous study reported that this plant contained flavones, alkaloids, triterpenoids and neolignans [73]. *T. angustifolia* contained all the phytochemicals except saponins. A previous phytochemical analysis of the methanol extract of *T. angustifolia* aerial part showed it contained flavonoids, alkaloids, phenolic compounds, steroids, tannins and saponins [74]. *T. micrantha* contained all the phytochemicals. The phytochemical profile of the 10% methanolic extract of *T. micrantha* leaf showed it contained flavonoids, terpenoids, saponins, steroids and tannins but not alkaloids. *S. sumatrensis* contained all the phytochemicals except saponins. Notably, no previous studies have phytochemically analysed *S. sumatrensis*. Based on these descriptions, we can conclude that slight differences in phytochemical content exist between plants from post-mining and non-post-mining landscapes.

Plants containing tannins are reported to have antioxidant, antimalarial and antimicrobial activities [75, 76]. In this study, alkaloids were present in the methanol extracts of most of the examined species. While we did not detect alkaloids in the methanol extract of *L. cernuum,* another study identified alkaloids in a crude extract of this plant prepared with the solvent methanol-tartaric acid-ether-chloroform in a multistage extraction process [77]. Many alkaloids have been reported to have antimalarial activity [78]. Alkaloids are also reported to have anticancer activity, inhibiting cell growth and inducing autophagic pathways and apoptosis [14]. Moreover, five of the eight species contained saponins. The reported biological effects of saponins include membrane permeabilisation, immunostimulation, cholesterol-lowering and anticancer [79, 80]. For example, an anticancer evaluation of the saponin group indicated that they induce apoptosis and inhibit cell growth in cancer cells [81]. Components containing saponins were also found to have anti-protozoal, analgesic, anti-nociceptive, antioxidant, antifungal and antiviral activities, impede protein digestion and cause hypoglycemia [82]. The phytochemical analysis data (Table 2) indicate that all species contained phenolic compounds, and almost all contained flavonoids, tannins, alkaloids, steroids, terpenoids and saponins. Therefore, each plant's antioxidant, antibacterial and antifungal activities warrant exploring.

3.3 Antioxidant activity

Each methanol extract's antioxidant activity was assayed using two methods: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and superoxide dismutase (SOD). The free-radical (DPPH) scavenging assay is an easy and rapid in vitro antioxidation test [83]; a compound's antioxidant activity is classified as very high if the half-maximal inhibitory concentration (IC_{50}) is < 10 µg/mL, high if the IC_{50} is 10–50 µg/mL, moderate if the IC_{50} is 50–100 µg/mL, low if the IC_{50} is 100–250 µg/mL, and inactive if the IC_{50} is > 250 µg/mL [84].

Table 3 Antioxidant activity of methanol extracts from plant species measured by DPPH and SOD assays. IC_{50} (half maximal inhibitory concentration)

No.	Sample	Part	IC_{50} (µg/mL)	
			DPPH*	SOD*
1	*Breynia cernua*	Leaf	141.33 ± 3.48	40 ± 3.61
2	*Chromolaena odorata*	Stem	52.94 ± 2.97	70 ± 3.60
3	*Dicranopteris linearis*	Aerial parts	73.66 ± 2.46	70 ± 5.03
4	*Melastoma malabathricum*	Stem	5.70 ± 0.34	35 ± 2.52
5	*Melastoma malabathricum*	Flower + leaf	18.39 ± 0.51	22 ± 2.52
6	*Lycopodium cernuum*	Aerial parts	1197.81 ± 11.38	520 ± 10
7	*Thypa angustifolia*	Aerial parts	87.83 ± 6.11	125 ± 2.65
8	*Trema micrantha*	Aerial parts	21.77 ± 2.96	60 ± 2.64
9	*Scleria sumatrensis*	Aerial parts	42.77 ± 2.55	142 ± 4.73

*DPPH = 2,2-diphenyl-1-picryl-hydrazyl-hydrate, SOD = Superoxide dismutase

B. cernua (leaf), *C. odorata* (stem), *D. linearis* (aerial parts), *M. malabathricum* (stem), *M. malabathricum* (flower & leaf), *L. cernuum* (aerial parts), *T. angustifolia* (aerial parts), *T. micrantha* (aerial parts) and *S. sumatrenis* (aerial parts)

The antioxidant activity results (Table 3) with the DPPH method revealed that all the methanol extracts except the one for *L. cernuum* had free-radical scavenging activity in vitro. Similarly, eight of the nine methanol extracts (88.9%) showed antioxidant activity in the SOD assay. The methanol extracts of *M. malabathricum* (flower + leaf), *M. malabathricum* (stem) and *B. cernua* (leaf) showed strong antioxidant activities (IC_{50} of 10–50 µg/mL; Table 3). The methanol extracts of *T. micrantha*, *C. odorata* and *D. linearis* showed moderate antioxidant activity in the SOD assay (IC_{50} of 50–100 µg/mL). The methanol extracts of *T. angustifolia* and *S. sumatrensis* showed low antioxidant activity (IC_{50} of 100–250 µg/mL)**.** The methanol extract of *L. cernuum* showed no antioxidant activity (Table 3).

The antioxidant activities of the examined methanol extracts of plants from a post-mining landscape in the DPPH assay were compared to those reported for plants from virgin lands. The methanol extract of *M. malabathricum* stems had very high antioxidant activity, with an IC_{50} of 5.70 µg/mL. The methanol extract of *M. malabathricum* flowers and leaves had high antioxidant activity, with an IC_{50} of 18.39 µg/mL (Table 3). This finding is comparable to the antioxidant activity previously reported for the ethanol extract of *M. malabathricum* flowers, which had an IC_{50} of 17.86 µg/mL [85]. A methanol extract of *M. malabathricum* was also reported to show high antioxidant activity at 200 µg/mL, with dosages of 250 and 500 mg/kg inducing hepatoprotective activity in rats [86].

The methanol extracts of *T. micrantha*, *S. sumatrensis*, *C. odorata, D. linearis* and *T. angustifolia* showed moderate antioxidant activities, with IC_{50} of 21.77, 42.77, 52.94, 73.66 and 87.83 µg/mL, respectively. A hydroethanolic extract of *T. micanthra* has been reported to show DPPH-scavenging activity, with an IC_{50} of 104.33 µg/mL [87]. *C. odorata* was reported to contain the flavonoids odoratenine, isosakuranetin and subscandenin and show antioxidant activity in the DPPH assay, with IC_{50} of 90.83, 57.26 and 188.61 µg/mL for dichloromethane, ethyl acetate, and methanol extracts, respectively [88]. The methanol extract of *D. linearis* showed 98.94% ± 1.14% antioxidant scavenging activity in the DPPH assay at 200 µg/mL [89]. Ethanol and water extracts of *T. angustifolia* were reported to have antioxidant activity in the DPPH assay, with IC_{50} of 9.51 and 50.85 µg/mL, respectively [51].

In this study, the methanol extract of *B. cernua* (flowers and leaves) showed low antioxidant activity, with an IC_{50} of 141.33 µg/mL. A previous study reported similar results for an ethanol extract of *B. cernua*, which showed 78.02% inhibitory activity at a high concentration (500 µg/mL) with the DPPH assay [47]. However, the methanol extract of *L. cernuum* had an IC_{50} of > 1,000 µg/mL, indicating that it does not have antioxidant activity. This finding is consistent with the qualitative phytochemical results, which showed that the tested *L. cernuum* methanol extract does not contain flavonoid or alkaloid compounds, which are both known to have antioxidant activity [90, 91]. Based on the results, it can be concluded that the methanol extracts of *M. malabathricum, T. micrantha, C. odorata, D. linearis* and *B. cernua* have more effective antioxidant activities than those from virgin lands with lower doses or IC_{50}.

As an additional indicator, we determined the SOD activity of the examined methanol extracts from the medicinal plants growing in the restored post-mining landscape. Consistent with the DPPH assay results, all the methanol extracts except the one from *L. cernuum* showed SOD activity.

3.4 Antibacterial activity against *S. aureus* (American Type Culture Collection [ATCC]: 25,923) and antifungal activity against *Candida albicans* (ATCC: 14,053)

Antibiotic resistance poses difficulties in the healthcare systems of developed and developing countries. Existing antibiotic therapies have been seriously challenged by the spread of multidrug-resistant organisms [92]. For example, the emergence of multidrug-resistant *S. aureus* and *C. albicans* poses the greatest threat [93]. Since plants contain diverse bioactive phytochemicals with proven medicinal properties, exploring antimicrobial molecules of plant origin has gained attention [94]. This study performed a preliminary analysis of the potential antimicrobial efficacies of various medicinal plant extracts against two common human pathogens.

C. albicans and *S. aureus* are responsible for most infections and are frequent sources of co-infection in critically ill patients. In the host environment, *C. albicans* mostly coexist with numerous microorganisms, including *S.aureus*, a Gram-positive bacterium with various virulence factors [95, 96]. *C. albicans* and *S. aureus* have been observed to interact synergistically, with potential adverse clinical implications, such as a higher mortality rate [97, 98]. In addition, oropharyngeal candidiasis appears to facilitate the spread of *S. aureus*. Therefore, immunocompromised individuals are at risk of both invasive oral *C. albicans* infection and *S. aureus* infection [99]. Consequently, research on obtaining drug candidates that have activity against *C. albicans* and *S. aureus* is vital.

The minimum inhibitory concentrations (MICs) of the methanol extracts of *C. odorata, T. micrantha, M. malabathricum* (flower and leaf) and *T. angustifolia* indicated that they had antibacterial activity against *S.aureus* (ATCC: 25,923; Table 4).

Table 4 MBC (minimum bactericidal concentration) and MIC (minimum inhibitory concentration) of methanol extracts from selected plants against *S.aureus*

No.	Plant extract	Part	MIC (mg/mL)	MBC (mg/mL)
1	*Breynia cernua*	Leaf	25.00 ± 2.08	50.00 ± 2.52
2	*Chromolaena odorata*	Stem	3.75 ± 0.29	15.00 ± 2
3	*Melastoma malabathricum*	Flower + leaf	8.33 ± 0.16	16.67 ± 0.64
4	*Melastoma malabathricum*	Stem	25.00 ± 3.06	50.00 ± 3.06
5	*Thypa angustifolia*	Aerial parts	8.33 ± 0.2	–
6	*Tremma micrantha*	Aerial parts	4.38 ± 0.25	–
7	*Scleria sumatrenis*	Aerial parts	8.93 ± 0.21	17.86 ± 0.36

Plant parts used in this study: *B. cernua* (leaf), *C. odorata* (stem), *M. malabathricum* (flower & leaf), *M. malabathricum* (stem), *T. angustifolia* (aerial parts), *T. micrantha* (aerial parts) and S. *sumatrenis* (aerial parts)

Table 5 MFC (minimum fungicidal concentration) and MIC of methanol extracts from selected plants against *C. albicans*

No.	Plant extract	Part	MIC (mg/mL)	MFC (mg/mL)
1	*Breynia cernua*	Leaf	6.25 ± 0.08	100.00 ± 3.61
2	*Chromolaena odorata*	Stem	15.00 ± 1.53	–
3	*Melastoma malabathricum*	Flower + leaf	4.17 ± 0.15	–
4	*Melastoma malabathricum*	Stem	12.50 ± 0.28	–
5	*Thypa angustifolia*	Aerial parts	8.33 ± 0.25	–
6	*Tremma micrantha*	Aerial parts	4.38 ± 0.15	–
7	*Scleria sumatrenis*	Aerial parts	4.46 ± 0.23	–

Plant parts used in this study: *B. cernua* (leaf), *C. odorata* (stem), *M. malabathricum* (flower & leaf), *M. malabathricum* (stem), *T. angustifolia* (aerial parts), *T. micrantha* (aerial parts) and S. *sumatrenis* (aerial parts)

However, only five of the seven plant extracts showed bactericidal activity. The minimum bactericidal concentration (MBC) of the methanol extracts of *T. angustifolia* and *T. micrantha* could not be determined, even at their highest concentration. The minimum fungicidal concentration (MFC) and MIC against *C. albicans* (ATCC: 14,053) of the plant methanol extracts dissolved in dimethyl-sulfoxide were determined using the microdilution method (broth microdilution). The MIC test (Table 5) showed that four of the seven methanol extracts inhibited *C. albicans* growth, including those from *M. malabathricum* (flower + leaf; 4.17 mg/mL), *T. micrantha* (4.38 mg/mL), *S. sumatrensis* (4.46 mg/mL) and *B. cernua* (leaf; 6.25 mg/mL). In addition, the MFC test showed that the methanol extract of *B. cernua* (leaf) could kill *C. albicans* at 100 mg/mL, while the other methanol extracts could not.

An extract of *Parkia timoriana* was considered to have effective antibacterial activity, with MICs below 8 mg/mL for bacteria such as *Bacillus subtilis, Bacillus pumilus, Pseudomonas aeruginosa* and *E. coli* [100]. In this study, the methanol extracts of *C. odorata* (stem), *T. micrantha, M. malabathricum* (flower + leaf) and *T. angustifolia* exhibited effective antibacterial activity, with MICs of 3.75, 4.38, 8.33 and 8.33 mg/mL (Table 4). An extract of *Persea americana* was also considered to have effective antifungal activity against *C. albicans*, with a MIC of 6.25 mg/mL [101]. In this study, the methanol extracts of *M. malabathricum* (flower + leaf), *T. micrantha, S. sumatrensis* and *B. cernua* (leaf) exhibited effective antifungal activity, with MICs of 4.17, 4.38, 4.46 and 6.25 mg/mL (Table 5).

The high MIC of the methanol extract of *M. malabathricum* against *S. aureus* and *C. albicans* might be because it lacks steroids. Nonpolar compounds such as steroids and cholesterol have been reported to exert antimicrobial activity by disrupting the permeability and integrity of cell membranes [102, 103]. Another study also found that the extracts of some studied plants from virgin lands also had antimicrobial activity. A methanol extract of *B. cernua* leaves and stems exhibited broad-spectrum antimicrobial activity against *S. aureus* and other Gram-positive and Gram-negative bacteria at 4 mg/disk. However, this extract was inactive against various microfungi, including *C. albicans* [68].

The methanol extract of *C. odorata* contains alkaloids, flavonoids, tannins and 4-hydroxybenzoic acid, which may inhibit the growth of *S. aureus, E. coli* and *P. aeruginosa* by inhibiting the dehydrogenase enzyme with IC_{50} of 208.49, 1361.01 and 903.08 µg/mL, respectively [104]. A water extract of *C. odorata* was reported to strongly inhibit *P. aeruginosa* and *C. albicans* at 200 mg/mL [105]. A methanol extract of *M. malabathricum* was reported to have a MIC of 3 mg/mL against clinical *S. aureus* strains [106]. An ethanol extract of *M. malabathricum* was also reported to have a MIC of 60 mg/

mL against *C. albicans* [107]. An extract of *T. angustifolia* was reported to inhibit *S. aureus, B. subtilis* and *C. albicans* [108]. An extract of *T. micrantha* showed slight inhibitory effects on Gram-negative and Gram-positive bacteria and various microfungi with MICs > 800 µg/mL and *E. faecalis* with a MIC of 200 µg/mL [87]. Based on these results, we can conclude that the tested methanol extracts of *B. cernua, C. odorata* and *M. malabathricum* have greater antifungal activity against *C. albicans* than previously reported plant extracts from virgin land.

In most cases, the antimicrobial activity of medicinal plants was associated with their essential oil content [109, 110]. Essential oils could be isolated using solvents such as ethanol [111] and methanol [112]. Therefore, the antimicrobial activities of the methanol extracts of the studied herbaceous plants might be derived from their essential oil content. The leaves of *C. odorata* reportedly contain essential oil, with α-pinene (42.2%) and β-pinene (10.6%) being major components [48, 113]. *C. odorata* roots were also reported to contain essential oil, with himachalol (24.2%) and 7-isopropyl-1,4-dimethyl-2-azulenol (17.6%) being the main constituents [114]. Among the plants examined in our study, the methanol extract of *C. odorata* had the most pronounced antibacterial activity, with the lowest MIC and MBC (Table 4). This finding suggests that the essential oil in this methanol extract might contribute to its antibacterial activity, which requires futher exploration.

Herbal medicine has been extensively studied as adjuvant therapy for numerous diseases such as cancer [115], osteonecrosis [116], adenomyosis [117], COVID-19 [118, 119] and type 2 diabetes mellitus [120]. The findings of this study demonstrate that land restoration after mining provides useful medicinal plants rich in bioactive compounds and with antioxidant and antimicrobial properties in vitro, making them promising candidates for adjuvant therapy in the future. One notable finding of this study was that the methanol extract of *S. sumatrensis* exhibits promising antioxidant, antibacterial and antifungal activities since phytochemical analysis and bioactivities are currently unavailable for this medicinal plant [121]. While few in vitro studies exist, *S. sumatrensis* has been traditionally used to treat many diseases, such as diabetes [122] and postpartum treatment [123].

We recommend further purification (fractionation and other methods) and examination of its chemical composition using chromatography (gas or liquid chromatography with mass spectrometry) to identify the active compounds contributing to its bioactivities. The results of this purification could be used to increase the product grade of standardised herbal medicines. In addition, when studying medicinal plants from restored lands after mining, it is crucial to ensure that the plant material is not contaminated with heavy metals and toxic elements. Therefore, we suggest that future studies must determine the presence of heavy metals and toxic elements in these plants and their extracts.

4 Conclusion

This study conducted a qualitative phytochemical analysis of eight plants from the restored post-mining landscape in South Kalimantan, Indonesia. It revealed that almost all the plant methanol extracts contained important phytochemicals such as phenolic compounds, flavonoids, tannins, alkaloids, steroids, terpenoids and saponins and exhibited promising antioxidant activity, except the one from *L. cernuum*. The methanol extracts of *C. odorata* (stem), *Tremma micrantha, M. malabathricum* (flower + leaf) and *T. angustifolia* exhibited antibacterial activity. In addition, the methanol extracts of *M. malabathricum* (flower + leaf), *T. micrantha, S. sumatrensis* and *B. cernua* (leaf) exhibited antifungal activity. The methanol extract of *M. malabathricum* (flower + leaf) has the greatest potential among the studied herbaceous plants since it exhibits the greatest antioxidant, antibacterial and antifungal activities. Further studies are needed to evaluate its antimicrobial activity against other common pathogenic bacteria and fungi and its antiviral, antiparasitic and anticancer activities. Future studies could also chemically profile this extract, conduct molecular docking, and isolate promising novel compounds derived from medicinal plants.

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Declarations

Conflict of interest According to this article, there are no possibly available competing interests, authorship, and/or publication of this article.

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