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FULL PAPER

Phytochemical screening and antioxidant activity of *Melastoma malabathricum* and *Chromolaena odorata* by DPPH radical scavenging method

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Abstract

Melastoma malabathricum and *Chromolaena odorata* are classified under broad-leaved weeds that are widely spread in the open land area. *Melastoma malabathricum* is commonly known as ''senduduk'', and *C. odorata* is locally known as "Pokok Kapal Terbang". Both weeds are categorized as potential weeds as they have high nutritive value and are rich in chemical compounds. This study aimed to determine their chemical constituents and possible potential for antioxidant activity as these weeds have been reported to possess antioxidant properties. Screening of the plants was performed using standard methods and revealed the existence of various secondary metabolites such as saponins, terpenoids, phenols, tannins, and flavonoids of both weed extracts. Antioxidant activity was validated by the DPPH radical scavenging assay of *M. malabathricum* and *C. odorata* crude ethanol extract. The IC50 values for the percentage radical scavenging effects for the extracts were determined. The IC50 value of *M. malabatrichum* extract was 81.116 µg/mL, *C. odorata* was 312.903 µg/mL, Vitamin C was 31.023 µg/mL and BHA was 71.521 µg/mL respectively. The study showed that the antioxidant activity of *M. malabatrichum* was more potent and better than *C. odorata*.

1. Introduction

Weeds are recognized as the unwanted and undesirable plant that grows at an open land area (Zimdahl, 2007). There are several morphological types' characteristics of weeds such as broadleaved weeds, grasses, sedges, and ferns (Barnes and Chan, 1990). Traditionally to treat human diseases, plant organic compounds of primary and secondary metabolite has been used due to their efficacy potential and have been confirmed safe for animal and human use. Some weeds are known as medicinal plants that have allelochemicals made up of secondary metabolites (Badmus and Afolayan, 2012). The chemical compounds that come from plants are a result of normal metabolic activities. These chemical compounds are classified into primary and secondary metabolites, and the secondary metabolites and other chemical constituents contribute to their medicinal value (Parivuguna, 2008). *Melastoma malabathricum* and *C. odorata* are chosen as they have chemical constituents that can contribute to their potential.

Phytochemicals that can be found in plants as plant chemicals have protective and preventive properties against diseases (Breslin, 2017). The presence of numerous bioactive chemicals in plants has been revealed by many studies including alkaloids, steroids, flavonoids, phenols, glycosides, and saponins (Banothu *et al.*, 2017). They play a very important aspect in the plant's protection such as antibacterial, antiviral, antifungal, and insecticidal agents (Hajlaoui *et al.*, 2009). *Melastoma malabathricum* and *C. odorata* are reported to have benefits and potential. *Melastoma malabathricum* is used as a folk medicine for the treatment of dysentery, haemorrhoids, diarrhoea, wounds, leucorrhoea, and cuts mainly in India, Malaysia, Indonesia, and other parts of the world (Joffry *et al.*, 2012). *Melastoma malabathricum* also showed bioactivity as an antibacterial (Choudhury *et al.*, 2011),

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antifungal (Johnny *et al.,* 2010), antiviral (Lohézic-Le Dévéhat *et al.*, 2002), anticoagulant (Manicam *et al.,* 2010), wound healing (Anbu *et al.*, 2008), antidiarrheal (Sunilson *et al.*, 2009), anti-inflammatory (Zakaria *et al.,* 2006) and antioxidant (Susanti *et al.*, 2007) which have been experimentally validated. Allelochemical that can be derived from *M. malabathricum* such as rutin hydrate, quercetin hydrate, nobotanin B, malabathrins B, malabathrins C, malabathrins D, strictinin, casuarictin, nobotanin G, nobotanin H, and nobotanin J (Yoshida *et al.,* 1992; Azahar *et al.,* 2020). While for *C. odorata*, it is used as a treatment of leech bites, burns, soft tissue injuries, and skin infections (Phan *et al.*, 2001). *Chromolaena odorata* also showed bioactivity as an antibacterial (Atindehou *et al.*, 2013), antifungal (Naidoo *et al.,* 2011), anti-inflammatory (Pandith *et al.*, 2013), anticancer (Kouamé *et al.*, 2013), antiplasmodial (Ezenyi *et al.*, 2014), antidiabetic (Onkaramurthy *et al.,* 2013), and antioxidant (Boudjeko *et al.*, 2015). Allelochemical that can be derived from *C. odorata* such as quercetin-4 methyl ether, aromadendrin-4'-methyl ether, taxifolin-7-methyl ether, taxifolin-4'-methyl ether, trans-caryophyllene, δ–cadinene, α copaene and caryophyllene oxide (Haji Jasnie *et al.,* 2009; Chakraborty *et al.,* 2011).

Every phytochemical compound has its potential. Saponins were reported to have toxicity towards insects (insecticidal activity), parasite worms (anthelmintic activity), molluscs (molluscicidal), fish (piscicidal activity), antifungal, antiviral, and antibacterial activities are well documented (Francis *et al.*, 2002). For tannins, it was reported to have antimicrobial effects (Stanley *et al.,* 2014), and the terpenoids showed the effect on the plant, fungi, and plant growth (Ling *et al.,* 2003). Flavonoids involve in the inactivation of microbial adhesins, enzymes, disrupting microbial membranes, and cell envelope transport proteins (Maji *et al.*, 2010). Flavonoids are also extensively distributed in plants and have been discovered to possess many biological properties such as antioxidant, free radical scavenging, anti-inflammatory, and anticarcinogenic properties (Eze *et al.,* 2013). The secondary metabolites reported being potent free radical scavengers that come from phenolic and flavonoids of plants. They are found in all parts of plants such as leaves, seeds, fruits, roots, and bark (Tiwary *et al.,* 2015). Phenolic compounds and flavonoids have been reported to be relatable with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Jørgensen *et al.,* 1999).

With the phytochemical's constituents in the weeds, it can be related ability for natural antioxidants. Natural antioxidants or phytochemical antioxidants are the secondary metabolites of plants (Walton and Brown, 1999). Antioxidants can be obtained synthetically or naturally. The antioxidant capacity can be measured in a rapid, simple, and inexpensive method by the use of the free radical, 2, 2-Diphenyl-1picrylhydrazyl (DPPH). This method is widely used to test the compounds' potential to perform as free radical scavengers or hydrogen donors and antioxidant activity evaluation (Kirtikar and Basu, 2006). The antioxidant is a bioactive substance, free radical scavengers, enzyme inhibitors, electron donors, and metal chelators that prevent the oxidation of harmful chemicals (Pisoschi *et al.,* 2016). The DPPH radical assay that was used in this study is one of the few stable organic nitrogen radicals, which carry a deep purple colour. This assay measures the ability of antioxidants reduction toward DPPH. The ability can be assessed through electron spin resonance (ESR) spectrometry or by measuring absorbance decrease value (Prior, 2005). The DPPH assay is a marginal reaction pathway that is expressed to be mainly dependent on an electron transfer (ET) reaction, and hydrogen-atom abstraction (Huang *et al.*, 2005). DPPH radicals are free radicals with high reactivity at room temperature.

The free radical scavenging activity of the extracts was measured based on the ability to scavenge the DPPH. The mechanism of radical scavenging is hydrogen donors and the high reactivity is caused by the delocalization of electrons around the molecules. When the DPPH radical is reacted with a substance that donates a hydrogen atom, DPPH radical is reduced into a non-radical DPPH. In the assay, this reaction is indicated by solution decolourization. It changes its colour from purple to yellow (Putri and Fatmawati, 2019). Substances that can perform this reaction are considered antioxidants or radical scavengers (Dehpour *et al.,* 2009). Antioxidative compounds (vitamin C, E, A, selenium, and carotenoids), ascorbic acid (Vitamin C) show a very strong intensity of antioxidative activities (Kaczmarski *et al.*, 1999). In most food industries, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), propyl gallate (PG), and others are used to prevent the rancidity of processed foods (Bhuiyan *et al.,* 2009).

Melastoma malabatrichum from Melastomataceae family and *C. odorata* from Asteraceae family was chosen to prove that these weeds have potential to be commercialized and can benefits to human through several bioactivities that can be done. Both weeds were selected to study their phytochemical constituents which can prove their contribution to several bioactivities and to determine the antioxidant activity by the DPPH scavenging method to possess antioxidant properties in these weeds. Thus, this study was carried out to screen

the phytochemicals present in the leaf and to determine the antioxidant potentials of *M. malabathricum* and *C.odorata* and evaluate which weeds have better antioxidant activity.

2. Materials and methods

2.1 Preparation of extracts

Melastoma malabathricum and *C. odorata* leaves washed under running tap water, dried under the shaded area, then were dried at 50°C for 24 hrs using the oven. The dried samples were prepared into powder using a mechanical blender. One hundred grams of powders of selected weed leaves were soaked in 500 mL of 95% ethanol at room temperature (27±1˚C) from day 1 to day 7. The extracts were then filtrated through Whatman No. 1 filter paper before evaporating the residual solvent using a rotary evaporator. The crude extracts were kept at 4˚C in a chiller until used.

2.2 Phytochemical screening

Phytochemical screening of selected weeds leaves were tested with modification for the presence of saponins, terpenoid, phenolic, tannins, and flavonoid. The qualitative results were expressed as (+) for the presence and (-) for the absence of phytochemicals. Test for saponin, about 0.5 g of each weed powder was shaken vigorously with 5 mL of distilled water in a test tube. The formation of stable foam was taken as an indication of the presence of saponins (Treas and Evans, 2002). Test for terpenoid, about 0.5 g of each weed powder was added with 2mL of chloroform, and 2mL of concentrated H_2SO_4. The reddish-brown colour was taken as an indication of the presence of terpenoid (Richardson, 1990). Test for phenolic, about 0.5 g of each weed powder was added with 5:5 of Methanol: distilled water, mixed with 2 drops 2% $FeCl_3$. The blue, green or purple colour was taken as an indication of the presence of phenols (Richardson, 1990). Test for tannin, about 0.5 g of each weed powder, was added with 0.5mL ethanol, 3.5mL deionized water and a few drops of 5% $FeCl_3$. The green or blue colour was taken as an indication of the presence of tannin (Richardson, 1990). Test for flavonoid, about 0.5 g of each weed powder, was added with 4mL ethanol and a few drops of 10 % $FeCl_3$ solution. The green or blue colour was taken as an indication of the presence of flavonoids (Brain and Turner, 1975).

2.3 Determination of antioxidant activity

The antioxidant activity of both weed leaf extract was determined by its DPPH radical scavenging activity. The method with modification by Zhang and Xu in 2015 for DPPH free radical scavenging was used in this study. 0.1 mM solution of DPPH in methanol was prepared. Five different extracts concentrations of 100, 200, 300, 400, and 500 ppm were prepared at 517 abs by using a spectrophotometer. Vitamin C and BHA were used as standard controls (Dehpour *et al.,* 2009). The percentage inhibition was calculated using the equation:

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of the sample) / (Absorbance of control)] × 100.

Half-maximal inhibitory concentration (IC50) values denote the concentration of the sample, which is needed to scavenge 50% of DPPH free radicals (Shekhar and Anju 2014).

3. Results and discussion

3.1 Phytochemical screening

The results of phytochemicals determine that phenolic and tannin were highly present for both weeds. *Melastoma malabatrichum* phytochemical test was highly present, followed by *C. odorata* (Table 1).

Table 1. Qualitative analysis of the phytochemicals of the leaves

	M. malabatrichum	*C. odorata*
Saponins	+++	+
Terpenoids	+++	+++
Phenols	+++	+++
Tannins	+++	+++
Flavanoids	+++	++

+, shows presence of phytochemical

Saponins, terpenoids, phenols, tannins and flavonoids were present in both samples. *Melastoma malabatrichum* showed positive results on saponins, terpenoid, phenolic, tannins, and flavonoid's existence of (3+). A similar result was obtained that revealed the presence of saponins, flavonoids, and tannin in *M. malabatrichum* leaves by Sulistyaningrum *et al.* (2018). This also has been proven by Sembiring *et al.,* 2018 that saponins, terpenoids, tannins, and flavonoids are present in the *M. malabatrichum* leaves. Zakaria *et al.* (2006) also revealed the existence of saponin (1+), flavonoids (2+), and tannins (2+) in *M. malabatrichum* leaves. Danladi *et al.* (2015) reported with no saponin, (3+) for tannins, (3+) for phenols, (1+) for terpenoid, and (2+) for flavonoids. For *C. odorata* leaves, a similar result was also obtained that revealed the presence of saponins, terpenoid, tannins, and flavonoids by Fauzi *et al.* (2020). Odutayo *et al.* (2017), also revealed the existence of (1+) for saponins, (2+) for terpenoids, (2+) for flavonoids, (1+) for tannins, and (1+) for phenols. Previous studies confirmed the presence of some phytochemical constituents in the leaves of both weeds. Thus, this supports the present results with the presence of

saponins, terpenoids, phenols, tannins and flavonoids in both weeds. With the present results that expressed with (+) and (-), its contribution for the new findings with (3+), shows the compound strongly present.

3.2 Antioxidant activity

The scavenging effect of *M. malabatrichum* and *C. odorata* with vitamin C and BHA were compared. On the DPPH radical, *M. malabatrichum* and *C. odorata* had significant scavenging effects with increasing concentration in the range of 100-500 ppm when compared with Vitamin C and BHA. The DPPH activity of *M. malabatrichum* and *C. odorata* was found to increase in a dose-dependent manner. The *M. malabatrichum* and *C. odorata* at the used concentrations showed the potential effect of DPPH activity as a percentage of free radicals inhibition. Antioxidant assay of *M. malabathricum* and *C. odorata* leaves extracts displayed the ability to inhibit DPPH free radical formation by 93% at 300 ppm concentration on *M. malabathricum* while the *C. odorata* by 79% at 500 ppm concentration (Figure 1).

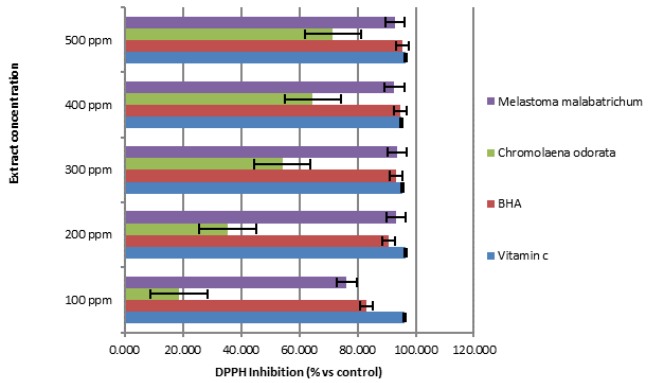

Figure 1. DPPH inhibition (%)

Radical scavenging activity in this study showed the ability of a compound to scavenge free radicals which in turn prevents their toxic effect on the cell. The phenolic compound can scavenge free radicals by donating hydrogen atoms (Riaz *et al.*, 2012). The results in (Figure 1) and (Table 2) show that *M. malabatrichum* has a DPPH radical inhibitory activity better than *C. odorata*. Vitamin C and BHA were noted as the reference antioxidant for this test. Scavenging of DPPH radical was found to rise with increasing concentration of the extracts.

The IC50 value 31.023 µg/mL obtained for Vitamin C and 71.521 µg/mL obtained for BHA as the standard was significantly lower than the values obtained for the extract of *M. malabatrichum* and *C. odorata*. The highest scavenging of extracts was observed with *M. malabatrichum* with an IC50 value of 81.116 µg/mL and as opposed to the IC50 value of standard Vitamin C and BHA which are well-known antioxidants (Table 3). The measured IC50 result indicates that the antioxidant activity of *M. malabatrichum* is greater than *C. odorata*. Therefore, the lower IC50 suggests that it has a higher antioxidant capacity. The result of the DPPH radical scavenging assay showed that *M. malabatrichum* extracts have a good DPPH scavenging effect and being the most active than *C. odorata*.

Table 3. IC50 Value (µg/mL)

	IC50 (µg/mL)
Vitamin C	31.023
BHA	71.521
M. malabatrichum	81.116
C. odorata	312.903

The results of antioxidant activity of *M. malabatrichum* leaves extracts showed that the higher concentration of the sample, the higher percentage of antioxidant activities. This is also due to the fact that the higher the concentration of extracts, the more concentrated the color of the sample, and the absorption of DPPH reduction is less than maximum. In this research, *M. malabatrichum* is known to have potent antioxidant properties, with the presence of flavonoids and tannins in the leaves. The result of the study also shows that the leaves extract of *M. malabatrichum* and *C. odorata* scavenged the free radicals by the DPPH method by a strong absorption band at 517 abs in the visible spectrum of deep violet colour. Free radicals are chemical compounds that have free electrons or unpaired electrons. Unpaired electrons are unstable which can easily bind to other molecules and unwanted reactions can be formed (Lingga, 2012). Unpaired electrons are the electrons in an atom that occur in an orbital alone. This means these electrons are not paired or occur as electron couples. Free radicals are when an atom or a molecule

Table 2. Percentage inhibition of DPPH free radical scavenging activity of *M. malabathricum* and *C. odorata* leaves extracts

Concentration (ppm)	Vitamin C (% Inhibition)	BHA (% Inhibition)	*C. odorata* (% Inhibition)	*M. malabatrichum* (% Inhibition)
100	96.04±0.92	83.20±4.35	35.37±15.37	73.11±6.96
200	96.64±0.69	90.71±3.97	52.35±12.50	93.24±0.72
300	95.70±0.66	93.18±0.82	52.35±12.50	93.47±0.27
400	96.03±0.72	94.58±1.17	64.14±22.78	93.24±0.68
500	97.08±0.25	95.90±0.59	79.86±10.57	92.92±0.41

Values are presented as mean±S.D

has this type of electron. The chemical elements having these electrons are highly reactive. This is because they tend to pair all their electrons to become stable and having an unpaired electron is unstable (Madhu, 2018). At higher concentrations, the plants' ability to scavenge DPPH radicals increases by increased percentage (%) inhibition. In this study, a higher percentage of the leaves extracts exhibited the ability to scavenge DPPH radical used in a concentration-dependent manner as its % Inhibition increased with the increase in concentration. *Melastoma malabatrichum* leaves compared with *C. odorata* revealed that the *M. malabatrichum* shows a strong activity as a radical scavenger using DPPH assay that indicates *M. malabatrichum* has a very strong ability to donate hydrogen when compared with standard, Vitamin C and BHA (Usunomena and Efosa, 2016). Our antioxidant result analyses of the leaves extract showed the presence of antioxidants and DPPH radical scavenging activity, which is denoted in phenolic compounds from plants and responsible for the radical scavenging activity (Oladipupo, 2014).

4. Conclusion

The study indicates that even though *M. malabatrichum* and *C. odorata* have been considered as an invasive weed, leaves of both weeds possess a valuable phytochemicals compound. The phytochemicals test (saponin, terpenoid, phenolic, tannin, and flavanoid) may relate to its importance in protective function, physical characteristics, and chemical characteristics of the weeds. The results also provide evidence that *M. malabatrichum* and *C. odorata* contain antioxidants properties. The results display that *M. malabatrichum* leaves have better antioxidant activity compared to *C. odorata*. Therefore, *M. malabatrichum* has a high potential to further study bioactivities.

Conflict of interests

The authors declare no conflicts of interest.

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