Antioxidant and Antibacterial Properties of Melastomataceae Species (*M. Malabathricum*, *M. Hirta,* and *M. Decemfidum*)

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Melastomataceae family species, commonly known as "senduduk" to the Malay community, have gained attention in scientific research as they provide many benefits. Traditionally, various parts of the plants, such as leaves, roots, and flowers, are used to cure diarrhea, accelerate wound healing, lower high blood pressure, and for post-natal care. This study explored the leaves of three different Melastomaceae species, namely *Melastoma malabathricum*, *Melastoma hirta*, and *Melastoma decemfidum* for their antioxidant and antibacterial properties. The results showed that all the *Melastoma* leaf extracts exhibited high antioxidant activities in the range of 39-93% and 4.47 - 9.82 mg/mL of ascorbic acid equivalents (AAE) in 2,2-azinobis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS) radical scavenging activity and total phosphomolybdate assay, respectively. The antibacterial properties of all the *Melastoma* leaf extracts showed *Staphylococcus aureus* and *Pseudomonas aeruginosa* were highly sensitive towards inhibition of studied samples. It was found that *M. hirta* exhibited the highest zones of inhibition and was the most sensitive towards target bacteria as compared to the other *Melastoma* leaves. Thus, it can be concluded that *Melastoma* plants, especially *M. hirta*, could be used as a potential source of antioxidants and antibacterial compounds for utilization in cosmeceuticals, nutraceuticals, and medicine.

Key words: Melastomataceae; *M. malabathricum*; *M. hirta*; *M. decemfidum*; antioxidant; antibacterial

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World Health Organization (WHO) reported that in tropical and developing countries various infectious diseases are responsible for over 50% of deaths worldwide [1]. For instance, numerous infectious diseases are predominantly caused by pathogenic bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. They are widely distributed in nature and responsible for morbidity and mortality in the population. It has been reported that some of these bacteria, such as *Staphylococcus aureus*, can cause a variety of serious infections like pneumonia, meningitis, mastitis, and urinary tract infections [2]. In addition, reported cases of food poisoning resulting from eating food contaminated with spoilage microorganisms have increased [3]. Hence, bacteria have become a big threat due to its harmful effects on living organisms. Therefore, to slow down the social and economic losses from health hazards, major research throughout the years have been focused on the identification and evaluation of natural sources, especially from plants, to prevent infectious diseases from perilous pathogens and to ensure the safety of consumers.

The potential of higher plants as a source for the development of new green medicine, especially to combat bacteria, is increasing worldwide. According to Shagufa *et al.* (2012), the antibacterial properties of plants were first documented in the late 19th century. For example, Bisht *et. al* (2012) reported that there are about 157 families of plant species extracts reported to be significant as active against microorganisms. A vast variety of plants such as *Punica granatum* (pomegranate), *Anacardium occidentale Linn* (cashew), *Cyclopia intermedia* (honeybush plant), and *Mimosa pudica* have been assessed and reviewed using extracts or essential oils to counter unsafe pathogens and bacteria [4–7]. It was reported these plants are rich in secondary metabolites or bioactive constituents belonging to the class of flavonoids, polyphenols, and tannins, which are significant as antibacterial agents to combat pathogens [8,9]. In addition, the use of natural sources as bio-preservatives has less negative side effects on human

health as compared to the use of chemical preservatives [10].

Melastomataceae is used in various human cultures around the world for medicinal purposes. According to Costa *et al.* (2015), this plant is widely distributed in tropical and subtropical areas and comprises about 170 genera and 4600 species in total. In Southeast Asia, particularly Malaysia, the genus *Melastoma* comprises 22 species, two subspecies, and three varieties, namely *M. malabathricum*, *M. hirta*, and *M. decemfidum*. They can be differentiated by the color of the flower petals, which is dark purple magenta for *M. malabathricum*, white for *M. decemfidum*, and white or pale pink for *M. hirta* [11]. Traditionally, members of this family are used to treat diarrhea, diabetes, high blood pressure, hepatitis, toothache, and even wounds [12–14]. Previous studies reported that these plants are rich in a wide variety of secondary metabolites, such as flavonoids, steroids, tannins, triterpenes, and saponins [15]. In addition, phytochemical study on one of these plants, *M. decemfidum*, revealed the presence of flavonoids naringenin and kaempferol, which are known to have anticancer and anti-inflammatory properties [16]. Hence, the abundance of phytochemical constituents found in these plants led us to the *in vitro* investigation of the leaves of three different *Melastoma* species (*M. malabathricum*, *M. hirta* and *M. decemfidum*) for their antimicrobial properties as well as their antioxidant potential to treat various diseases caused by pathogenic bacteria.

EXPERIMENTAL

Chemicals

The chemicals used in this work were ABTS (2,2'-azino-bis (3- ethylbenzothiazoline -6- sulfonic acid) radical solution (Sigma Aldrich), potassium persulphate ($K_2S_2O_8$), sulfuric acid (Merck), sodium phosphate, ammonium molybdate (Sigma Aldrich), ascorbic acid (Sigma Aldrich). All other chemical reagents used in this study were of analytical grade and double distilled water was used throughout the experiment.

Plant Materials

The leaves of *Melastoma malabathricum* (mm) (purple petals) and *Melastoma hirta* (mh) (pale pinkish petals) were collected from their natural habitat at Ayer Hitam Forest Reserve, Puchong, Malaysia from August 2017 to October 2017. Meanwhile, *Melastoma decemfidum* (md) (white petals) was collected in Johor, in the south of Peninsular Malaysia. All leaves were further identified by Dr. Paiman bin Bawon, a senior lecturer from the Faculty of Forestry, Universiti Putra Malaysia (UPM), Serdang, Malaysia. A voucher herbarium specimen for each sample was deposited at the Institute of Bioscience (IBS), Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia with voucher no. SK3377/18, SK 3378/18, and SK3379/18 for *M. malabathricum*, *M. hirta*, and *M. decemfidum*, respectively.

Preparation of Samples and Plant Extraction

All plant materials were thoroughly washed and dried in a shaded area for two weeks. According to Zakaria *et al.* (2011), this process does not affect the bioactive compounds of the leaves of the plants, as proven by the availability of antioxidant, anti-nociceptive, anti-inflammatory and antibacterial activities. Next, the dried leaves were ground into powder using a mechanical grinder machine. The whole leaf powder samples were packed in sealed plastic bottles until extraction. The extraction of the plants was performed at solid/liquid ratio of 1:10 (w/v) into three different solvent extractions (hexane, ethyl acetate, and methanol) by maceration for three consecutive days at room temperature. Next, the mixtures were filtered using a filter paper (Whatman No.1) and evaporated until dry using rotary evaporator (Yamato, Rotary Evaporator, model RE 801, Japan).

Determination of Antioxidant Assay

ABTS (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical assay

The ABTS radical assay of the leaves of three different *Melastoma* species (*M. malabathricum*, *M. hirta*, and *M. decemfidum*) was evaluated according to the decolorization of the ABTS radical cation ($ABTS^{\bullet+}$) procedures by Shalaby and Shanab (2013) [17]. First, the ABTS radical cation was produced by reacting 10 mL of 7 mM ABTS stock solution with 10 mL of 2.45 mM potassium persulfate ($K_2S_2O_8$) and allowed to stand in a dark place at ambient temperature for 4 to 16 hours until the reaction was complete. Next, the generated $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm for measurements (Microplate reader Infinite M200, Tecan). Then, 50 µL of each extract (100 µg/mL) was mixed with the generated $ABTS^{\bullet+}$ solution and the resulting mixture was vortexed and allowed to stand in the dark for 15 minutes. Finally, the absorbance of the reaction mixtures was recorded at 734 nm spectrophotometrically. The antioxidant activities of each *Melastoma* leaf extract were expressed as the percentage of ABTS radical scavenging by using Eq.1.

$$\text{ABTS radical scavenging activity} = \frac{\text{Abs}_{\text{negative/control}} - \text{Abs}_{\text{extract}}}{\text{Abs}_{\text{negative/control}}} \times 100 \qquad \text{(Eq. 1)}$$

Total phosphomolybdate assay

The total antioxidant activities in each sample of the extracts were determined by the green phosphomolybdenum complex formation, according to the method by Prieto *et al.* (1999) [18]. An aliquot of 0.1 mL of sample (1000 µg/mL) was mixed in an Eppendorf tube with 1 mL of reagent solution containing 600 mM sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. Next, the reaction mixture was incubated in a water bath at 95ºC for 90 minutes. After the samples had cooled to room temperature, the absorbance of each extract was measured at 695 nm against a blank. A standard and positive control of ascorbic acid was used in this study. The total antioxidant activities of the *Melastoma* plant extracts were expressed as mg/mL of ascorbic acid equivalents (AAE).

Antimicrobial assay

The three different *Melastoma* leaf extracts (*M. malabathricum*, *M. hirta* and *M. decemfidum*) were tested for activities against bacteria and fungi using modified agar-well diffusion procedures. Four bacterial strains were used in this study: gram positive bacteria, namely *Staphylococcus aereus* and *Bacillus subtilis*; and gram negative bacteria, *Pseudomonas aeruginosa* and *Esherichia coli.* Meanwhile, *Aspergillus brasiliensis* was used as the target fungus. The target microbes were obtained from the Microbial Culture Collection Unit (UNiCC), Institute of Bioscience, Universiti Putra Malaysia, Malaysia. The tests were carried out by placing 6 mm in diameter paper discs containing respective extracts and antibiotics onto plates which individual microbes were growing. The microbe cultures were standardized to 0.5 McFarland standard, which was approximately 10^8 cells. Streptomycin and Nystatin were used as standards against bacteria and fungi, respectively. Next, the plates containing bacterial strains were inverted and incubated at 30-37ºC for 18 to 24 hours, 24 to 48 hours or until sufficient growth has occurred. After incubation, each plate was examined for antimicrobial activities. The zones of inhibition were measured to the nearest whole millimeter using a ruler. All experiments were performed in triplicate and the means of the diameter of the inhibition zones were calculated to minimize test error.

RESULTS AND DISCUSSION

Antioxidant Activity of *Melastoma* Leaf Extracts

More than 35,000 plant species are being used in various human cultures around the world for medicinal purposes [19]. There are about 1,200 species of higher plants in Peninsular Malaysia and 2,000 species in Sabah and Sarawak reported to exhibit various medicinal values and have been used for generations for various ailments as traditional remedies. To date, several assays have been used for the measurement of the total antioxidant activity in various medicinal plant parts such as leaves, stem, bark, flower, roots, and so forth. According to Re *et al.* (1999), two types of approaches have been used and they are inhibition assay where the extent of the scavenging by hydrogen or electron donation of a pre-formed free radical is observed, and assays involving the presence of antioxidant systems during the generation of the radical. In this present work, the radical scavenging activity of the three different *Melastoma* leaf extracts (*M. malabathricum*, *M. hirta* and *M. decemfidum*) was determined by the decolorization of the $ABTS^{\bullet+}$ radical cation. Meanwhile, the total phosphomolybdate assay was based on the reduction of Mo(VI) to Mo(V) complex at 695 nm by the antioxidant compounds in the studied samples [20].

The three *Melastoma* species leaves of *M. malabathricum*, *M. hirta*, and *M. decemfidum* showed high antioxidant activities in ABTS radical scavenging and total phosphomolybdate assay in the range of 39.0-93.0% and 4.47-9.82 mg/mL AAE, respectively (Table 1). The extract from *M. hirta* was recorded to have the highest radical inhibition which showed 93.0% total inhibition as compared to the other *Melastoma* plants. Meanwhile, the highest total phosphomolybdate activity exhibited in *M. hirta* leaf extract was 9.82 mg/mL AAE. In addition, as can be seen in the data, the antioxidant activity of all the *Melastoma* leaf extracts was influenced by the type of solvent extraction used. For instance, in ABTS radical scavenging activity, most of the methanolic extracts of all the *Melastoma* plants exhibited the highest inhibition activity towards ABTS radical. This was followed by the ethyl acetate extracts, while the least inhibition was from the hexane extracts (Figure 1). Meanwhile, for total phosphomolybdate activity, the highest antioxidant activity was recorded by the methanolic extracts for both *M. malabathricum* and *M. decemfidum.* However, for *M. hirta*, the ethyl acetate extract gave a slightly higher inhibition rate as compared to the methanolic extract (Figure 2). Apart from that, it could be seen that the hexane extract showed the least inhibition for total antioxidant in phosphomolybdate activity. This highlighted that methanol (polar solvent) and ethyl acetate (semi polar solvent) produced higher activity extracts from the *Melastoma* plants as compared to non-polar solvents. Therefore, it shows that different extraction solvents play a significant ability of *Melastoma* leaf extracts to produce valuable non-polar compounds.

A study done by Alam *et al.* (2018) found four antioxidant compounds, which were flavonoid constituents (kaempferol-3-*O*-[2',6'-di-*O*-*p*-trans-coumaroyl, kaempferol-3-*O*-D-glucoside, naringenin and kaempferol) from the leaf extract of *M. decemfidum* that were extracted with polar solvents [16,21]. The significant bioactive constituents of the non-polar compound of naringenin have been reported to have a potent anti-cancer effect against MCF7, a human breast cancer cell line, while kaempferol was documented to have an anti-inflammatory activity and various pharmacological activities, including antibacterial activity [13,16]. Moreover, research on *M. malabathricum* revealed that its bioactive constituents contain new complex tannins, namely ellagitannin, flavan-3-ol, quecertin, quecitrin, and rutin [22]. Last but

not least, *M. hirta* develops high antioxidant activity due to the abundance of phenolics and flavonoids constituents [14]. It is well reported that phenols are the type of compounds that have the ability to destroy radicals because they contain hydroxyl [23]. Thus, it can be concluded that different solvent extractions significantly contribute to a high percentage of antioxidant activities in the extract and produce significant bioactive compounds that can inhibit and slow down the radical activity.

Table 1. ABTS radical scavenging and total phosphomolybdate activity of *Melastoma* leaf extract

Samples	ABTS radical scavenging activity (%)	Total phosphomolybdate activity (mg/mL AAE)
MM-Hex	43.14	5.41± 1.0
MM-Ea	77.43	6.63± 1.3
MM-MeOH	87.00	7.51± 2.6
MH-Hex	40.43	5.84± 2.5
MH-Ea	91.57	9.82± 2.0
MH-MeOH	93.57	9.74± 2.1
MD-Hex	39.00	4.47± 2.9
MD-Ea	62.71	6.71± 0.8
MD-MeOH	93.00	8.82± 0.6

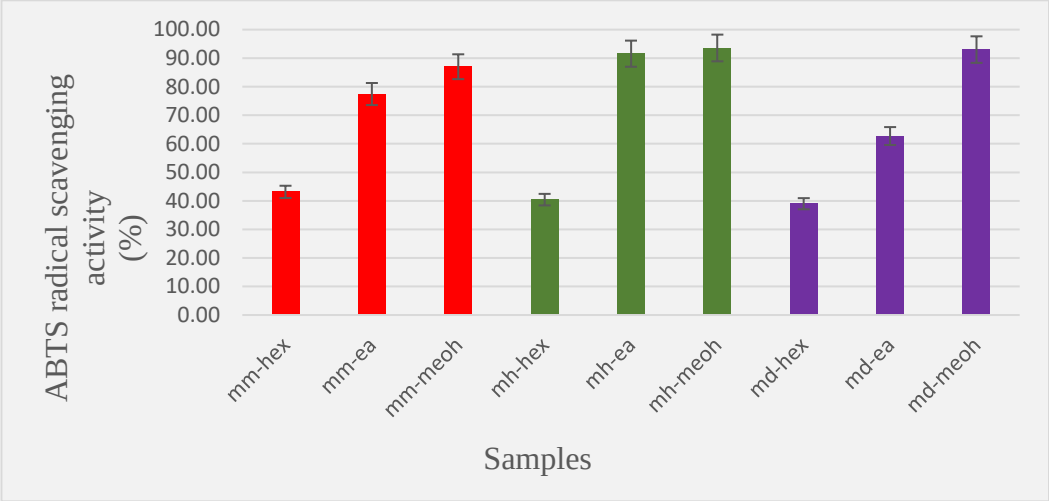

Figure 1. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation scavenging activity (%) of *Melastoma* leaf extracts

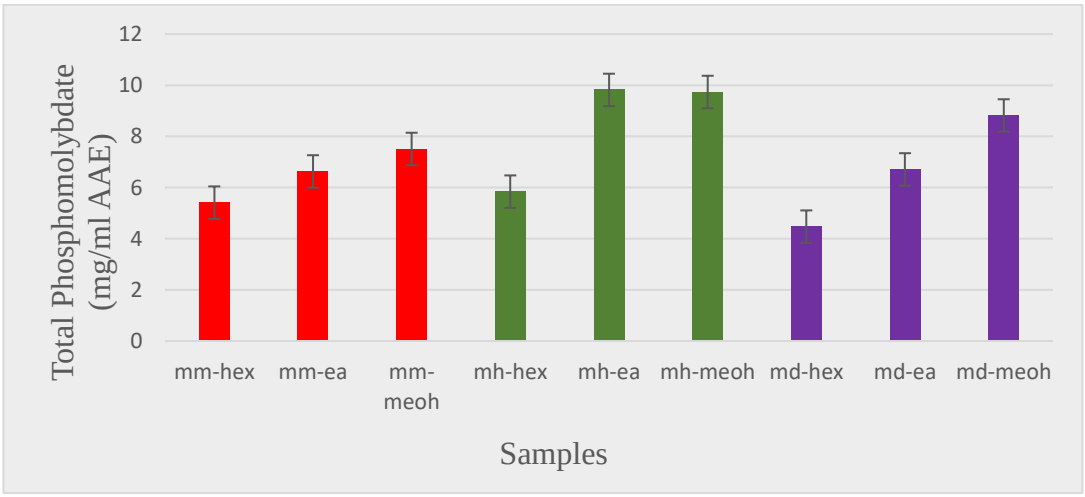

Figure 2. Total phosphomolybdate activity (mg/mL AAE) of *Melastoma* leaf extracts

Antibacterial Analysis of *Melastoma* Leaf Extracts

Infectious diseases are one of the current issues faced by countries worlwide, and more so by the developing ones. Various pathogenic microbes such as bacteria, fungi, parasites, and viruses are in our surrounding. For example, it has been reported that microbes of *E. coli* and *Bacillus subtilis* have been isolated from various environments to cause several deaths such as *Bacillus subtilis* may be expected to temporarily inhabit the gastrointestinal tract and skin of humans [2]. Hence, major precautions and safety from antimicrobial properties have been developed for mankind, especially using natural plant sources. In this present work, we had explored Melastomataceae leaf extracts of *M. malabathricum*, *M. hirta*, and *M. decemfidum*. The therapeutic antimicrobial properties of these plant extracts were evaluated against gram positive and gram negative bacteria and fungi using different solvent extracts, namely hexane, ethyl acetate, and methanol. Based on the results, as shown in Figure 3, only two of the target bacteria; *Staphylococcus aureus* and *Pseudomonas aeruginosa*, were found to be sensitive towards inhibition. Meanwhile, all the bacteria and the fungus were sensitive to the positive controls, streptomycin for bacteria and nystatin for fungi. It has been reported by Mele´ndez and Capriles (2006) that *Staphylococcus aureus* is one of the bacteria most susceptible to plant extracts. In addition, from this study, it was found that different solvent extractions used influenced the sensitivity against bacteria. For instance, the methanolic extract of all the *Melastoma* plants (*M. malabathricum*, *M. hirta*, and *M. decemfidum*) was the most sensitive against *Staphylococcus aereus* and *Pseudomonas aeruginosa*, as presented in Figure 3. Meanwhile, the ethyl acetate and hexane extracts showed moderate and negative results toward the target bacteria and fungus, respectively, as they did not show any antimicrobial properties.

The inhibition zone (in mm) exhibited the ethyl acetate and methanolic leaf extracts of *M. hirta* presented the highest inhibition activity, which was 20 mm against *Staphylococcus aureus*, as compared to other *Melastoma* plants. The methanolic extract of *M. decemfidum* showed 13 mm of inhibition zone, followed by *M. malabathricum*, with the least inhibition zone activity at 10 and 11 mm for the ethyl acetate and methanolic extracts, respectively (Table 2). It was also found that *M. hirta* leaf extracts in ethyl acetate and methanol had significant effects towards *Pseudomonas aeruginosa*, which exhibited 10 mm of inhibition zone, as compared to *M. malabathricum* and *M. decemfidum* leaf extracts.

A research done by Sarbadhikary *et al.* (2015) also showed similar results as our study, as the ethanolic leaf extract of *M. malabathricum* inhibited *Staphylococcus aureus* and *Pseudomonas aeruginosa*, both at 18 mm in diameter. Meanwhile, the inhibitory zones of the methanolic extract of *M. hirta* reached 22 mm, which was slightly higher than our studied samples, however, it was found that they also showed no inhibition activity for *E. coli* [24]. Meanwhile, a study done by Elkington *et. al* (2009) found that the antimicrobial properties from the extract of *M. decemfidum* leaves also had positive inhibition towards *Staphylococcus aureus* and *E. coli* at 16.58% and 34.52%, respectively. Ismail *et al.* (2017) reported that the inhibition zone for the methanolic extract of *M. decemfidum* increased as the concentration of the extract was increased, where the inhibition zone at 100 µg/mL for *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa* were 9.8, 12.4 and 12.6 mm, respectively. Based on our study, it can be seen that our findings were significantly different from previous studies as this can be explained by the different types of solvents, extraction methods, and types of plants used in this experiment, which significantly influenced the target results. Apart from that, interestingly, it can be seen that positive comparisons were obtained between the studied extracts (*M. malabathricum*, *M. hirta*, and *M. decemfidum*) with other *Melastoma* species, namely *Melastoma candidum*, which had shown similar anti-microbial properties [25].

The potential of *Melastoma* leaf extracts (*M. malabathricum*, *M. hirta*, and *M. decemfidum*) acting as antibacterial agents can be supported by the antioxidant activity screened earlier, which were able to scavenge radical species and present high total inhibition activities from the ABTS radical assay. Moreover, the presence of phytochemicals such as saponins, flavonoids, terpenoids, tannins, phenolic acid, alkaloids, and polyphenols in the *Melastoma* leaf extracts could significantly increase its antibacterial activities. According to Wang *et al.* (2008), plants show antimicrobial properties because it can synthesize antimicrobial compounds called phytoalexins as a defense mechanism. A previous study had found that the antibacterial properties demonstrated by *M. decemfidum* was identified from two flavonoids constituents, which are naringenin and kaempferol [16]. Therefore, the abundance of phytochemicals in all *Melastoma* extracts causes various pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, and so forth, which can treat various diseases. Hence, from the results, the high antioxidant activities in all the *Melastoma* leaf extracts, especially in the ethyl acetate and methanolic extracts, may attribute to their high antimicrobial activities. Moreover, according to Oussaid *et al.* (2017), antimicrobial and antioxidant agents have been incorporated into foods to extend their shelf life and at the same time to prevent lipid peroxidation and foodborne illnesses due to pathogen growth.

Table 2. Antibacterial and antifungal inhibition zone (in mm) of *Melastoma* leaf extracts

Samples	Target Microbes				
	Bacillus subtilis	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Aspergillus brasiliensis*
MM-Hex	-	-	-	-	-
MM-Ea	-	10	-	-	-
MM-MeOH	-	11	-	-	-
MH-Hex	-	-	-	-	-
MH-Ea	-	20	10	-	-
MH-MeOH	-	20	10	-	-
MD-Hex	-	-	-	-	-
MD-Ea	-	-	-	-	-
MD-MeOH	-	13	-	-	-
+ve (Streptomycin)	26	25	32	29	-
+ve (Nystatin)	-	-	-	-	20

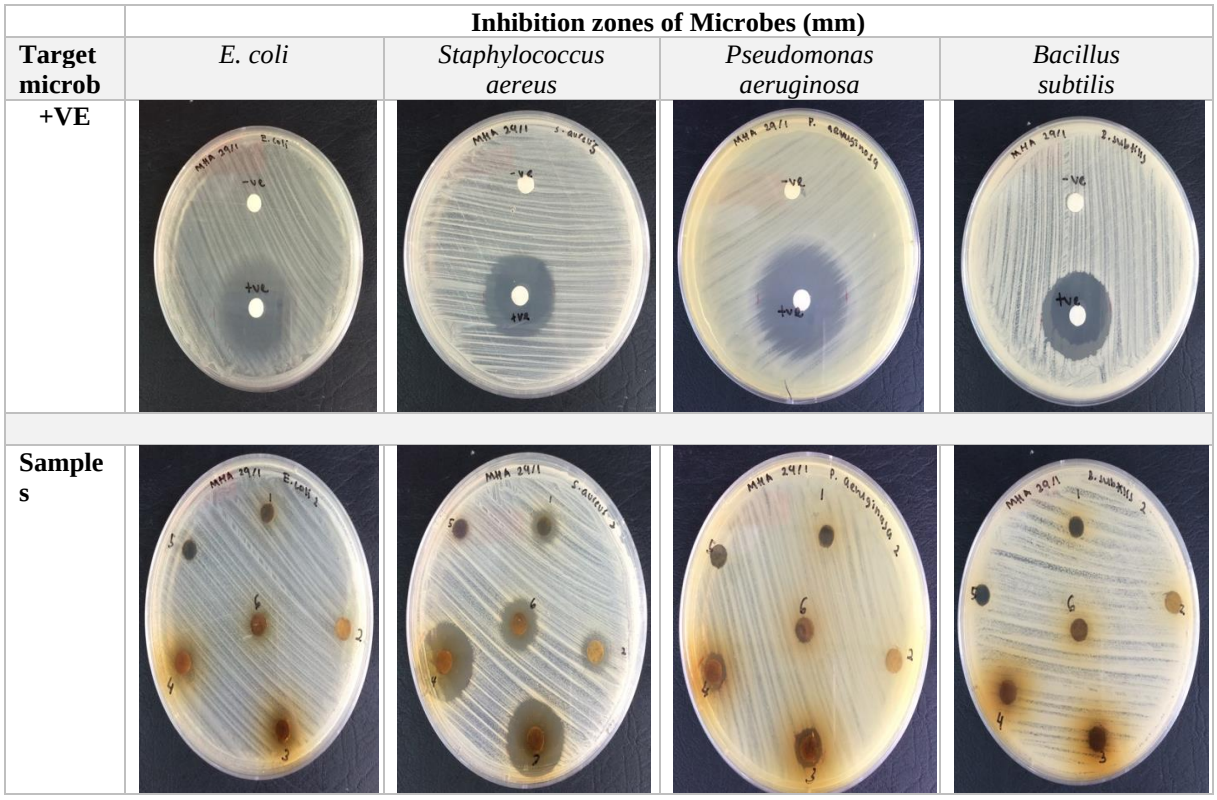

Figure 3. The inhibition zones (mm) of microbes and positive control
+ve standard; Streptomycin (bacteria)
**Fungi and hexane extract of all Melastoma leaf extracts are excluded in Fig. 3, as they showed no inhibition activity*
Samples ; no. 1 and 2 indicate ethyl acetate and methanolic extracts of *M. Malabathricum*
; no. 3 and 4 indicate ethyl acetate and methanolic extracts of *M. hirta*
; no. 5 and 6 indicate ethyl acetate and methanolic extracts of *M.decemfidum*

CONCLUSION

In this study, *M. malabathricum*, *M. hirta*, and *M. decemfidum* leaf extracts were successfully evaluated for their antioxidant potential and antibacterial properties. Based on the results, *M. hirta* was recorded to exhibit the highest antioxidant activity and the most sensitive towards the inhibition of targeted bacteria as compared to other *Melastoma* leaf extracts. It can be seen that different solvent extraction plays an important role in both assays as a more polar solvent extract, such as ethyl acetate and methanolic extract, significantly increased the value of antioxidant activity and zones of inhibition of the studied bacteria. Thus, it can be concluded that the good antioxidant potential found in *Melastoma* species of *M. malabathricum*, *M. hirta*, and *M. decemfidum*, incorporated with antibacterial properties, can significantly enhance therapeutic medicine and provide good value in pharmaceutical, cosmetic, and agrochemical ingredients for future uses against various diseases.

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