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Antioxidant and Antimicrobial Activity of the Extracts from Different Parts of *Etlingera sayapensis* (Zingiberaceae)

(Aktiviti Antioksida dan Antimikrob bagi Ekstrak daripada Bahagian Berbeza *Etlingera sayapensis* (Zingiberaceae))

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ABSTRACT

The rhizomes, stems and leaves of Etlingera sayapensis *were extracted with ethyl acetate, acetone, ethanol and methanol to give 12 extracts. The total phenolic content (TPC) of the extracts was measured in this study, together with their antioxidant activity. Assays used were radical scavenging activity (RSA), β-carotene bleaching (BCB), ferrous ion chelating (FIC) and ferric reducing antioxidant power (FRAP). Among the assays, the leaf methanolic extract showed the highest activity with 35.67 mg GAE/g (mg of gallic acid per gram of extract) for TPC,* IC_{50} *of 53.43 μg/mL for RSA, 83.24% for BCB,* IC_{50} *of 242.43 μg/mL for FIC (second highest) and 19.53 mg TrE/g (mg of Trolox per gram of extract) for FRAP. The measured BCB values for the four leaf extracts were higher than those of the positive controls of butylated hydroxytoluene (BHT), gallic acid (GA) and ascorbic acid (AscA) and slightly lower than that of* α-tocopherol *(Toc). The antimicrobial activity of the extracts was also measured using disc-diffusion (DD) and minimum inhibition concentration (MIC) assays. Among the extracts, only ethanolic and methanolic extracts showed specific bacterial inhibition against* Bacillus subtilis. *Only stem methanolic extract exhibited specific fungal inhibition against* Candida parapsilosis. *The highest activity was shown by this with 9.9 mm and 1.04 mg/mL for DD and MIC assays respectively.*

Keywords: *Antimicrobial activity; antioxidant activity;* Etlingera*; Zingiberaceae*

ABSTRAK

Rizom, batang dan daun Etlingera sayapensis *telah diekstrak dengan etil asetat, aseton, etanol dan metanol untuk memberikan 12 ekstrak. Kandungan fenolik jumlah (TPC) ekstrak telah diukur dalam kajian ini, bersama-sama dengan aktiviti antioksida mereka. Asai yang digunakan adalah aktiviti penskavenjing radikal (RSA), pelunturan β-karotena (BCB), pengkelatan ion ferus (FIC) dan kuasa antioksida penurunan ferik (FRAP). Dalam kalangan asai ini, ekstrak metanol daun menunjukkan aktiviti tertinggi dengan 35.67 mg GAE/g (mg asid galik per gram ekstrak) bagi TPC,* IC_{50} *53.43 μg/*mL *bagi RSA, 83.24% bagi BCB,* IC_{50} *242.43 μg/mL bagi FIC (kedua tertinggi) dan 19.53 mg TrE/g (mg Troloks per gram ekstrak) bagi FRAP. Nilai BCB yang diukur bagi empat ekstrak daun adalah lebih tinggi daripada nilai kawalan positif hidroksitoluena terbutil (BHT), asid galik (GA), asid askorbik (AscA) dan rendah sedikit daripada nilai bagi α-tokoferol (Toc). Aktiviti antimikrob ekstrak juga telah diukur menggunakan asai peresapan-cakera (DD) dan kepekatan perencatan minimum (MIC). Dalam kalangan ekstrak, hanya ekstrak etanol dan metanol menunjukkan perencatan bakteria yang spesifik melawan* Bacillus subtilis. *Hanya ekstrak metanol batang memperlihatkan perencatan fungus yang spesifik menentang* Candida parapsilosis. *Aktiviti tertinggi telah ditunjukkan olehnya dengan 9.9 mm dan 1.04 mg/mL bagi asai DD dan MIC.*

Kata kunci: *Aktiviti antimikrob; aktiviti antioksida;* Etlingera*; Zingiberaceae*

INTRODUCTION

Antioxidants are considered inhibitors of living tissue damage via different mechanisms such as neutralization of free radicals or metal chelating. Due to this, there has been a growing interest in the use of natural antioxidants as food additives. This growing interest is also encouraged by the knowledge that synthetic antioxidants like BHT, butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) tend to have unhealthy effects (Kulisic et al. 2004; Liu & Nair 2010; Moon & Shibamoto 2009; Singh & Rajini 2004; Zhu et al. 2004). The search for new antimicrobial drugs continues largely due to the rise of resistance against pathogenic microorganisms, as well as the side effects that antibiotics produce (Kokoska et al. 2002). From ancient times, humans discovered the existence of microbes and have used plants to cure common infectious illnesses (Ríos & Recio 2005). This interest has intensified in recent years with many research groups around the world focusing on the search for new antioxidants and antimicrobial agents from natural sources. The plant kingdom with its great diversity in producing secondary metabolites has attracted particular interest to exploit new antioxidants and antimicrobial agents (Ebrahimabadi et al. 2010). Zingiberaceae with 53 genera

and over 1200 species is known as the largest family of the order Zingiberales. It is widely distributed in the tropics especially in Southeast Asia (Kress et al. 2002). Antioxidant and antimicrobial activity of the genera of *Alpinia*, *Curcuma*, *Elettariopsis*, *Hedychium*, *Kaempferia* and *Zingiber* have been reported by different authors (Al-Reza et al. 2010; El-Ghorab et al. 2010; Elzaawely et al. 2007; Habsah et al. 2000; Kader et al. 2011). *Etlingera* is another genus of Zingiberaceae family, which has been identified as a candidate for the measurement of its antioxidant and antimicrobial bioactivity. The genus is an Indo-Pacific genus that includes more than 100 species. *Etlingera* consists of terrestrial and evergreen herbs and grows from sea level to the altitude of 2500 m (Poulsen 2006). The bioactivity from different parts of various *Etlingera* species such as *E. brevilabrum, E. elatior*, *E. rubrostriata*, *E. littoralis*, *E. fulgens* and *E. maingayi* have been reported in literature (Abdelwahab et al. 2010; Chan et al. 2007; Lachumy et al. 2010; Mahdavi et al. 2013, 2012; Sulaiman et al. 2011; Wijekoon et al. 2011). In this study, we measured the antioxidant and antimicrobial activities of the rhizomes, stems and leaves of *E. sayapensis* which were extracted by using ethyl acetate, acetone, ethanol and methanol.

Materials and Methods

Plant Parts

The studied plant parts were the rhizomes, stems and leaves of *Etlingera sayapensis*. They were collected on May 2011 from its natural habitat in Sabah, Malaysia. The plant species was identified by Mr. Sani Miran, a botanist from Universiti Kebangsaan Malaysia. A voucher specimen of WAY 503 for the plant was deposited at the Universiti Kebangsaan Malaysia Herbarium (UKMB).

Plant Extracts

The dried parts of *Etlingera sayapensis* which included rhizomes (100 g), stems (150 g) and leaves (150 g) were ground and extracted with different solvents, namely; ethyl acetate, acetone, ethanol and methanol. The powder formed was then macerated non-consecutively in each solvent at room temperature for 72 h. After filtration, the solvents were evaporated under reduced pressure using Heidolph evaporator (Laborota 4000 eco).

Antioxidant Activity Assays

The procedures discussed next are based on methods previously described (Mahdavi et al. 2013).

Determination of Total Phenolic Content

A 0.5 mL of FCR (10% in distilled water) was added to a vial containing 0.5 mL of each extract (1000 μg/mL in methanol) and 1.5 mL of distilled water; the mixture was then shaken vigorously. After 5 min, 2 mL of 10% sodium carbonate solution was added and the mixture was shaken again. The mixtures were incubated in the dark for 2 h at room temperature. The absorbance was measured at 760 nm with the Varian (Cary 50 conc) UV-vis. spectrophotometer. The analyses were carried out in triplicate. The total phenolic content was estimated as gallic acid equivalent (GAE), that is, mg of gallic acid per gram of extract (mg GAE/g extract).

Determination of Radical Scavenging Activity

A 1.5 mL aliquot of each extract in methanol at 20, 100, 500 and 1000 μg/mL, respectively, were added to 1 mL of 0.1 mM DPPH in methanol. The mixture was shaken for 1 min and allowed to stand in the dark for 90 min at room temperature; the absorbance was read at 517 nm. Positive controls of AscA, BHT, GA and Toc were used. All measurements were carried out in triplicate over 3 days. The radical scavenging activity (RSA) was calculated according to the following equation:

$$RSA\% = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control (DPPH solution without extract); and A_s is the absorbance of the extract (extract with DPPH solution).

Determination of Antioxidant Activity with the Β-Carotene Bleaching Assay

A 5 mL of β-carotene solution in chloroform (1 mg/mL) was added to a flask containing 50 μL of linoleic acid and 500 μL of Tween 40. The chloroform was evaporated under vacuum at 45°C for 10 min, then 125 mL of oxygenated water was added and the mixture was vigorously shaken to form an emulsion. 2.5 mL of the emulsion was then added to a 0.2 mL of extract solution (1000 μg/mL in methanol) and the absorbance was immediately read at 470 nm. The mixtures were incubated at 50°C; the absorbance was measured at 45 min intervals up to 180 min. All measurements were carried out in triplicate. Using the following formula, the antioxidant activity (AA) was evaluated in terms of bleaching of β-carotene:

$$AA\% = [1-(A^s_0 - A^s_t)/(A^c_0 - A^c_t)] \times 100,$$

where A^s_0 and A^c_0 are absorbance of the extract and control (2.5 mL of the emulsion and 0.2 mL of methanol) at zero time; and A^s_t and A^c_t are absorbance of the extract and control after 180 min.

Ferrous Ion Chelating Ability Assay

A 50 μL $FeSO_4$ (2 mM) was added to a vial containing 1 mL of each extract in methanol at 2000 μg/mL and 2 mL of distilled water. The reaction was initiated by addition of 100 μL of ferrozine (5 mM); the reaction mixture was shaken well and incubated at room temperature for 10 min. The absorbance was measured at 562 nm. All

measurements were run in triplicate. Positive controls of EDTA (disodium salt), citric acid (CitA) and AscA were used. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_c - A_s)/A_c] \times 100,$$

where A_c is the absorbance of the control (contains 50 μL of the $FeSO_4$, 100 μL of the ferrozine and 1 mL of methanol); and A_s is the absorbance of the extract.

FERRIC-REDUCING ANTIOXIDANT POWER ASSAY

The ferric reducing antioxidant power (FRAP) of the extracts was carried out by reduction of Fe^{3+} to Fe^{2+} method as described previously with some modifications (Musa et al. 2011). To prepare the fresh FRAP reagent, 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tri(2-pyridyl)-*s*-triazine) in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ in the ratio of 10:1:1 were mixed. 3,900 μL of the FRAP reagent was warmed at 37°C and mixed with 100 μL of the samples (1000 μg/mL), standard (0.25 - 250 μg/mL), or extraction solvent (as reagent blank). After 30 min, the absorbance was measured at 595 nm wavelength. All measurements were run in triplicate. The FRAP value was estimated as Trolox equivalent (TE), that is, mg of Trolox per gram of extract (mg TE/g extract).

ANTIMICROBIAL ACTIVITY ASSAYS

The procedures discussed next are based on methods previously described (Mahdavi et al. 2012).

MICROORGANISMS

The test bacterial strains of six Gram-positive namely *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 11774, *Bacillus thuringiensis* ATCC 10792, *Enterococcus faecalis* ATCC 14506, *S. epidermidis* ATCC 12228 and Methicillin Resistant *S. aureus* (MRSA) were obtained from the Microbiology Laboratory culture collection, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia and verified by standard microbiology method. Ten Gram-negative namely *Aeromonas hydrophila* ATCC 7966, *Enterobacter aerogenes* ATCC 13048, *Escherichia coli* ATCC 10536,*Proteus vulgaris* ATCC 33420, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 10145, *Salmonella typhimurium* ATCC 51812, *Serratia marcescens* ATCC 13880, *Shigella sonnei* ATCC 29930 and *Vibrio parahaemolyticus* ATCC 17802; and two *Candida* species of *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 were also tested.

DISC DIFFUSION ASSAY

Microorganism suspensions containing 10^8 CFU/mL were loaded on a sterile cotton swabs and streaked over the dried surface of Mueller-Hinton agar plates for inoculation. Sterile filter paper discs with 6 mm in diameter were impregnated with 20 μL (2×10 μL) of each extract (100 mg/mL) and then placed on the inoculated agar. The plates were incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the diameter of inhibition zone against the microorganisms. The inhibition zone was measured in millimeters including the disc diameter. All tests were carried out in triplicate. Chloramphenicol 30 μg and nystatin 30 μg were used as the positive controls. A disc which was impregnated with 20 μL of solvent was used as the negative control. For this assay, the extracts of the *Etlingera sayapensis* were dissolved in acetone or methanol.

MINIMUM INHIBITORY CONCENTRATION ASSAY

The 96-well plate (8×12 wells) was filled with 100 μL of the culture media Mueller-Hinton broth (MHB); the first well was charged with 100 μL of DMSO solution of the extracts; 100 μL from each of their serial dilutions was transferred into consecutive wells; each well was charged with 50 μL of the MHB and 50 μL of the bacteria or fungi inoculums described earlier. The final volume in each well was 200 μL with concentrations of 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 mg/mL for each extract and concentrations of 100, 50, 25, 12.5, 6.25, 3.12, 1.56 and 0.78 μg/mL for the positive controls (chloramphenicol against the bacteria and nystatin against the fungi). Each plate was used for one microorganism, respectively. Three wells in the last line of the plate were used as growth controls by filling the wells with 50 μL of inoculums and 150 μL of MHB; also three wells were used as negative control by filling them with 200 μL of MHB. The covered plates were incubated at 37°C for 24 h in an incubator. The turbidity of each well was then observed and recorded. The minimum inhibitory concentration was assessed as the minimum concentration that resulted in no visible growth. All tests were carried out in triplicate.

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVITY TOTAL PHENOLIC CONTENT

According to the results shown in Table 1, the polarity of extracting solvents [Ea (ethyl acetate), A (acetone), M (methanol) and Et (ethanol)] to some extent influence the values of the extracts [R (rhizomes), S (stems) and L (leaves)] total phenolic content (TPC). For each part, methanol and ethyl acetate extracts exhibited the highest and lowest TPC, respectively. The decreasing order of the TPC values for different parts/solvents were highest for the leaf extracts (LM, LA, LEt, LEa; 35.67±1.58, 31.33±1.93, 28.44±1.43, 26.47±0.67 mg GAE/g), medium for the stem extracts (SM, SA, SEt, SEa; 24.21±1.00, 21.28±1.03, 21.02±1.37, 15.85±0.97 mg GAE/g), and lowest for the rhizome extracts (RM, RA, REt, REa; 23.88±0.19, 14.12±0.35, 12.03±0.689.31±1.34 mg GAE/g).

Our previous measurements on the TPC of stem and leaf ethanolic extracts from *Etlingera brevilabrum* (31.08±0.76 and 12.82±0.86 mg GAE/g) (Mahdavi et al. 2013) showed higher and lower TPC compared to the respective parts from *E. sayapensis* (21.02±1.37 and 28.44±1.43). Andarwulan et al. (2010) reported the TPC of the leaf ethanolic extract of *E. elatior* from Indonesia with value of 0.81±0.10 mg GAE/g which was much lower compared to the similar parts of *E. sayapensis* and *E. brevilabrum*. The TPC values of the leaf methanolic extracts of five *Etlingera* species obtained by Chan et al. (2007) were in decreasing order of *E. elatior*, *E. rubrostriata*, *E. littoralis*, *E. fulgens* and *E. maingayi* (23.90±3.29, 22.50±1.13, 21.50±0.94, 12.80±1.44, and 11.10±0.93 mg GAE/g). Our measured TPC of the leaf methanolic extract of *E. sayapensis* was far higher (35.67±1.58 mg GAE/g) than the five mentioned above.

RADICAL SCAVENGING ACTIVITY

The radical scavenging activity (RSA) values of the non-consecutive extracts from three different parts of *Etlingera sayapensis* are shown in Table 1. The leaf, steam and rhizome extracts obtained with extracting solvent from the four showed the respective highest, medium, and lowest activity. For all the extracts, RSA results are concentration-dependent. The IC_{50} of different extracts for each plant part were also shown to be dependent on the solvent polarity; they were in decreasing order of methanol, ethanol, acetone and ethyl acetate. The lowest and highest IC_{50} belonged to the leaf methanolic extract (53.43±6.63 μg/mL) and rhizome ethyl acetate extract (534.99±8.57 μg/mL), respectively. All the extracts showed lower RSA activity than the positive controls of BHT, AscA and GA. The measured IC_{50} for the leaf ethanolic extract of *E. sayapensis* (84.55±7.29 μg/mL) was more active than the same reported previously from some of *Etlingera* species of *E. rubrostriata*, *E. littoralis*, *E. fulgens* and *E. brevilabrum* (90, 120, 160 & 264 μg/mL, respectively) but less active than that of *E. elatior* (80 μg/mL) (Chan et al. 2007; Mahdavi et al. 2013). The rhizome methanolic extracts from *E. elatior* and *E. maingayi* exhibited very low RSA (with estimated IC_{50} values for both being 2000 μg/mL) (Chan et al. 2008).

ANTIOXIDANT ACTIVITY WITH THE β-CAROTENE BLEACHING ASSAY

The β-carotene bleaching (BCB) results for the 12 extracts of *Etlingera sayapensis* as shown in Table 1 shows that increasing the solvent polarity in non-consecutive extraction leads to higher ability of the extracts to inhibit BCB. All the methanolic extracts of rhizomes, stems, and leaves (73.45±2.22, 73.29±2.49, 83.24±2.36%) were more active than the positive controls of AscA, GA and BHT but less active than α-tocopherol. Chan et al. (2007) found that for the leaf methanolic extracts, the BCB activity was in the order of *E. maingayi* > *E. elatior* > *E. rubrostriata* > *E. littoralis*. The leaf extract of *E. elatior* was more active than the rhizome extract. The BCB activity of the leaf methanolic extract of *E. sayapensis* was higher than *E. rubrostriata* and lower than *E. elatior*. The BCB activity of the leaves and stems of *E. sayapensis* was more than those of *E. brevilabrum* that were reported previously (Mahdavi et al. 2013). According to the literature, there was no correlation between DPPH radical scavenging and BCB activity since their reaction mechanisms are different. This may be due to additive, antagonistic or synergistic effects between the components in the complex chemical composition of the plant extracts (Chan et al. 2007; Lim & Quah 2007). However in the case of *E. sayapensis* extracts, there seems to be correlation between DPPH radical scavenging and BCB activity.

FERROUS ION CHELATING ABILITY

The ferrous ion chelating (FIC) ability of the *Etlingera sayapensis* extracts is shown in Table 1. For each plant part, the FIC ability decreased with decreasing polarity of solvents used in the extraction in order of methanol, ethanol, acetone and ethyl acetate. This indicates that the more polar solvent provided the extracts with compounds of higher FIC ability. All the extracts possessed higher ability in FIC than the positive controls of AscA and CitA but less than EDTA. The FIC ability of the ethanolic extracts from the leaves and stems of *E. sayapensis* (IC_{50}=249.23±8.61, 315.63±3.35 μg/mL) (these data are for leaves and stem of *E.sayapensis,* respectively) were more than those of leaves and stems of*E. Brevilabrum* (IC_{50}=267.65±4.79, 279.49±5.32 μg/mL) (Mahdavi et al. 2013). Comparison of our results with those obtained by Chan et al. (2007) indicated that the FIC ability of the leaf methanolic extracts of *E. sayapensis* and *E. brevilabrum* are more than and in the order of *E. maingayi* > *E.* fulgens >*E. elatior* > *E. littoralis*>*E. rubrostriata*. The rhizome extracts of *E. maingayi* and *E. elatior* also showed weaker FIC ability than those of *E. sayapensis*. However, the other Zingiberaceae species of *Curcuma longa*, *Kaempferia galanga*, *Alpinia galanga* and *Zingiber spectabile* showed higher FIC ability than that of *E. sayapensis* and *E. brevilabrum* (Chan et al. 2008).

FERRIC-REDUCING ANTIOXIDANT POWER

According to the results in Table 1, within similar extraction solvents, the leaf extract of *Etlingera sayapensis* showed the highest ferric-reducing antioxidant power (FRAP) value followed by the stem extract and then the rhizome extract. For all the parts, the polarity of the extracting solvents strongly influenced the FRAP values; so that the decreasing order was methanol > ethanol> acetone > ethyl acetate. In our previous study, we found out that the leaf ethanolic extract of *E. brevilabrum* showed higher reducing power than the base and stem extracts (37.55, 4.56 and 3.13 mg AscAE/g) (Mahdavi et al. 2013). Chan et al. (2007) reported the FRAP values of the leaf extracts from some *Etlingera* species in decreasing order of *E. elatior*> *E. rubrostriata*> *E. littoralis*> *E. fulgens*> *E. maingayi*; with

respective values of 19.6, 16.6, 11.6, 9.4 and 4.9 mg GAE/g. Andarwulan et al. (2010) reported of 37.2 μmol TrE/gas the FRAP value for the Indonesian *E. elatior.*

ANTIMICROBIAL ACTIVITY

The antimicrobial activity of the extracts from the rhizomes, stems and leaves of *Etlingera sayapensis* obtained using disc diffusion and minimum inhibitory concentration assays is shown in Table 2. These results illustrate the weak activity of two active microbes (out of 18 tested) against two active ethanolic and methanolic extracts (out of four used for each plant part). These extracts specifically prevent the bacterial growth of *Bacillus subtilis* with weak inhibition zones compared to that of the positive control. The related MIC results demonstrated that the leaf methanolic extract with value of 1.17 mg/mL was the most active extract. For the antifungal activity, only the stem methanolic extract gave inhibition against specific *Candida parapsilosis* with moderate inhibition zone of 9.9 mm and MIC value of 1.04±0.45 mg/mL. The antimicrobial activity of *E. sayapensis* was more significant than the activity of the extracts from different parts of *E. brevilabrum* obtained in our previous study. *Etlingera brevilabrum* extracts inhibited the growth of Gram-positive bacteria MRSA, *S. aureus*, *S. epidermidis*, *B. thuringiensis* and Gram-negative bacteria *A. hydrophila*, *V. parahaemolyticus* and *S. sonnei* (Mahdavi et al. 2012). Comparing the antimicrobial activities with other *Etlingera* species showed that the leaf methanolic extracts of *E. elatior, E. fulgens*, *E. maingayi*, *E. rubrostriata* and *E. littoralis* moderately prevented the growth of *S. aureus* strain (Chan et al. 2007).

CONCLUSION

According to the results of this study, the leaf extracts of *Etlingera sayapensis* showed the highest antioxidant activity followed by the stem and then the rhizome extracts. It should be noted that the polarity of the solvent extraction exerts a direct effect on the antioxidant activity of the extracts, thus the methanolic and ethyl acetate extracts exhibited the highest and lowest antioxidant activity, respectively. Although the leaf methanolic extract showed lesser RSA than all the positive controls, but the extract showed more FIC than those of AscA and CitA and also more BCB than those of BHT, GA and AscA. Among the extracts used and microbes tested, only the ethanolic, methanolic extracts of the four for each plant part and only *B. subtilis* and *C. parapsilosis* of the 18 were found to be moderately to weakly active in antimicrobial activity.

TABLE 1. Total phenolic content (TPC), DPPH radical scavenging activity (RSA), β-carotene bleaching (BCB), ferrous ion chelating ability (FIC), and ferric reducing antioxidant power (FRAP) of different extracts of rhizomes (R), stems (S) and leaves (L) of *Etlingera sayapensis* obtained by using Ea (ethyl acetate), A (acetone), M (methanol), and Et (ethanol) against standard of butylated hydroxytoluene (BHT), gallic acid (GA), α-tocopherol (Toc), ascorbic acid (AscA), citric acid (CitA) and EDTA

Extract	TPC mg GAE/g extract	RSA IC_{50} (μg/ml)	BCB (%)	FIC IC_{50} (μg/ml)	FRAP mg TrE/g extract
Rhizome					
REa	9.31±1.34d	534.99±8.57d	41.10±0.71d	437.68±8.49d	2.91±0.35d
RA	14.12±0.35b	519.24±3.50c	54.34±0.66c	363.59±11.63c	4.33±0.75c
REt	12.03±0.68c	438.72±11.32b	56.19±0.43b	323.50±8.08b	6.56±0.57b
RM	24.21±1.00a	353.18±7.70a	73.45±2.22a	227.44±4.88a	9.24±0.65a
Stem					
SEa	15.85±0.97c	440.10±11.89c	42.27±1.26d	443.10±8.576d	3.81±0.24c
SA	21.28±1.03b	307.81±8.30b	59.44±1.17c	384.96±5.39c	6.59±0.91b
SEt	21.02±1.37b	309.86±5.01b	66.89±3.13b	315.63±3.35b	8.31±0.90b
SM	23.88±0.19a	285.42±7.45a	73.29±2.49a	284.17±4.32a	11.68±1.47a
Leaf					
LEa	26.47±0.67d	184.23±11.64d	72.75±3.27c	386.67±9.39d	7.46±0.53c
LA	31.33±1.93b	129.53±6.66c	80.04±4.23b	322.60±6.30c	11.67±1.12b
LEt	28.44±1.43c	84.55±7.29b	83.05±2.93a	249.23±8.61b	13.63±0.70b
LM	35.67±1.58a	53.43±6.63a	83.24±2.36a	242.43±4.91a	19.53±2.70a
Standard					
BHT	-	14.89±0.89b	71.99±4.44	-	-
GA	-	7.86±1.23a	44.24±1.33	-	-
Toc	-	-	87.90±2.53	-	-
AscA	-	40.28±1.06c	21.83±2.04	1426.64±67.43	-
CitA	-	-	-	1446.78±88.25	-
EDTA	-	-	-	67.23±5.21	-

*Values are presented as means ± SD (*n*=3). Means with different letters are significantly different in each column for any part and standards

TABLE 2. Antimicrobial activity of the rhizome (R), stem (S), and leaf (L) extracts of *Etlingera sayapensis* obtained from Ea (ethyl acetate), A (acetone), M (methanol) and Et (ethanol) using disc-diffusion (DD) and minimum inhibition concentration (MIC) assays against 16 bacterial and two fungal strains[a]

Extract	*Bacillus subtilis*		*Candida parapsilosis*	
	DD (mm)	MIC mg/mL	DD (mm)	MIC mg/mL
Rhizome				
REt	9.2±0.7[b,c]	6.25±0.00	-	-
RM	11.0±0.7	2.60±0.90	-	-
Stem				
SEt	8.9±0.5	8.33±3.61	-	-
SM	9.4±0.6	4.16±1.81	9.9±0.5	1.04±0.45
Leaf				
LEt	7.9±0.7	6.25±0.00	-	--
LM	9.6±0.6	1.17±0.67	-	-
Controls				
Positive[d]	28.9±0.8	0.02±0.01	14.1±0.4	0.02±0.01
Negative[e]	-	-	-	-

a: Others are: *Staphylocoocus aureus*, *Bacillus thuringiensis*, *Enterococcus faecalis*, *S. epidermidis*, Methicillin Resistant *S. aureus* (MRSA), *Aeromonas hydrophila*, *Enterobacter aerogenes*, *Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhimurium*, *Serratia marcescens*, *Shigella sonnei, Vibrio parahaemolyticus* and *Candida albicans*; b: Values are presented as means ± SD (n = 3); c: size of discs (6 mm), non-active; d:Chloramphenicol (disc:30 µg) for bacteria and nystatin (disc:30 µg) for fungal; e:Solvent

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