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Original Research Article

Antioxidant and antimicrobial activities of aqueous & ethanol crude extracts of 13 Thai traditional plants

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A B S T R A C T

Aqueous and ethanol crude extracts from thirteen Thai traditional plants were screen for their total phenolic contents, antioxidant and antibacterial properties. Folin-Ciocalteu method was used to quantify total phenolic contents. The antioxidant activities were evaluated using antioxidant assay kit and antimicrobial activities against four indicator strains were tested by disc diffusion and broth dilution method. The highest total phenolic contents and antioxidant activity obtained from aqueous crude extracts of *Syzygium cumini* (L.) Skeels was at 358.250±0.014mgGAE/g_{dw} and 332.425±0.21mMTEAC/g_{dw}, respectively. *Piper betle* Linn. ethanol crude extracts was found to have the highest phenolic contents of 474.083±0.005mgGAE/g_{dw}. *Anacardirm occidentale* Linn. ethanol crude extracts showed the highest antioxidant activity at 411.916±0.05mMTEAC/g_{dw}. Almost plants demonstrated the correlation between total phenolic contents and antioxidant capacity. The antimicrobial activity was found in seven aqueous crude extracts and two ethanol crude extracts. Aqueous crude extract of *Syzygium cumini* (L.) Skeels showed the highest inhibition zone against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* (MRSA) at the concentration of 100mg/ml. Minimal inhibitory concentration (MIC) and minimal bacteriocidal concentration (MBC) of *Syzygium cumini* (L.) Skeels aqueous crude extracts against *S. aureus* and MRSA were 6.25 and 12.5mg/ml, respectively. Thai traditional plants, especially *Syzygium cumini* (L.) Skeels. and *Piper betle* Linn. were possessed high phenolic contents, antioxidant and antimicrobial activities which demonstrated that all these plants can be potential sources of natural products for side dishes, dietary supplement product and medicinal uses.

Keywords

Antimicrobial activity; antioxidant activity; phenolic contents; Thai traditional plants

Introduction

Phenolic compounds are secondary metabolites which synthesize in plants. They possess beneficial biological properties such as antioxidant, anti-

apoptotic activities, anti-aging, anticarcinogen, anti-inflammation, anti-artherosclerosis, cardiovascular protection, improvement of the endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological actions have been attributed to their intrinsic reducing capabilities (Han *et al*., 2007). Several studies have indicated that the antioxidant activities of some fruits, vegetables and herbs were highly correlated with their total phenolic contents. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). Antioxidants compounds can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*., 1998; Emmons *et al*., 1999).

Plants, which are rich in phenolic components, are of interest as sources of natural antioxidants. Phenolics display a wide variety of structures, ranging from simple moieties containing a single hydroxylated aromatic ring to highly complex polymeric substances (Delgado-Adamez *et al*., 2012). The biosynthesis of phenolic compounds and related substances is derived from some proteins, including tyrosine and tryptophan in the shikimic acid pathway. In addition, the phenolic usually occur in bound form such as flavonoid glycosides and phenolic acid derivatives, which are synthesized from sugar. More than 3,000 plant species including Thai traditional plants have been documented to have high phenolic contents, antioxidant properties, antimutagenic properties (108 species) (Trakoontivakorn *et al*., 2001; Nakahara *et al*., 2002), and activity related to inflammation (four Thai plants) (Laupattarakasem *et al*., 2003), which were related to their antioxidant properties. Phenolics which occur in plant respiration and other phenolics also arise from this pathway and subsequent reaction such as cinnamic, p-coumaric, caffeic, furulic, chlorogenic, protocatechuic and gallic acids. These are derived from phenylalanine and tyrosine, which are amino acids. There are several processes the translocation carbohydrate from leave to various sink organs during photosynthesis (Maisuthisakul *et al*., 2008).

In addition to the properties of plants, people are taking it as a choice for treatment of infection disease. Normally, both of gram-positive and gram-negative bacteria are food poisoning originating from contaminated foods which causes concern to society and to industry. A major problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the insufficiency of antimicrobial treatment (Valero & Salmeroj, 2003). Especially for Methicillin-resistant *Staphylococcus aureus* infections can cause a broad range of symptoms depending on parts of the body that are infected. Therefore, three strains of human pathogenic bacteria, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, and one of resist strain, Methicillin-resistant *Staphylococcus aureus*, are proposed for the test of new antimicrobial substances from various sourced as novel antimicrobial chemotherapeutic agents and the biologically active compounds derived from plants . The aimed of this study was to evaluate the *in vitro* of total phenolic contents, antioxidant capacity and

antibacterial activity of thirteen Thai traditional plants. These data should be useful for screening plants as potential sources and safe natural antioxidants and antibacterial compounds.

Materials and Methods

Preparation of crude extracts

Thirteen Thai traditional plants in Table 1 were collected during March-May in 2012 from different natural habitats at Nakhon Si Thammarat, Thailand. The fresh leaves or fruits were rinsed with distilled water. Aqueous crude extracts were prepared using 100g of fresh leaves, chopped and homogenized in 150ml of distilled water for 1min. Then, the suspension was filtered by Whatman No.1 and then lyophilized using freeze dryer at –20^{o}C for 20hrs. The powder was stored at 4^{o}C until used. To prepare ethanol crude extracts, leaves were air dried and ground to fine powder. 100g of samples were soaked in 500ml of 95% ethanol at room temperature for a weeks, the supernatant was filtered and evaporated by vacuum rotary evaporator at 50^{o}C. The samples were kept at 4^{o}C until used.

Preparation of crude extracts for antimicrobial activity

The crude extracts were dissolved with the same extraction solvent (distilled water or ethanol), centrifuged at 6,000rpm for 30min at room temperature and then filtered with sterilized pyrogen free membrane No. 0.22µm. The MIC and MBC values were evaluated and two-fold serial dilutions method was used (Zaidi *et al*., 2009). Final concentrations of the extract were 100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.20mg/ml. Four environmental isolated bacterial strains [*Staphylococcus aureus* (SA), Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (EC) and *Pseudomonas aeruginosa* (PA)] were supplied by Microbiology Laboratory of Science and Technology Faculty of NSTRU University, Nakhon Si Thammarat, Thailand. Bacterial were maintained on nutrient agar (NA).

Determination of total phenolic contents and antioxidant activity

The amount of total phenolics in aqueous/ethanol extract of thirteen Thai traditional plants were determined with the Folin-Ciocalteu reagent (Lister & Wilson, 2001) as modified by this study. Briefly, 20µl of sample was added into 100µl of 2N Folin-Ciocalteu's reagent and incubated at room temperature for 5min. Then, 300µl of Na_2CO_3 (25% w/v) was added and incubated at 45^{o}C for 30min. The absorbance was read at 765nm using UV–visible spectrophotometer. Results were expressed as milligram of gallic acid equivalent per gram of dry weight (mgGAE/g_{dw}). The antioxidant capacity of water/ ethanol extracts was determined by antioxidant assay kit (Sigma, St Luis, USA) and expressed in millimole of trolox equivalent per gram of dry weight (mM TEAC/g_{dw}). The total phenolic content and antioxidant activity of thirteen Thai traditional plants were obtained from three separated experiments and values are expressed as mean±S.E.M.

Antimicrobial activity

Disc diffusion assay and broth dilution assay were used in this study. For the first assay, the extracts were subjected by disc diffusion assay (Jorgensen *et al*., 1999) with minor modifications. Briefly, bacterial strains were grown in trypticase

soy broth (TSB) at 37°C for 16hrs and cells were suspended in TSB to get 10^6cfu/ml by using McFarland No. 0.5. Each bacterial test strains were swab onto Mueller- Hinton agar (MHA) medium. Then, 6mm diameter filter paper discs with 50µl of various concentrations of crude extracts were placed onto MHA. After incubation at 37°C for 24hrs, the antibacterial activity was measured in the diameter (mm) of clear zone of growth inhibition. Aqueous or ethanol was used as negative control. Vancomycin (320µg/ml, 50µl/disc) and gentamycin (40µg/ml, 50µl/disc) were used as positive reference standard drug to determine the sensitivity of gram positive and gram negative bacteria, respectively. The broth dilution assay were used for initial screening, the extracts that were shown no visual growth of bacterial colonies compared to positive control were further evaluated for MIC and MBC values. 1ml of crude extract was two-fold serial diluted in TSB. The bacterial culture (10^6cfu/ml) was added into the mixture as the equal volume and incubated at 37°C for 24hrs. Vancomycin and gentamycin were used as positive drug references. After incubation, the concentration of last tube that showed no bacterial growth was determined as MIC values. To evaluate the MBC values, 0.1ml from the tube which absents of the bacterial growth was spread on trypticase soy agar (TSA) and incubated at 37°C for 24hrs. The MBC was defined as the test samples which there were less than 5 colonies of bacterial at the latter cultivation.

Results and Discussion

Total phenolic content and antioxidant activity

The highest total phenolic content of thirteen species of plants was found in the aqueous crude extract of *Syzygium cumini* (L.) Skeels. and the ethanol crude extract of *Piper betle* Linn. at 358.250±0.014 and 474.083±0.005mgGAE/$g_{dw,}$ respectively. The highest antioxidant in aqueous and ethanol crude extract was found in *Syzygium cumini* (L.) Skeels. and *Anacardirm occidentale* Linn. at 332.425±0.21 and 411.916±0.05mM TEAC/g_{dw}, respectively (Table 2).

The correlation between the antioxidant activity and total phenolic contents was determined. The antioxidant capacity of aqueous crude extracts appears to be largely influenced by the contents of total phenolic compounds (r^2=0.899) than ethanol crude extracts (r^2=0.852) (Fig. 1). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma and flavor and also in providing health benefit effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive, prevent molecular damage and disrupt by microorganisms, insect, and herbivores (Vaya et al., 1997). However, the correlation between phenolic compounds and antioxidant activities are not clear. In this study, almost of the extracts were correlation between total phenolic content and antioxidant activity which depend on total phenolic and flavonoid compounds, whereas the total phenolic compounds correlated weakly with other components except flavonoid contents (Maisuthisakul *et al*., 2008). The correlation results which were not related in some plants may possibly due to the presence of some other phytochemicals such as ascorbic acid, tocopherol and pigments as well as the synergistic effects among them, which also contribute to the total antioxidant capacity and using of Folin-Ciocalteu methods is not an absolute measurement of the amount of phenolic materials even it

Table. 1 Thirteen species of Thai traditional plants

Scientific name	Family	Traditional name	Part of use
Anacardirm occidentale Linn.	Anacardiaceae	Yaruang	Leaves
Clausena cambodiana Guill.	Rutaceae	Samui	Leaves
Ficus racemosa L.	Moraceae	Maduar	Fruits
Glochidion wallichianum Muell.	Euphorbiaceae	Munpu	Leaves
Litsea petiolata.	Lauraceae	Tummung	Leaves
Ocimum basilicum Linn.	Labiatae	Horapa	Leaves
Ocimum canum Sims.	Lamiaceae	Mangluk	Leaves
Piper betle Linn.	Piperaceae	Plu	Leaves
Piper sarmentosum Roxb.	Piperaceae	Chaplu	Leaves
Pseuderanthemum palatiferum (Nees) Radlk.	Acanthaceae	Phrayavanon	Leaves
Spondias pinnata Kurz.	Anacardiaceae	Makok	Leaves
Syzygium cumini (L.) Skeels.	Myrtaceae	Wa	Leaves
Syzygium gratum (Wight) S.N. Mitra var. gratum	Myrtaceae	Samedshun	Leaves

Table.2 Total phenolic contents and antioxidant activity of thirteen Thai traditional plants.

Thai traditional plants	Phenolic content (mgGAE/g_{dw})		Antioxidant (mMTEAC/g_{dw})	
	Aqueous	Ethanol	Aqueous	Ethanol
Anacardirm occidentale Linn.	99.917±0.003	305.750±0.008	63.177±0.12	411.916±0.05
Clausena cambodiana Guill.	130.750±0.001	38.250	4.921±0.30	9.274±0.13
Ficus racemosa L.	317.417±0.010	ND	209.630±0.16	ND
Glochidion wallichianum Muell.	163.250±0.003	225.750±0.004	160.572±0.10	341.055±0.15
Litsea petiolata	183.250±0005	44.917±0.001	58.165±0.05	6.912±0.04
Ocimum basilicum Linn.	4.083±0.001	8.250±0.001	2.430±0.06	ND
Ocimum canum Sims.	ND	20.750	ND	ND
Piper betle Linn.	25.750±0.002	474.083±0.005	8.759±0.01	12.491±0.025
Piper sarmentosum Roxb.	14.083±0.006	35.750±0.001	ND	ND
Pseuderanthemum palatiferum (Nees) Radlk.	30.750±0.003	29.083±0.001	7.926±0.01	ND
Spondias pinnata Kurz.	33.250±0.002	70.750±0.005	13.968±0.05	21.099±0.19
Syzygium cumini (L.) Skeels.	358.250±0.014	75.750±0.001	332.425±0.21	25.664±0.06
Syzygium gratum (Wight) S.N. Mitra var. gratum.	111.583±0.001	84.917±0.001	38.375±0.16	5.731±0.16

Values are mean±S.E.M. of triplicates and ND is undetected of phenolic compound and antioxidant activity.

was claimed to be the best method for determine the amount of total phenolic content which was published on the screening of natural antioxidants (Spigno *et al*., 2007). The extracts from plants possibly contain different types of phenolic compounds, which have different antioxidant capacities (Javanmardi *et al*., 2003; Sengul *et al*., 2009). This able to suggests that phenolic compounds do not make a major contribution to the antioxidant activity of the extracts. Therefore, this results of some plants were similar to the report that no correlation between the antioxidant activity and total phenolic contents (Modorresi & Shaida Fariza Sulaiman, 2009).

Antimicrobial activity

The antimicrobial activities of thirteen Thai traditional plants were tested by disc diffusion assay at different concentrations (100, 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 and 0.20mg/ml). Seven species of aqueous crude extract showed the inhibitory activities against the tested bacteria. *Syzygium cumini* (L.) Skeels. at 100mg/ml was showed the highest diameter of inhibition zone at 12.48mm against *Staphylococcus aureus*. The antimicrobial activity of *Syzygium cumini* (L.) Skeels. against Methicillin-resistant *Staphylococcus aureus* was ranged from 8.46 to 13.85mm which was more sensitive than *Staphylococcus aureus*. The ethanol crude extracts of *Anacardirm occidentale* Linn. and *Piper betle* Linn. were possessed the highest antimicrobial activity against all tested strains. The ethanol crude extracts of *Piper betle* Linn. was inhibited all indicator strains tested and at 100mg/ml was showed the highest inhibition zone against *Staphylococcus aureus* and gave the diameter ranged from 9.00 to 22.06mm at the concentration of 12.5 to 100mg/ml, respectively. The ethanol crude extract of *Piper betle* Linn. revealed the sensitivity to Methicillin-resistant *Staphylococcus aureus* and also showed the highest inhibition zone at the concentration of 100mg/ml. Moreover, at the concentration of 50 and 100mg/ml were inhibited the growth of both *Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activities of aqueous and ethanol crude extract of thirteen species Thai traditional plants were presented in Table 3.

Antioxidant substances, hydroxyl (^{o}OH) group in phenolic compounds were claimed as a largely responsible for their antioxidant and antimicrobial actions. The potentially antimicrobial mechanisms of phenolic compounds include the interruption of function of bacterial cell membranes and disrupt enzyme system. The ^{o}OH groups in phenolic compounds are highly reactive under aqueous conditions and react with several biomolecules, causing deformation of these molecules, which results in retardation of growth and bacterial growth (Kim *et al*., 2013). According to our results, the aqueous and ethanol crude extracts of *Syzygium cumini* (L.) Skeels. were shown the highest total phenolic contents and antibacterial activity against *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus*. In Brazil, the bark, fruits, seeds and leaves of *Syzygium cumini* (L.) Skeels. were used for the treatment of diabetes and administered in various pharmaceutical preparations (Braga *et al*., 2007). Seeds of *Syzygium cumini* (L.) Skeels. possesed hypoglycemic and antioxidant activities. Bark is also used for dysentery and diarrhea. Moreover, *Syzygium cumini* (L.) Skeels. had sedative and anticonvulsant

Table.3 Antimicrobial activities of aqueous and ethanol extracts of thirteen Thai traditional plants by disc diffusion assay

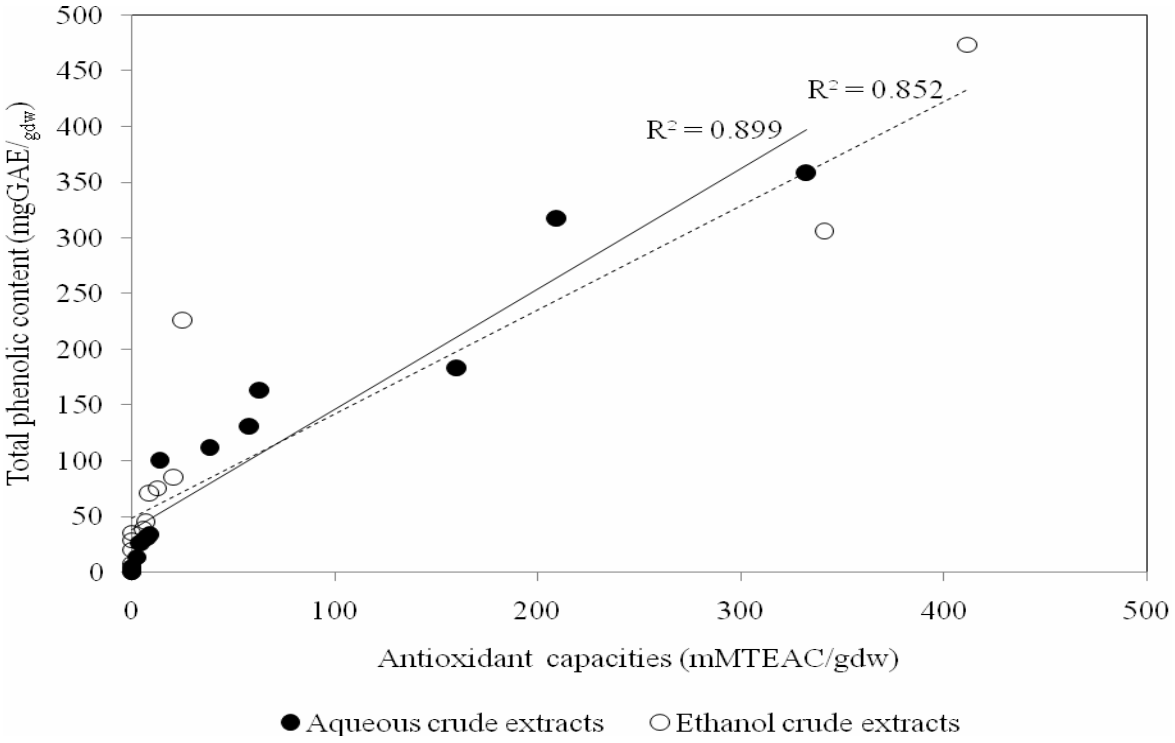
Crude extracts	Bacterial indicator	Diameter of inhibition zone (mm) (50µL/disc)					
		Concentration of crude extracts (mg/mL)					
		100	**50**	**25**	**12.5**	**6.25**	**3.125**
Negative control Distilled water	SA	-					
	MRSA	-					
	EC	-					
	PA	-					
Ethanol	SA	-					
	MRSA	-					
	EC	-					
	PA	-					
Positive Control Vancomycin (320 µg/mL.)	SA	14					
	MRSA	13.5					
Gentamycin (30 µg/mL.)	EC	21.5					
	PA	24.2					
Aqueous extract *Anacardirm occidentale* Linn.	SA	8.3	7.35	6.26	-	-	-
	MRSA	8.78	7.94	6.88	-	-	-
	EC	7.42	-	-	-	-	-
	PA	8.76	7.25	-	-	-	-
Clausena cambodiana Guill.	SA	6.10	-	-	-	-	-
	MRSA	6.10	-	-	-	-	-
Ficus racemosa L.	SA	7.00	6.42	6.12	-	-	-
	MRSA	7.09	6.35	-	-	-	-
Glochidion wallichianum Muell.	MRSA	8.80	7.39	6.24	-	-	-
Litsea petiolata	SA	8.26	6.45	-	-	-	-
	MRSA	6.92	6.10	-	-	-	-
Spondias pinnata Kurz.	SA	7.39	-	-	-	-	-
	MRSA	7.75	-	-	-	-	-
	PA	6.10	-	-	-	-	-
Syzygium cumini (L.) Skeels.	SA	12.48	9.13	8.00	7.13	6.92	-
	MRSA	13.85	12.84	11.51	9.45	8.46	-
Ethanol extracts *Anacardirm occidentale* Linn.	SA	6.38	-	-	-	-	-
	MRSA	6.60	-	-	-	-	-
	EC	6.40	-	-	-	-	-
	PA	6.57	-	-	-	-	-
Piper betle Linn.	SA	22.06	16.52	14.20	9.00	-	-
	MRSA	16.53	16.47	15.50	10.06	-	-
	EC	11.00	8.00	-	-	-	-
	PA	9.22	7.25	-	-	-	-

- = no inhibition zone

Table.4 Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) of Thai traditional plants by broth dilution assay

Crude extracts	MIC (mg/mL)				MBC (mg/mL)			
	SA	**MRSA**	**EC**	**PA**	**SA**	**MRSA**	**EC**	**PA**
Aqueous extracts								
Anacardirm occidentale Linn.	25	25	100	50	25	25	100	100
Clausena cambodiana Guill.	100	100	-	-	100	100	-	-
Ficus racemosa L.	25	50	-	-	50	50	-	-
Glochidion wallichianum Muell.	-	25	-	-	-	25	-	-
Litsea petiolata	50	50	-	-	100	100	-	-
Spondias pinnata Kurz.	100	100	-	100	100	100	-	100
Syzygium cumini (L.) Skeels.	6.25	6.25	-	-	12.5	12.5	-	-
Ethanol extracts								
Anacardirm occidentale Linn.	100	100	100	100	100	100	100	100
Piper betle Linn.	12.5	12.5	50	50	12.5	12.5	50	50

Figure.1 Correlation between antioxidant capacities and total phenolic content of aqueous and ethanol crude extracts of thirteen Thai traditional plants.



effects and a potent central nervous system depressant effect (Pepato *et al*., 2004).

The highest total phenolic content and antibacterial activity of the ethanol crude extracts were found in *Piper betle* Linn. The leaves of the *Piper betle* Linn. was used in traditional medicine and possess antioxidant, antibacterial, antifungal, anti-diabetic, radioprotective and anti-allergic activity (Wirotesangthong *et al*., 2008). At Tunisia, they were evaluated on antimicrobial and antioxidant activities of some vegetables *in vitro*, they found that antioxidant and antimicrobial activities of aqueous extracts could be derived from their compounds such as flavonoids, polyphenol compounds and vitamin C (Edziri *et al*., 2012).

It is indicated that Thai traditional plants are rich in potential use as shown in a term of high phenolic contents, antioxidant and antimicrobial function that could be used to find out the new and effective drugs from natural plants. However, the toxicity of plant extracts should be tested to confirm their safety use and should be purified to clarify the pharmacological properties for pharmaceutical application.

Minimal inhibitory concentration (MIC) and Minimal bactericidal concentration (MBC) determination

The active extracts from disc diffusion assay were subsequently subjected for MIC and MBC. The MIC and MBC of active extract on different tested strains were shown in Table 4. The results indicated that *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus* were susceptible to the aqueous crude extract of *Syzygium cumini* (L.) Skeels. at MIC and MBC values of 6.25 and 12.5mg/ml, respectively. The ethanol crude extract of *Piper betle* Linn. was showed the highest activity against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at MIC of 12.5, 12.5, 50 and 50mg/ml, respectively and showed the activity against *S. aureus* and MRSA at MBC of 12.5mg/ml and against to *Escherichia coli* and *Pseudomonas aeruginosa* at MBC value of 50mg/ml.

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