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Full Length Research Paper

Antibacterial activities and phytochemical screening of the acetone extract from *Euphorbia hirta*

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Euphorbia hirta **is widely used as traditional medicine. The aim of the present study was to evaluate *E. hirta* parts and whole plants for the antibacterial activities plus phytochemical exist through acetone extracts. Antibacterial assay was carried out *via* agar well diffusion assay for screening purpose and finally through micro dilution method in order to determine minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) values against 10 selected bacteria. Gram positive bacteria were more susceptible compared to Gram negative bacteria *via* agar well diffusion assay. Whole plants *E. hirta* extracts very useful for inhibitory bacterium purpose. Whilst, all *E. hirta* extracts exhibited the bactericidal effect towards the ten bacteria tested. All bacteria tested susceptible to the roots extract based on the MBC/MIC value which is less than or equal to 4 (≤4). Phytochemical screening of *E. hirta* extracts revealed the presence of carbohydrates, lipids, protein, flavonoids, alkaloids, saponins, resins, steroids, acidic compounds, tannins, glycosides, phenols and terpenoids.**

Key words: *Euphorbia hirta*, antibacterial activities, agar well diffusion assay, micro dilution method, phytochemical screening.

INTRODUCTION

The emergence and spread of antibiotic resistant bacteria pose increasingly difficult therapeutic problems, particularly in hospitals. Intensive research efforts have been devoted to the development of alternative antimicrobial agents to combat this problem (Darwish et al., 2002). Plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies (Nascimento et al., 2000). The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and the environment (Joy et al., 2001). The characteristics of the plants that inhibit microorganisms are important for human health which have been researched in laboratories since 1926 (Ates and Erdogrul, 2003). Antimicrobial compounds from plants represent a potentially novel source of antimicrobial substances since they act against bacteria via mechanisms that are different from those of currently used antibiotics and may thus have a clinical value in the treatment of antibiotic-resistant antimicrobial strains (Eloff, 1998). The majority of antimicrobial plant compounds are identified as secondary metabolites, mainly being of terpenoid or phenolic biosynthetic origin. The rest are hydrolytic enzymes (glucanases and chitinases) and proteins acting specifically on membranes of invading microorganisms (Shafiur, 2007). Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Nascimento et al., 2000). *Euphorbia hirta* is widely used as a traditional medicine herb in all the tropical countries (Loh et al., 2009). This herb is categorized as anthropogenic herb which is commonly seen occupying open waste spaces, roadsides, pathways, and as a weed of cultivation (Adedapo et al., 2005). According to Upadhyay et al. (2010), different parts of *E. hirta* are used for curing various ailments. The aerial parts of the plant

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are harvested when in flower during the summer and dried for later use. The stem is used as a treatment for asthma, bronchitis and various other lung complaints. The whole plant is decocted and used in the treatment of athlete's foot, dysentery, enteritis, and skin conditions. Thus, these findings suggested that some components of *E. hirta* extracts are active in the treatment of various diseases and ailments. Recently, this herb is reported to show analgesic, anti-pyretic, anxiolytic, sedative, anti-inflammatory activities, inhibitory action on platelet aggregation, antimutagenicity (Loh et al., 2009) and antioxidant (Basma et al., 2011). In this context, *E. hirta* is the potential herb to identify the new drugs for the development of novel therapeutic agents in the antibiotic treatments. However the studies on the antibacterial properties of this herb are still limited. Therefore, this study was undertaken to investigate the antibacterial properties of *E. hirta*.

MATERIALS AND METHODS

Test bacteria

Five selected Gram positive bacteria were used in this study namely *Bacillus cereus* (ATCC 13061), *Bacillus subtilis* (ATCC 11774), *Enterococcus faecalis* (ATCC 12228) *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228). Whilst, five selected Gram negative bacteria used were *Klebsiella pneumoniae* (ATCC 13883), *Salmonella typhimurium* (ATCC 13331), *Shigella dysentriae* (ATCC 11311), *Shigella sonnei* (ATCC 9290) and *Serratia marcescens* (ATCC 13880). All the bacteria strains were obtained from Microbiology Laboratory, Faculty of Science and Technology, USIM. The bacteria were cultured in Mueller-Hinton broth (MHB) overnight at 37ºC. For determining the density of bacterial growth, biophotometer were used. The concentration of each bacterial inoculum was determined by referring to McFarland Standard Formula and then diluted to the concentration of 10^7cells/ml for further experiment.

Extraction of *Euphorbia hirta*

Fresh *E. hirta* were collected from Nilai, Negeri Sembilan at the middle of July 2012. The test sample preparation procedures were according to Ehsan et al. (2009) with modifications. Fresh plants were washed thoroughly two to three times with running tap water and then with sterile water followed with the separation of the plants into whole plants and parts needed (flowers, stems, leaves and roots), shade-dried for two weeks, powdered and finally used for extraction. 100 g of powder of plants were soaked with 1000 ml of acetone (extract/solvent ratio = 1:10 w/v) for seven to eight days at room temperature with frequent agitation. Following filtration of the suspension through a Buckner funnel and Whatman filter paper #1, the crude acetone extracts were evaporated in rotary evaporator at 40°C with 65 rpm. The crude extract obtained was then weighed and prepared for stock solution at a concentration of 1000 mg/ml by diluting a 1000 mg of crude extract in 1 ml of 99.5% Dimethylsulfoxide (DMSO). The stock solution was then preserved at 4°C in airtight bottle until further used.

Agar-well diffusion method

Agar-well diffusion assay was done based on Adegoke et al., (2010). Cultures of the bacteria were inoculated separately on the solidified Mueller Hinton agar (MHA) on each Petri dish by streaking using sterilized cotton swabs and allowed to set. Wells of 5 mm diameter and 5 mm depth were made in the solidified agar using a sterile borer. About 10 µl of test samples (1000 mg/ml) were dispensed into the wells and allowed to stand about 15 minutes for pre-diffusion of samples. As control, 10 µl of chloramphenicol at a concentration of 5 mg/ml (positive control) and 99.5% of DMSO (negative control) were also loaded into respective wells for each agar plates. The plates were then incubated at 37ºC for 24 hours. The sensitivity of the test bacteria to the extracts were determined by measuring the diameters of the zone of inhibition surrounding the wells in millimeter (mm). All the tests were performed in duplicate.

MIC and MBC value *via* microdilution method

Micro dilution method was done based Adegoke et al., (2010) with modifications. 100 µl of overnight bacterial inoculums was added into each wells of 96-well microtitre plate containing 100 µl of the test samples with the concentrations (ranging from 500 to 7.82 mg/ml) and chloramphenicol 5 mg/ml (positive control). The test samples (100 µl) at the selected concentrations without bacteria also added into other wells which were served as negative controls. Then, microtitre plates were further incubated for an overnight at 37°C. The wells were then observed for visible growth based on turbidity. MIC value was recorded as the lowest concentration that showed no bacterial growth (non-turbid). MBC value then determined by streaking onto the MHA plates each of the test samples from the MIC test wells with then incubated for an overnight at 37°C. The lowest concentration of test

Table 1. Agar well diffusion assay for test bacteria to the crude extracts of *E. hirta.*

Test bacteria	Zone of inhibition (mm) of *Euphorbia hirta* crude extracts[b]					
	Whole plant	**Flowers**	**Stems**	**Leaves**	**Roots**	**C5[a]**
B. cereus	11.00 ± 0.00	8.50 ± 2.12	12.00 ± 0.00	12.50 ± 0.71	13.50 ± 0.71	28.00 ± 0.00
B. subtilis	11.00 ± 0.00	12.50 ± 0.71	13.00 ± 0.00	15.00 ± 0.00	14.50 ± 0.71	31.00 ± 0.00
E. faecalis	11.50 ± 0.71	10.00 ± 0.00	12.00 ± 1.41	14.50 ± 0.71	11.50 ± 0.71	28.00 ± 0.00
S. aureus	12.50 ± 0.71	10.00 ± 0.00	13.00 ± 0.00	14.00 ± 1.41	13.00 ± 0.00	28.00 ± 0.00
S. epidermidis	11.50 ± 0.71	11.00 ± 0.00	12.00 ± 0.00	14.00 ± 0.00	13.00 ± 0.00	28.00 ± 0.00
K. pneumonia	6.50 ± 0.71	5.75 ± 0.35	6.00 ± 0.00	7.50 ± 0.71	5.35 ± 0.21	28.00 ± 0.00
S. typhimurium	6.00 ± 0.00	5.75 ± 0.35	6.00 ± 0.00	8.50 ± 0.71	6.00 ± 0.00	33.00 ± 0.00
S. marcescens	6.00 ± 0.00	7.00 ± 0.00	5.35 ± 0.21	10.00 ± 0.00	6.00 ± 0.00	24.00 ± 0.00
S. dysentriae	10.50 ± 0.71	10.50 ± 0.71	12.00 ± 0.00	13.00 ± 1.41	12.50 ± 0.71	31.00 ± 0.00
S. sonnei	9.50 ± 0.71	12.00 ± 0.00	11.00 ± 0.00	14.50 ± 0.71	13.00 ± 0.00	27.00 ± 0.00

[a] Chloramphenicol (5 mg/ml)
[b] (≥7mm) : Strains susceptible to the extracts; (<7mm) : Strains resistant to the extracts

Table 2. MIC values of E. hirta extracts.

Test bacteria	Minimum inhibition concentration values (mg/ml)				
	Whole plant	Flowers	Stems	Leaves	Roots
B. cereus	62.500 ± 0.000	62.500 ± 0.000	31.250 ± 0.000	125.000 ± 0.000	62.500 ± 0.000
B. subtilis	62.500 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	125.000 ± 0.000
E. faecalis	15.625 ± 0.000	31.250 ± 0.000	15.625 ± 0.000	62.500 ± 0.000	31.250 ± 0.000
S. aureus	31.250 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	62.500 ± 0.000
S. epidermidis	31.250 ± 0.000	62.500 ± 0.000	15.625 ± 0.000	62.500 ± 0.000	125.000 ± 0.000
K. pneumonia	31.250 ± 0.000	31.250 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	125.000 ± 0.000
S. typhimurium	62.500 ± 0.000	125.000 ± 0.000	125.00 ± 0.000	125.00 ± 0.000	250.000 ± 0.000
S. marcescens	62.500 ± 0.000	125.000 ± 0.000	125.00 ± 0.000	125.00 ± 0.000	125.000 ± 0.000
S. dysentriae	31.250 ± 0.000	62.500 ± 0.000	62.500 ± 0.000	31.250 ± 0.000	62.500 ± 0.000
S. sonnei	31.250 ± 0.000	31.250 ± 0.000	31.250 ± 0.000	31.250 ± 0.000	62.500 ± 0.000

samples that showed no bacterial growth was recorded as MBC value. All the tests were performed in duplicate.

Phytochemical screening

Phytochemical screening was carried out according to the standard procedures described by Manjamalai et al., (2010) and Tiwari et al., (2011) in order to identify the constituents present in acetone extract of *E. hirta* parts and whole plants.

STATISTICAL ANALYSIS

The data for agar well diffusion assay and micro dilution method were analyzed by simple arithmetic means and standard deviations (n = 2) of the extracts. Analysis on susceptibility and tolerance of the strains towards the extracts were done according to the ratio of MBC over MIC values (Canillac and Mourey 2001). No other statistical test was applied for the phytochemical studies to show significance since the extracts were either positive or negative

RESULTS

The results show the wide variation in the antibacterial properties of *E. hirta* from acetone extraction. According to the results from agar well diffusion assays (Table 1), the crude leaves extract exhibited the highest antibacterial activity (15.00 ± 0.00 mm) towards *B. subtilis*. Whilst, the lowest antibacterial activity (5.35 ±

Table 3. MBC values of E. hirta extracts.

Test bacteria	Minimum bactericidal concentration values (mg/ml)				
	Whole plant	Flowers	Stems	Leaves	Roots
B. cereus	250.000 ± 0.000	125.000 ± 0.000	125.000 ± 0.000	500.000 ± 0.000	125.000 ± 0.000
B. subtilis	500.000 ± 0.000	500.000 ± 0.000	500.000 ± 0.000	250.000 ± 0.000	500.000 ± 0.000
E. faecalis	62.500 ± 0.000	125.000 ± 0.000	31.250 ± 0.000	62.500 ± 0.000	62.500 ± 0.000
S. aureus	31.250 ± 0.000	250.000 ± 0.000	250.000 ± 0.000	62.500 ± 0.000	125.000 ± 0.000
S. epidermidis	31.250 ± 0.000	62.500 ± 0.000	15.625 ± 0.000	62.500 ± 0.000	125.000 ± 0.000
K. pneumonia	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000
S. typhimurium	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000
S. marcescens	500.000 ± 0.000	500.000 ± 0.000	250.000 ± 0.000	500.000 ± 0.000	500.000 ± 0.000
S. dysentriae	250.000 ± 0.000	250.000 ± 0.000	500.000 ± 0.000	250.000 ± 0.000	250.000 ± 0.000
S. sonnei	125.000 ± 0.000	125.000 ± 0.000	125.000 ± 0.000	125.000 ± 0.000	125.000 ± 0.000

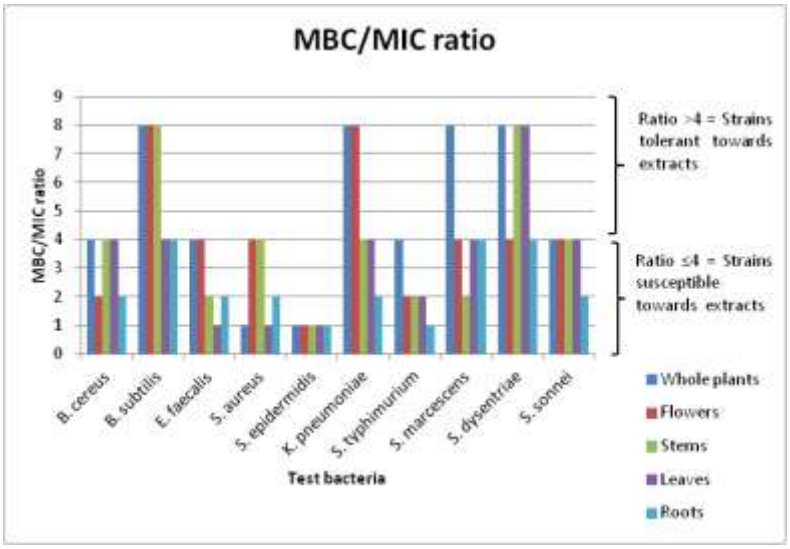

Figure 1. MBC/MIC ratio values of *E. hirta* extracts.

0.21 mm) was recorded by the crude stems extract against *S. marcescens* and similarly on the crude roots extract towards *K. pneumoniae*. Table 2 and Table 3 showed MIC and MBC values of the test bacteria to the *E. hirta* extracts respectively from micro dilution test.

Treatments of whole plants and stems extracts against *E. faecalis* gave the lowest MIC value which was 15.625 ± 0.000 mg/ml. The lowest MIC value also showed by the stems extract against *S. epidermidis*. Meanwhile, the highest MIC value (250.000 ± 0.000 mg/ml) was recorded by the roots extract towards *S. thyphimurium*. Stems extract indicated the highest bactericidal activity against *S. epidermidis* with low MBC value of 15.625 ± 0.000 mg/ml. Treatment of whole plants, flowers and roots extracts against *B. subtilis* and *S. marcescens* exhibited the lowest bactericidal activity with MBC value of 500.000 ± 0.000 mg/ml. Likewise, stems extract indicated the lowest bactericidal activity towards *B. subtilis* and *S. dysentriae*. Leaves extracts also showed the lowest bactericidal activity against *B. cereus* and *S. marcescens*. Analysis on susceptibility and tolerance of the test bacteria to the *E. hirta* extracts as described by MBC/MIC ratio were shown in Figure 1. Generally, all bacteria tested susceptible to the *E. hirta* extracts with MBC/MIC value is less than or equal to 4 (≤4) except on certain extracts towards *B. subtilis*, *K. pneumoniae*, *S. marcescens*

Table 4. Phytochemical screening of crude extract of *E. hirta.*

Phytochemicals	Whole plant	Flowers	Stems	Leaves	Roots
Carbohydrates	+	+	+	+	+
Protein	+	+	+	+	+
Lipid	+	+	+	+	+
Flavonoids	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Pholabatannins	-	-	-	-	-
Resins	+	+	+	+	+
Sterols	+	-	-	-	+
Steroids	+	+	+	+	+
Acidic compounds	+	+	+	+	+
Tannins	+	+	+	+	+
Glycosides	+	+	+	+	+
Anthraquinone	+	+	-	+	-
Phenols	+	+	+	+	+
Terpenoids	+	+	+	+	+

+ Present - Absent

and *S. dysentriae.* All bacteria tested susceptible to the roots extract. Phytochemical screening of the crude extracts from whole plant and different parts of *E. hirta* were shown in Table 4. Mostly, the results of phytochemical were positive. However, the crude extracts of *E. hirta* gave negative results on pholabatannins test. Whilst, flowers stems and leaves crude extracts gave negative result on sterols. Besides, the crude extracts of stems and roots gave negative result on anthraquinone.

DISCUSSION

The susceptibility of the test microorganism is related to the inhibition zone size in millimeters *via* agar well diffusion assay. Microorganisms are termed susceptible to the plant extract when the zone inhibition is equal to or more than 7 mm (≥7) in diameter, or resistant with a zone of inhibition less than 7 mm (<7) therapies (Nascimento et al., 2000). Generally, Gram negative bacteria are more resistant to the *E. hirta* extracts compare to the Gram positive bacteria. Gram negative organisms are less susceptible to the action of antimicrobials since they possess an outer layer of membrane which could protect cell wall and restrict hydrophobic compounds from diffuse through the lipopolysaccharide covering (Vaara, 1992). The crude leaves extracts exhibited the highest antibacterial activities against all bacteria tested *via* agar well diffusion assays. Besides, the extraction of this part of *E. hirta* crude extracts produced very sticky black green extracts with the highest in percentage yield (11.12%) compare to the others. The abundant of phytochemicals in leaves like alkaloids, phenols, tannins and the others as proof from Table 4 might contribute to the highest result of antibacterial activities. However, the formation of a clear zone of inhibition from agar-well diffusion assay could not indicate the effectiveness of the antibacterial activity since this assay only useful as screening tools in antibacterial assays (Friedman et al., 2002). The micro dilution method has provided a potentially useful technique for quantitative antibacterial assays (Ncube et al., 2007). In general, MIC values shown that all the test bacteria except *B. cereus* and *S. epidermidis* are most susceptible towards whole plant extracts compare with single part of *E. hirta* extracts tested. This data proof that the whole plants of *E. hirta* extracts very useful for inhibitory bacterium purpose. According to the data from Table 4, out of 16 phytochemicals test, the whole plants only gave the negative result on pholabatannins. The presence of abundant phytochemicals might contribute to the best inhibitory result. Thus, these findings support the useful of whole plant to treat a lot of ailments like the treatment of athlete's foot, dysentery, enteritis, and skin conditions. Basically, all *E. hirta* extracts exhibited the bactericidal effect towards the ten bacteria tested. However, the data on MBS values cannot simply conclude on which part of tested herbs gave the best result since all parts had the highest values of MBC (500.000 ± 0.000 mg/ml) Different parts of *E. hirta* extracts possesses different constituents

and in different concentration, which accounts for differential bactericidal effects. The abilities of this herb extracts to kill totally certain bacteria like *B. subtilis* with the low concentration of the extracts as MIC values somehow difficult due to the resistance factors of the bacteria. Nascimento et al. (2000) reported that *B. subtilis* has the protective endospores so that it can tolerate in the extreme conditions easily. According to Canillac and Mourey (2001), MBC/MIC value is less than or equal to 4 (≤4) the strains is considered to be susceptible, while if the ratio is greater than 4 (>4) then the strains is considered to be tolerant. In general, Gram positive bacteria more susceptible against *E. hirta* extracts compare to the Gram negative bacteria. Among the strains tested, *S. epidermidis* was very susceptible towards *E. hirta* extracts. All the strains tested also most susceptible to the roots extract since the minimum concentration values between inhibitory and bactericidal in the best ranges to obey the standards of susceptible (MBC/MIC ≤4). However, the spread antibiotic-resistant bacteria plus the complexity of the phytochemical itself might be the reasons for certain of the *E. hirta* extracts susceptible or tolerant to the some bacterial tested.

CONCLUSION

Gram positive bacteria more susceptible against *E. hirta* extracts compare to the Gram negative bacteria. Whole plants *E. hirta* extracts is very useful for inhibitory bacterium purpose. Whilst, all *E. hirta* extracts exhibited the bactericidal effect towards the ten bacteria tested. The results presented above indicated that *E. hirta* extracts exhibited antibacterial properties, thus justifying scientifically their traditional used as medicinal plants. Further study on isolated or purified antibacterial compound from *E. hirta* kindly should be done.

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