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In vitro antimicrobial activity of aqueous and ethanol extracts of *Euphorbia hirta*

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SUMMARY

Euphorbia hirta, locally called 'ara tanah' or 'susun nabi' in Malaysia is a small annual herb common to the tropical countries and belongs to the same family as the tic and tapioca. *E. hirta* has had a long history of usage in the treatment of various ailments. In this current study, *in vitro* sensitivity test of crude aqueous and ethanol extracts of leaves and barks of *E. hirta* was carried out against bacteria (*Escherichia coli*, *Salmonella enteritidis*, *Staphylocccus aureus* and *Bacillus subtilis*) and fungi (*Microsporum canis*, *Aspergillus fumigatus*, *Candida albicans* and *Candida tropicalis*) using the discs diffusion method. The extract-impregnated discs (20, 40 and 80 μg/μl), the *E. hirta* extracts inhibited the growth of all the bacteria tested. The growth of *C. albicans* was inhibited in a concentration dependent manner by the aqueous leaves and barks extracts. *C. tropicalis* was found to be sensitive to the aqueous leaves extracts. The results were compared to antibacterial drugs of chloramphenicol, ampicilin, penicillin G, and enrofloxacine; and to antifungal drug of ketoconazole, itraconazole and miconazole. In this current study, it can be concluded that this plant has antimicrobial activity that is as potent as the standard antimicrobial drugs against certain microorganisms.

Key words: *Euphorbia hirta*; *In vitro*; Ethanol/aqueous extract; Antimicrobial

INTRODUCTION

For the past few years, a number of studies have been reported, dealing with antimicrobial screening of extracts from medicinal plants (Khan *et al*., 1980; Perumal Samy and Ignacimuthu, 2000; Portillo *et al*., 2001; Somchit *et al*., 2001). *Euphorbia hirta*, one of the medicinal plants from the *Euphorbiacea* family is widely distributed in tropical countries. Its a small plant that can easily be found on the roadsides, fields and abandon areas. A common characteristic of *E. hirta* is the production of a milky-looking sap that the stems and leaves secrete. The leaves are small, oppositely arranged and up to 5 cm long; the flowers are small, numerous and consists of tiny flowers in clustered form that the clusters themselves resemble flowers (Seaforth *et al*., 1985). *E. hirta* is also known as Dudhi (Hindi), Chara (Sanskrit), Boro keruic (Bengali), Amampetchaiarisi (India), Kaka wie adwe (Ghana) and Ara tanah/Susun nabi (Malaysia), (Somchit *et al*., 2001).

E. hirta is known to contain chemical compounds such as gallic acid, quercitrin, myricitrin, 3,4-di-galloylquinic acid, 2,4,6-tri-O-galloyl-D-glucose and 1,2,3,4,6-penta-O-gallyl-beta-D-glucose (Chen *et al*., 1991). A number of other compounds that have been isolated and chemically characterised include cycloarternol, 24-methylene-cycloarternol, β-sitosterol, euphorbol hexacozonate, β-amyrin acetate, 1-hexacosanol, ingenotriacetate, tinyaloxin, campesterol, stigmasterol and quercitrin (Galvez *et al*., 1993). The *E. hirta* leaves showed that it has relatively high amino acids composition of lysine, threonine, valine, methionine, isoleucine and leucine, with also high mineral ion concentration of calcium, copper, iron, zinc, magnesium and lead. (Wallace *et al*., 1998). However, despite the array of chemical compounds characterised, very few pharmacological evaluations have been investigated to ascertain the

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traditional usage and the efficacy of this plant.

This plant commonly used as traditional medicine in Australia, India, Africa and in Malaysia for the treatment of coughs, chronic bronchitis, asthma and other pulmonary disorders due to its antispasmodics and anti-asthmatic principles (Wong-Ting-Fook, 1980). In East and West Africa, extracts of the decoction of the flowering fruiting plants are used in the treatment of asthma and respiratory tract infections and sometimes the extracts are combined with bronchial sedatives like *Grindelia robusta* in preparations for inhalation (Kokwaro, 1976). It is also used for relieving hay fever and catarrh (Le Strange, 1977). In northern Nigeria, in addition to it use in the treatment of asthma, *E. hirta* is commonly applied to eczematous skin (Johnson, 1999). The Swahilis and Sukumas in East Africa use *E. hirta* as a diuretic agent (Watt and Brandwijk, 1962). *E. hirta* mixed with *Phyllanthus niruri* are used for oliguria. The latex from a freshly broken stem is used to treat ringworm by its application around the area to stop the spread of the infection (Seaforth, 1985). This plant is used in treatment of chronic diarrhoea and dysentery. It is also applied topically to ulcers and in a case of oedema. The juice is considered tonic, narcotic and anti-asthmatic (Kirtikar Basu *et al*., 1975).

This study is focusing on the investigation of *E. hirta* extracts against gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*E. coli* and *Salmonella enteritidis*), yeast (*Candida albicans* and *Candida tropicalis*) and moulds (*Microsporum canis* and *Aspergillus fumigatus*).

MATERIALS AND METHODS

Plant materials and extraction

Euphorbia hirta was obtained at the Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. The plant was identified and voucher specimen was submitted at the Phytomedicinal herbarium of Institute of Bioscience, Universiti Putra Malaysia (Voucher No: SK 186/02).

The leaves and barks of *Euphorbia hirta* were separated and oven dried over a period of 24 hours at 45°C. It was then ground into powder and extracted using Soxhlet apparatus with absolute ethanol and distilled water as solvent. The resultant extraction of ethanol were completely evaporated by using rotary evaporator, while resultant distilled water extraction were frozen and freeze dried for 24 to 48 hours.

Microorganisms and medium

The bacteria (*Escherichia coli*, *Salmonella enteriditis*, *Staphylococcus aureus*, *Bacillus subtilis*) and fungi (*Candida albicans*, *Candida tropicalis*, *Microsporum canis*, *Aspergillus fumigatus*) used in this study were from clinical isolates and identified at the Department of Pathology and Microbiology, Universiti Putra Malaysia. The bacteria were inoculated in Muller Hinton agar medium (Merck, Germany), as the fungi were grown and maintained in Yeast and Mould agar (Merck, Germany).

Antimicrobial sensitivity testing

The antibacterial and antifungal activities were demonstrated using disc diffusion method test (Somchit *et al*., 2003). Briefly, sterile blank disc (6 mm diameter, Oxoid, UK) impregnated with various solvent extracts concentration (20, 40 and 80 μg/μL) were placed on the Muller Hinton or Yeast and Mould agar surface previously inoculated with bacteria/fungi. Blank discs without plant extracts served as the negative control. Commercial antibiotics disc which consists of penicillin G (10 μg/μl), chloramphenicol (30 μg/μl), enrofloxacine (5 μg/μl) and ampicilin (10 μg/μl) were used as reference. Standard antifungal drugs of ketoconazole, itraconazole and miconazole diluted in dimethyl sulfoxide were impregnated onto sterile blank discs with the concentration of 80 μg/ml respectively. The bacteria inoculated plates were incubated at 37°C for 24 h to 72 h and the fungi inoculated plates were stored in dark place for 7-10 days in order to observe the formation of inhibition zones around the disc.

Statistical analysis

Results were expressed as mean ± S.D of 3 separate experiments. Statistical significance ($p<0.05$) was determined by analysis of variance. Duncan Multiple Post-test was performed for the significant treatment means.

RESULTS

The results obtained showed that the *E. hirta* aqueous and ethanol extracts both exhibit antibacterial activity as presented in Table 1. It could be observed that the leaves of aqueous and ethanol extracts inhibited the growth of all the bacteria. The most marked effects was observed against *S. aureus* with average inhibition zones range from 9.3 to 19.7 mm and from 9 to 13.3 mm for the aqueous and ethanol extracts respectively. The aqueous extracts of *E. hirta* bark had inhibition zones against *Bacillus subtilis* that was statistically bigger than penicillin G and ampicilin.

Table 2 presents the result for antifungal activity of *E. hirta* against the yeast and moulds. We observed that the aqueous extract of *E. hirta* leaves and barks were marked with average inhibition zones range from 13.2 to 15.6 mm and 12.7 to 14.2 mm against *C. albicans* respectively. These inhibition zones were statistically bigger to the antifungal drug, itraconazole. The *E. hirta* leaves aqueous

Table 1. Antibacterial activities of *Euphorbia hirta* extracts and standard antibiotics

Samples		Concentration (mg / ml)	Microorganism			
			E.c	S.e	S.a	B.s
Aqueous	Leaf	20	-	-	9.3±0.6^{a}	8.7±1.4^{a}
		40	8.3±2.3^{a}	-	13.1±1.0^{b}	14.2±1.1^{b}
		80	12.7±3.7^{b}	10.3±1.1^{bc}	19.67±1.1^{c}	18.67±0.6^{c}
	Bark	20	-	-	-	8.3±2.1^{a}
		40	-	-	-	8.7±1.2^{a}
		80	9.1±1.1^{a}	9.3±0.6^{ab}	8.3±1.5^{a}	12.0±2.1^{b}
Ethanol	Leaf	20	-	-	9.2±1.0^{a}	-
		40	-	7.0±0.1^{a}	11.7±0.6^{a}	10.3±1.2^{a}
		80	8.7±0.6^{a}	8.3±0.6^{a}	13.3±0.6^{b}	12.7±0.6^{b}
	Bark	20	-	-	-	-
		40	-	-	-	-
		80	-	-	-	-
Penicillin G		10	-	15.0±1.0^{c}	37.0±1.0^{f}	8.7±0.6^{a}
Chloramphenicol		30	20.3±0.6^{c}	21.7±0.6^{d}	23.0±1.0^{d}	22.7±0.6^{d}
Enrofloxacine		5	26.0±1.0^{c}	27.0±1.0^{e}	25.0±1.0^{d}	25.0±1.0^{d}
Ampicilin		10	-	20.7±0.6^{d}	41.0±1.0^{e}	10.3±0.6^{a}

- : No inhibition zone, E.c: *Escherichia coli*, S.e: *Salmonella enteriditis*, S.a: *Staphylococcus aureus*, B.s: *Bacillus subtilis*, Values are mean±S.D. (mm) of four separate experiments. a-f; Mean with different superscript differ significantly (p<0.05) in the same column.

Table 2. Antifungal activity of *Euphorbia hirta* extracts and standard antifungal drugs

Sample		Concentration (mg / ml)	Microorganism	
			Candida albicans	*Candida tropicalis*
Aqueous	Leaf	20	13.2±0.1^{a}	-
		40	14.3±0.3^{b}	-
		80	15.6±0.2^{c}	8.7±1.5^{a}
	Bark	20	12.7±0.5^{a}	-
		40	13.4±0.1^{a}	-
		80	14.2±0.2^{b}	-
Ketoconazole		80	26.7±2.1^{d}	30.3±2.5^{b}
Miconazole		80	25.2±1.3^{d}	28.3±1.5^{b}
Itraconazole		80	12.0±1.1^{a}	-

- : No inhibition zone, Values are mean±S.D. (mm) of four separate experiments. a-d; Mean with different superscript differ significantly (p<0.05) in the same column.

extract was also detected with antifungal activity against *C. tropicalis* at 80 μg / ml discs concentration. No inhibition zones was visible with the ethanol extracts on the yeast growth (data not shown). The growth of *M. canis* and *A. fumigatus* were not affected by any of the extracts (data not shown).

DISCUSSION AND CONCLUSIONS

This study reveals that the *E. hirta* aqueous and ethanol extracts shows a remarkable antimicrobial activity. The aqueous extracts show a potential antibacterial activity against the *S. aureus, B. subtilis, E. coli* and *S. enteritidis* growth with the leaves and barks extracts. Ethanol extracts on the other hand exhibit antibacterial activity only with the leaf extracts of the plant. The results indicated that gram-positive bacteria (*S. aureus* and *B. subtilis*) appeared to be more sensitive to both aqueous and ethanol extracts when compared with the inhibition zones measured on the growth of gram-negative bacteria (*E. coli* and *S. enteritidis*). The aqueous extracts of *E. hirta* leaves were also found to show antifungal activity against the *C. albicans* and *C. tropicalis* growth. The extracts antifungal activity detected on *C. tropicalis* is a new finding since no study was done previously against this yeast. The data obtained suggested significant concentration-dependent inhibition zones on some of the bacteria and fungi growth. The outcomes revealed that the extracts had better antibacterial and antifungal activity than some of the commercial standard antibacterial (penicillin G and ampicilin) and antifungal (itraconazole) drugs against certain microorganisms respectively.

Previously we reported that the aqueous and ethanol extracts of *E. hirta* leaves, stem and flower did not exhibit any antibacterial activity against the *S. aureus* and *E. coli* tested, but did inhibit the growth of *C. albicans* (Somchit *et al*., 2001). The antimicrobial test carried out in the previous study was lower extract concentrations (5 to 30 μg / μl). In this current study, higher extract concentrations were used (20 to 80 μg / μl). Therefore, more bacteria and fungi were more susceptible to the extracts. Perumal Samy *et al.* (1999) and Srinivasan *et al.* (2001) also reported that no inhibition zones was observed and concluded that there was no antibacterial activity against *S. aureus.*

The methanol extracts of *E. hirta* L. were studied against dysentery causing *Shigella* spp. using the Vero cell line. Cytotoxicity studies of the extracts were performed using the cell line and the non-toxicity concentration of the extract was tested for antibacterial activity against the cytopathic dose of the pathogen. This extract was found to be non-cytotoxic and effective antibacterial agents (Vijaya *et al*., 1995). Previous research revealed the presence of catechic tannins, flavonoids compound, organic acids (Lanhers *et al*., 1990), and also a number of amino acids composed in *E. hirta*, which are believed to be responsible for the antibacterial and antifungal activity.

In general, commercial antibiotic and antifungal drugs cause side effects such as liver, kidney and gastrointestinal tract toxicity (Somchit *et al*., 2003). Restrepo *et al.* (1986) reported, 7% of patients receiving continuos itraconazole therapy had elevated liver enzymes. Severe hepatotoxicity had also been reported in patients undergoing anti-fungal drug therapy (Tucker *et al*., 1990). However, herbal remedies often do not produce any side effects (Perry, 1980). Therefore, alternative medicine has become a popular remedy to various types of ailments. Though *E. hirta* has the potential to be an antimicrobial agent, further laboratory and clinical studies are required in order to understand its principles. The discovery of a potent herbal remedy that is safe will be a big advancement in bacterial and fungal infection therapies.

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