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Full Length Research Paper

In vitro antimicrobial activity of leaves of *Acalypha indica* Linn. (Euphorbiaceae)

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The antimicrobial activity of water, ethanol and chloroform extracts of *Acalypha indica* was tested against four bacterial and fungal strains using the disc diffusion method. The antibacterial activity against gram positive bacteria was more pronounced ($p < 0.05$) in water and ethanol extracts. Antifungal activity was more significant ($p < 0.05$) only in chloroform extract. This antimicrobial activity was compared to standard antibiotics (penicillin, enrofloxacin, ampicillin and chlorampenicol) and antifungal drugs (ketoconazole, itraconazole and fluconazole). Findings from current study support the use of *Acalypha indica* in traditional medicine for the treatment of various bacterial and fungal infections.

Key words: Antifungal, antibacterial, disc diffusion assay.

INTRODUCTION

Acalypha indica Linn. of the family Euphorbiaceae is a common weed in many parts of Asia including India, Pakistan, Yemen, Sri Langka and throughout Tropical Africa and South America (Ramachandran, 2008). It is an annual herb, about 80 cm high and commonly found in waste places or fields (Burkill, 1985). It is locally known as “kucing galak” or “rumput lis-lis”, “kuppaimeni” in India and “t’ie han tsai” in China (Kirtikar and Basu, 1975).

This plant is used as diuretic, anthelmintic and for respiratory problems such as bronchitis, asthma and pneumonia (Varier, 1996). The roots of *A. indica* is used as laxative and leaves for scabies and other cutaneous diseases (Perry, 1980). Major phytochemicals identified from *A. indica* are acalyphine, cyanogenic glycoside, inositol, resin, triacetomamine and volatile oils (Winter and Griffith, 1998). This plant has been used extensively in herbal medicine in many tropical and sub tropical countries (Kirtikar and Basu, 1975; Ramachandran, 2008).

Previous studies on *A. indica* revealed that this plant has antibacterial activity against several gram positive bacteria (Govindarajan et al., 2008; Krishnaraj et al., 2010). Others have shown that plants in the same genus has potential anti-microbial properties (Alade and Irobi, 1993). Recently, Rahman et al. (2010) reported *A. indica* having analgesic and anti-inflammatory effects. In Malaysia, *A. indica* is used for generations for the treatment of superficial fungal and several other bacterial infections (Abdul Rahman, 1996). Thus, the objective of this current study was to evaluate the antibacterial and antifungal activities of water, ethanol and chloroform extracts of *A. indica* and compare the anti-microbial activity with standard antibiotics and antifungal drugs.

MATERIALS AND METHODS

Leaves of mature *A. indica* plants (5 kg wet weight) were collected in the State of Selangor (Western Malaysia) and identified. A

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Table 1. Antibacterial activity of *A. indica* extracts and standard antibiotics.

Samples	Concentration (mg/ml)	Bacteria			
		E. coli	***S. enteriditis***	***S. aureus***	***B. subtilis***
Water	10	-	-	7.3 ± 0.4^{a}	8.9 ± 0.2^{a}
	20	-	-	14.2 ± 1.0^{b}	12.1 ± 2.1^{b}
	30	11.2 ± 0.7^{b}	10.1 ± 0.9^{a}	23.8 ± 2.1^{c}	20.7 ± 2.6^{c}
Ethanol	10	-	-	6.5 ± 0.3^{a}	-
	20	7.1 ± 0.1^{a}	-	10.7 ± 0.9^{a}	11.0 ± 1.3^{a}
	30	12.7 ± 0.3^{b}	9.3 ± 0.2^{a}	14.3 ± 0.2^{b}	12.3 ± 0.7^{ab}
Chloroform	10	-	-	9.2 ± 0.5^{a}	-
	20	-	-	-	-
	30	-	-	-	-
Penicillin G	10	-	15.0 ± 2.0^{b}	37.0 ± 4.2^{d}	8.8 ± 0.3^{a}
Chloramphenicol	30	20.3 ± 1.6^{c}	22.7 ± 1.6^{cd}	23.2 ± 1.6^{c}	22.3 ± 0.9^{c}
Enrofloxacin	5	26.0 ± 1.0^{c}	28.0 ± 1.2^{d}	25.4 ± 1.2^{c}	25.0 ± 1.3^{c}
Ampicilin	10	-	20.7 ± 0.6^{c}	40.3 ± 5.7^{e}	10.3 ± 0.6^{a}

Values are mean ± sd (mm) of 4 separate experiments. – No inhibition zone. $^{a-e}$ Means within a column with different superscripts differ significantly ($p \leq 0.05$) using ANOVA and Duncan multiple post test.

voucher specimen (Voucher number SK 1631/2007) has been deposited at the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia. Leaves of *A. indica* were washed, oven dried at 45°C overnight, then grounded into powder form and extracted using Soxhlet apparatus with either chloroform, ethanol or distilled water as solvent for 12 h. The solvent was concentrated under vacuum using a rotary evaporator. The yields were 2.57, 4.25 and 7.9% respectively. The solid residues were stored at -20°C prior to use.

Sterile 6.0 mm diameter blank discs (Oxoid, UK) were used to impregnate four different dilutions of the extracts as follows: 0, 10, 20 and 30 mg/mL extract (n = 4/extract). Discs were stored at –5°C prior to use. Tests were performed by the disc diffusion method (Somchit et al., 2004) and experiments were conducted four separate times.

Bacteria (*Escherichia coli*, *Salmonella enteritidis*, *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, Faculty of Veterinary Medicine, Universiti Putra Malaysia. Detailed method was published previously (Somchit et al., 2003). These micro-organisms are commonly seen in both human and veterinary medicine in Malaysia.

Commercial antibiotics disc which consists of penicillin G (10 mg/ml), chloramphenicol (30 mg/ml), enrofloxacine (5 mg/ml) and ampicilin (10 mg/ml) were used as reference. Standard antifungal drugs of ketoconazole, itraconazole and fluconazole diluted in dimethyl sulfoxide were impregnated onto sterile blank discs with the concentration of 30 mg/ml respectively.

The results are presented as mean±standard deviation (SD). All data obtained were analyzed using One-way analysis of variance (ANOVA) with Duncan post hoc test using SPSS v. 17 and the result will be considered significant if $p < 0.05$.

RESULTS AND DISCUSSION

Antibacterial activity of *A. indica* is listed in Table 1 and Figure 1. All extracts of *A. indica* showed varying degrees of antibacterial activity against all microorganisms tested. The gram positive bacteria are more susceptible than the gram negative bacteria. These different antibacterial activities could be due to the nature and concentration of antibacterial compounds plus its/their mode of action (Tortora et al., 2001). Polar extract (water) and the semi-polar extract (ethanol) revealed more potent antibacterial activity than the non-polar extract chloroform. The antibacterial activity of water extract at 30 mg/mL against *S. aureus* and *B. subtilis* was statistically ($p > 0.05$) similar to the control antibiotics chloramphenicol and enrofloxacin. Interestingly, this activity was more potent than penicillin G and ampicillin (Table 1).

There are many reports of plants in the family Euphorbiaceae possessing anti-microbial activity (Perez et al., 1997; Awoyinka et al., 2007; Falodun et al., 2008). Interestingly, Irobi et al. (1994) reported that water and ethanol extracts of *Bridelia ferruginea* (Euphorbiaceae) produced *in vitro* antimicrobial activities mainly against bacteria against hospital strains similar to this current study. They concluded from their preliminary phytochemical analysis that phenols and tannins detected in the extracts may contribute to the antimicrobial effect. This may be the reason why *A. indica* also showed similar anti-microbial activity. Indeed, previous study on *A. indica* revealed this plant has anti-bacterial property against other bacteria (Govindarajan et al., 2008).

The antifungal activity of *A. indica* is shown in Table 2 and Figure 2. Only the non-polar extract showed antifungal action and at 30 mg/mL chloroform extract, the activity was statistically similar to the antifungal drug

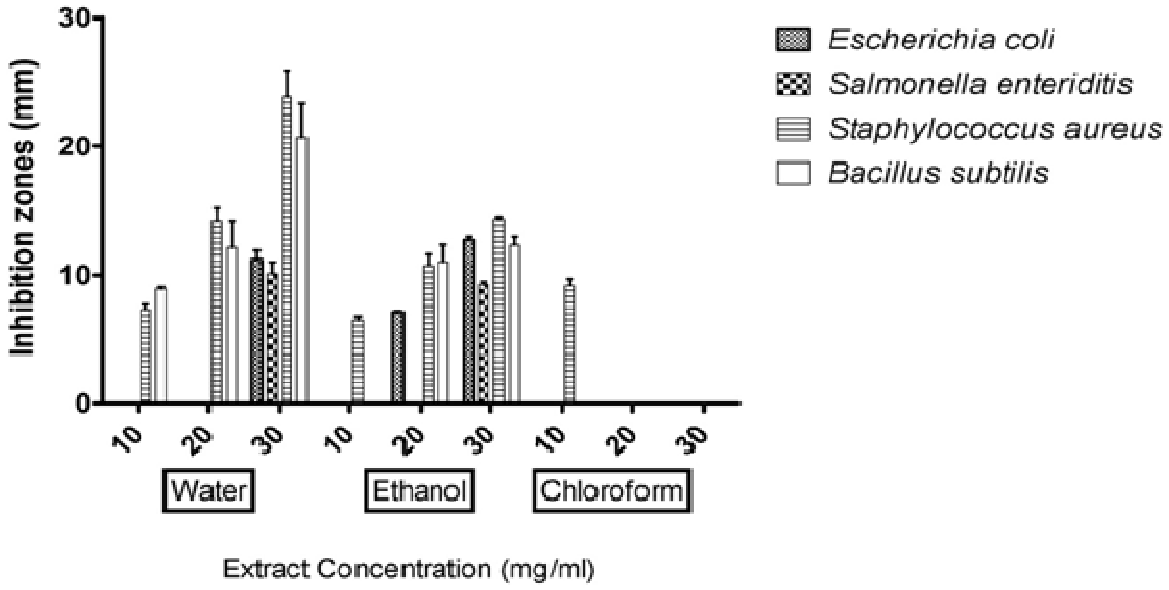

Figure 1. Antibacterial activity of *Acalypha indica* extracts. Values are mean ± S.d (mm) of 4 separate experiments.

Table 2. Antifungal activity of *A. indica* extracts and standard antifungal drugs.

Sample	Concentration (mg/ml)	Fungi			
		C. albicans	***C. tropicalis***	***M. canis***	***A. fumigatus***
Water	10	-	-	-	-
	20	-	-	-	-
	30	-	-	-	-
Ethanol	10	-	-	-	-
	20	-	-	-	-
	30	8.7 ± 0.6 [a]	-	9.3 ± 0.6 [a]	-
Chloroform	10	-	-	-	-
	20	8.3 ± 2.3[a]	-	9.3 ± 0.6 [a]	-
	30	12.7 ± 3.7 [b]	10.3 ± 1.1 [a]	13.0 ± 1.5 [b]	8.7 ± 1.4 [a]
Ketoconazole	30	13.3 ± 1.8 [b]	-	-	17.7 ± 2.6 [b]
Fluconazole	30	21.3 ± 0.7 [c]	15.7 ± 3.6 [b]	17.0 ± 1.9 [c]	-
Itraconazole	30	25.6 ± 1.7 [c]	17.0 ± 1.2 [b]	19.2 ± 3.0 [c]	22.0 ± 1.1 [c]

Values are mean±Sd (mm) of 4 separate experiments. – No inhibition zone. [a-c] Means within a column with different superscripts differ significantly ($p \leq 0.05$) using ANOVA and Duncan multiple post test.

ketoconazole. There is no previous study conducted evaluating the anti-fungal property of *A. indica*. Oksana et al. (2007) reported that flavonoids (quercetin, kaempferol, isorhamnetin, isoquercitrin), phenolic derivatives (gallicin, gallic, syringic, and caffeic acids), and coumarin (scopoletin) have potent anti-fungal activity against *Microsporum* spp. and *Trichophyton* spp. Interestingly, Ogunwenmo et al. (2007) stated that Euphorbiaceae showed high concentrations of flavonoids, phenols and alkaloids. These may be responsible for the potent anti-fungal activity of *A. indica* reported in this current study.

Results obtained revealed potent selective antimicrobial activity in all extracts of *A. indica*. The water and ethanol extracts exhibited better antibacterial activity against gram positive bacteria and this was as potent as several commercial antibiotics. The chloroform extract however, revealed antifungal activity mainly against *M. canis* and *C. albicans*. This antifungal activity was as

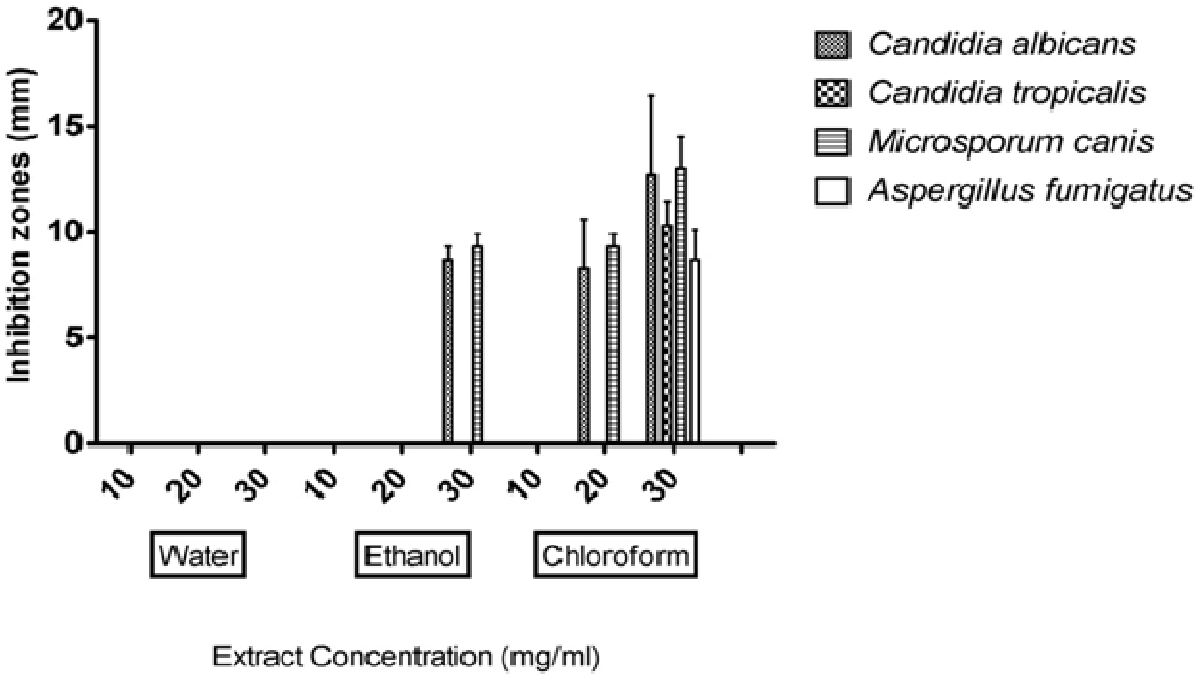

Figure 2. Antifungal activity of *Acalypha indica* extracts. Values are mean ± Sd (mm) of 4 separate experiments.

potent as ketoconazole and fluconazole. Hence, they can be used in treatment of infectious diseases caused by tested strains and potential antimicrobial agents may be developed. However, further studies must be performed to identify the specific principles responsible for the anti-microbial activity of *A. indica*.

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