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In vitro antimicrobial activity of *Cassia auriculata*

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SUMMARY

Ethanol and aqueous extracts of *Cassia auriculata* were tested *in vitro* against fungi (*Candida albicans* and *Microsporum canis*) and bacteria (*Escherichia coli, Salmonella enteriditis, Staphylococcus aureus* and *Bacillus subtilis*). *M. canis* showed dose-dependent susceptibility only towards ethanol leaf and bark extracts. *C. albicans,* were resistant to all types of plant extracts. Results were statistically smaller to antifungal drug ketoconazole and miconazole at equivalent concentration. Both ethanol and aqueous extracts of *Cassia auriculata* leaves and barks exhibit antibacterial activity against *S. aureus* and only the ethanol extracts of leaf and bark were detected against *Bacillus subtilis.* The results were compared to antibacterial drugs chloramphenicol, ampicillin, penicillin G, and enrofloxacin. The antibacterial activity was statistically similar to penicillin G. Based on the current findings, it can be concluded that this plant has antimicrobial activity, which is as potent as standard antimicrobial drugs.

Key words: *Cassia auriculata;* Antimicrobial activity

INTRODUCTION

In recent years there has been a growing interest to evaluate plants possessing antimicrobial activities for various diseases (Clark and Hufford, 1993). A number of studies have been reported, dealing with antimicrobial screening of extracts of medicinal plants (Perumal Samy and Ignacimuthu, 2000; Pertillo *et al.,* 2001; Somchit *et al.,* 2001). Plant derived drugs have become a popular alternative medicine in developing countries. Synthetic antifungal/ antibacterial drugs are widely used at present are causing toxicity adverse drug reactions (Somchit *et al.,* 2002). Furthermore, herbal medicines and supplementation are considered less toxic than the synthetic compounds (Perry, 1980). However, folk medicine has not been studied extensively.

Cassia auriculata L. (Ceasalpiniaceae), locally known as avaram, aroda or tarwar (India), Tanner's Cassia (Australia), ranawara or matara tea tree (Sri Lanka), Kiang Meng (China), gelam tangedu (Indonesia) and avaram (Malaysia). It is widely distributed in the tropical countries from the dry regions of the central province and the west of India desert, Indonesia and Malaysia (Grosvenor *et al.,* 1995). The bark and root is considered as astringent and used as an alterative. The seeds are applied to the eyes in chronic purulent conjunctivitis. In Sri Lanka, the leaves were infused as a substitute for tea. The leaves have medicinal values which are good for ulcers, act as antihelmintic and used for skin diseases (Kirtikar and Basu, 1975). Recently, flowers from *Cassia auriculata* have been reported

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to posses' anti-diabetic and anti-lipideamic properties (Pari and Latna, 2002). Interestingly, extracts of *Cassia* species had been demonstrated to be potent antimicrobial agents (Somchit *et al.*, 2003; Somchit *et al.*, 2004). Hence, an attempt has been made to study the *in vitro* antimicrobial activity of the plant *Cassia auriculata.*

MATERIALS AND METHODS

Plant materials and extractions

Cassia auriculata L. leaves and barks were obtained from Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. The plant specimens were identified and voucher specimens were submitted to the Phytomedicinal Herbarium, Institute of Bioscience, Universiti Putra Malaysia (Voucher No: SK 338/02).

The leaves and barks of *Cassia auriculata* were oven dried over a period of 24 hours at 45°C. They were ground into powder form 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 media

Four different bacteria and two fungi were used as test organisms, *Escherichia coli* and *Salmonella enteriditis* (Gram-negative bacteria), *Staphylococcus aureus* and *Bacillus subtilis* (Gram-positive bacteria), *Microsporum canis* (mold) and *Candida albicans* (yeast). All microorganisms were clinical isolates and identified at the Department of Pathology and Microbiology, Universiti Putra Malaysia. The bacteria were inoculated in Müller Hinton agar medium (Merck, Germany). *C. albicans* and *M. canis* were inoculated and grown in Yeast and Mould agar (Merck, Germany).

Antimicrobial sensitivity test

The antibacterial and antifungal activities were demonstrated using disc diffusion method. The tests were done in triplicates. Sterile blank disc (6 mm diameter) impregnated with various solvent extracts concentration (0, 20, 40 and 80 mg/mL) were placed on the Müller Hinton or Yeast and Mould agar surface previously inoculated with microorganism. Respective solvents without plant extracts served as negative control. Commercial antibiotics disc used were chloramphenicol (30 mg/mL), ampicillin (10 mg/mL), penicillin G (10 mg/mL), and enrofloxacin (5 mg/mL) were used as references. Standard antifungal drugs ketoconazole and miconazole diluted in dimethyl sulfoxide and impregnated onto sterile blank discs with the concentration 40 mg/mL were used. Plates were incubated at 37°C for 24 h to 72 h to observe the formation of clear zone 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 or student *t*-test.

RESULTS

The results of the antibacterial activity by the disc diffusion method of *C. auriculata* ethanol and aqueous extracts were presented in Table 1. It was observed that the ethanol extracts of *C. auriculata* leaves and barks showed antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis.* The inhibition zones of extracts studied were in the range 7-10.3 mm (Fig. 1). No activity was seen against *E. coli* and *S. enteriditis.* When we compared ethanol leaf and bark extracts at concentration of 80 mg/ml to commercial antibiotic, ampicillin, we found that against *B. subtilis,* both extracts exhibit similar results 10.3 ± 1.2 mm and 10.3 ± 0.6 mm in diameter inhibition zones respectively. Again, on comparison of the ethanol extracts, at a concentration 80 mg/ml to penicillin, and found that the extracts exhibit statistically larger inhibition zone than penicillin against the same organism i.e. 10.3 ± 0.6

Table 1. Antibacterial activities of *Cassia auriculata* extracts and standard antibiotics

Samples		Concentration (mg/ml)	Diameter of Inhibition zone (mm)			
			E.c	S.e	S.a	B.s
Ethanol	Leaf	20	-	-	9.7 ± 1.5	2.7 ± 2.6
		40	-	-	9.7 ± 0.6	7.0 ± 0.1
		80	-	-	11.0 ± 1.0	10.3 ± 1.2
	Bark	20	-	-	7.7 ± 0.6	7.2 ± 0.3
		40	-	-	10.1 ± 1.1	9.3 ± 0.6*
		80	-	-	10.3 ± 0.6	10.3 ±0.6*
Aqueous	Leaf	20	-	-	-	-
		40	-	-	-	-
		80	-	-	-	-
	Bark	20	-	-	-	-
		40	-	-	-	-
		80	-	-	-	-
Penicillin G		10	-	17.4 ±1.0	34.0 ± 1.4	8.3 ± 0.6
Chloramphenicol		30	19.3 ±0.6	22.3 ±0.6	22.8 ± 1.0	24.2 ± 0.6
Enrofloxacin		5	24.0 ±1.9	24.0 ±1.2	22.7 ± 1.2	25.9 ± 1.0
Ampicillin		10	-	19.8 ±0.6	45.1 ± 3.5	10.3 ± 0.6

–; 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 three separate experiments. Statistical value: *$P<0.05$ when compared to Penicillin G.

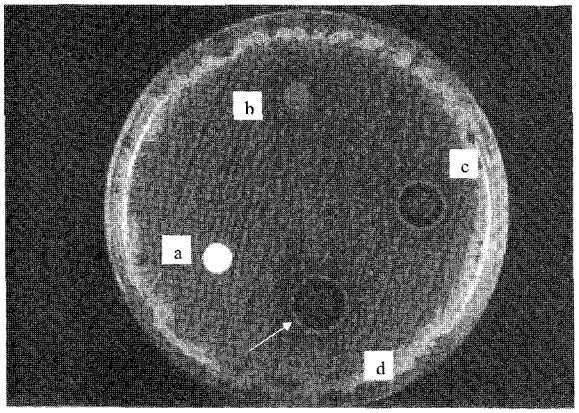

Fig. 1. Inhibition zones of *Cassia auriculata* bark extracts against *Bacillus subtilis*.
Arrow: showing inhibition zone. a: Control; b: 20 mg/ml extract; c: 40 mg/kg extract and d: 80 mg/kg extract.

mm and 8.7 ± 0.7 mm in diameter, respectively (Table 1).

It was observed that the ethanol extracts exhibited antifungal activity against *M. canis* in a dose dependent manner. The ethanol leaf extracts exhibit a statistically larger inhibition zone when compared with the ethanol bark extracts (Table 2). When compared to the standard commercial antifungal drugs, the ethanol leaf extract with concentration of 40 mg/ml and 80 mg/ml exhibit value close to ketoconazole against *M. canis* (20.0 ± 3.0 mm and 22 ± 4.0 mm respectively). The aqueous extracts of both leaves and barks of *C. auriculata* were devoid of any antifungal activity against the tested organism (data not shown).

DISCUSSION

The antimicrobial activity of the extracts of *Cassia auriculata* was studied by the disc diffusion methods against six microorganisms. Our results showed a remarkable antibacterial activity of the ethanol leaves extract of *C. auriculata* against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and moulds (*Microsporum canis*). The antimicrobial activity expressed with the

Table 2. Antifungal activity of *Cassia auriculata* extracts and standard commercial antifungal drugs

Sample		Concentration (mg/ml)	Diameter of Inhibition zone (mm)	
			Microsporum canis	Candida albicans
Ethanol	Leaf	20	16.7 ± 4.6	-
		40	20.0 ± 3.0	-
		80	22.0 ± 4.0	-
	Bark	20	-	-
		40	5.3 ± 9.2	-
		80	6.7 ± 11.5	-
Ketoconazole		40	37.5 ± 3.8	33.7 ± 1.3
Miconazole		40	22.1 ± 1.9	13.7 ± 1.5

–; No inhibition zone. Values are mean ± S.D. (mm) of three separate experiments.

ethanol leaf extracts against *B. subtilis* and *M. canis* are comparable to those shown by penicillin and ketoconazole respectively.

As evident from the results, the antibacterial action of the extracts is more pronounced on Gram-positive than on Gram-negative bacteria and these findings correlate with the observation of the previous screenings of medicinal plants for antibacterial activity, where most of the active plants showed activity against Gram-positive strains only (Ali *et al.*, 1999; Perumal Samy *et al.*, 2000). The result obtained from the antifungal activity against *M. canis* is in agreement with the previous screening of antimicrobial activity of Caesalpiniaceae (Ali *et al.*, 1999).

Cassia occidentalis, Cassia fistula and *Cassia tora* are known to be active against bacteria, fungi, protozoa and virus (Dhar *et al.*, 1968). More recently, we have reported *Cassia alata* (Somchit *et al.*, 2002) and *Cassia tora* (Somchit *et al.*, 2004) having potent antimicrobial activity. A majority of *Cassia* species has been studied for a large variety of active principles, including chrysorabin, anthraquinones, flavonoids, polysaccharides and essential oils (Nageswara *et al.*, 2000). Preliminary phytochemical research revealed the presence of tannins, flavonoids and anthraquinones in *C. auriculata* (Nageswara *et al.*, 2000). The presence of these vital phytochemicals may be responsible for the antibacterial and antifungal properties reported here.

Toxicological studies on other *Cassia* species suggest that the active component mentioned above does not cause toxicity when consumed (Thamlikitul *et al.*, 1990). In general, commercial synthetic antibiotic and antifungal drugs cause side effects such as liver, kidney and gastrointestinal tract toxicities (Somchit *et al.*, 2003). Herbal remedies often do not produce any side effects (Perry, 1980). Alternative medicine has become a popular remedy to various types of ailments.

Cassia auriculata has the potential to be used as an antibacterial agent against gram-positive bacteria. Further laboratory and clinical studies of *Cassia auriculata* are required in order to understand better antimicrobial principles, which will allow the scientific community to recommend their use as an accessible alternative to synthetic drugs.

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