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Antimicrobial Activity Evaluation of *Cassia spectabilis* Leaf Extracts

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Abstract: The aim of the present study was to characterize the antimicrobial properties of various crude extracts of the *Cassia spectabilis* leaf against bacteria and yeast. Acetone, n-hexane, dichloromethane, ethyl acetate and methanol extracts of *C. spectabilis* leaves were evaluated *in vitro* against Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*), Gram negative bacteria (*Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeroginosa*) and yeast (*Candida albicans*). The Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) were determined using micro dilution assay. Methanol showed the highest yield (14.12%) followed by dichloromethane (8.37%), acetone (6.66%), ethyl acetate (4.76%) and n-hexane (1.80%). Acetone and methanol crude extracts showed a good antimicrobial activity with MIC values ranging from 0.625 to 2.5 mg mL^{-1} and MBC or MFC values ranging from 1.25 to 5 mg mL^{-1}. The MIC, MFC and MBC values of these extracts were 10 to 80 times less potent than standard antimicrobial drugs, amoxilin and miconazole nitrate.

Key words: Bacteria, yeast, minimum inhibitory concentration, crude extracts

INTRODUCTION

Medicinal plants for the past two decades have been of keen interest as potential major source of new drugs mainly due to presence of various bioactive secondary metabolites such as alkaloids, phenolics compounds, terpenoids and essential oils. Hence, natural products in particular medicinal plants remain as a potential source of new antimicrobial agents since microbes has been found increasingly resistant to clinically used antimicrobial drugs (Cowan, 1999). Medicinal plants become a good choice as these natural products have ordinary less side effects, are costless and effective against broad spectrum of antibiotic resistant microbes (Motamedi *et al*., 2010).

The genus *Cassia* is well known due to their excellent medicinal values. It has been dispersed broadly in the tropical countries such as, India, Thailand, Malaysia, Indonesia and certain region in Australia (Chukeatirote *et al*., 2007). It consists of 600 species, for example *Cassia spectabilis*, *Cassia alata* and *Cassia tora* and owing to their beautiful flowers; these plants are used as ornamental plant (Pivatto *et al*., 2005).

They are reported to have laxative, purgative, antimicrobial, antipyretic, anti-inflammatory agent and antiviral properties (Silva *et al*., 2005; Viegas *et al*., 2007). In Brazil, the crushed leaves tea of *Cassia* species is used to treat throat inflammation and diarrhea (Viegas *et al*., 2007). Malaysia is endowed naturally with diverge flora and among the promising crops that should receive more attention is *C. spectabilis* (sin *Senna spectabilis*) (DC) Irwin et Barn. It is traditionally known in Malaysia as kasia kuning.

The leaves and pods of *C. fistula*, *C. spectabilis* and *C. podocarpa* possess laxative and antimicrobial activities (Ayo *et al*., 2007). However the in depth antimicrobial properties of *C. spectabilis* plant has not been well characterized except for the antimicrobial study reported by Sangetha *et al*. (2008) and (Chukeatirote *et al*., 2007). With this in view, the present study was undertaken to characterize the *in vitro* antimicrobial properties of various crude extracts of the *C. spectabilis* leaf against bacteria and yeast.

MATERIALS AND METHODS

Plant material: The fresh leaves of *C. spectabilis* were collected from Penang, Malaysia in 2009. The plant material was examined and washed to remove dirt before oven dried at 40°C for 3 days and ground into powder form.

Chemical and reagents: Sabouraud 4% Dextrose Agar (SDA), Sabouraud 2% Dextrose Broth (SDB), Mueller-Hinton Agar (MHA), ethyl acetate (EtOAc), ethanol 96%

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(EtOH), methanol (MeOH), n-hexane, acetone, dichloromethane (DCM) and sulphuric acid (H_2SO_4) were purchased from Merck (Darmstadt, Germany), while Mueller-Hinton broth (MHB) from Becton, Dickson and Company (Le Pont de Claix, France). Barium chloride was obtained from BDH laboratory Supplies (Poole, England). All other chemicals and reagents used are of analytical grade.

Extraction of plant material: Briefly, 10 g of the dried powdered plant materials were extracted separately in 50 mL of acetone, n-hexane, DCM, EtOAc and MeOH for 4 days. Then, the whole extracts were decanted and filtered using Whatman No. 1 filter paper. This process was repeated three times for each solvent. The filtrates obtained were concentrated under vacuum with rotary evaporator (BUCHI R-110, USA) at 40°C to obtain the crude extracts. The extracts were subsequently freeze dried.

Antimicrobial activity evaluation

Test microorganism and growth media: All bacteria (*Bacilus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeroginosa*) and yeast (*Candida albicans*) were obtained from the laboratory stock culture. The bacteria were cultured on MHA slants while *C. albicans* was cultured on SDA slants at 37°C for 18 h.

Minimum Inhibition Concentration (MIC): The Minimum Inhibitory Concentration (MIC) was performed based on a microdilution method in 96 multi-well microtiter plates according to Valgas *et al.* (2007). The crude extracts were dissolved in 50% acetone, respectively. The use of acetone as a solvent was necessary for the miscibility of the extracts. The stock concentrations of the samples were 10 mg mL^{-1}. Then the stock concentration was serially diluted two-fold by using MHB as diluents. There after, each well was inoculated with 5 μL of suspension containing 10^8 cfu mL^{-1} (equivalents to McFarland 0.5) of the culture and incubated at 37°C overnight. To account for the inhibitory effect of the solvent, a negative control was included for all pathogens. This was achieved by preparing the control acetone (25%) sample with broth in place of test plant material. The commercial drugs, amoxillin and miconazole nitrate serve as positive controls for bacteria and yeast respectively. Microorganisms growth was detected by addition of 40 μL of 0.2 mg mL^{-1} of p-iodonitrotetrazolium violet (INT) dissolved in water into each of the microplate wells (Eloff, 1998). The covered microplates were incubated further for 30 min at 37°C. The MIC was recorded as the lowest concentration of the extract that inhibited the microorganisms' growth after 24 h.

Determination of Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC): The MBC and MFC were determined for each of the extracts by sub-culturing the media from each well showing no visible growth onto MHA for bacteria and SDA plates for yeast. The plates were incubated at 37°C until growth was seen in the control plates. The MBC/MFC was defined as the consequent concentrations required killing 99.9% of the cells (Scorzoni *et al.*, 2007).

Determination of Total Activity (TA): The total activity of a plant is the quantity of material extracted from one gram of dried plant material divided by the minimum inhibitory concentration value using the formula following formula described by Eloff (2004).

$$\text{Total activity (mg L}^{-1}) = \frac{\text{Amount extracted from 1 g (mg g}^{-1})}{\text{MIC (mg mL}^{-1})}$$

The units mL g^{-1} indicate the amount to which the active extracts, fractions or compounds in one gram of plant material can be diluted and still inhibit the growth of the test organism.

RESULTS AND DISCUSSION

The purpose of this study was to determine the antimicrobial activity of various organic solvent extracts of *C. spectabilis* leaf. Organic solvents of different polarities namely n-hexane, DCM, EtOAc, acetone and MeOH were used in this study. The degree of extraction of chemical constituents from *C. spectabillis* leaf depends on the solubility of the solvents used. In general, studies have shown that n-hexane extracts wax, lipids, fat soluble oils and ester; DCM extracts terpenoids; EtOAc and acetone are used for ester extraction and MeOH for extraction of alkaloids (Samy and Gopalakrishnakone, 2008).

The solvent extraction yield decreased in the following order: MeOH>DCM>acetone>EtOAc>n-hexane (Table 1). MeOH resulted in a higher yield when compared to other solvents which was in agreement with reported by Masoko and Eloff (2005). They reported earlier higher yield and recovered more chemical compounds when MeOH was used as extractant for leaves of *Combretum*

Table 1: Percentage yield of various organic extracts of *Cassia spectabilis* leaves

Solvents	Yield (%)
n-hexane	1.80
DCM	8.37
EtOAc	4.76
Acetone	6.66
MeOH	14.12

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)/Minimum Fungicidal Concentration (MFC) activity of various organic extracts of *Cassia spectabilis* leaves

		Crude extracts (mg mL^{-1})						
Bacterial species	Activity	n-hexane	DCM	EtOAc	Acetone	MeOH	Amoxillin (μg mL^{-1})	Miconazole nitrate (μg mL^{-1})
Gram positive bacteria								
Bacilus subtilis	MIC	NA	NA	5	0.625	2.5	62.5	ND
	MBC	NA	NA	>5	1.25	5.0	125	ND
	MBC/MIC	ND	ND	ND	2.0	2.0	2.0	ND
Staphylococcus aureus	MIC	NA	5	5	1.25	1.25	62.5	ND
	MBC	NA	>5	>5	2.5	2.5	125	ND
	MBC/MIC	ND	ND	ND	2.0	2.0	2.0	ND
Gram negative bacteria								
Escherichia coli	MIC	NA	NA	NA	1.25	2.5	31.25	ND
	MBC	NA	NA	NA	2.5	5.0	62.5	ND
	MBC/MIC	ND	ND	ND	2.0	2.0	2.0	ND
Salmonella typhi	MIC	NA	NA	NA	1.25	1.25	62.5	ND
	MBC	NA	NA	NA	2.5	2.5	125	ND
	MBC/MIC	ND	ND	ND	2.0	2.0	2.0	ND
Pseudomonas aeroginosa	MIC	NA	NA	5	1.25	2.5	12.5	ND
	MBC	NA	NA	>5	2.5	5.0	25.0	ND
	MBC/MIC	ND	ND	ND	2.0	2.0	2.0	ND
Yeasts								
Candida albicans	MIC	NA	5	5	1.25	1.25	-	25
	MFC	NA	>5	>5	2.5	2.5	-	50
	MFC/MIC	ND	ND	ND	2.0	2.0	ND	2

NA: Not active at concentration 5 mg mL^{-1}; ND: Not determined

species. In the present study, the MeOH, n-hexane, DCM, EtOAc and acetone extracts were re-dissolved in acetone for further use in bioassay. The highest acetone concentration used was 25% and was found not to be inhibiting the growth of any of the tested bacteria and yeast. Acetone appears to be good solvent for use in bioassays and most pathogens are found to be resistant to acetone even at concentration of 51% as postulated by Eloff *et al.* (2007).

Susceptibility of the microorganisms was determined quantitatively using 96 wells plate method. Acetone and MeOH extracts of *C. spectabilis* leaf showed a potentially good antimicrobial activity. The MIC values of acetone extract range from 0.625 to 1.25 mg mL^{-1} for the tested bacteria and *C. albicans* whereas the corresponding MIC values of MeOH extract range from 1.25 to 2.5 mg mL^{-1} (Table 2). The MIC values of these extracts were 10 to 80 times less potent than standard antimicrobial drugs, amoxilin and miconazole nitrate. Acetone and methanol crude extracts showed MBC or MFC values in range of 1.25 to 5 mg mL^{-1} which was 10 to 80 times less potent than standard antimicrobial drugs used. The variation between plant extracts and standard antimicrobial drugs may due to the mixtures compound in the plant extracts compared to pure compound in standard (Gatsing *et al*., 2010).

The acetone extract was most active against *Bacillus subtilis* with MIC of 0.625 mg mL^{-1} which was only 10 times less potent than the standard antimicrobial drug, amoxilin (62.5 μg mL^{-1}). The DCM and EtOAc extracts of *C. spectabilis* leaf were less active against most of the tested pathogens including *C. albicans* with MIC value 5 mg mL^{-1} or more. On the other hand n-hexane extract was not active (MIC>5 mg mL^{-1}) against the tested microorganisms. Antimicrobial substances are considered as bacteriostatic agents when the ratio MBC/MIC>4 and bactericidal agents when the ratio MBC/MIC≤4 (Gatsing *et al*., 2009). In present study, MeOH and acetone showed the ratio MBC/MIC≤4, suggesting that these extracts may be classified as bactericidal agent. Whereas for DCM, EtOAc and n-hexane the MBC/MIC was not determined.

Earlier Chukeatirote *et al.* (2007) showed both ethanol and water crude extracts of various parts (leaf, flower, stem and pod) of *Senna spectabilis* (synonym of *C. spectabilis)* had no activity against *C. albicans* at 75 mg mL^{-1} concentration using the agar disk diffusion method. Only crude water extracts of *S. spectabilis* showed inhibitory effect on *B. cereus* growth with a MIC of 30 mg mL^{-1} while it was inactive against *E. coli.* In contrast, using disk diffusion technique and broth dilution method, Sangetha *et al.* (2008) reported that *C. spectabilis* leaf extracts has favorable antifungal activity against *C. albicans* with MIC of 6.25 mg mL^{-1}. Present study showed that *C. spectabilis* methanol and acetone crude extracts was active against *C. albicans* or other microbes with a smaller MIC values.

Though ethanol (polarity index: 5.2; viscosity: 1.2) and water (polarity index: 9; viscosity: 0.89) have high polarity but they are highly viscous than methanol (polarity index: 5.1; viscosity: 0.6). Methanol with low viscosity has low density and high diffusivity and can

Table 3: Total Activity (TA) of various organic extracts of *Cassia spectabilis* leaves

	Total activity (mL g^{-1})					
Microorganisms	n-hexane	DCM	EtOAc	Acetone	MeOH	Average
Bacilus subtilis	ND	ND	9.54	106.67	56.48	57.56
Staphylococcus aureus	ND	16.75	9.54	53.34	112.97	48.15
Escherichia coli	ND	ND	ND	53.34	56.48	54.91
Salmonella typhi	ND	ND	ND	53.34	112.97	83.15
Pseudomonas aeroginosa	ND	ND	ND	53.34	56.48	54.91
Candida albicans	ND	16.75	9.54	53.34	112.97	48.15
Average	ND	16.75	9.54	62.23	84.73	

ND: Not determined

easily able to diffuse into the pores of the plant materials (Hemwimol *et al*., 2006). Methanol was found to be quantitatively the best extractant, extracting a greater quantity of plant material than any of the other solvents used. This observation suggest that the bioactive(s) responsible for tested microorganism could be to high polarity properties.

The effectiveness of the plant materials against microorganisms not only determined based on the MIC values but also based on the TA of the plant. TA in mL g^{-1} is a measurement of potency of the plant to inhibit the microbial growth. These values indicated the volume that can be added in dried plant material and still kill the microorganisms (Eloff, 2004). Based on average TA, MeOH has the highest value (84.73 mL g^{-1}) followed by acetone (62.23 mL g^{-1}) and these extracts were considered the best to work with for detailed antimicrobial evaluation (Table 3).

CONCLUSION

A good antimicrobial activity of methanol and acetone *C. spectabilis* leaf extracts was observed when compared to those leaf extracted with low polarity solvents. This study supports the use of *C. spectabilis* leaf as medicinal plant by traditional healers. However further work is warranted to fractionize, isolate and characterize the bioactive(s) responsible for the antimicrobial activity.

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REFERENCES

Ayo, R.G., J.O. Amupitan and Y. Zhao, 2007. Cytotoxicty and antimicrobial studies of 1, 6, 8-trihydroxy-3-methyl-anthraquinone (emodin) isolated from leaves of *Cassia nigricans* Vahl. Afr. J. Biotechnol., 6: 1276-1279.

Chukeatirote, E., P. Hanpattanakit, A. Kaprom and J. Tovaranonte, 2007. Antimicrobial activity of *Senna spectabilis* and *S. tora*. J. Plant Sci., 2: 123-126.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimum inhibitory concentration of plant extracts for bacteria. Planta Med., 64: 711-713.

Eloff, J.N., 2004. Quantification the bioactivity of plant extract during screening and bioassay guided fractionation. Phytomedicine, 11: 370-371.

Eloff, J.N., P. Masoko and J. Picard, 2007. Resistance of animal fungal pathogens to solvents used in bioassays. South Afr. J. Bot., 73: 667-669.

Gatsing, D., V. Tchakoute, D. Ngamga, J.R. Kuiate and J.D.D. Tamokou *et al*., 2009. *In vitro* antibacterial activity of *Crinum purpurascens* Herb. leaf extract against the *Salmonella* species causing typhoid fever and its toxicological evaluation. Iran. J. Med. Sci., 34: 126-136.

Gatsing, D, C.F.N. Nkeugouapi, B.F.N. Nkah, J.R. Kuiate and F.M. Tchouanguep, 2010. Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extract of *Alchornea cordifolia* (Euphorbiaceae). Int. J. Pharmacol., 6: 173-182.

Hemwimol, S., P. Pavasant and A. Shotipruk, 2006. Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. Ultrasonics Sonochem., 13: 543-548.

Masoko, P. and J.N. Eloff, 2005. The diversity of antifungal compounds of six South African *Terminalia* species (Combretaceae) determined by bioautography. Afr. J. Biotechnol., 4: 1425-1431.

Motamedi, H., E. Darabpour, M. Gholipour and S.M. Seyyed Nejad, 2010. Antibacterial effect of ethanolic and methanolic extracts of *Plantago ovata* and *Oliveria decumbens* endemic in iran against some pathogenic bacteria. Int. J. Pharmacol., 6: 117-122.

Pivatto, M., A.E.M. Crotti, N.P. Lopes, I. Castro-Gamboa and A. Rezende *et al*., 2005. Electrospray ionization mass spectrometry screening of piperidine alkaloids from *Senna spectabilis* (Fabaceae) extracts: Fast identification of new constituents and co-metabolites. J. Braz. Chem. Soc., 16: 1431-1438.

Samy, R.P. and P. Gopalakrishnakone, 2008. Therapeutic potential of plants as anti-microbials for drug discovery. Evid Based Complement Alternat. Med.

Sangetha, S., Z. Zuraini, S. Sasidharan and S. Suryani, 2008. Fungicidal effect and oral acute toxicity of *Cassia spectabilis* leaf extract. Jap. J. Med. Mycol., 49: 299-304.

Scorzoni, L., T. Benaducci, A.M.F. Almeida, D.H.S. Silva, V.S. Bolzani and M.J.S.M. Gianinni, 2007. The use of standard methodology for determination of antifungal activity of natural products against medical yeasts *Candida* sp. and *Cryptococcus* sp. Braz. J. Microbiol., 38: 391-397.

Silva, G.H., H.L. Teles, H.C. Trevisan, V.S. Bolzani and M.C.M. Young *et al*., 2005. New bioactive metabolites produced by *Phomopsis cassiae*, an endophytic fungus in *Cassia spectabilis*. J. Braz. Chem. Soc., 16: 1463-1466.

Valgas, C., S.M. Souza, E.F.A. Smania and A. Smania Jr., 2007. Screening method to determine antibacterial activty of natural products. Braz. J. Microbiol., 38: 369-380.

Viegas, C. Jr., D.H.S. Silva, M. Pivatto, A. Rezende, I. Castro-Gambo, V.S. Bolzani and M.G. Nair, 2007. Lipoperoxidatin and cyclooxygenase enzyme inhibitory piperidine alkaloids from *Cassia spectabilis* green fruits. J. Nat. Prod., 70: 2026-2028.