Mal J Nutr 15(2): 223 - 231, 2009

Antimicrobial Activity of *Emilia sonchifolia* DC., *Tridax procumbens* L. and *Vernonia cinerea* L. of *Asteracea* Family: Potential as Food Preservatives

Yoga Latha L[1], Darah I[1], Sasidharan S[2] & Jain K[3]

[1] *School of Biological Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia*
[2] *Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia 11800 Minden, Penang, Malaysia,*
[3] *School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia*

ABSTRACT

Chemical preservatives have been used in the food industry for many years. However, with increased health concerns, consumers prefer additive-free products or food preservatives based on natural products. This study evaluated antimicrobial activities of extracts from *Emilia sonchifolia L.* (Common name: lilac tassel flower), *Tridax procumbens L.* (Common name: tridax daisy) and *Vernonia cinerea L.* (Common name: Sahadevi), belonging to the *Asteracea* family, to explore their potential for use against general food spoilage and human pathogens so that new food preservatives may be developed. Three methanol extracts of these plants were tested *in vitro* against 20 bacterial species, 3 yeast species, and 12 filamentous fungi by the agar diffusion and broth dilution methods. The *V. cinerea* extract was found to be most effective against all of the tested organisms and the methanol fraction showed the most significant ($p < 0.05$) antimicrobial activity among all the soluble fractions tested. The minimum inhibitory concentrations (MICs) of extracts determined by the broth dilution method ranged from 1.56 to 100.00mg/mL. The MIC of methanol fraction was the lowest in comparison to the other four extracts. The study findings indicate that bioactive natural products from these plants may be isolated for further testing as leads in the development of new pharmaceuticals in food preservation as well as natural plant-based medicine.

Keywords: Antimicrobial activity, food preservation, plant extracts

INTRODUCTION

The growth of food spoilage and food-borne pathogens in food can reduce the nutritional quality of the food as the pathogens utilise the fat, protein and carbohydrate that are present in the food for their metabolism. This may lead to food discoloration, mustiness, deleterious biochemical changes and accumulation of toxic substances. Some species of food-spoilage and food-borne pathogens are capable of producing highly toxic compounds such as aflatoxin, ochratoxin A and cyanogenic glycosides in food, which are harmful to consumer health (Li & Yi, 2003; You, 2006). An ideal food

Correspondence author: Dr S Sasidharan; Email: *srisasidharan@yahoo.com*

preservative should be inexpensive, corrosion-free, low in toxicity, and have good antimicrobial activity. Currently, the food industry uses chemical food preservatives as the main way to inhibit microbial growth. As safety concerns with chemical preservatives have received extensive attention worldwide, natural preservatives derived from natural resources such as herbal plants have high potential as food preservatives for the food industry (Sasidharan *et al.*, 2008; Li & Yi, 2003; Xie *et al.*, 2001).

Extracts from many plants have been known to possess antimicrobial effects and are used in food preservation and for medicinal purposes (Cowan, 1999; Lee *et al.*, 2007; Rý´os & Recio, 2005). Malaysia, being one of the 12 mega-diversity centres of the world, is rich in all three levels of biodiversity namely, species diversity, genetic diversity and habitat diversity, with many plants used for medicinal and nutritional purposes. This study focuses on the antimicrobial activities of extracts from *Emilia sonchifolia L.* (Common name: lilac tassel flower), *Tridax procumbens L.* (Common name: tridax daisy) and *Vernonia cinerea L.* (Common name: Sahadevi), that belongs to the *Asteracea* family against general food spoilage and human pathogens so that new food preservatives may be developed using these natural resources.

MATERIALS AND METHODS

Materials

The aerial parts of *Emilia sonchifolia L.*, *Tridax procumbens L.* and *Vernonia cinerea L.* were collected in Penang, Malaysia in December 2005, and authenticated by the School of Biological Sciences, Universiti Sains Malaysia, Penang.

Preparation of extracts

Approximately 100g of dried plant material was boiled in a soxhlet with 200ml of methanol for 24 hours at 80°C. All three methanol extracts from *E. sonchifolia*, *T. procumbens* and *V. cinerea* were evaporated in vacuo at 50°C. The concentrated extracts were partitioned successively with four solvents: chloroform, diethyl ether, ethyl acetate and butanol (50mL each), consecutively; diethyl ether was added after eluting chloroform fraction; and ethyl acetate was added after eluting diethyl ether fraction. Finally, butanol was added for further partition. All four fractions and methanol extracts were concentrated in vacuo to one-fifth volume in a centrifugal evaporator (Buchi rotary evaporator, Switzerland) at 50°C and then sterilised by filtration using a 0.22-mm membrane for antimicrobial assay. Other sets of the same fractions were evaporated to dryness to determine amounts of solids in concentrated materials.

Test microorganisms and growth media

The Gram-positive and Gram negative bacteria, yeasts, and molds, obtained from the Fermentation and Enzyme Technology Laboratory, Universiti Sains Malaysia, were used in this study as listed in Table 1. The bacteria (nutrient agar (NA; Difco) slants), fungi including the yeasts (Sabouraud dextrose agar (SDA; Difco) slants) were maintained (short term storage) at 4°C.

Antimicrobial disk diffusion assay

Antibacterial and antifungal activities of the three plant extracts were investigated by the disk diffusion method (Bauer *et al.*, 1966; Alzoreky & Nakahara, 2003). The Mueller Hinton Agar (MHA) plates, containing an inoculum size of 10^6 colony-forming units (CFU) / mL of bacteria, and Sabouraud dextrose agar (SDA) and Potato dextrose agar (PDA) plates containing an inoculum size of $2x10^5$ CFU / mL yeast cells or molds spores, were spread on the agars with an L-shaped glass rod. Then, disks (6.0mm diameter) impregnated with 25μL of each extract at a concentration of 10.0mg / mL were placed on the inoculated plates. Similarly, each plate was placed with a

sterile disk impregnated with kanamycin sulfate or nystatin (50mg / mL and 100mg / mL repectively) as positive control for bacteria and fungi or solvent (v / v) as negative control. All of the plates were incubated at 37°C for 18 hours for bacteria and at 28°C for 48 hours for fungi. The zones of growth inhibition around the disks were measured after 18 hours of incubation at 37°C for bacteria and 48 hours for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks. All of the experiments were performed in triplicate. The results are reported as the average of three experiments.

Determination of minimum inhibitory concentration (MIC)

MIC was determined by both broth dilution method (NCCLS, 2002). The extracts were dissolved in water 120 + 2% dimethyl sulfoxide (DMSO). Twofold serial dilutions (1.275 to 200.0mg / mL) of the five extracts were prepared in Mueller-Hinton broth for bacteria and Saboraud glucose broth for fungi. Subsequently, 0.1mL of standardised suspension of bacteria (10^6 CFU / mL) and fungal cell or spores ($5x10^5$ CFU / mL) was added to each tube (containing five extracts at a final concentration of 1.275 to 200.0mg / mL) and incubated at 37°C for bacteria for 18 hours or at 28°C for fungi for 48 hours. The control tube contained only organisms and not the plant extract. MICs were taken as the average of the lowest concentration showing no growth of the organism and the highest concentration showing visible growth by macroscopic evaluation (Burrowes *et al*., 2004). Each assay was performed in triplicate.

Statistical Analysis

The triplicate data were subjected to an analysis of variance for a completely random design using Statistical Analysis System (SPSS version 12). Multiple-range test was used to compare the differences amongst means at the level of 0.05.

RESULTS AND DISCUSSION

The results of the antimicrobial screening assay of the extracts of all species of plants are shown in Table 1. All the species were found to be active in at least one of the microbial strains. In general, bacteria were found to be more susceptible to the test agents than fungi ($P<0.05$). The preliminary disk diffusion assay of the three species of plant extracts against bacteria showed that the *V. cinerea* extracts had the most distinct effect on most of the tested microorganisms, in inhibiting 17 out of 20 test micro-organisms with 8-17mm zone of inhibition. This was followed by the *E. sonchifolia,* which inhibited 17 out of 20 test microorganism with 8-14mm zone of inhibition and *T. procumbens,* which inhibited 16 out of 20 test microorganism with 8-14mm zone of inhibition .

The antimicrobial screening assay of the three plant extracts against yeasts showed all the extracts inhibited *C. albicans* and *C. neoformans* with 9-20mm zone of inhibition. However, as shown in Table 1 none of the extracts used had an inhibitory effect on all 12 fungal strains tested. The antimicrobial activities of the herbal drugs (*E. sonchifolia, T. procumbens* and *V. cinerea* at a concentration of 10.0mg / ml) were found to be less effective to the positive controls (kanamycin sulfate, 50.0mg / ml for bacteria and nystatin, 100.0mg / ml for fungus). The negative control methanol was devoid of any antimicrobial activity.

Different crude preparations of the *Asteracea* family showed differing efficacy against the tested microorganisms ($P<0.05$). In the disk diffusion method, the micro-organisms tested produced zones of inhibition of about 9-14 mm diameter. The agar dilution method showed MIC values of 1.56 to 100.00mg / mL for each of the microorganisms that were inhibited. These

Table 1. Antimicrobial activities of *Vernonia cinerea*, *Tridax procumbens*, and *Emilia sonchifolia* extracts[a]

Microorganisms	*Zones of inhibition (mm)*																
	Vernonia cinerea					*Tridax procumbens*					*Emilia sonchifolia*					*Controls*	
	ME	*CE*	*DEE*	*EAE*	*BE*	*ME*	*CE*	*DEE*	*EAE*	*BE*	*ME*	*CE*	*DEE*	*EAE*	*BE*	*Kan*	*Nys*
Bacteria																	
Staphylococcus aureus	8	11	8	10	10	8	9	9	10	9	8	10	9	10	9	23	ND
Pseudomonas aeruginosa	10	11	12	11	9	13	11	10	11	10	13	12	10	13	12	23	ND
Citrobacter freundii	14	10	10	11	11	10	10	9	9	8	13	11	10	11	10	23	ND
Escherichia coli	12	11	12	10	10	12	13	12	10	10	12	12	8	9	9	26	ND
Acinetobacter calcoaceticus	13	12	9	10	12	-	-	-	-	-	13	10	8	8	10	24	ND
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	25	ND
Acinetobacter anitratus	14	12	11	10	10	13	10	10	9	10	11	10	9	10	10	21	ND
Bacillus licheniformis	12	11	10	10	9	10	11	9	9	9	11	9	8	8	9	18	ND
Micrococcus spp	12	11	10	11	10	12	10	11	10	11	12	11	11	10	10	15	ND
Staphylococcus epidermidis	9	10	12	12	11	9	9	9	8	9	9	9	8	9	8	16	ND
Salmonella typhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16	ND
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	ND
Erwinia spp	12	10	11	9	9	9	11	9	10	9	11	11	10	11	10	24	ND
Bacillus cereus	17	11	10	12	10	12	10	11	10	10	14	12	11	14	13	25	ND
Serratia marcescens	11	8	10	8	11	10	10	9	8	8	8	9	8	9	8	21	ND
Staphylococcus saprophyticus	10	9	11	9	10	10	9	9	10	11	10	9	9	10	9	18	ND
Yersinia enterocolitica	13	10	10	8	9	11	11	12	11	10	13	12	11	11	10	15	ND
Shigella sonnei	15	11	10	11	10	14	14	13	10	10	10	11	9	10	9	16	ND
Morganella morganii	8	12	9	9	9	10	12	13	11	12	8	10	8	9	9	16	ND
Enterobacter aerogenes	10	11	9	9	9	11	10	11	10	10	10	8	8	11	8	24	ND
Yeast																	
Candida albicans	20	20	16	17	16	20	20	15	18	16	20	20	16	17	15	ND	32
Rhodotorula rubra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31
Cryptococcus neoformans	13	12	15	13	10	13	11	12	13	10	9	10	10	9	9	ND	32

Table 1. Continued

Fungi																		
Trichoderma viride	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Rhizophus spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Mucor spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	30	
Penicillium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	32	
Fusarium spp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Trichophyton rubrum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	32	
Microsporum canis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Trichophyton mentagrophytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	30	
Fusarium oxysporium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	32	
Aspergillus niger	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Aspergillus flavus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	31	
Microsporum gypseum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	ND	30	

[a]Values, including diameter of the disk (6.0 mm), are the mean of three replicates. Concentrations of methanol (ME), chloroform (CE), diethyl ether (DEE), ethyl acetate (EAE), and butanol (BE) extract, were 10.00 mg / mL; concentrations of controls, kanamycin sulfate (Kan) was 50.00mg / mL and nystatin (Nys) was 100.00mg / mL; 25 µL of solution was applied to each disk. Dashes indicate no inhibition. ND: not done. (P<0.05)

differences could be due to the nature and levels of the antimicrobial agents present in the extracts and their mode of action on the different test microorganisms (Barbour *et al.*, 2004). In addition, the extracts tested in this study showed a wide range of antimicrobial activities with differences in susceptibility between Gram-positive and Gram-negative bacteria (Table 2). The differences in sensitivity between Gram-positive and Gram-negative bacteria might be explained by the differences in morphological structure between these microorganisms; Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances in plant extracts. The Gram-positive bacteria, on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeable barrier. Thus, the cell walls of Gram-negative organisms, which are more complex than the Gram-positive, act as a diffusional barrier making them less susceptible to the antimicrobial agents than Gram-positive bacteria (Nostro *et al*., 2000; Hodges, 2002). In spite of these permeability differences, some of the extracts showed a good degree of inhibition against Gram-negative organisms as well.

Review of the literature on the phytochemical constituents of the plants tested revealed that steroids, triterpenoids, sesquiterpenes, flavonoids and tannins are the major components of *V. cinerea* (Gupta *et al*., 2003); alkyl esters, sterols, flavonoids, pentacyclic triterpenes, fatty acids and polysaccharides are the major components of *T. procumbens* (Ali, Ravinder & Ramachandram, 2001); whereas pyrro-lizidine alkaloids and flavonoids are the major components of *E. sonchifolia* (Shylesh & Padikkala, 1999). Most of these compounds are highly polar and may be responsible for the observed antimicrobial effects in this study.

Based on the initial antimicrobial screening assay, those extracts that showed positive results were selected for further studies for the determination of MIC. The MICs of the extracts are shown in Table 2. The MIC values of *V. cinerea* extracts ranged from 1.56mg / ml to 50mg / ml and were found to be active against bacteria such as *Pseudomonas aeruginosa*, *E. coli*, *Yersinia enterocolitica* and *Shigella sonnei* with MIC values of 3.13mg / ml to 6.25mg / ml and lower MIC values for yeast *C. albicans* (1.56mg / ml). Meanwhile, the MIC values of *T. procumbens* extract ranged from 1.56mg / ml to 100.00 mg / ml and was found to be active against bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Bacillus cereus*, and *E. coli* with MIC values of 3.13mg / ml to 12.50mg / ml and lower MIC values for yeast *Candida albicans* (1.56mg / ml). The MIC values for *E. sonchifolia* extract also ranged from 1.56 mg / ml to 50.00mg / ml and was found to be active against bacteria such as *Yersinia enterocolitica*, *Shigella sonnei* and *Bacillus subtilis* with MIC values of 3.13mg / ml to 6.25mg / ml and also lower MIC values for yeast *Candida albicans* (1.56mg / ml). The MIC values of the three species seem to be relatively higher. However, being crude extracts, the overall antimicrobial activity screening results are only indicative of the potential of these herbal drugs for the purpose of food preservation and medicinal purposes.

Comparison of the antimicrobial activities of the different solvent systems with that of the crude extract indicate that the polar fractions are stronger in all cases. These results are expected because methanol, being highly polar, can extract as much of the active compounds (which are likely to be polar) as can be extracted with less polar solvents like ethyl acetate and butanol. In addition, these results also support the fact that the active compounds are concentrated more in methanol fraction. It also emphasises the importance of testing

Table 2. Minimum inhibitory concentration (MIC) of *Vernonia cinerea, Tridax procumbens,* and *Emilia sonchifolia* extracts[a]

Microorganisms	*MIC (mg/mL)*																
	Vernonia cinerea					*Tridax procumbens*					*Emilia sonchifolia*					*Controls*	
	ME	CE	DEE	EAE	BE	ME	CE	DEE	EAE	BE	ME	CE	DEE	EAE	BE	Kan	Nys
Bacteria																	
Staphylococcus aureus	6.25	6.25	6.25	6.25	12.5	6.25	6.25	6.25	50.0	25.0	6.25	6.25	6.25	6.25	12.5	0.01	ND
Pseudomonas aeruginosa	3.13	3.13	6.25	6.25	12.5	6.25	6.25	12.5	3.13	50.0	6.25	6.25	6.25	6.25	12.5	0.01	ND
Citrobacter freundii	6.25	6.25	6.25	12.5	12.5	6.25	12.50	12.5	12.5	50.0	6.25	6.25	6.25	6.25	25.0	0.02	ND
Escherichia coli	3.13	3.13	6.25	3.13	25	12.5	12.50	12.5	6.25	50.0	12.5	12.5	12.5	12.5	25.0	0.01	ND
Klebsiella pneumoniae	-	-	-	-	-	-	-	-	-	-	6.25	6.25	12.5	6.25	12.5	0.01	ND
Acinetobacter calcoaceticus	6.25	6.25	6.25	6.25	12.5	-	-	-	-	-	-	-	-	-	-	0.01	ND
Acinetobacter anitratus	6.25	6.25	50.0	25.0	50.0	12.5	12.50	12.5	50.0	50.0	3.13	12.5	25.0	25.0	25.0	0.05	ND
Bacillus licheniformis	6.25	6.25	12.5	12.5	25.0	6.25	12.50	25.0	50.0	50.0	6.25	6.25	12.5	25.0	50.0	0.05	ND
Micrococcus spp.	6.25	6.25	25.0	6.25	50.0	6.25	12.50	50.0	100.0	-	6.25	6.25	25.0	25.0	25.0	0.05	ND
Staphylococcus epidemidis	3.13	6.25	6.25	6.25	50.0	3.13	3.13	50.0	50.0	50.0	-	-	-	-	-	0.05	ND
Bacillus subtilis	-	-	-	-	-	-	-	-	-	-	3.13	6.25	6.25	6.25	50.0	0.02	ND
Erwinia spp.	3.13	6.25	6.25	12.5	25.0	6.25	12.50	12.5	25.0	25.0	3.13	6.25	6.25	12.5	25.0	0.02	ND
Bacillus cereus	3.13	6.25	12.5	25.0	50.0	12.5	12.50	12.5	50.0	-	12.5	6.25	12.5	25.0	25.0	0.01	ND
Serratia marcescens	3.13	3.13	3.13	12.5	12.5	3.13	6.25	6.25	12.5	25.0	3.13	3.13	3.13	12.5	12.5	0.01	ND
Staphylococcus saprophyticus	6.25	6.25	12.5	12.5	25.0	6.25	6.25	12.5	12.5	50.0	6.25	6.25	6.25	12.5	25.0	0.01	ND
Yersinia enterocolitica	3.13	3.13	12.5	3.13	12.5	3.13	3.13	3.13	12.5	12.5	1.56	3.13	3.13	3.13	12.5	0.01	ND
Shigella sonnei	3.13	6.25	6.25	3.13	12.5	1.56	3.13	3.13	6.25	12.5	3.13	3.13	6.25	6.25	12.5	0.01	ND
Morganella morganii	6.25	6.25	6.25	6.25	25.0	6.25	12.50	50.0	50.0	50.0	6.25	6.25	12.5	12.5	25.0	0.02	ND
Enterobacter aerogenes	3.13	12.5	12.5	25.0	50.0	25.0	25.00	100.0	100.0	25.0	6.25	6.25	25.0	25.0	25.0	0.05	ND
Yeast																	
Candida albicans	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56	ND	0.01
Cryptococcus neoformans	6.25	6.25	12.5	12.5	12.5	3.13	12.5	12.5	12.5	12.5	12.5	12.5	12.5	3.13	12.5	ND	0.01

[a] Results are the average of records determined by both agar and broth dilution methods. ME, methanol; CE,chloroform; DEE, diethyl ether; EAE, ethyl acetate; BE, butanol; Kan, kanamycin sulfate; Nys, nystatin. Dashes indicate no inhibition. ND: not done. (P<0.05)

activities of the different fractions before reporting that such type of extracts are inactive by simply looking at the results of the crude extract, especially for those plants with a long history of use in conventional medical practices. There are a number of natural preservatives available on the market as a permitted preservative such as grapefruit seed extract, resveratrol, goji juice, astragalus, vanilla extract, curcumin, green tea extract, aloe vera juice and artemisinin.

CONCLUSION

In conclusion, all the plants investigated possessed activity against at least one strain of bacteria and fungi. Further studies aimed at the isolation and identification of active substances from the methanol fractions of *E. sonchifolia*, *T. procumbens* and *V. cinerea* may disclose other compounds with better value for food preservation.

REFFERENCES

Ali M, Ravinder E & Ramachandram R (2001). A new flavonoid from the aerial parts of *Tridax procumbens*. *Fitoterapia* 72: 313-315.

Alzoreky NS & Nakahara K (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int J Food Microbiol.* 80: 223–230.

Barbour E, Sharif MA, Sagherian VK, Habre AN, Talhouk RS & Talhouk SN (2004). Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J. Ethnopharmacol.* 93: 1-7.

Bauer AW, Kirby WMM, Sherris JC & Turck M (1966). Antibiotic susceptibility testing by standardized single disc method. *Am J Clin Pathol* 36: 493–496.

Burrowesn OJ, Hadjicharalambous C, Diamond G & Lee TC (2004). Evaluation of antimicrobial spectrum and cytotoxic activity of pleurocidin for food applications. *J Food Sci* 69: FMS66-FMS71.

Cowan MM (1999). Plant products as antimicrobial agents. *Clin Microbiol Rev* 12: 564–582.

Gupta MU, Mazumder K, Manikandan L, Haldar PK, Bhattacharya S & Kandar CC (2003). Antibacterial activity of *Vernonia cinerea*. *Fitoterapia* 74: 148-150.

Hodges N (2002). Pharmaceutical applications of microbiological techniques. In: *Pharmaceutics: The Science of Dosage Form Design*. Aulton, ME (ed.), 2nd ed., 606p. Harcourt Publishers Limited, London.

Lee SH, Chang KS, Su MS, Huang YS & Jang HD (2007). Effects of some Chinese medicinal plant extracts on five different fungi. *Food Control* 18: 1547-1554 .

Li YY & Yi ZY (2003). Present situation and development of food antistaling agent and preservatives. *J Beijing Institute of Petro-chem Technol* 11: 18–23.

National Committee for Clinical Laboratory Standards (NCCLS) (2002). Reference method for 261 broth dilution antifungal susceptibility testing of filamentous fungi. Approved 262 Standard.Wayne, Pennsylvania. NCCLS Document M38-A.

Nostro A, Germano MP, D'Angelo V, Marino A & Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol* 30: 379–384.

Rý´os JL & Recio MC (2005). Medicinal plants and antimicrobial activity. *J Ethnopharmacol* 100: 80–84.

Sasidharan S, Zuraini Z, Yoga Latha L, Sangetha S & Suryani S (2008). Antimicrobial activities of *Psophocarpus tetragonolobus* (L.) DC extracts. *Foodborne Pathog Dis* 5: 303-309.

Shylesh BS & Padikkala J (1999). Antioxidant and anti-inflammatory activity of *Emilia sonchifolia. Fitoterapia* 70: 275-278.

Xie JJ, Zhong QP, Xu Y & She SW (2001). Application of natural antimicrobials in food preservation. *China Food Add* 1: 27–29.

You X (2006). Food safety and food additive of antiseptics. *Food Sci Technol* 1: 1–4.