American Journal of Plant Sciences, 2015, 6, 718-724
Published Online March 2015 in SciRes. http://www.scirp.org/journal/ajps
http://dx.doi.org/10.4236/ajps.2015.65077

Determination of Malaysian Herbs and Spices as Biopreservative Agents in Food Products

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Received 12 February 2015; accepted 18 March 2015; published 23 March 2015

Abstract

Preservatives are usually added to food products to ensure longer shelf life and prevent decomposition process and microbial growth. However, synthetic food preservatives can also give negative side effect to health and are harmful to human and animal physiology. Based on the potential of herbs and spices as antimicrobial agent, the purpose of this study is to identify antibacterial activity from extracts of some local herbs and spices: *Phaeomeria speciosa* (*P. speciosa*), *Aquilaria subintegra* (*A. subintegra*), *Polygonum minus* (*P. minus*), *Syzygium aromaticum* (*S. aromaticum*), *Cinnamomum verum* (*C. verum*) and *Piper nigrum* (*P. nigrum*) against food bacteria using disc diffusion method. Results revealed that dichloromethane extracts of *C. verum*, hexane extracts of *S. aromaticum* and *P. minus* showed the most active antibacterial against tested bacteria. The Minimal Inhibitory Concentrations (MIC) ranged from 25 to 75 mg/ml for dichloromethane extract of *C. verum*, hexane extract of *S. aromaticum* and *P. minus.* Therefore further research should be pursued to identify the chemical structure of antibacterial agents from the active extracts as an alternative source of natural preservatives.

Keywords

Preservative Agents, Antibacterial Activity, Disc Diffusion Assay, MIC

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How to cite this paper: Azziz, S.S.S.A., Talip, M.A., Wong, C.F., Alimon, H., Bakar, N.A., Mahamod, W.R.W., Yahaya, R., Ahmad, M.S. and Juahir, Y. (2015) Determination of Malaysian Herbs and Spices as Biopreservative Agents in Food Products. *American Journal of Plant Sciences*, **6**, 718-724. http://dx.doi.org/10.4236/ajps.2015.65077

1. Introduction

Preservatives are crucial in food manufacturing, cosmetics, medicine and pharmaceutical products to extend their shelf life and avoid the growth of bacteria. Besides that, preservation also plays an important role in the taste, colour and texture as well as improves nutrition value of the foods. The source of preservatives can be natural or synthetic. Preservatives from natural sources such as herbs and spices have long been practiced in traditional food and medicinal preparations, and recent studies have shown their potential as antibacterial, antifungal, antiviral and antioxidant agents [1]. Essential oils from herbs and spices such as clove, coriander, garlic, rosemary, thyme, onion and sage had been found to have strong antimicrobial activities [2]. Cinnamon and clove extracts also showed significant inhibition zones against *Staphylococcus aureus* by using disc diffusion technique [1]. Antimicrobial property of an extract is determined by the chemical composition, structure and functional group of a compound [2]. Most phenolic compounds, polyphenols, alkaloids, terpenes and flavonoids from plant extract had been reviewed as antimicrobial agents [3]-[5].

On the other hand, synthetic preservatives synthesized through chemical reactions produce cheaper alternative and now are becoming more reliable and in favour. Previous studies have shown that without proper management, synthetic food preservatives could give negative effect not only to human and animal health but also to the physiology of other living organisms, damage the biology system and pollute the environment [6]. A common example of synthetic preservative—sodium benzoate is a salt from benzoic acid widely used to preserve pickle, sauce and fruit juice. However, this substance can cause skin irritation and cancer [7]. A study on synthetic preservative had been done on rats and found that sodium benzoate gives bad effect to their nervous system [8]. In comparison, natural preservative had been reported to be safer and more effective [9]. Nevertheless to optimize the action of natural preservatives, the extraction process is imperative.

Solvent used for plant extraction will determine the compound extracted and its bioactivity. The solvent can be grouped into different polarities which are low, medium and high polarity [10]. Solvent in low polarity hexane is usually used to extract waxes, fats and volatile oils. Medium polarity dichloromethane solvent is used to extract alkaloids, aglycones and volatile oil while high polarity solvent, such as methanol and water, is used to extract sugars, amino acids and also glycosides. Hence, this study will work on different polarities of solvent used for plant extraction and investigate the potential of each extract against common bacteria that usually are found in spoilt foods such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

2. Materials and Method

2.1. Plant Material

P. speciosa, *A. subintegra*, and *P. minus* were collected around Tanjung Malim, Perak while *S. aromaticum*, *C. verum* and *P. nigrum* were purchased from the local market in Behrang, Perak in 2013. Plant species and their parts used in this study were summarized in **Table 1**. Plant materials were separated, cut into small pieces and oven-dried at 40˚C. The dried plant materials were ground into powder using grinder machine.

2.2. Plant Extraction

Powdered samples were sequentially extracted with hexane, dichloromethane and methanol by maceration tech-

Table 1. Plant species and the plant parts used.

Plant species	Parts used	Remarks
Phaeomeria speciosa	Flowers	Only widely open petals were chosen
Aquilaria subintegra	Young leaves	Healthy young leaves were chosen
Polygonum minus Huds.	Leaves	Only healthy and green leaves were chosen
Syzygium aromaticum	Buds	Brownish and dry buds were chosen
Cinnamomum verum	Stem barks	Light reddish brown barks were chosen
Piper nigrum	Drupes	Only dried and black drupes were chosen

nique at room temperature for 72 hours and then extract with water by reflux technique for two hours to avoid deterioration of the chemical compounds. The organic extracts (18 extracts) were evaporated in vacuum by using rotary evaporator at 40˚C while the water extracts (6 extracts) were freeze-dried. The dried extracts were stored in glass bottle at 4˚C for further analysis.

2.3. Antimicrobial Activity

2.3.1. Microorganisms

Bacteria cultures of *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi* were obtained from culture collection centre, Department of Biology, Universiti Pendidikan Sultan Idris, Perak, used for antibacterial test microorganisms. All bacteria strains were maintained and grown according to standard procedures, unless otherwise stated.

2.3.2. Disc Diffusion Method

After overnight incubation in nutrient broth at 37˚C, a sterile cotton swab was dipped into each nutrient broth suspension containing the tested bacteria; *E. coli*, *B. cereus*, *P. aeruginosa*, *S. aureus* and *S. typhi*, were swab on the agar surface. Then the 5 mm-diameter sterile filter paper discs were impregnated with different concentration of extracts and a control. The antibacterial test was done at concentration 25, 50, 75 and 100 mg/ml. Methanol and distilled water that used to dilute the extract were choose as the control. Then, they were placed on the NA plate with suitable distance from each other. The discs were gently pressed down by using sterile forcep to ensure complete contact with agar surface. The plates were then sealed with parafilm and incubated at 37˚C overnight in inverted to prevent the moisture build up on the lid from dripping onto the bacteria. Observations were recorded after 24 hours incubation. Antibacterial activities were interpreted from the diameters of inhibition zone around the disc and done in triplicate [11].

2.4. Determination of Minimal Inhibitory Concentration (MIC)

The minimal inhibitory concentration was performed on extracts which showed zone of inhibition in the preliminary screening by using dilution method according to Nascimento *et al*. [12]. MIC of selected extracts was determined by measuring the optical density at 620 nm by comparing the reading of nutrient broth added with extract and inoculated with tested bacteria, with the nutrient broth added with extract without the tested bacteria as control. Each MIC determination was carried out in triplicate.

3. Results and Discussion

3.1. Disc Diffusion Test

The disc diffusion method was used to evaluate the antibacterial activity. In this study, 18 organic extracts and 6 water extracts from *P. speciosa*, *A. subintegra*, *P. minus*, *S. aromaticum*, *P. nigrum* and *C. verum* were tested against Gram negative bacteria, *E. coli*, *S. typhi*, *P. aeruginosa* and Gram positive bacteria, *B. cereus* and *S. aureus* which commonly found in food such as chilli sauce. Four out of six herbs and spices tested in this study have shown antibacterial activity by using disc diffusion method (**Table 2**). Nor organic neither water extracts from *P. speciosa* and *P. nigrum* showed any antibacterial activity. Only methanol extracts of *A. subintegra* at higher concentration (75 mg/ml and 100 mg/ml) showed inhibition zone to only *P. aeruginosa*. Aqueous extracts of *P. minus* did not show any antibacterial activities against all bacteria tested.

A study carried out on pure piperine isolated from *P. nigrum*'s drupes showed zone of inhibition against *E. coli*, *P. aeruginosa* and *S. aureus* [13]. *P. nigrum*'s extracts in this study were found not active against all tested bacteria. The difference result obtained probably caused by antagonistic effect of the compound where the pure single compound inhibition effect is greater than the combination of compound mixture in an extract [14].

Other than environmental condition, genetic factor, solvent used for extraction also affects the degree of antibacterial activity [15] [16]. Previous study of water extract from *P. speciosa* showed inhibition against *S. aureus* and other bacteria including *Staphylococcus xylosus* and Micrococcus species [17]. However, *P. speciosa* from this study were found not active against all the tested bacteria. Method of extraction in this study might be affecting the antibacterial activity of *P. speciosa* because the compound had been extracted serially in different polarity solvents. The composition of bioactive compounds from plant material will depend on the types and

Table 2. Antibacterial activity of different extracts from *P. minus* Huds., *S. aromaticum* and *C. verum*.

Plants	Extracts	Test bacteria	Conc. (mg/ml)/Zone of inhibition (mm ± s.d)				
			0	25	50	75	100
A. subintegra	MeOH	*E. coli*	-	-	-	-	-
		S. aureus	-	-	-	-	-
		S. typhi	-	-	-	-	-
		B. cereus	-	-	-	-	-
		P. aeruginosa	-	-	-	6.67 ± 0.58	7.00 ± 0.00
	Hex	*E. coli*	-	-	-	-	-
		S. aureus	-	6.67 ± 0.58	7.33 ± 0.58	7.67 ± 0.58	7.67 ± 0.58
		S. typhi	-	-	-	-	-
		B. cereus	-	7.00 ± 0.00	8.00 ± 0.00	8.67 ± 0.58	9.33 ± 0.58
		P. aeruginosa	-	7.67 ± 0.58	8.67 ± 0.58	9.00 ± 1.00	9.33 ± 0.58
P. minus Huds.	DCM	*E. coli*	-	-	-	-	-
		S. aureus	-	-	-	-	-
		S. typhi	-	-	-	-	-
		B. cereus	-	-	-	6.67 ± 0.58	6.67 ± 0.58
		P. aeruginosa	-	-	6.33 ± 0.58	6.67 ± 0.58	7.67 ± 0.58
	MeOH	*E. coli*	-	-	-	-	-
		S. aureus	-	-	-	6.67 ± 0.58	7.33 ± 1.15
		S. typhi	-	-	-	-	-
		B. cereus	-	-	-	6.33 ± 0.58	6.67 ± 0.58
		P. aeruginosa	-	-	6.67 ± 0.58	6.67 ± 0.58	7.00 ± 0.00
	Hex	*E. coli*	-	7.33 ± 0.58	7.00 ± 1.00	7.60 ± 0.58	8.00 ± 0.00
		S. aureus	-	7.00 ± 0.00	8.67 ± 0.58	9.00 ± 1.00	9.00 ± 1.00
		S. typhi	-	6.33 ± 0.58	7.00 ± 0.00	7.67 ± 1.15	9.00 ± 1.00
		B. cereus	-	7.00 ± 0.00	8.33 ± 0.58	8.33 ± 0.58	9.00 ± 0.00
		P. aeruginosa	-	6.33 ± 0.58	7.00 ± 0.00	7.60 ± 0.58	8.00 ± 0.00
S. aromaticum	DCM	*E. coli*	-	-	-	-	7.00 ± 0.00
		S. aureus	-	-	-	-	7.00 ± 1.00
		S. typhi	-	-	-	-	-
		B. cereus	-	-	-	-	-
		P. aeruginosa	-	-	7.00 ± 1.73	6.67 ± 1.15	7.67 ± 0.58
	Hex	*E. coli*	-	-	-	7.00 ± 1.00	8.00 ± 0.00
		S. aureus	-	7.67 ± 0.58	7.67 ± 0.58	7.67 ± 1.15	8.00 ± 1.00
		S. typhi	-	7.00 ±0.00	7.33 ± 0.58	8.33 ± 0.58	9.00 ± 1.00
		B. cereus	-	7.00 ± 0.00	7.67 ± 0.58	11.00 ± 0.00	10.00 ± 0.00
		P. aeruginosa	-	7.00 ± 0.00	8.67 ± 0.58	11.00 ± 0.00	10.67 ± 0.58
C. verum	DCM	*E. coli*	-	-	-	-	-
		S. aureus	-	-	7.00 ± 0.00	8.33 ± 0.58	9.00 ± 1.00

Continued

		S. typhi	-	-	-	6.67 ± 0.58	7.67 ± 0.58
		B. cereus	-	8.00 ± 1.00	10.67 ± 0.58	14.00 ±1.73	13.67 ± 1.15
		P. aeruginosa	-	9.00 ± 0.00	11.33 ± 0.58	13.33 ± 2.52	14.33 ± 1.53
		E. coli	-	-	-	-	-
		S. aureus	-	6.00 ± 0.00	7.00 ± 1.00	7.33 ± 0.58	7.00 ± 0.00
	MeOH	*S. typhi*	-	-	-	-	-
C. verum		*B. cereus*	-	6.33 ± 0.58	7.00 ± 1.00	7.67 ± 0.58	8.67 ± 0.58
		P. aeruginosa	-	7.33 ± 0.58	9.00 ± 1.00	10.33 ± 0.58	10.33 ± 0.58
		E. coli	-	-	-	-	-
		S. aureus	-	-	7.00 ± 1.00	8.33 ± 0.58	8.67 ± 0.58
	Aqueous	*S. typhi*	-	-	-	-	-
		B. cereus	-	8.00 ± 0.00	8.67 ± 0.58	9.67 ± 0.58	10.00 ± 0.00
		P. aeruginosa	-	7.00 ± 0.00	8.33 ± 0.58	9.33 ± 0.58	9.33 ± 0.58

Key: Hex = Hexane, DCM = Dichloromethane, MeOH = Methanol (n = 3), (-) = No zone of inhibition.

polarity of solvent used for extraction. A polar compound will extracted by polar solvent and non-polar compound will extracted by non-polar solvent. Thus, the biological activity shown may be different between extracts since different compounds will be extracted [18]. For example, Qader *et al*. [19] reported that *P. minus* have antibacterial properties against *H. pylori* when extract using petroleum ether, chloroform and methanol. However, aqueous extract of *P. minus* showed no inhibition against *H. pylori*.

Interestingly, hexane extract of *C. verum* and *S. aromaticum* showed antibacterial properties against all five tested organisms while hexane extract from *P. minus* gave positive result against three bacteria; *S. aureus*, *B. cereus* and *P. aeruginosa*. Even though essential oil is usually extracted by steam distillation technique, in this study, essential oil, waxes and fats might be dissolved in non polar solvent such as hexane [10] (Peter and Amala, 1998) and showed good antibacterial activity based on the zone of inhibition exhibited, range from 7 mm to 11 mm in diameter (**Table 2**). In previous study, ethanol extract of *P. minus* was found to be active as antimicrobial against Gram-positive bacteria; *Bacillus cereus* and *Bacillus megaterium*, two Gram-negative bacteria; *Escherichia coli* and *Pseudomonas aeruginosa*, and two fungi; *Aspergillus ochraceous* and *Cryptococcus neoformans* [20] (Macken *et al*., 1997). In present study, dichloromethane extracts from *P. minus* showed inhibition against *B. cereus* and *P. aeruginosa* while *S. aromaticum* showed positive results against *E. coli*, *S. aureus* and *P. aeruginosa*. In addition, dichloromethane extract from *C. verum* gave positive results against *S. aureus*, *S. typhi*, *B. cereus* and *P. aeruginosa.* Methanol extracts from *C. verum* and *P. minus* showed antibacterial activity against *S. aureus*, *B. cereus* and *P. aeruginosa* while methanol extract of *S. aromaticum* showed no inhibition against all the tested bacteria. Water extract from *C. verum* showed inhibition against *S. aureus*, *B. cereus* and *P. aeruginosa.*

Previous studies indicate that eugenol and cinnamaldehyde, were two major chemical components found in the spice oil of *Cinnamom* species and *S. aromaticum* that showed inhibition against gram negative and gram positive bacteria [21] [22]. Eugenol is a phenolic compound with-OH group, which gives hydrophobicity of the molecule that enables to penetrate the lipopolysaccharide of the gram negative bacterial cell membrane and disturbed the cell structures [23]. Hence, this lead to the leakage of ions and other cell contents, which consequently inhibit the bacterial growth.

3.2. Minimum Inhibition Concentration (MIC)

The results from disc diffusion methods show three extracts that exhibited very active antibacterial activity; dichloromethane extract of *C. verum*, hexane extract of *S. aromaticum* and hexane extract of *P. minus* Huds. leaves (**Table 2**). They were further tested to determine the Minimal Inhibitory Concentration (MIC). The MIC values for the extracts were shown in **Table 3**. Plant extract of *C. verum* in dichloromethane showed MIC of 25

Table 3. Minimum inhibitory concentraction (MIC) of dichloromethane extract of *C. verum*, hexane extract of *S. aromaticum* and hexane extract of *P. minus* Huds. from leaves.

Test organisms	Extracts/Minimum inhibition concentration (mg/ml)		
	DCM extract of *C. verum*	Hex extract of *S. aromaticum*	Hex extract of *P. minus* Huds.
E. coli	-	25	-
S. aureus	25	25	25
S. typhi	50	75	-
B. cereus	25	50	25
P. aeruginosa	25	25	25

mg/ml against tested bacteria *S. aureus*, *B. cereus* and *P. aeruginosa*, whereas 50 mg/ml for *S. typhi*. Hexane extract of *S. aromaticum* showed MIC of 25 mg/ml against *E. coli*, *S. aureus* and *P. aeruginosa*, whereas 50 mg/ml against *B. cereus* and 75 mg/ml against *S. typhi*. The MIC of 25 mg/ml was obtained against *S. aureus*, *B. cereus* and *P. aeruginosa* when tested with hexane extract of *P. minus* Huds. From the results, antibacterial activity of the selected herbs and spices against both gram positive and gram negative bacteria show the presence of broad-spectrum antibacterial properties. Therefore, it is hypothesized that this local herbs and spices can be used as biopreservative agent in food products.

4. Conclusion

Herbs, spices and edible plants have long been given most attention for their medicinal properties and low toxicity to human. They also show potential as antibacterial agents that are relatively safer than synthetic alternatives like sodium benzoate. From the findings, the most significant antibacterial activities were found in all four extracts of *C. verum*, hexane extracts of *S. aromaticum* and *P. minus* Huds. which can form basis information for further studies. Hence, these herbs and spices that are of interest for antibacterial agents are suggested for further study on toxicity testing, further isolation of active compounds, identification of its chemical structure and evaluation against a wider range of biological activities like *in vivo* testing with the aim for use in food preservation.

Acknowledgements

The authors acknowledge Ministry of Higher Education, Malaysia and Universiti Pendidikan Sultan Idris (UPSI) for provision of financial support for this research work, FRGS (2014-0030-101-02) and GPU (2013-0068-102-01).

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