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Full Length Research Paper

Antibacterial activity of several Malaysian leaves extracts on the spoilage bacteria of yellow alkaline noodles

Teza Alfindo Rosyid[1], Roselina Karim[1]*, Noranizan Mohd Adzahan[1] and Farinazleen Mohd Ghazali[2]

[1]Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, My, 43400, Malaysia.
[2]Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, Serdang, My, 43400, Malaysia.

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Yellow alkaline noodle (YAN) is very susceptible to spoilage and has a short shelf life due to the high moisture content. This study was conducted to isolate and identify spoilage bacteria of YAN in an attempt to apply local plant extracts that possess antibacterial activity in extending the shelf life of YAN. Thirty colonies were isolated from spoiled YAN and were identified using the Biolog GEN III system. Eight bacteria which consisted of five Gram-positive (GP) and three Gram-negative (GN) (*Bacillus pumilus, Clavibacter agropyri, Corynebacterium urealyticum, Corynebacterium jeikeium, Enterobacter cloaceae, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus sciuri)* were identified. *E. cloaceae* and *S. sciuri* were the most abundant bacteria on YAN with percentage values of 23 and 26%, respectively. The antibacterial activities of ethanol and water extracts of six types of leaves (*Centella asiatica*, *Jasminum sambac*, *Pereskia bleo*, *Cosmos caudatus caudatus*, *Murraya koenigii*, and *Melicope lunu*) against all the identified bacteria were studied. The ethanol extracts of *M. koenigii* are most effective extract that possess the highest antibacterial activity against all the eight spoilage bacteria studied.

Key words: Yellow alkaline noodle, spoilage bacteria, plant extracts, antibacterial activity.

INTRODUCTION

Yellow alkaline noodle (YAN) is one of the popular food in Malaysia, Indonesia and Singapore. The noodle is made usually from wheat flour, water, salt and alkaline salt (Miskelly, 1996; Hou and Kurk, 1998). The addition of alkaline salt confers a unique flavor and quality of YAN, which is absent in pasta or other type of noodles. Besides producing noodles with a pH range of 9.0 to 10.0, microbial or fungal growth causes development of sliminess on the surface of noodles at this pH range. Yellow alkaline noodles are partially boiled wet noodles with moisture content of 50 to 60% (Karim, 1989), hence they are very susceptible to spoilage and have a shelf life of 1 to 1.5 days. The spoilage is not only due to visible growth of microorganisms, but also to the production of end metabolites which results in off-odors, gas and slime production (Forsythe, 2000). According to Ray (2001) the highest incidence of spoilage in processed foods is caused by bacteria followed by yeast and moulds.

Many studies have reported on application of additives free methods to extend the shelf life of fresh pasta and noodles products. McGuire et al. (1989) applied hurdle technology by using combination of dough pasteurization, modified atmosphere packaging, and chilling to preserve fresh pasta and succeeded in extending the shelf life to 120 days. Jianming (1998) discovered that irradiation by 10 kGy of ^{60}Co-γ rays could increase the shelf life of fresh noodles up to 10 days when stored at room temperature. Fu et al. (2007) discovered that the shelf life of fresh noodles with neutral pH could be extended up to 10 days when stored at 37°C using food grade Monolaurin Microemulsion System (MMEs). Several researchers

*Corresponding author. E-mail: rosaz@putra.upm.edu.my. Tel: +60389468372. Fax: +60389423552.

have used organic acid solutions in combination with heat treatment that is (pasteurization) to increase the shelf life of fresh pasta and noodles (Nagao, 1996; Wu et al., 1998; O'Rourke et al., 2003). Suitable conventional organic acids that were used include citric, malic, acetic, fumaric, tartaric, adipic, lactic, ascorbic, sorbic, propionic, and erythrobic acids (Howard et al., 1994). Saito (2003) had proposed on the use of biological methods in extending the shelf life of noodles. He discovered that addition of *B. helvolum* B8 and *Arthrobacter* sp. B25 improved the quality of yellow alkaline noodles especially the color and it extend the shelf life up to 7 days.

Many new natural food preservation methods have been investigated by researchers. Nobile et al. (2009) has proven that natural antibacterial compounds from lemon extracts could improve the microbiological stability of refrigerated amaranth-based homemade fresh pasta. In fact, it could delay the growth of mesophilic and psychrotrophic bacteria. Zainol (2004) reported was develop and optimize *C. asiatica* leaf applied into the noodles, which will be an herbs noodle containing flavonoids as bioactive compounds to alleviate or prevent chronic diseases and cancer. Khan (2001) reported that ethanol extracts of *C. alata* leaves inhibited many types of bacteria, including *Enterobacter cloaceae, Escherichia coli and Staphylococcus aureas*. A monomeric protein with molecular mass that isolated from Murraya koenigii leaf was potentially posseses antibacterial activity against all the human pathogenic bacteria, this study were investigate by Ningappa et al. (2009) that leaf was effectively inhibit Escheria coli, Staphylococcus aeureus and Salmonella typhi. Previous studies, according to Fatouma (2010) and Nor (2010), showed that the natural plant extracts were more widely used to inhibit some antibacterial activity, and the use of natural plant was more safe than the use of chemicals. Although the antibacterial activity of component from traditional herbs and spices is well reported, here is still considerable opportunity to identify and evaluate antibacterial properties from other native plants that may be potentially beneficial as food preservatives. Potentially useful antibacterial plant compounds or extracts should ideally display activity against a wide range of microorganisms, as foods are seldom contaminated or spoiled by a single species (Gram et al., 2002). As a result, there has been a great interest in naturally produced antibacterial agents. The wide variation in levels and range of activity, however also indicates that application of the herbs or plant extracts will strongly depend on the specific problem to be addressed. Even though the antibacterial effect of several types of herbs or leaves extracts have been reported, however at present, not much work had concentrate on the application or usage of these extracts in extending the shelf life of YAN has been studied. Therefore the objective of this study was to isolate and identify spoilage bacteria on YAN and to screen the antibacterial activity of six types of local leaves extracts on the identified spoilage bacteria.

MATERIALS AND METHODS

Preparation of YAN

Wheat flour that is, (PEN 'M' brand) was bought from Aik Seng Edar Sdn. Bhd in Selangor Darul Ehsan. Noodles were made in laboratory using 100 parts of wheat flour, 34 parts of water, 1 part of sodium chloride (NaCl), and 1 part of alkaline salt comprising of 60% sodium carbonate (Na_2CO_3) and 40% potassium carbonate (K_2CO_3). All the ingredients were mixed using a mixer (Kitchen Aid Model 5K5SS, Michigan USA) at speed 1 for 20 min and at speed 2 for 5 min to form a crumbly mixture. The mixture was compressed into a dough slab (dimension: 600 x 25 x 1.6 mm) using a fabricated compressor before it was passed through a pair of noodle roller for seven successive sheeting steps. Immediately after the final sheeting, the dough was cut into noodles strips using a cutting roll. Raw noodles were boiled in boiling water (1 part of noodles in 10 part of water) at 98±0.5℃ for 50 s. They were then cooled immediately under tap water for 1 min, drained, dried under the fan for 10 min and oiled with 3% (w/w) of edible oil to prevent sticking. The YAN were packed in polyethylene bags (100 g/bag) and stored at room temperature (28±2℃) prior to analysis.

Microbial analysis

Isolation of YAN

Yellow alkaline noodles were allowed to spoil by placing them in polyethylene bags at room temperature (28±2℃) for two days. Five pack of noodles (100) were mixed thoroughly and 10 g of the sample were taken and homogenized in 90 ml of sterile peptone water (Merck, Germany) in a sterile stomacher bag using a stomacher machine (Lab-blender 400, Seward Laboratory, UK) for 60 seconds. Eight-fold dilution series were prepared with sterile peptone water for plating. The PCA media for total mesophilic bacteria were incubated at 37℃ for 48 h. Colonies were enumerated using the Whitely Acolyte Automated Colony Counter. All experiments were conducted in duplicates. The single colonies were picked based on differences in morphology from the plate count agar (PCA, Merck) and transferred to nutrient agar (NA, Merck) to obtain pure isolate culture. Then, the isolates were transferred to NA slant and kept at freezer -20˚C±2˚C until further analysis.

Identification of spoilage bacteria in YAN using BIOLOG GEN III microplate system

Gram-staining method was conducted on each of the isolate to observe the morphology. Catalase test were done on Gram positive (GP) bacteria, whereas oxidase test was carried out to differentiate between Gram negative-non enteric (GN-NENT) from Gram negative-enteric (GN-ENT) bacteria. Positive reaction in oxidase test indicates that the culture is a GN-NENT bacteria.

Each bacteria strain from the NA slant was cultured onto NA plate and incubated for 24 hat 35℃. After incubation about 3 to 5 colonies of bacteria were swabbed from the surface of agar, and was inoculated into the inoculating fluid-A (IF-A, FocusBIOTECH). The turbidity of bacteria suspension was controlled using a turbidity meter (BIOLOG, Inc). Based on the previous result of Gram staining method, GP and GN bacteria were swabbed from the surface of the agar and suspended into IF-A at cell densities of 90 to 98%. Following this, 100 µl of the bacterial suspension were pipetted into each reservoir of the GEN III microplates using a digital multichannel pipette then incubated into the OmniLog system (incubator) for 30 to 36 hat 35℃. The MicroPlates were read using the BIOLOG OmniLog GEN III system with its equivalent software

and the database obtained was used to identify individual bacteria.

Preparation of plant extract

The six types of leaves were collected from the University Park of Agriculture, Universiti Putra Malaysia. These include *Centella asiatica* (pegaga), *Jasminum sambac* (melur), *Pereskia bleo* (jarum tujuh bilah), *Cosmos caudatus* (ulam raja), *Murraya koenigii* (kari), and *Melicope lunu* (tenggek burung). The freshly collected leaves were washed thoroughly with running tap water followed by rinsing with distilled water. The leaves were air-dried at room temperature for 8 h and then oven dried at 40°C overnight. The fully dried leaves were ground into powder using a dry blender (Lab-blender, Waring Commercial USA) at speed 1 for 180 s. Extracts using ethanol (polar solvent) and water (polar solvent) were prepared by adding 1g of dried powder herb to 5 ml of each of the solvents and extraction was done for 7 days with occasional shaking and the process was repeated for three times. The extract mixtures were vacuum filtered through Whatman® No.541 filter paper. The filtrates were evaporated by rotary evaporators (Buchi Model R-210, Switzerland) at 40°C for ethanol extracts and at 60°C for water extracts to remove the solvents. The yield of the extracts were calculated and stored in amber bottle at -20±2°C until further analysis.

Bacterial strains

The antibacterial activity of extract was tested against the identified spoilage bacteria of YAN which comprised of five Gram-positive strains (*Bacillus pumilus, Clavibacter agropyri, Staphylococcus sciuri, Corynebacterium urealyticum and Corynebacterium Jeikeium*) and three Gram-negative strains *(Enterobacter cloaceae, Pseudomonas aeruginosa and Serratia marcescens)*

Antibacterial assay (disc diffusion method)

The antibacterial effect of the extracts was carried out by determining the zone of inhibition using the paper disc (6 mm in diameter, Whatman No.1) diffusion method (Sahoo et al., 2006). The bacterial strains identified in this study were inoculated in Petri dishes containing nutrient agar and incubated at 37°C overnight and were referred to as seeded agar. Four or five colonies were transferred to a tube of sterile saline. The density of bacterial suspension was compared to the 0.5 McFarland standard turbidity. The turbidity standard should be agitated on a vortex mixer immediately prior to use. The ethanol and water extracts of six leaves were dissolved in 0.1% dimethyl sulfoxide (DMSO, Sigma) to a concentration of 50 mg/ml. The disk was impregnated with 20 µl of ethanol and water extracts to give a final concentration of 1 mg/disc were placed in Mueller-Hinton Agar (MHA, Merck). Negative controls were prepared with the same solvent used to dissolve the leaves extracts. Tetracycline (30 µg/disc Oxoid, UK) as standard antibiotics was used as positive control to determine sensitivity of strain isolate in each test. The inoculated plates were incubated at 35 to 37°C for 18 h. Antibacterial activities were indicated by the presence of clear zone of inhibition. The appearance of zones of inhibition was regarded as positives for the presence of antibacterial action in the test substance. All experiments were carried out in triplicate.

Statistical analysis

The experiment was performed in triplicate and repeated two to ensure reproducibility. Statically significant of data obtained was determined using the SAS software (Version 9.2) (SAS Institute, Cary, NC). Analysis of variance was carried out and Duncan's was used for differentiation means

RESULTS AND DISCUSSION

Isolation and identification of spoilage bacteria from YAN

A total of 30 isolates were obtained from the spoiled YAN. They comprised of five GP bacteria which were *Bacillus pumilus, C. agropyri, C. urealyticum, C. jeikeium, S. sciuri* and three GN bacteria which were *E. cloaceae, P. aeruginosa, S. marcescens.* Table 1 shows that the most abundant of spoilage bacteria on YAN were *E. cloaceae* and *S. sciuri* and their similarity index were 0.58 and 0.59 at probability of 90 and 80%, respectively. Both bacteria had the highest percentage of occurrence from the total of 30 isolates bacteria with 23 and 26%, respectively. Other spoilage bacteria identified were *P. aeruginosa, C. jeikeium, C. agropyri,* and *B. pumilus* with similarity index of 0.74, 0.55, 0.85, and 0.68 and at probability of 96, 92, 100 and 99%, respectively. The least dominant spoilage bacteria found in YAN were *S. marcescens* and *C. urealyticum* with similarity index of 0.58 and 0.75 and at probability of 94 and 99%, respectively.

Table 2 shows the basic characteristic of bacteria isolated and identified from YAN during storage at room temperature (28±2°C). *S. sciuri* is a GP bacteria with coccus cell shape with width of 1 to 2 mm white to yellow colonies and show positive results for the catalyst test. The *E. cloaceae* is a GN bacteria possessing basic characteristic of rod cell shape colonies of 2 to 3 mm in diameter with light yellow in color and showed negative result for oxidase test. This result is consistent with the fact that the genus *E. cloaceae* grew at pH 9.5 (Mundt, 1986) whereas the pH of YAN is in the range 9.0 to 10.0.

Among all the 30 isolates, the BIOLOG results showed that five of them were GP bacteria, whereas only three were identified as GN bacteria. The spoilage of yellow alkaline noodles could be caused by the interplay of the activities of these bacteria. Findings from this study showed that the major groups of bacteria identified were *S. sciuri* and *E. cloaceae.* The *S. scuri* is widespread in nature and is associated with a variety of domestic and wild animals (Kloss et al., 1976; Stepanovic et al., 2001). A study by Nagase et al. (2002) showed that *S. sciuri* is among the predominant bacteria isolates in horse, cows and rodents. Previous study by Caceras (1997) on carbon sources utilization support this present study on bacteria identification which stated that L-arabinose, D-cellobiose, D-fructose, D-galactose, glycerol, lactose, maltose, D-mannitol, D-mannose, D-melezitose, salicin,

Table 1. Identification of bacteria isolated from spoiled Yellow Alkaline Noodles by BIOLOG Microplate GEN III System

Bacterial Identified	Probability (%)	Similarity index	No[a]	%[b]
Bacillus pumilus	99	0.68	3	10
Clavibacter agropyri	100	0.85	3	10
Corynebacterium urealyticum	99	0.75	1	3
Corynebacterium jeikeium	92	0.55	3	10
Enterobacter cloaceae[2]	90	0.58	7	23
Pseudomonas aeruginosa	96	0.74	3	10
Serratia marcescens	94	0.58	2	6
Staphylococcus sciuri[1]	80	0.59	8	26

[a] denotes number of isolates from total thirty unidentified isolates, [b] denotes percentages (%) of occurrence from total of thirty unidentified isolates.

Table 2. Basic characteristics of bacteria isolated and identified from spoiled yellow alkaline noodles after 2 days of storage at room temperature (28 ± 2°C).

Bacterial identified	Gram	Colony morphology	Cell shape	Catalase test	Oxidase test
Bacillus pumilus	+	Medium, white, yellow	Rod	Positive	NA
Clavibacter agropyri	+	White yellow	Rod	Positive	NA
Corynebacterium urealyticum	+	Small yellow	Rod	positive	NA
Corynebacterium jeikeium	+	Small yellow	Rod	Positive	NA
Enterobacter cloaceae	-	Light yellow	Rod	NA	Negative
Pseudomonas aeruginosa	-	Dry, wrinkle, yellow	Rod	NA	Negative
Serratia marcescens	-	Light yellow	Coccus	NA	Negative
Staphylococcus sciuri	+	Small, white cream, yellow	Coccus	Positive	NA

*NA denotes that test was not performed as it is not applicable in the Biolog GEN III System identification process. Positive, -negative.

sucrose, D-trehalose and D-xylose found in MicroPlates were among the carbon sources utilized by *S. sciuri.* The growth of aerobic mesophilic bacteria in such *S. sciuri* was more rapid compared to *E. cloaceae.* Kloos *et* al. (1976) considered *S. sciuri* as one of the most ancestral and dispersed staphylococcal species, with a wide range of habitats that includes the skin of several animals as well as environment reservoirs, such as soil, sand and water. Members of the *S. sciuri* group (*S. sciuri, S. lentus, and S. vitulinus*) are nevobiocin resistant, oxidase positive, coagulase-negative *staphylococci* and are widespread in nature. *S. sciuri* also can be found in YAN probably through manual contact during semi-manual processing stage such as sheeting of dough and cutting of noodle sheet into strands. Therefore, if the environment of the processing and equipment is not carefully controlled, spoilage of noodles would be likely to occur. *E. cloaceae* has the basic characteristic of rod cell shape and showed negative result for oxidase test. *E. cloaceae* is classified as GN bacteria and it belongs to one of the coliform bacteria. Coliform bacteria are used as indicator of hygiene and sanitation during food processing and preparation. The bacterial is widely distributed in nature, water, sewage, soil, plants and food - notably dairy products, meats, vegetable salads and spices (Barbara et al., 2000). These findings showed that GP bacteria were the most abundant bacteria in YAN followed by the GN bacteria. According to Garbut (1997), high moisture content foods (a_w 0.95-0.90) are generally spoiled by GP bacteria. YAN contained high moisture and high a_w and, hence it could easily be spoiled by both GP and GN bacteria. The ability of *S. sciuri* and *E. cloaceae* to metabolize the substrates available in noodle samples during storage suggested that it is possible that both bacteria may contribute to spoilage of YAN as observed in this study.

Antibacterial activity (disc-diffusion)

The antibacterial activity of six extracts which consisted of the leaves of *Centella asiatica* (pegaga), *Jasminum sambac* (melur), *Pereskia bleo* (jarum tujuh bilah), *Cosmos caudatus* (ulam raja), *Murraya koenigii* (kari), and *Melicope lunu* (tenggek burung) were tested on all the isolated bacteria. Table 3 clearly shows that the ethanol extracts of *M. koenigii* was high potentially active against all GP and GN bacteria represent zone of

Table 3. Antibacterial activity (inhibition zone in mm) of six ethanol and water leaves extracts against eight spoilage bacteria of YAN.

Microorganisms	Extracts												Antibiotic tetracycline
	Centella asiatica (Pegaga)		*Jasminum sambac* (Melur)		*Pereskia bleo* (Jarum tujuh bilah)		*Cosmos caudatus* (Ulam raja)		*Murraya koenigii* (Kari)		*Melicope lunu* (Tenggek burung)		
	EtOH*	H_2O*	EtOH*	H_2O*	EtOH*	H_2O*	EtOH*	H_2O*	EtOH*	H_2O*	EtOH*	H_2O*	
Bacillus pumilus	9.73±0.46	8.80±0.10	9.00±0.00	8.73±0.46	8.37±0.23	6.70±0.61	8.47±0.5	7.73±0.23	17.80±0.72	14.33±0.49	9.00±0.95	8.00±0.00	25.00±2.23
CLavibacter agropyri	8.57±0.21	0.00±0.00	8.67±0.15	0.00±0.00	6.90±0.20	0.00±0.00	0.00±0.00	0.00±0.00	15.70±0.44	11.1±0.6	9.53±0.45	0.00±0.00	0.00±0.00
Corynebacterium urealyticum	7.63±0.57	7.47±0.40	9.07±0.06	6.60±0.26	7.30±0.26	7.33±0.58	7.37±0.64	7.10±0.17	16.20±0.36	13.37±0.21	8.00±0.00	6.80±0.35	17.00±1.00
Corynebacterium jeikeium	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	16.33±0.68	11.96±0.05	0.00±0.00	0.00±0.00	19.00±0.00
Enterobacter cloaceae	9.57±0.51	9.00±0.00	8.67±1.15	7.47±0.25	7.67±0.58	7.20±0.44	8.93±1.05	7.80±0.26	19.40±0.36	14.10±0.26	7.00±0.10	6.43±0.58	20.0±0.00
Pseudomonas aeruginosa	8.33±0.25	7.30±0.20	7.60±0.36	6.87±0.81	9.43±0.38	7.00±1.00	6.93±0.81	6.80±0.53	15.47±0.38	12.27±0.47	7.87±0.32	6.63±0.47	18.00±0.00
Serratia marcescens	7.80±0.10	7.23±0.23	7.63±0.15	6.93±0.90	9.63±0.64	7.00±0.00	7.67±0.49	7.40±0.35	17.67±0.51	14.45±0.85	8.67±0.58	7.90±0.20	25.67±1.15
Staphylococcus sciuri	8.27±0.31	7.17±0.21	7.30±0.52	7.10±0.10	9.13±0.23	8.73±0.38	7.37±1.10	6.67±0.58	20.63±1.10	14.00±1.21	8.47±0.67	7.93±0.80	22.00±0.00

Values are mean ± S.D (mm), *Data represents a means of 3 replicates. Ethanol and water extracts: 20 µl/disc. Positive control: Tetracycline 30 µg/disc

inhibition ranging from 15.47 to 20.63 mm. On the other hand, other leaves in ethanol extracts also capable to inhibit all GP and GN bacteria, but with lower degree of inhibition from (6.90 to 9.37 mm), except for extracts *C. caudatus* which showed no antibacterial activity. The water extracts of *M. koenigii* was able to inhibit the GP and GN bacteria with zone of inhibition ranging from 11.1 to 14.45 mm. In general, the inhibition zone diameter (IZD) for all bacteria tested using the ethanol leaves extracts range from 6.90 to 20.63 mm, whereas for the leaf water extracts it was 6.43 to 11.1 mm, except for bacteria *C. agropyri* that showed no inhibition. In this study for ethanol and water leaves extracts highly inconvenient to inhibit *C. jeikeium* bacteria, except *M. koenigii* and antibiotic tetracycline. Compared to all the ethanol and water extracts, standard antibiotic such as tetracycline gives the highest zone of inhibition from 17.0 to 25.67 against all GP and GN bacteria, except *C. agropyri* that no showed inhibition.

The ethanol extracts of *M. koenigii* showed the highest inhibition against all GP and GN bacteria. This is supported by Goutam (1994) who reported that the crude extracts of *M. koenigii* leaf possess highest strong antibacterial and antifungal activity when tested in microorganisms. The leaves are rich in vitamins A and B, mineral and calcium, and it also contains amino acid such as glycine, serine and glutamic acid. The leaves have been used as antimicrobial, antidiabetic, antioxidant and anti inflammatory (Mallavapura et al., 1999) agent. In this study the *C. jeikeium* is apparently resistant to all of ethanol and water extracts, except *M. koenigii*. This may caused too many species normal commensalism of the human skin including *C. jeikeium*, as well as the non-pathogenic soil bacteria including *C. glutamicum* and *C. efficiens* that are widely used in biotechnological production processes of food and feed additives (Fudou et al., 2002). In this study was assessed that all of leaves extracts have an important role to inhibit the spoilage bacteria in YAN. All of crude extracts were able to inhibit all GP and GN bacteria probably because the crude leaves extracts contain alkaloids, glycoside, flavonoid, terpenes, tannin, resin, and salicylic acid which possess antibacterial activity against food-borne pathogens (Fatouma et al., 2010). Joy (2008) had showed that the extracts of *J. sambac* contain antibacterial activity against *S. typhii* and *S. aureus.* Besides that, these leaves also contain antibacterial effect against *S. aureas.* Extracts of *M. lunu* showed moderate test results. The ethanol and water extracts showed moderate activity and these results are in agreement with previous report by McCormick et al. (1996). According to Wiart (2006), the leaf of *P. bleo* are simple spiral, glossy and succulent, it is believed to have anti-cancer, anti-tumour and anti-rheumatic also to treat diabetes and hypertension (Tan et al., 2004). Leaf extracts of *C. caudatus* showed varying degrees of antibacterial activity against all microorganisms tested. The GP bacteria were more susceptible than GN bacteria (Tortora et al., 2001). There are many types of active components in a particular extracts, but not all are able to inhibit bacteria. Only certain

types of active component were able to inhibit certain bacteria (Sawai et al., 2002). Based on this observation, it can be concluded that ethanol extracts of *M. koenigii* (kari) are the most effective leaf extracts that possess the highest antibacterial activity against all the eight spoilage bacteria isolated from YAN.

Conclusion

Generally, the extracts of ethanol from all the leaves studied were able to inhibit GPand GN bacteria that spoiled yellow alkaline noodles more effectively than the crude water extracts. The ethanol extracts of *M. koenigii* contributed the strongest antibacterial activity against GP and GN bacteria of YAN. These leaf could be added in YAN as an alternative source of natural antibacterial substances to prevent the growth of food-borne bacteria and to extend the shelf-life of YAN.

However, further works need to be carried out to identify the active component of these plants that is responsible to inhibit the spoilage bacteria during the storage period. The wide variation in levels and range of activity, however also indicated that application of these plants will strongly depend on the specific problem to be addressed.

REFERENCES

Abdoul-Latif F, Edou P, Eba F, Mohamed N, Ali A, Djama S (2010). Antimicrobial and antioxidant activities of essential oil and methanol extract of *Jasminum sambac* from Djibouti. Afr J. Plant Sci., 4(3): 38-43.

Barbara ML, Tony CB (2000). Grahame Warwick Gould. The microbial safety and quality of food. Springer Publisher, pp. 1396-1398.

Brul S, Coote P (1999). Preservative agents in foods. Mode of action and microbial resistance mechanisms. Int. J. Food Microb., 50: 1-17.

Cáceres P, Castillo D, Pizarro M (1997). Secondary flora of Casar de Cáceres cheese: characterization of Micrococcaceae. Int. Dairy J*., 7*(8-9): 531-536.

Del Nobile M, Di Benedetto N, Suriano N, Conte A, Lamacchia C, Corbo, M (2009). Use of natural compounds to improve the microbial stability of Amaranth-based homemade fresh pasta. Food Microb*.,* 26(2): 151-156.

Forsythe SJ (2000). The Microbiology of Safe Food. Blackwell Science, Oxford.

Fu X, Huang B, Feng F (2008). Shelf life of Fresh Noodles as Affected by the Food Grade Monolaurin Microemulsion System. J. Food Proc. Eng., 31(5): 619-627.

Fudou R, Jojima Y, Seto A, Yamada K, Kimura E, Nakamatsu T (2002). Corynebacterium efficiens sp. nov., a glutamic-acid-producing species from soil and vegetables. Int. J. Syst. Evol. Microb*.,* 52(4): 1127.

Garbutt J (1997). Essential of Food Microbiology. Arnold, London.

Gram L, Ravn L, Rasch M, Bruhn JB, Christensen AB, Givskov M (2002). Food spoilage-interactions between food spoilage bacteria. Int. J. Food Microb*.,* 78: 79-97.

Goutam MP, Purohit RM (1994). 'Antimicrobial activity of the essential oil of the leaves of *Murraya koenigii* (Linn.) Spreng (Indian Curry leaf), Indian J. Pharm*.,* 36(1): 11-12.

Howard JG, Reiter HJ, Fritsch D, Scheren B, Lafleur TJ (1994). Acid Stabilized Pasta. United State Patent: US 5332587.

Hou G, Kruk M (1998). Asian Noodle Technology. Asian Noodle Technical Bulletin., 20(12): 1-10.

Huis in't Veld JHJ (1996). Microbial and biochemical. spoilage of foods: an overview. Int. J. Food Microb., 33: 1-18.

Jianming C (1998). Preservation of fresh Noodles by Irradiation. Radiation Phys. Chem*.,* 52(1-6): 35-38.

Joy P, Raja D (2008). Anti-Bacterial Activity Studies of *Jasminum grandiflorum* and *Jasminum sambac.* Ethnobotanical Leaflets, 1: 59.

Karim R (1989). Textural Studies on Chinese Wet Noodles (Hokkien Styles Noodles). Thesis, Universiti Putra Malaysia.

Kloos W, Schleifer K, Smith R (1976). Characterization of *Staphylococcus sciuri* sp and its subspecies. Int. J. Syst. Evol. Microb., 26(1): 22.

Mallavapura GR, Ramesh S, Syamasundar KV, Chandrashekara RS (1999). Composition of Indian curry leaf oil', J. Ess. Oil Res., 11(2): 176 178.

McCormick JL, Mckee TC, Cardelina HJ, Boyd MR (1996). Inhibitory natural products: quinoline alkaloids from Euodia roxburghiana, J. Natural Prod., 59: 469- 471.

McGuire M, DiGiacomo R, Palmer M, Ligget L (1989). Method for Preparing and Perserving Fresh Pasta. United State Patent: US, 4 876-104.

Miskelly DM (1998). Modern Noodle Based Foods-Raw Material Needs. In Blakeney, A. B., and O'Brien, L. Pasific People and Their Food St. Paul, Minnesota: America Association of Cereal Chemist, Inc., pp. 123-142.

Mundt JO, Hinkle NF (1976). Bacteria within ovules and seeds. Appl. Environ. Microbiol., 32(5): 694.

Nagao S (1996). Processing Technology of Noodle Product in Japan. In J. E. Kruger, R. B. Matsuo, and J. W Dick. Pasta and Noodle Technology St. Paul, Minnesota: America Association of Cereal Chemist, Inc., pp. 169-194.

NCCLS (2000). National Committee for Clinical Laboratory Standards. Approved Standard M7-A5: Methods for Dilution Antimicrobial Susceptibility Test for Bacteria that Grow Aerobically, 5th Ed.

Nagase N, Sasaki A, Yamashita K, Shimizu A, Wakita Y, Kitai S (2002). Isolation and species distribution of staphylococci from animal and human skin. J. Vet. Med. Sci./Jpn Soc. Vet. Sci., 64(3): 245.

O'Rourke J, Tunstall JA, Howe SC, Moberg LJ (2003). Method for Thermal Processing and Acidification of Pasta Product. United State Patent: US 6576279.

Ray B (2001). Fundamental Food Microbiology. CRC Press, pp. 97-228.

Sahoo S, Kar D, Mohapatra S, Rout S, Dash S (2006). Antibacterial activity of Hybanthus enneaspermus against selected urinary tract pathogens. Indian J. Pharm. Sci., 68(5): 653.

Saito K, Nishio Z, Takata K, Kuwabara T, Yokota A, Yamauchi H (2003). Bacterial Adjunct to Improve the Quality of Alkaline Noodles. Food Sci. Technol. Res., 9(1): 40-44.

Sawai J, Doi R, Maekawa Y, Yoshikawa T, Kojima H (2002). Indirect conductimetric assay of antibacterial activities. J. Indust. Microb. Tech., 29: 296-298.

Tan ML, Sulaiman SF, Najimuddin N, Samian MR, Tengku Muhammad TS (2004). Methanolic extracts of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. J. Ethnopharmacol., 96: 287-294.

Tortora GJ, Funke BR, Case CL (2001). Microbiology: An Introduction.7th edition Benjamin Cummings Publishing. San Fransisco, USA, p. 88.

Wiart C (2006). Medical plants of the Asia-Pacific Drugs for the Future. World Scientific Publisihing, pp. 98-99.

Wu TP, Kuo WY, Cheng MC (1998). Modern Noodles Based Foods-Product Range and Production Methods. In Blakeney, A. B., and O'Brien, L. Pasific People and Their Food St. Paul, Minnesota: America Association of Cereal Chemist, Inc., pp. 37-90.

Zainol M, Khairi M (2004). Determition of Flavonoids In Centella Asiatica (L.) Urban And Their Utilization In Herbal Noodle. PhD Thesis. Universiti Putra Malaysia.