Malaysian Journal of Microbiology, Vol 12(3) 2016, pp. 284-290
http://dx.doi.org/10.21161/mjm.83816

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Screening of phytochemical properties and antimicrobial activity of Malaysian medicinal plants against aquatic bacteria

Sharifah Raina, Manaf[1, 3] and Hassan, Mohd. Daud[1, 2]*

[1]Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2]Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[3]Faculty of Plantation and Agrotechnology, Universiti Technology Mara (Melaka), Jasin Campus, 77300 Merlimau, Melaka, Malaysia.
Email: hassanmd@upm.edu.my

Received 7 March 2016; Received in revised form 3 June 2016; Accepted 29 June 2016

ABSTRACT

Aims: Quantitative screening of phytochemical properties and antimicrobial activities were done on some plants of importance in human medicine as traditional herbs to treat fish diseases in Malaysia. Six extracts of *Vitex trifolia, Aloe vera*, *Strobilanthes crispus*, *Clinacanthus nutans, Pereskia grandifolia* and *Peperomia pellucida* were determined for phytochemical properties and their antibacterial activities against common freshwater pathogens i.e. *Streptococcus agalactiae, Aeromonas hydrophila* and *Enterobacter cloacae.*
Methodology and results: Qualitative screening of phytochemical properties in herbs were determined using conversional method for flavonoids, tannins, saponin, alkaloids steroid and glycoside The results showed flavonoid was presence in all plant extracts. For the antimicrobial activity, the aqueous and methanolic extracts were tested by using disk diffusion method. Antimicrobial assay of methanolic crude extracts (25 to 100 mg/mL) showed effectiveness against the pathogenic bacteria. Comparatively, all aqueous extracts did not show any antimicrobial activity. Strong antibacterial activity was shown by the methanolic extracts of *V. trifolia, A. vera* and *S. crispus* while moderate antimicrobial activity was shown by *C. nutans, P. grandifolia* and *P. pellucida*.
Conclusion, significance and impact study: The current results indicated that the studied plants might indeed be potential sources of natural antimicrobial agents to control fish diseases.

Keywords: Vitex trifolia, Aloe vera, Strobilanthes crispus, phytochemical, antimicrobial

INTRODUCTION

Medicinal plants (herbal remedies) are a profoundly rooted component of the cultural heritage of numerous people from diverse cultures and countries and are, as such, closely linked to the maintenance of good health (Galina *et al*., 2009). Malaysia is rich in biodiversity encompassing a variety of herbs and shrubs with potential medicinal properties. In Peninsular Malaysia, there are about 1,200 species of higher plants which have been reported to have medicinal properties for treatment of various diseases and ailments. But, so far only about a hundred have been investigated fully for their medicinal potential (Jamal *et al*., 2010).

The total freshwater aquaculture production in Malaysia showed an increase of 19.7% in 2013 (Annual Statistics, Department of Fisheries Malaysia). Overall, tilapia production reached 52,000 MT in 2013. The increasing world-wide importance of cultured tilapia as a food fish has prompted considerable research on improvement of growth performance in tilapia. However, due to intensive culture practices, serious economic downfall to the industry was contributed by microbial diseases. In tilapia, a bacteria disease caused by *Streptococcus agalactiae* has been reported to cause fatal mortality and morbidity of tilapia culture worldwide. The medicinal plants have strong antibacterial effects of active compounds known to play an important role in preventing bacterial infections (Iruthayam *et al*., 2014). It can be used not only as remedies, but also as growth promoters and stress resistance boosters. Hence, medicinal plants in disease management are gaining success, because they are cost effective, eco-friendly and have minimal side effects. A large portion of the world population, especially in developing countries depends on the traditional system of medicine for a variety of diseases (Govind *et al*., 2012). Amongst the many hundreds of potential medicinal plants, for this study we have selected six such as plants namely *V. trifolia* (simpleleaf chastetree), *A. vera* (crocodile tongue), *S. crispus* (yellow strobilanthus), *P. pellucida* (shiny bush), *C. nutans* (Sabah snake grass) and *P. grandifolia* (rose cactus).

***Corresponding author**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

This selection is based on the commonly used plants in traditional human medicine for the treatment and control of several diseases and availability to obtain them.

One mililiter Currently, research has focus for alternatives of antibiotics to prevent and control of diseases in aquaculture. The herbs and plant extracts (phytobiotics) has reported to contain compounds with antibacterial properties, which can be use as preventive and control measure for diseases in aquatic organisms. Accordingly, present study was performed to determine the phytochemical constituents and the antibacterial activity of *V*. *trifolia*, *A*. *vera*, *S*. *crispus*, *P*. *pellucida*, *C*. *nutans* and *P*. *grandifolia* plants towards selected aquatic pathogenic bacteria.

MATERIALS AND METHODS

Preparation of herbs

V. *trifolia*, *A*. *vera*, *S*. *crispus*, *P*. *pellucida*, *C*. *nutans* and *P*. *grandifolia* were obtained from Universiti Agriculture Park, Universiti Putra Malaysia, Selangor, Malaysia. Fresh healthy leaves, stems and including flowers were collected in morning or afternoon and washed under running tap water to remove dirt particles. They were allowed to dry in a draught oven at a temperature of 65 °C for 48 h. They were then chopped into small pieces and grounded into powder using mechanical grinder (Panasonic, MY333). The powdered plant materials were keep in airtight bottles prior to extraction in room temperature.

Preparation of extraction

For aqueous extraction, a 100 g of the powdered plant were mixed into 1 L of deionised distilled water in a 2 L of conical flask. While, for methanol extracts was prepared by adding 100 g of plants powder into 1 L of 70% of methanol. The conical flasks were concealed using aluminium foil and homogenised for 3 days at room temperature using shaking incubator. Then, the mixture was filtered through 11 µm membrane filter paper (Whatman® No. 1). Then, the extracts were evaporated using rotary evaporator at 40 °C. The stock solution (0.5 g/mL) of the methanol extracts were prepared by dissolving in 70% methanol and for the aqueous extracts in deionised distilled water. The extracts were stored at 20 °C until further use.

Qualitatively screening of phytochemical

Phytochemical screening for flavonoids, tannins, saponin and alkaloids using methods as described by Parekh and Chanda (2008) and Aiyegoro and Okoh (2010). Screening of the presence of steroid was performed as described by (Kumar *et al*., 2009) and glycoside (Edeoga *et al*., 2010) were made on the plant's extract. To screen flavonoids, 1 g of plant extract was dissolved in 10 mL dH_2O and then filtered. Ten mg magnesium were added into 1 mL of the filtrate and followed by addition of 0.05 mL concentrated sulphuric acid (1 N). The presence of flavonoids would showed magenta red within three minutes. While, to test tannins, about 0.5 g of the dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. The presence of blue-black precipitates resulting after the addition a few drops of ferric chloride (0.01 g/mL) reagent and indicated the presence of tannins. Frothing test was used to determine the presence of saponins. About 2 g of the plant extract was boiled in 20 mL of distilled water in a water bath and filtered. Ten mililiter of the filtrate was mixed with 5 mL of distilled water and shaked vigorously for a stable persistent of froth. The froth produced were mixed with 3 drops of olive oil and shaked vigorously, then observed for the formation of emulsion. The presence of honey-comb froth indicated the existence of saponins. The presence of alkaloids were determined by dissolving 0.02 g of extract in 1 mL methanol and filtered through 11 µm membrane filter. This followed by boiling the extract after adding 2 mL of 1% hydrochloric acid for 5 minutes. 4 to 6 drops of Dragendorff's reagent was then added into the extract preparation, and the formation of orange precipitates indicated the presence of alkaloids. Although, to check for the steroid, 0.5 g of plant extract was dissolved in 2 mL methanol and filtered through 11 µm membrane filter. One mililiter of chloroform and 1 mL of concentrated sulphuric acid (1 N) were then added into 1 mL filtrate by dropping at the side of the tube and the presence of yellow with green fluorescence at the sulphuric acid layer indicated the presence of steroids. Keller-Killani test was used to determine the presence of glycoside. Five mL of plant extracts was treated with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1 mL of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides.

Antibacterial assays using disk diffusion method

The antibacterial assays of the plant extracts was performed using a slightly modified agar disc-diffusion method as described by Bauer *et al*. (1966). The plants extracts were loaded onto sterile Whatman No. 1 membrane filter paper discs (6 mm diameter) and left to dry in oven at 30 °C. A loop of bacteria was inoculated in TSB until it reached Mc Farland standard needed. One hundred µl of bacteria were spread onto the surface of Muller-Hinton agar (MHA) plates. Paper discs containing the plant extracts were carefully placed on the surface of each plate. Oxytetracycline (OTC) served as positive control and solvent as negative control. The plates were left at 4 °C for an hour to allow diffusion of extracts before they were incubated for 24 h at 37 °C. Microbial inhibition was indicated by measuring the diameter of the clear zone around the discs and recorded as diameter on inhibition zone in millimetre. The diameter of the inhibition zones was measured and the results were expressed as mean of the three independent readings. The test was repeated three times. Antibacterial activity was assessed qualitatively as: (−) no inhibition; (+) zone of inhibition. The strength of activity was classified as strong for

ISSN (print): 1823-8262, ISSN (online): 2231-7538

inhibition zone diameters ≥ 20 mm, moderate for diameters ranging from 10 to 19 mm and weak for diameters ranging from 1 to 9 mm (Shahidi, 2004).

Minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC), quantitative serial dilutions of aqueous and methanol extracts were tested against bacteria. Extracts of plant that showed positive antimicrobial activities were used for the study using broth dilution method. The plant extracts were diluted to the highest concentrations i.e. 50 mg/mL. Then serial two-fold dilutions were made in concentrations ranging from 0.625 mg/mL to 50 mg/mL. Six different concentrations of the plant extracts were tested. The final concentration of bacteria culture of 10^8 cells/mL was dispensed into tubes. All inoculated tubes were incubated at 37 °C for 24 h. The concentration at the lowest serial dilution of the extracts at which growth did not occur on broth was regarded as the MIC.

Statistical analysis

The significance differences ($P<0.05$) of bacterial inhibition zones was analysed using Two Way ANOVA.

RESULTS

Phytochemical screening of plant extractsand

The phytochemical screening of extracted plants showed the presence of alkaloids, saponin, tannins, flavanoid, steroid, and glycoside (Table 1). Flavonoid were abundantly present in all of the extracted samples. Furthermore, phenolic compounds were present in the extracts of high polarity solvents (MeOH and water) for each part the plant whereas flavonoids and tannins which are commonly distributed groups of plant phenolic were absent in the medium polar solvent accept in leaves as compared to tannin, that were only present in leaves for all solvent. The phytochemical of *V*. *trifolia* methanol extracts showed tannins, flavonoid and glycoside. While, *A*. *vera* and *S*. *crispus* methanol extracts contained of alkaloids, tannins and flavonoid. *P*. *pellucida* showed positive results having all variable tested. In *P*. *grandifolia* and *C. nutans* showed had alkaloids and flavonoid.

Table 1: Preliminary phytochemical screening of plant extracts.

Variable tested	*V. trifolia*	*Aloe vera*	*S. crispus*	*P. pellucida*	*C. nutans*	*P. grandifolia*
Alkaloids	−	+	+	+	+	+
Saponin	−	−	−	+	+	−
Tannins	+	+	+	+	−	−
Flavonoid	+	+	+	+	+	+
Steroid	−	−	−	+	−	−
Glycoside	+	−	−	+	−	−

*+ Presence; - Absence

Antimicrobial screening of plant extracts

In the present study, the *in vitro* antibacterial activities of the plants extract against the pathogenic bacteria were examined by the presence or absence of inhibition zones.. The bacterial identifies were confirmed using conventional bacteriology identification methods including Gram stain and oxidase test (Frerichs and Millar, 1993). Biochemical profiles for *A*. *hydrophila* was standardized following the manufacturers guidelines for the API 20E (BioMerieux®, U.K.). For *S*. *agalactiae* and *E*. *cloacae* BBL CrystalTM E/NF were used.

There was a large variability in the antimicrobial activities showed by the tested herbs. The activity was classified as weak, medium, strong and very strong, as followed by the US National Committee for Clinical Laboratory Standards (Cockerill *et al*., 2012). All six extracts were tested on selected Gram-positive and Gram-negative bacteria (Table 2). Results showed that all plant water extracts showed weak reaction against all bacteria tested. The methanol and aqueous extracts of all plants screened showed various inhibitory effects (9 – 15 mm) and (7 – 9 mm), respectively. *A. vera* and *P. pellucida* aqueous extracts showed maximum inhibition against bacteria showing an inhibition zone of 9 mm. The largest inhibition zone was observed from *V*. *trifolia* methanol and aqueous extracts against *A. hydrophila* (15 mm) and *S*. *agalactiae* (11 mm), respectively. The *A vera* extracted in methanol showed inhibition against *A*. *hydrophila* (13 mm) and *S*. *agalactiae* (11 mm).However, the former showed no inhibition against *E*. *cloacae*. While, *S*. *crispus* methanol extracts largest inhibition zone was observed in *S*. *agalactiae* and showed 11 mm against *A*. *hydrophila* and *E*. *cloacae*. *C. nutans* methanol extracts only gave effect towards *A. hydrophila* and its aqueous extracts showed weak activity against the other bacteria. Whereas, in *P*. *pellucida* methanol extract inhibited *E*. *cloacae* and *A*. *hydrophila* growth, showing inhibition zones of 9 and 12 mm respectively. Different results were observed in *P*. *grandifolia* methanol extracts which were12 mm and 9 mm against *A*. *hydrophila* and *S*. *agalactiae,* respectively. Antibiotics oxytetracycline, chloramphenicol, thrimethoprim, streptomycin, penicillin, amoxycillin and ampicillin were used as the controls. Oxytetracycline showed inhibition against *A*. *hydrophila* (27 mm), *S*. *agalactiae* (20 mm) and *E*. *cloacae* (24 mm). While, chloramphenicol, thrimethoprim and streptomycin showed 27 mm, 18 mm, 12mm inhibition zone, respectively against *A*. *hydrophila*.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Response of different solvents of plants extracts and controls.

Bacteria	Zone of inhibition (mm)					
	A. hydrophila		*S. agalactiae*		*E. cloacae*	
Plant	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
A. vera	9	13	7	11	8	-
S. cripus	8	11	-	13	7	11
V. trifolia	8	15	8	11	7	-
P. pellucida	9	12	8	-	7	9
C. nutans	-	11	7	-	8	-
P. grandifolia	-	12	7	9	8	-
Oxytetracycline (OTC30C)–30 µg	27		20		24	
Chloramphenicol (C10C)–10 µg	27		-			
Thrimethoprim (TM2.5C)–2.5 µg	18		-			
Streptomycin (S10C)–10 µg	12		-			
Penicillin g (PG1C)–1 unit	-		-			
Amoxycillin (A10C)–10 µg	-		-			
Ampicillin (AP10C)–10 µg	-		-			

-, no inhibition

Minimum inhibitory concentration

Table 3 presented the MIC values of methanolic plant extracts. The range of MIC obtained was from 6.25 to 50 mg/mL. The MIC for *A. vera* and *V. trifolia* were 6.25 mg/mL for both *A. hydrophila* and *S. agalactiae* bacteria. While, *S. crispus* and *P. pellucida* values were 6.25 mg/mL against *A. hydrophila* and 12.5 mg/mL against *S. agalactiae*. The value of MIC for *C. nutans* and *P. grandifolia* was 25 mg/mL against *A. hydrophila* and 50 mg/mL against *S. agalactiae*.

Table 3: Minimum inhibitory concentration of methanolic plant extracts.

Methanolic Plant Extract / (mg/mL)	Bacteria	
	A. hydrophila	*S. agalactiae*
A. vera	6.25	6.25
S. cripus	6.25	12.5
V. trifolia	6.25	6.25
P. pellucida	6.25	12.5
C. nutans	25	50
P. grandifolia	25	50

DISCUSSION

A number of different alternative antimicrobial compounds are widely used today to control major aquatic pathogens. They are a good alternative source for synthetic drugs and antibiotics in the aquaculture industry, and reduce the side effects observed with synthetic compounds and antibiotics. Plants are rich in a broad diversity of secondary metabolites with antimicrobial properties, and are thus a major source of antimicrobial compounds. Plants are the storehouses and sources of safer and cheaper chemicals (Prasad and Variyur, 1993); of the 80% of pharmaceuticals derived from plants, very few are presently used as antimicrobials (Perumal and Gopalakrishnakone, 2008).

Medicinal plants synthesize antimicrobial compounds as part of their defence against invasion by microbial pathogens. It is estimated that almost 50% of synthetic medicines are derived from or patterned after phytochemicals (Canadian Pharmaceutical Association, 1988). In the medicinal plant family secondary metabolites such as alkaloids, phenolics and other compounds have contributed the largest number of antimicrobial drugs in the pharmacological industry. The safer, biodegradable plant-derived compounds offer a promising solution to the problem of resistant microbes (Citarasu, 2010).

The selection of medicinal plant for present study is based on the commonly used plants in traditional human medicine for the treatment and control of several diseases and availability to obtain them. *Vitex trifolia* is an aromatic shrubby or small tree which can grow up from 1 to 4 m high, with the stems covered by soft hairs (tomentose), sometimes prostate or ascending in habitat (Rajan *et al*., 2012). *Aloe vera* (Xanthorrhoeaceae) is a succulent, almost sessile perennial herb. Their leaves 30 - 50 cm long and 10 cm wide at the base; colour in pea-green (when young they are flecked with white) and bright yellow tubular flowers, 25 – 35 cm in length arranged in a slender loose spike. It contains a colourless mucilaginous gel called *A. vera* gel (Bruneton, 1999). *Strobilanthes crispus* is bush-like plant, attaining a maximum height of 0.5 - 1.0 m. It can be found on riverbanks or abandoned fields (Maznah *et al*., 2000). The leaves are oblong-lanceolate, rather obtuse and shallowly crenate crispate and have a rough surface, covered with short hairs (Backer and Bakhuizen, 1963; Sunarto, 1977). *Pereskia grandifolia* is a shrub or small tree, 2 – 5 m tall with a rounded crown, with a greyish-brown trunk up to 20 cm in diameter. The spine's range from black to brown and their number at each areole gradually increases with age, the leaves are edible and vary in size from 9 to 23 cm long and the shapes range from elliptic to ovate and obovate-lanceolate (Anderson, 2001). *Peperomia pellucida* (L.) is an herbaceous plant with succulent, alternate oval leaves,

ISSN (print): 1823-8262, ISSN (online): 2231-7538

inflorescences in terminal spikes axillary and opposite to the leaves, which grows well in loose and humid soils under the shade of trees (Majumder *et al*., 2011). *Clinacanthus nutans* is a shrub or perennial herbs that can grow up to 1 m in height with pubescent branches. The stem is torete, striate and glabrescent. The leaves are simple, opposite, narrowly elliptic-oblong or lanceolate (2.5 - 13 cm long × 0.5 - 1.5 cm wide) (Panyakom, 2006).

The assessment of phytochemicals in the current study revealed that the chemical constituents and the bioactive constituents of the tested herbal plant have proved why they acquired a lot of attention all over the world. It has been reported that biological activities in the selected plants were exhibited by different classes of phytochemicals. The present study included the phytochemical screening of the plants *V. trifolia*, *A. vera*, *S. crispus*, *C. nutans*, *P. grandifolia* and *P. pellucida*. Results from the phytochemical screening test indicated the presence of alkaloids, saponin, tannin, flavonoid, steroid and glycoside. Similarly Pappachen and Annam (2013) and Majumder *et al*. (2011) had reported that the methanolic extract of *P*. *pellucida* showed the presence of carbohydrates, flavonoids, steroids, triterpenoids tannins, glycosides and alkaloids. Compounds in *P. pellucida*, is known to have astringent properties which hasten the healing of wound and inflamed mucous membranes (Fatan, 1990). These must be the reason why it has been practiced by the Orang Asli Temuan tribe for snake bite healing (Faridah and Nurulhuda, 1999).

In the present study showed *V*. *trifolia* methanol extracts showed tannins, flavonoid and glycoside. Similarity, Aditya and Ravi (2014) and Rajan *et al*. (2012) showed that *V*. *trifolia* methanolic extract positive for alkaloids, flavonoids and antraquinone glycosides, but negative for saponin and cardiac glycosides. In addition, *A*. *vera* and *S*. *crispus* contain alkaloids, tannins and flavonoid. Furthermore, Dahiya and Purkayastha (2012) concluded that the phytochemical analysis of the *A*. *vera* methanolic extracts revealed the presence of reducing sugar, anthraquinones, glycoside, saponins and absence of tannins, flavonoids, phlobatannins, steroids and terpernoids. While, *S*. *crispus* contains polyphenols, catechins, alkaloids, caffeine, tannins, vitamins (C, B1 and B2) and also high mineral content (Maznah *et al*., 2000). Liza *et al*. (2010) have identified eight flavonoid compounds from the leaves of *S*. *crispus*. Whereas, *P*. *grandifolia* methanolic extract showed positive result of the analyses for flavonoids, tannins, alkaloids and saponinic heterosides (Kazama *et al*., 2012).

Alkaloids are a diverse group of heterocyclic nitrogen compounds with a bitter taste. They have been found to have good antimicrobial effects against bacterial pathogens and protozoan parasites (Ghoshal *et al*., 1996). *Clinacanthus nutans* showed had alkaloids and flavonoid. While saponins are phytosterol compounds derived from natural plants and occur in small quantity and also a glycoside of triterpens in which most of anticancer agents come from (Bryan, 2005). Jamaludin *et al*. (2011) stated that the extracts in non-polarity and low polarity solvent showed a positive presence of steroids in three parts of the *Donax grandis* i.e. fruits, leaves and stems. Cardiac glycosides only present in stems in a low polarity solvent whereas saponins were present in extracts of high polar solvents. Moreover, saponin is a glycoside of triterpenes in which most of anticancer agents coming from this group of compound (Bryan, 2005). The presence of saponins was confirmed to be the highest in percentage in fruits (2.39%) as compared to the other parts of the plant.

In antimicrobial assay, aqueous extracts of herbs did not show or showed little activity against all bacterial species tested. As we know, water is considered to have large dipole molecules and a high dielectric constant (Thabile, 2008). Thus, water is very polar and only miscible in itself. Water extracts of lemongrass stem did not show any antimicrobial because main compounds of the lemongrass are oily substances (Thabile, 2008). Water has been used by many researchers as an extract solvent and most of their results indicated that water extracts gave little or no inhibitory effect (Siripongvutikorn *et al*., 2005; Aliero and Afolayan, 2006). One important factor of solvent effectiveness is the active components of the plants. According to a study by Rojas *et al*. (2006), some of the water extracts of some plants such as *Bidens pilosa* L., *Jacaranda mimosifolia* D. Don and *Piper pulchrum* had antimicrobial activity, but others such as *Justica secunda* did not. This means that based on the compounds' miscibility in water, some will have antimicrobial properties.

Based on our current findings, *V. trifolia* methanolic extracts were found to be the most effective against *A. hydrophila*. The herb was shown to produce antibacterial activity against all test organisms except *E. clocae*. Hossain *et al*. (2001) reported in other studies the ethanol extracts of *V*. *trifolia* leaves was tested for Gram-positive and Gram-negative bacteria and showed moderate inhibiting activity. Inhibition zones were seen in *A. vera*, *V. trifolia* and *P. grandifolia* methalonic extracts. There were clear antimicrobial reaction against *S. agalactiae*. All extracts showed moderate to strong inhibition for *A. hydrophila*. The antimicrobial effect of methanolic extracts against some bacteria might be due to the ability of the methanol to extract some of the active properties of these plants like saponin and other secondary metabolites which were reported to be antimicrobial (Cowan, 1999; Okwu and Josiah, 2006). Only in *S. crispus* and *P. pellucida* showed inhibition against *E*. *cloacae*. It showed that not all extracts were active against Gram negative bacteria. This could be due to the presence of outer membrane as a permeability barrier provided by the cell wall or to the membrane accumulation mechanism (Chew *et al*., 2011).

CONCLUSION

The phytochemical of screening of the methanol extract of *V*. *trifolia*, *A*. *vera*, *S*. *crispus*, *C*. *nutans, P*. *pellucida* and *P*. *grandifolia* showed the presence of secondary metabolites including alkaloids, saponin, tannins, flavanoid, steroid, and glycoside. Among the aqueous and

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

methanol extracts, the antimicrobial activity of *V. trifolia, A. vera*, *S. crispus*, *C. nutans, P. pellucida* and *P. grandifolia* showed *V. trifolia*, *S. crispus* and *A. vera* had the strongest activity against the tested bacteria such as in *S. agalactiae*, *A. hydrophila* and *E. cloacae*. *A. hydrophila* was the most sensitive bacteria tested, with the highest inhibition zone in the presence and low value of MIC 6.25 µg/mL of the methanol extracts of *V. trifolia*. The results indicated the positive potential of *V. trifolia, A. vera and S. crispus* as environmental friendly therapeutants as they showed no side effect in the intended fish. Also, they showed significant improvement of *Oreochromi*s sp. performance as diet supplement.

ACKNOWLEDGEMENT

The authors would like to acknowledge University Technology Mara (UiTM) for the scholarship to pursue post graduate research programme.

REFERENCES

Aditya, K. and Ravi, K. A. (2014). Phytochemical evaluation of *Vitex leuocoxylon*, *Vitex negundo* and *Vitex trifolia*. *Indian Journal of Research in Pharmacy and Biotechnology* **2(2), 1106-1108.**

Aiyegoro, O. A. and Okoh, A. I. (2010). Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine* **10(21), 1-8.**

Aliero, A. A. and Afolayan, A. J. (2006). Antimicrobial activity of *Solanum tomentosum*. *African Journal of Biotechnology* **5(4), 369-372.**

Anderson, E. (2001). The Cactus Family. Timber Press. USA. **pp. 566-568.**

Backer, C. A. D. and Bakhuizen, R. C. V. (1963). Flora of Java (Spermatophytes only). Vol. II. Noordhoff Publishers, Groningen. **pp. 561-562.**

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *American Journal of Clinical Pathology* **36, 493-496.**

Bruneton, J. (1999). Pharmacognosy, phytochemistry, medicinal plants. Lavoisier Publisher, France. **pp. 1136.**

Bryan, A. H. (2005). Understanding medicinal plants: Their chemistry and therapeutic action. The Haworth Herbal Press, USA. **pp. 83-118.**

Canadian Pharmaceutical Association (CPA). (1988). Self medication. Ottawa, Canada.

Cockerill, F. R., Wikler, M. A., Alder, J., Dudley, M. N., Eliopoulos, G. M., Ferraro, M. J. and Zimmer, B. L. (2012). Performance standards for antimicrobial disk susceptibility tests; approved standard. *Clinical and Laboratory Standards Institute-NCCLS* **32(1), 1-58.**

Chew, Y. L., Chan, E. W. L., Tan, P. L., Lim, Y. Y., Stanslas, J. and Goh, J. K. (2011). Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of *Leguminosae* medicinal plants in Peninsular Malaysia. *BMC Complementary and Alternative Medicine* **11(12), 1-10.**

Citarasu, T. (2010). Herbal biomedicines: A new opportunity for aquaculture industry. *Aquaculture International* **18(3), 403-414.**

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiololgy Reviews* **12(4), 564-582.**

Dahiya, P. and Purkayastha, S. (2012). Phytochemical screening and antimicrobial activity of some medicinal plants against multi-drug resistant bacteria from clinical isolates. *Indian Journal of Pharmaceutical Sciences* **74(5), 443-450.**

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2010). Phytochemical constituents of some nigerian plants. *African Journal of Biotechnology* **4(7), 685-688.**

Faridah, H. I. and Nurulhuda, H. (1999). The use of medicinal plant species by the temuan tribe of Ayer Hitam Forest, Selangor, Peninsular Malaysia. *Pertanika Journal Tropical Agriculture Science* **22(2), 85-94.**

Fatan, H. Y. (1990). Etiologi penyakit di kalangan masyarakat Melayu: Satu kajian kes di mukim Sanggang, Temerloh Pahang. Tesis Sarjana. Universiti Sains Malaysia, Malaysia.

Frerichs, G. N. and Millar, S. D. (1993). Manual of the isolation and identification of fish bacterial pathogens. Pisces Press, Stirling. **pp. 60.**

Galina, J., Yin, G., Ardo, L. and Jeney, Z. (2009). The use of immunostimulating herbs in fish: An overview of research. *Fish Physiology and Biochemistry* **35(4), 669-676.**

Ghoshal, S., Krishna, P. B. N. and Lakshmi, V. (1996). Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica in vitro* and *in vivo*. *Journal of Ethnopharmacology* **50(3), 167-170.**

Govind, P., Madhuri, S. and Mandloi, A. K. (2012). Medicinal plants useful in fish diseases. *Plant Archives* **12(1), 1-4.**

Hossain, M. M., Paul, N., Sohrab, M. H., Rahman, E. and Rashid, M. A. (2001). Antibacterial activity of *Vitex trifolia*. *Fitoterapia* ***72*(6), 695-697.**

Iruthayam, V. K., Gurusamy, C., Thangapandi, V., Syed, H. P. S. and Jayaraj, M. (2014). Medicinal plants as immunostimulants for health management in Indian cat fish. *Journal of Coastal Life Medicine* **2(6), 426-430.**

Jamal, P., Barkat, A. A. and Amid, A. (2010). Distribution of phenolics in various malaysian medicinal plants. *Journal of Agricultural Sciences* **10(21), 2658-2662**.

Jamaludin, M. D., Husna, H. M. H., Ridzwan, H. and Muhammad, T. (2011). Phytochemicals screening and antioxidant activities of Malaysian *Donax grandis* extracts. *European Journal of Scientific Research* **61(4), 572-577.**

Kazama, C. C., Uchida, D. T., Canzi, K. N., Souza, P. D., Crestani, S., Gasparotto, J. A. and Laverde, J. A. (2012). Involvement of arginine-vasopressin in the diuretic and hypotensive effects of *Pereskia grandifolia* Haw. (Cactaceae). *Journal of Ethnopharmacology*

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

144, 86-93.

Kumar, A., Ilavarasan, R., Jayachandran, T., Decaraman, M., Aravindhan, P., Padmanabhan, N. and Krishnan, M. R. V. (2009). Phytochemicals investigation on a tropical plant, *Syzgium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. *Pakistan Journal of Nutrition* **8(1), 83-85.**

Liza, M. S., Abdul Rahman, R., Mandana, B., Jinap, S., Rahmat, A., Zaidul, I. S. M. and Hamid, A. (2010). Supercritical carbon dioxide extraction of bioactive flavonoid from *Strobilanthes crispus* (pecah kaca). *Food and Bioproducts Processing* **88, 319-326.**

Majumder, P., Abraham, P. and Satya, V. (2011). Ethno-medicinal, phytochemical and pharmacological review of an amazing medicinal herb *Peperomia pellucida* (L.) HBK. *Research Journal of Pharmaceutical, Biological and Chemical Sciences* **2, 358-364.**

Maznah, I., Manickam, E. D. A. M., Asmah, R. and Asmah, Y. (2000). Chemical composition and antioxidant activity of Strobilanthes crispus leaf extract. *The Journal of Nutritional Biochemistry* **11, 536-542.**

Okwu, D. E. and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology* **5(4), 357-361.**

Panyakom, K. (2006). Structural elucidation of bioactive compounds of *Clinacanthus nutans* (Burm. f.) Lindau leaves. M. Sc. Thesis, Suranaree University of Technology Nakhon Rathchasima, Thailand.

Pappachen and Annam. (2013). Preliminary phytochemical screening and *in-vitro* cytotoxicity activity of *Peperomia pellucida* Linn. *Pharmacie Globale*: *International Journal of Comprehensive Pharmacy* **4(8), 1-5.**

Parekh, J. and Chanda, S. (2008). *In vitro* antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African Journal of Biotechnology* **7(23), 4349-4353.**

Perumal, S. R. and Gopalakrishnakone, P. (2008). Therapeutic potential of plants as anti-microbials for drug discovery. *Evidence-Based Complementary and Alternative Medicine* **7(3), 283-294.**

Prasad, S. and Variyur, P. K. B. (1993). Chemical investigation of some commonly used spices. *Aryavaidyan* **6(4), 262-267.**

Rajan, D., Suresh, J., Paramakrishnan, N., Sri Vasavi Reddy, A. and Nayeem, M. (2012). Review on phytochemical and pharmacological properties of *Vitex trifolia* linn. *Research Journal Pharmacognosy and Phytochemistry* **4(2), 124-129.**

Rojas, J. J., Ochoa, V. J., Ocampo, S. A. and Munoz, J. F. (2006). Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: a possible alternative in the treatment of non-nosocomial infections. *BMC Complementary and Alternative Medicine* **6(2), 1-6.**

Shahidi, B. G. H. (2004). New approaches in screening for antibacterials in plants. *Asian Journal of Plant Sciences* **3(1), 55-60.**

Siripongvutikorn, S., Thummaratwasik, P. and Huang, Y. (2005). Antimicrobial and antioxidant effects of Thai seasoning, Tom-Yum. *Journal of Food Science and Technology* **38(4), 347-352.**

Sunarto, P. A. (1977). Materia Medika Indonesia. Penerbit Ditektorat Jenderal Pengawasan Obat dan Makanan, Jakarta, Indonesia. **pp. 95-99.**

Thabile, P. N. (2008). Antimicrobial properties of selected Asian herbs. M.Sc. Thesis. University of Florida, USA.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538