The aim of this work was to evaluate the antibacterial activity of Brazilian plants extracts against fish pathogenic bacteria. Forty six methanolic extracts were screened to identify their antibacterial properties against *Streptococcus agalactiae*, *Flavobacterium columnare* and *Aeromonas hydrophila*. Thirty one extracts showed antibacterial activity.

**Key words:** plant extracts, bacteria, fish.

Fish are susceptible to several bacterial infections, mainly when reared in high densities conditions. Diseases outbreaks are responsible for elevated mortality rates and decrease of the productivity efficiency, causing high economic losses to the fish farmers (6,10). The use of antibiotics is the main treatment applied to control bacterial illness in fish farms. Due to the use of a wide variety of antibiotics, aquaculture has been implicated as potential environment to the development and selection of resistant bacteria and a source of these pathogens to other animals and humans (10,19). The adoption of same antibiotics in different fields (veterinary and human medicine) improves the emergence and occurrence of the resistance phenomenon. Some bacterial fish pathogens are also associated to diseases in humans, making the aquaculture products a potential risk to the customers (zoonotic or foodborne diseases) (24).

*Streptococcus agalactiae* is a dangerous pathogen to freshwater and marine fish. The infection is characterized by brain invasion, nervous signs and septicemia (13,18). These bacteria can infect humans, causing mainly pneumonia and meningitis in newborns (17). *Aeromonas hydrophila* is responsible for cases of skin infections, septicemia and gastroenteritis in fish and human (25). *Flavobacterium columnare* is pathogenic only to freshwater fish species and shows low environmental fitness, when compared with other aquatic bacteria. Even though, this agent is highly virulent to young fish (fry and fingerling), causing skin lesions and high mortality, generally associated with poor environmental conditions (7,8). Regarding the problem of microbial resistance, there is an urgent need to establish the rules to the rational use of antibiotics and the discovery of new drugs and alternative therapies to control bacterial diseases. Owing the ability to synthesize many different substances, the plants are one of the top richest sources of new drugs (1,5). Extracts of Brazilian methanolic plants have a high potential as an alternative source of antibacterial compounds (15,16,20,23). Therefore, the aim of this study was to investigate the *in vitro* antibacterial activity of Brazilian plants extracts against three fish pathogenic bacteria.

During period 2002-2004, forty six native plants (Table 1) of southeast region of Brazil (San Francisco Valley and Lavras city) were sampled and identified by comparison with available specimens in the Herbarium of the Federal University of Lavras (UFLA). The fresh material were washed with distilled water, dried at 40ºC for 48h and triturated into small particles. To the extraction, 300g of particulate material was suspended in 600 ml
Table 1. Bacterial inhibition zone (mm) of methanol extracts (0.4 mg.mL⁻¹) in agar diffusion assay.

| Species                           | Family            | Plant part assayed | Inhibition zones (mm) |
|-----------------------------------|-------------------|--------------------|-----------------------|
| Actinostemon concolor (Sprengel) Müll. Arg. | Euphorbiaceae      | Leaves             | 22                    |
| Allophylus edulis (A.St.-Hil., Cambess. & A.Juss) Radlk | Sapindaceae      | Leaves             | -                     |
| Amaioua guianensis Aubl.          | Rubiaceae         | Leaves             | 11                    |
| Andira fraxinifolia Benth.        | Fabaceae          | Leaves             | 14.5                  |
| Bathysa meridionalis L.B Sm. & Downs | Rubiaceae      | Leaves             | 11                    |
| Bauhinia longifolia (Bongard) Steudel | Fabaceae          | Leaves             | 11                    |
| Cabralea canjerana (Vell.) Mart.  | Meliaceae         | Stem barks         | 11                    |
| Calyptranthes clusifolia (Miq.) O. Berg | Myrtaceae      | Leaves             | 8                     |
| Cariniana legalis (Mart.) Kuntze  | Lecythidaceae     | Stem barks         | 13                    |
| Celtis iguanaea Jacq. Sar.        | Cannabaceae       | Stem barks         | 17                    |
| Celtis iguanaea Jacq. Sar.        | Cannabaceae       | Leaves             | -                     |
| Croton floribundus Spreng.        | Euphorbiaceae      | Stem barks         | 14                    |
| Croton floribundus Spreng.        | Euphorbiaceae      | Leaves             | 17.5                  |
| Cryptocarya aschersoniana Mez     | Lauraceae         | Stem barks         | 15.5                  |
| Cupania vernalis Cambess.         | Sapindaceae       | Stem barks         | 12                    |
| Erythrina falcata Benth.          | Fabaceae          | Leaves             | -                     |
| Eugenia florida DC.               | Myrtaceae         | Leaves             | 12                    |
| Eugenia handroana D. Legrand      | Myrtaceae         | Leaves             | 12                    |
| Guarea guidonia (L.) Sleumer      | Meliaceae         | Stem barks         | 17                    |
| Heisteria silvianii Schwace        | Olacaceae         | Leaves             | 8                     |
| Inga marginata Wild.              | Fabaceae          | Stem barks         | -                     |
| Machaerium hirtum (Vell.) Stellfeld | Fabaceae         | Leaves             | -                     |
| Matayba elaegnoides Radlk.        | Sapindaceae       | Leaves             | -                     |
| Matayba elaegnoides Radlk.        | Sapindaceae       | Stem barks         | -                     |
| Maytenus glazioviana Loes.        | Celastraceae       | Leaves             | -                     |
| Maytenus glazioviana Loes.        | Celastraceae       | Stem barks         | 14                    |
| Merremia tomentosa (Choisy) Hallier | Convolvulaceae    | Leaves             | 7.5                   |
| Mollinedia argyrogyra Perkins     | Monimiaceae       | Stem barks         | -                     |
| Mollinedia argyrogyra Perkins     | Monimiaceae       | Leaves             | -                     |
| Myrcia tomentosa (Aublet) DC.     | Myrtaceae         | Leaves             | 15.5                  |
| Myrcia velutina O.Berg            | Myrtaceae         | Stem barks         | 15                    |
| Pera glabrata (Schott) Poepp.     | Euphorbiaceae     | Leaves             | -                     |
| Platycyamus regnellii Benth.      | Fabaceae          | Leaves             | -                     |
| Protium heptaphyllum (Aubl.) Marchand | Burseraceae      | Leaves             | 10                    |
| Protium spruceanum (Benth.) Engl. | Burseraceae       | Leaves             | 13.5                  |
| Protium spruceanum (Benth.) Engl. | Burseraceae       | Stem barks         | 10                    |
| Ruprechtia laxiflora Meisn.       | Polygonaceae      | Leaves             | -                     |
| Schinus terebinthifolia Raddi     | Anacardiaceae     | Stem barks         | 12                    |
| Securinega guarauna Kühlm.        | Euphorbiaceae     | Leaves             | 9                     |
| Siparuna guianensis Aubl.         | Siparunaceae      | Stem barks         | 11.5                  |
| Siparuna guianensis Aubl.         | Siparunaceae      | Leaves             | -                     |
| Swartzia apetalata Raddi.         | Fabaceae          | Folhas             | -                     |
| Virola sebifera Aubl.             | Myristiaceae      | Leaves             | 14.5                  |
| Xylosma sp. I                     | Salicaceae        | Leaves             | -                     |
| Xylosma sp. II                    | Salicaceae        | Leaves             | 12                    |
| Zanthoxylum riedelianum Engl.     | Rutaceae          | Leaves             | 7                     |

(-) Inhibition not observed; *1 Streptococcus agalactiae; *2 Flavobacterium columnare; *3 Aeromonas hydrophila.
of methanol during 48 hours. Therefore, the suspension was filtered and the extraction procedure was repeated twice to vegetable residues. The solvent was vacuum evaporated at 45°C. The plant extracts were lyophilized and stored at -20°C until use.

*S. agalactiae* (strain SA 16-06), *F. columnare* (strain BZ 01-02) and *A. hydrophila* (strain AE 255-03), isolated from *Oreochromis niloticus* (Linnaeus, 1758) were selected to the antibacterial assays (6,7,11). *Escherichia coli* ATCC 25922 was used as control (3,4).

Agar diffusion assay was performed according to the guidelines “Susceptibility testing of bacteria isolated from aquatic animals” of the Clinical and Laboratory Standards Institute (4). The strains were maintained at -70°C. To the tests the strains were thawed and recovered by streaking onto agar plates. *A. hydrophila* and *S. agalactiae* were incubated at 30°C for 24 hours in Mueller-Hinton (MH) Agar (Difco, USA). MH Agar was supplemented with 10% of equine blood to the cultivation of *S. agalactiae*. *F. columnare* was growth onto Medium of Hsu-Shotts (MHS) (0.3% collagen, 0.2% tryptone, 0.05% yeast extract, 0.03% calcium chloride) plates (2) for 48 hours at 26°C. *A. hydrophila* and *S. agalactiae* suspensions were prepared in sterile 0.85% saline solution, adjusted to a turbidity of 0.5 McFarland scale, equivalent to 10⁶ CFU.mL⁻¹ (4). MHS broth was used to prepare the *F. columnare* suspension. The concentration of the suspension was standardized using spectrophotometer SP 11-05 (Spectrum, China) to an absorbance of 0.230, corresponding to 10⁶ CFU.mL⁻¹.

The suspensions were streaked onto agar plates using sterile cotton swabs. Seven wells each with 6 mm of diameter were made in agar and 40 μL of the different extracts, diluted in ethanol: water (7:3) solutions (10 mg.mL⁻¹) were applied in the wells. Plates with *S. agalactiae* and *A. hydrophila* were incubated for 24 hours at 30°C and plate with *F. columnare* for 48 hours at 26°C. The inhibition zone was measured with a millimeter ruler. The assay was made in duplicate and the extracts solvent was used as control.

The extracts with antibacterial activity in agar diffusion assay were selected to determination of the minimum inhibition concentration (MIC) against the same strains. MH Broth (Difco, USA) supplemented with the divalent cations Ca²⁺ and Mg²⁺ (CAMHB) was used for strains AE 255-03 and SA 16-06 incubated at 30°C for 24 hours. Additionally, for the cultivation of SA 16-06 CAMHB was supplemented with 2.5% of lysed horse blood (3). MHS broth was used for strain BZ 01-02 incubated at 26°C for 48 hours.

The bacterial suspensions were prepared as described in agar diffusion assay and diluted 10 times in CAHMB (3). Extract solutions were prepared in dimethyl sulfoxide (10 mg.mL⁻¹). The solutions were twofold serially diluted in CAMHB, ranging from 3000 μg.mL⁻¹ to 11.71 μg.mL⁻¹ and 5μL of bacterial suspension was inoculated per well.

After incubation, aqueous solution of 2, 3, 5-Triphenyltetrazolium chloride (Merck, Germany), 2 mg.mL⁻¹, was added 25 μL to each well to check the bacterial growth, indicated by the pink colour formation. Florfenicol and dimethyl sulfoxide (solvent) were used as controls.

The results of the antimicrobial screening by agar diffusion are showed in Table 1. Thirty one methanolic extracts presented antibacterial activity for at least one strain tested. *F. columnare* was the most susceptible organism, being inhibited by 31 extracts. *S. agalactiae* and *A. hydrophila* were inhibited by five and four plant extracts, respectively.

Table 2 shows the MIC values for the extracts tested. These ranged from 93.75 μg.mL⁻¹ to 1500 μg.mL⁻¹ for *F. columnare*, *Calyptrothamnus clusiifolia* (Miq.) O.Berg and *Merremia tomentosa* (Choisy) Hallier inhibited the growth of *S. agalactiae*, presenting a MIC of 1500 μg.mL⁻¹. Methanolic extracts of *Cariniana legalis* (Mart.) Kutze, *Croton floribundus* Spreng. and *Myrica velutina* O. Berg inhibited the growth of *A. hydrophila*, with MICs values ranged from 187.5 μg.mL⁻¹ to 375 μg.mL⁻¹.

A variety of plant species are capable of synthesizing many substances with antibacterial activity. These properties have been described to extracts of many plants found in Brazilian flora (1,16,20). However, to the plants analyzed in this work, there aren’t previous studies evaluating this characteristic, except to *Schinus terebinthifolia* Raddi and *Xylosma* sp. (9,12). The extract of *Schinus terebinthifolia* Raddi presented antimicrobial activity against fluoroquinolone-resistant and macrolide-resistant *Staphylococcus aureus* strains. Chemical analysis showed the presence of phenols, pentacyclic, triterpenes and anthraquinonas in the extract of *Schinus terebinthifolia* Raddi (12). Species of *Xylosma* also showed the capacity of inhibit the growth of *Staphylococcus aureus* and *Candida albicans*, presenting MIC values of 2.5 mg.mL⁻¹ and 1.2 mg.mL⁻¹ respectively (14). Antioxidant effect, cytotoxicity and antimicrobial activity of diterpene esters and phenolic glycosides isolated from plants of the family Flacourtiaceae have been identified. However, there are no data describing the chemical composition of the other plants tested here. No one of the extracts analyzed here showed antibacterial activity against *A. hydrophila* and *S. agalactiae*, simultaneously. The occurrence of antibiotic resistant strains of bacteria has been described in aquaculture systems (6,11). Probably, the same mechanism involved in the antibiotic resistance should inhibit the deleterious action of the extracts on the bacterial cells. Even though, some extracts were effective against the pathogens, being a potential alternative to the therapy of fish diseases.

*F. columnare* was the microorganism most susceptible to major of tested extracts. In contrast to its high virulence to young fish, this bacterium is sensible to the main disinfectants used in fish farms, such potassium permanganate, hydrogen
Inhibition of fish pathogens

 Despite of their common use, these compounds may be dangerous to fry and aquatic environment. The plant extracts can be applied as an alternative to prevent and control outbreaks of columnaris, mainly in hatchery. Since these substances are natural, their hazardous potential is lower when compared with other products. The results show that analyzed plants presented a high potential as alternative therapy of bacterial fish diseases currently observed in Brazilian fish farming.

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**Table 2.** Minimal inhibitory concentration of plant methanolic extracts to selected fish bacterial pathogens.

| Species                              | Family          | Plant part     | MICs values (µg.mL⁻¹) |
|--------------------------------------|-----------------|----------------|------------------------|
| Actinostemon concolor (Sprengel) Müll. Arg. | Euphorbiaceae   | Leaves        | * 93.75 *              |
| Amaioua guianensis Aubl.             | Rubiaceae       | Leaves        | * 375 *                |
| Andira fraxinifolia Benth.           | Fabaceae        | Leaves        | * 375 *                |
| Bathysa meridionalis L.B. Smith & Downs | Fabaceae        | Leaves        | * 375 *                |
| Bauhinia longifolia (Bongard) Steudel | Fabaceae        | Leaves        | * 750 *                |
| Cabralea canjerana (Vell.) Mart.     | Meliaceae       | Stem barks    | * 187.5 *              |
| Calyptranthes clusifolia (Miq.) O. Berg | Myrtaceae      | Leaves        | 1500 187.5 *           |
| Cariniana legalis (Mart.) Kuntze     | Lecythidaceae   | Stem barks    | * 93.75 187.5          |
| Celtis iguanaea Jacq. Surg.          | Cannabaceae     | Stem barks    | * 187.5 *              |
| Croton floribundus Spreng.           | Euphorbiaceae   | Stem barks    | * 93.75 375            |
| Croton floribundus Spreng.           | Euphorbiaceae   | Leaves        | - 93.75 *              |
| Cryptocarya aschersoniana Mez         | Lauraceae       | Stem barks    | * 93.75 *              |
| Cupania vernalis Cambess.             | Sapindaceae     | Stem barks    | * 750 *                |
| Eugenia florida DC.                  | Myrtaceae       | Leaves        | * 375 1500             |
| Eugenia handroana D. Legrand         | Myrtaceae       | Leaves        | * 187.5 *              |
| Guarea guidonia (L.) Sleumer         | Meliaceae       | Stem barks    | * 187.5 *              |
| Heisteria silvianii Schwacke         | Oligaeaceae     | Leaves        | - 750 *                |
| Maytenus glazioviana Loes.           | Celastraceae    | Stem barks    | * 187.5 *              |
| Merremia tomentosa (Choisy) Hallier  | Convolvulaceae  | Leaves        | 1500 93.75 *           |
| Mollinedia argyrogyna Perkins        | Monimiaceae     | Leaves        | * 375 *                |
| Myrcia tomentosa (Aublet) DC.        | Myrtaceae       | Leaves        | * 93.75 *              |
| Myrcia velutina O.Berg               | Myrtaceae       | Stem barks    | * 187.5 375            |
| Protium heptaphyllum (Aubl.) Marchand| Burseraceae     | Leaves        | * 750 *                |
| Protium spruceanum (Benth.) Engl.    | Burseraceae     | Leaves        | * 375 *                |
| Protium spruceanum (Benth.) Engl.    | Burseraceae     | Stem barks    | * 375 *                |
| Schinus terebinthifolia Raddi        | Anacardiaceae   | Stem barks    | * 187.5 *              |
| Securinega guaraia Kuhlm.            | Euphorbiaceae   | Leaves        | * 1500 *               |
| Siparuna guianensis Aubl.            | Siparunaceae    | Stem barks    | * 93.75 *              |
| Virola sebifera Aubl.                | Myristiaceae    | Leaves        | * 93.75 *              |
| Xylosma sp. II                      | Salicaceae      | Leaves        | * 375 *                |
| Zanthoxylum riedelianum Engler       | Rutaceae        | Leaves        | - 1500 *               |
| Florfenicol                          | -               | 2 1 2         |

(*) MIC assay not done; (-) No MIC values established; *1 Streptococcus agalactiae; *2 Flavobacterium columnare; *3 Aeromonas hydrophila.

peroxide, chloramines and salt (21,22). Despite of their common use, these compounds may be dangerous to fry and aquatic environment. The plant extracts can be applied as an alternative to prevent and control outbreaks of columnaris, mainly in hatchery. Since these substances are natural, their hazardous potential is lower when compared with other products. The results show that analyzed plants presented a high potential as alternative therapy of bacterial fish diseases currently observed in Brazilian fish farming.

**RESUMO**

*Atividade antibacteriana de extratos de plantas do Brasil contra bactérias patogênicas para peixes*

O objetivo deste trabalho foi avaliar a atividade antibacteriana de extratos de plantas brasileiras contra bactérias patogênicas para peixes. A atividade antibactériana de quarenta e seis extratos metanólicos de plantas foi avaliada contra os agentes *Streptococcus agalactiae*, *Flavobacterium columnare* e *Aeromonas hydrophila*. Trinta e um extratos apresentaram atividade antibacteriana.

**Palavras-chave:** extratos de plantas, bactérias, peixes.
REFERENCES

1. Antunes, R.M.P.; Lima, E.O.; Pereira, M.S.V.; Camara, C.A.; Arruda, T.A.; Catão, R.M.R.; Barbosa, T.P.; Nunes, X.P.; Dias, C.S.; Silva, T.M.S. (2006). Atividade antimicrobiana “in vitro” e determinação da concentração inibitória mínima (CIM) de fitoconstituintes e produtos sintéticos sobre bactérias e fungos levaduriforme. Rev. Bras. Farmacogn., 16, 517-524.

2. Bader, J.A.; Nusbaum, K.E.; Shoemaker, C.A. (2003). Comparative challenge model of Flavobacterium columnare using abraded und unabraded channel catfish, Ictalurus punctatus (Rafinesque). J. Fish Dis., 26, 461-467.

3. Clinical and laboratory standards institute. National Committee for Clinical Laboratory Standards, Methods for broth dilution susceptibility testing of bacteria isolated from aquatic animals, approved guideline. Wayne, PA: 2006. (CLSI/NCCLS Document M49-A).

4. Clinical and laboratory standards institute. National Committee for Clinical Laboratory Standards. Methods for Antimicrobial Disk Susceptibility Testing of Bacterial Isolated from Aquatic Animals, approved guideline. Wayne, PA: 2006. (CLSI/NCCLS Document M42-A).

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

6. Figueiredo, H.C.P.; Carneiro, D.O.; Faria, F.C.; Costa, G.M. (2006). Streptococcus agalactiae associado à meningocenefalite e infecção sistêmica em tilápia-do-Nilo (Oreochromis niloticus) no Brasil. Arq. Bras. Med. Vet. Zootec., 58, 678-680.

7. Figueiredo, H.C.P.; Klesius, P.H.; Arias, C.R.; Evans, J.; Shoemaker, C.A.; Pereira Junior, D.J.; Peixoto, M.T. (2005). Isolation and characterization of strains of Flavobacterium columnare from Brazil. J. Fish Dis., 28, 199-204.

8. Grabowshi, L.D.; LaPatra, S.E.; Cain, K.D. (2004). Systemic and mucosal antibody response in tilapia, Oreochromis niloticus (L.), following immunization with Flavobacterium columnare. J. Fish Dis., 27, 573-581.

9. Guerra, M.J.M.; Barreiro, M.L.; Rodrigues, Z.M.; Rubalcaba, Y. (2000). Actividad antimicrobiana de um extrato fluido al 80% de Schinus terebinthifolius Raddi (Copal). Rev. Cub. Plant. Med., 5, 23-25.

10. Hatha, M.; Vivekanandhan, A.A.; Joice, G.J. (2005). Christol. Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. Int. J. Food Microbiol., 98, 131-134.

11. Hirsch, D.; Pereira Junior, D.J.; Logato, P.V.R.; Picolli-Valle, R.H.; Figueiredo, H.C.P. (2006). Identificação e resistência a antimicrobianos de espécies de Aeromonas móveis isoladas de peixes e de ambientes aquáticos. Cienc. Agrotecnol., 30, 1211-1217.

12. Lima, M.R.F.; Luna, J.S.; Santos, A.F.; Andrade, M.C.C.; Sant’Ana, A.E.G.; Genet, J.P.; Marquez, B.; Neuville, L.; Moreau, N. (2006). Anti-bacterial activity of some Brazilian medicinal plants. J. Ethnopharmacol., 105, 137-147.

13. Miller, J.D.; Neely, M.N. (2004). Zebrasfish as a model host for streptococcal pathogenesis. Acta Trop., 91(1), 53-68.

14. Mosaddik, M.A.; Banbury, L.; Forster, P.; Booth, R.; Markham, J.; Leach, D.; Waterman, P.G. (2004). Screening of some Australian Flaccoustriaceae species for in vitro antioxidant, cytotoxic and antimicrobial activity. Phytomedicine, 11, 461-466.

15. Nascimento, G.G.F.; Locatelli, J.; Freitas, P.C.; Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Braz. J. Microbiol., 31, 247-256.

16. Oliveira, D.F.; Pereira, A.C.; Figueiredo, H.C.P; Carvalho, D.A.; Silva, G.; Nunes, A.S.; Alves, D.S.; Carvalho, H.W.P. (2007). Antibacterial activity of plant extracts from Brazilian southeast region. Fitoterapia, 78, 142-145.

17. Pettersson, K. (2007). Perinatal infection with group B streptococci. Semin. Fetal Neon. Med., 12, 193-197.

18. Russo, R.; Mitchell, H.; Yanong, R.P.E. (2006). Characterization of Streptococcus iniae isolated from ornamental cyprinid fishes and development of challenge models. Aquaculture, 256, 105-110.

19. Serrano, P.H. (2005). Responsible use of antibiotics in aquaculture. In: Food and Agriculture Organization (FAO) Fisheries Technical Paper, 469, Roma, 97 p.

20. Sartoratto, A.; Machado, A.L.M.; Delarmelina, C.; Figueira, G.M.; Duarte, M.C.; Rehder, V.L.G. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. Braz. J. Microbiol., 31, 247-256.

21. Soumalainen, L.R.; Tiirola, M.; Valtonen, E.T. (2005). Treatment of columnaris diseases of rainbow trout: low pH and salt as possible tools? Dis. Aquat. Org., 65 (2), 115-120.

22. Thomas-Jinu, S.; Goodwin, A.E. (2004). Acute columnaris infection in channel catfish, Ictalurus punctatus (Rafinesque): efficacy of practical treatments for warmwater aquaculture ponds. J. Fish Diseases., 27 (1), 23-28.

23. Ushimaru, P.I.; Silva, T.N.; Di Stasi, L.C.; Barbosa, L.; Fernandez Junior, A. (2007). Antibacterial activity of medicinal plant extracts. Braz. J. Microbiol., 38, 717-719.

24. Yanong, R.P.E.; Francis-Floyd, R. (2006). Streptococcal infections of fish. The institute of food and agricultural sciences. Circular. 57, 1-6.

25. Yu, B.H.; Kaur, R.; Lim, S.; Wang, X.H.; Leung, K.Y. (2007). Characterization of extracellular proteins produced by Aeromonas hydrophila AH-1. Proteomics. 7, 436-449.