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CASE STUDY

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# SCREENING THE SENSITIVITY OF MEDICINAL PLANTS AGAINST FISH PATHOGENS

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***Abstract***

Aquaculture is a remarkable economic sector in many countries.The biggest problem faced by the aquaculture industry worldwide is diseases due to various biological and nonbiological agents. Due to the increased resistance of many microorganisms towards the currently available commercial antibiotics. Hence an attempt has been made to study the disease causing bacteria. The study reaveled that from the diseased fish *Aeromonas hydrophila* and vibrio sp were isolated.The extract of Andrographis paniculata showed maximumzone of inhibition against.parahaemolyticus(17± 0.62) and was followed by V.cholerae(15± 0.4).The SDS-PAGE analysis MX -0825KDa bands.

***Key Words:*** Fish Andrographis paniculata Vibrio Aeromonas sp

## INTRODUCTION

Aquaculture is the farming of economically important aquatic animals and plants under controlled conditions. Aquaculture is an ancient practice. Aquaculture includes freshwater culture, brackish water culture, mariculture and methaline culture. Aquaculture is a remarkable economic growth sector in many countries. Its growth is expected to continue in most parts of the world as the gap between supply and demand for fish products are widening. Aquaculture is a broad term covering, culturing of a variety of animals such as fish, seaweed, molluscs, shrimps, etc., in water most of the global aquaculture production is from semi-intensive and intensive management farms. They follow a combination of pond fertilizationl and supplementary feed inputs or compounds aqua feeds.

The aquaculture is one of the high profitable businesses now a days. The aquaculture is operating in a number of countries including India. It is production of biological organism from aquatic system and then marketed. Developed countries can offer the necessary investment on the research and training programme, whereas developing countries like India tend to concentrate thus limited fund on aquaculture (Banerjee and Packrasi, 1986). Apart from feed, the physico-chemical parameters such as salinity, pH, dissolved oxygen, turbidity, $CO_2$ and water temperature of the culture pond directly or indirectly affect the life activities and growth of fishes. The water quality management and daily water exchange improve the fish production. Changes in the equilibrium such as deterioration in the water quality can result in fish becoming “stressed” and vulnerable to disease. Intensive culture system offer the ideal environment for disease outbreak, because such systems have stressed the host and virulent pathogen. Depending on the nature the disease may cause mass mortality of the affected population in a short time, produced reduced growth make the culture fish unsuitable for human consumption.

The biggest problem faced by the aquaculture industry worldwide is diseases caused due to various biological and non-biological agents. Among the groups of microorganisms that cause serious tosses in shrimp culture, the best known are bacteria because of the devastating economic effects they have on affected forms, microbes mainly cause the disease. Microorganisms are ubiquitous in nature, extremely diverse group and are microscopic in nature which includes viruses, bacteria, fungi, protozoa and some algae.

Vibrio is caused by a number of *Vibrio* sp. of bacteria including *Vibrio harveyi*, *Vibrio parahaemolyticus, Vibrio vulnificus, Vibrio alginolyticus* and *Vibrio penaeicida* (Brock and Lightner, 1999; Shimaru *et al*., 1995(. There have been occasional reports of vibriosis caused by *Vibrio damsela, Vibrio fluvialis* and other undefined *Vibrio species* (Lightner, 1996). *Vibrio species* are ubiquitous in the aquatic environment. They appear at particularly high densities in marine organisms including corals, fish, mollusks, sea grass, sponges, shrimps and zooplankton (Thompson *et al*., 1997). Among the major disease caused by *Vibrio* species is cholera, which occurs when *Vibrio cholerae* colonizes in the small intestine and releases on enterotoxin (Gopal *et al*., 2005). Bacterial disease in aquaculture are mainly controlled by antibiotics. However, continuous intensive use of antibiotic is undesirable as this leads to the development of drug resistance and thereby to a reduced efficacy of the drugs.

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In the public health context, antibiotic resistance can be transferred to environmental and human pathogenic bacteria (Alderman and Hastings, 1998). In addition, antibiotics accumulate in the environment and fish, posing a potential risk to consumers and to the environment in general. Antibiotic such as oxytetracycline, erythromycin and tetracycline are widely used to prevent bacterial disease in fish and other aquatic organisms.

Traditional medicines represented mainly by plants have become an alternative as they are considered synthetic antibiotics. Hence, the need to increase the relatively safer and more affordable when compared to body of knowledge on the antimicrobial activities of some traditional medicinal plants towards curbing the effects of antibiotic resistance in *Vibrio* species become imperative. In recent years, herbal plant supplements have been developed as an innovation and could provide a reliable long term answer for the industry facing the antibiotics ban. The medicinal herbs contain physiologically active principles that over the years exploited in traditional medicine for the treatment of various ailments as they have antimicrobial properties (Kelmanson *et al*., 2000).

They are several reports indicating a variety of pharmacological activities of herbs such as antioxidant (Masuda *et al*., 2001), antimicrobial (Negi *et al*., 1999), anti-allergic (Yano *et al*., 2000), anti-inflammatory (Ammon and Wahl, 1991), hepatoprotective (El-Ansary *et al*., 2006). However, few of the medicinal plants are several investigated for their antimicrobial of these species and their essential oil reported to passes antimicrobial activities including garlic savory, basil, zinger, laurel, mint, cumin, turmeric It is easily cultivated from seeds on all types of soil, the herb, *Andrographis paniculata* is the main source of the bitter principle Andrographolide *Andrographis paniculata* or Kalmegh is one of the most widely used plants in ayurvedic formulations (Jarukamjo, 2008; Chandran *et al*., 2009). *Andrographis paniculata* Nees (Acanthaceae) commonly known as Nilavembu is an annual herb. It is found in wild throughout the plains of India especially in Tamil Nadu, Karnataka, Maharashtra, Orissa and Uttar Pradesh. Various medicinal properties like antidiarrhoel, anti-inflammatory, choleretic and immunostimulant have been attbitued to this plant in the traditional system of Indian medicine. Further reported activities are antimalarial, antihypertensive, antipyretic, antithrombotic, antidote and hepatoprotective. (Arora and Kaur, 1999).

Trivedi and Rawal (2000) reported by Hepatoprotective and toxicological evaluation of *Andrographis paniculata* on severe liner damae. Calabnese, Berman and Babish (2000) studied a phase I trial of andrographolide in HIV positive patients and normal volunteers phytotherapy resPravjal, Roy and Doy (2003) studied by antimicrobial activity of *Andrographis paniculata* pitoerapia. Du, Jerz and Wiinterbatter (2003) reported by separation of andrographolide and neoandrographolide from the leaves of *Andrographis paniculata* by micellar electrokinetic chromatography research. Coon and Ernst (2004) *Andrographis paniculata* in the treatment of upper respiratory tract infections. A systematic review of safety and efficacy. Poolsup *et al*. (2004) *Andrographis paniculata* in the systematic treatment of uncomplicated upper respiratory tract infections. Yutaka Yano *et al*. (2006) studied the antimicrobial effect of species and herbs on *Vibrio parahaemolyticus*. Abubacker *et al*. (2010) studied the antibacterial activity of ethanolic leaf extract of *Andrographis paniculata* Nees (Acanthaceae) and its bioactive compound Androgra Sharmila and Subburathinam (2013) studied the effect of signal compounds on Andrographolide in the hairy root culture of *Andrographis paniculata*. Sharmila and Subburathinam (2013) studied the effect of signal compounds on Andrographolide in the hairy root culture of *Andrographis paniculata*. Ghasemi Pirbalouti *et al*. (2011) studied the inhibitory activity of Iranian endemic medicinal plants against *Vibrio parahaemolyticus* and *Vibrio harveyi*.

Sharmila and Subburathinam (2013) studied the effect of signal compounds on Andrographolide in the hairy root culture of *Andrographis paniculata*.

## MATERIALS AND METHODS

### *Plant collection*

*Andrographis paniculata* plant leaves were collected from college campus, Kundhavai Naachiyar Govt. Arts College for women (Autonomous), Thanjavur, Tamil Nadu.

### *Preparation of plant leaves powder*

The collected plant leaves were washed thoroughly using water and proffered to the laboratory the plant material was shade dried for three days. After during, plant material powdered with the help of mixer grinder. The fine particles were separated and stored in clean container. It was used for further analysis (Fig.1).

### *Preparation of acetone extract*

5 gm of plant powder sample was soaked in 20 ml of acetone for 12 hrs. After soaking the extracts were filtered using Whatman filter paper No.42 (125 mm).

### *Phytochemical study (Plate I)*

Phytochemical compounds were qualitatively analysed method by Harborne (1973), Trease and Evans (1989) and Sofowara (1993).

### *Test for tannins*

0.5 ml of plant extract was taken in test tubes and was few drops of 0.1 per cent ferric chloride mixed, and appearance of brownish green or a blue black colour was observed on indicate as tannins positive.

### *Test for pholobatanins*

0.5 ml plant extract was taken in test tubes in add dissolved distilled water and the filtered. In filtrate boiled with 2 per cent HCl solution added. The appearance of red precipitate observed on indicate as pholobatannins positive.

### *Test for saponin*

0.2 ml of plant extract was taken in test tubes. And add 5 ml of distilled water and shaken well. The ready to heated to boil. The appearance of frothing (creamy mars small bubbles) observed on indicate as saponin positive.

### *Test for flavonoids*

0.2 ml of extract was taken in test tubes in add dissolved in diluted NaOH. In again HCl was added. The appearance of yellow solutions turns colourless observed on indicate as flavonoids negative.

### *Test for steroids*

0.2 ml of plant extract was taken in the test tube and added 2 ml of acetic anhydrine and 0.5 ml of methanol extract. The extract sample containing add 2 ml of $H_2SO_4$ added. The colour changed from, appearance of violet to blue r green observed on indicate as steroids negative.

### *Test for terpenoids (Salkowski test)*

0.5 ml extract was taken in the test tube and mixed in 2 ml of chloroform and concentrated $H_2SO_4$ (3 ml) was carefully added to form a layer. The appearance of reddish brown colouration observed on indicate as steroids negative.

### *Test for cardiac glycosides (Keller-Killani test)*

0.5 ml of plant extracts was taken in the test tube was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. The appearance of brown ring formation observed on indicate as cardiac glycosides negative.

### Test for carbonyl

2 ml of extract was treated with add few drops of 2, 4 dinitriophenol hydrazine and well shaken; appearance of orange red precipitate observed on indicate as Alkaloids positive.

### Test for reducing sugar

Plant extract 0.1 ml and shaken with add 5 ml distilled water. After boiled with drops of Fehling solution A&B 2 minutes. Appearance of red precipitate observed on indicate as reducing sugar positive.

### Test for alkaloids

0.2 ml of extract and 2 ml of 2% $H_2SO_4$ added and few drops of Dragencloff's reagent add and appearance of orange red precipitate observed on indicate as alkaloids positive.

### Assay of antimicrobial activity (Plate II)

5 gms of powdered plant materials was mixed with 20 ml of acetone solvent. The extracts prepared in succession from powdered leaf material, by soaking method, the extract preparation were done by (Baker, 1983). The collected extracts were stored in a vial for further studies.

### Disc preparation

The 6 mm (diameter) discs were prepared from Whatman No.1 filter paper and the discs were sterilized by autoclave at 121°C. After sterilization the moisture in the discs were dried on hot air oven at 50°C. The respective solvent extracts were added to the disc (0.2 mg/disc) individually and aseptically. After drying they were used for screening the anti microbial activity (De.Boer *et al*., 2005).

### *Microorganisms used*

The bacterial cultures of *Vibrio cholera, Vibrio parahaemolyticus* and *Aeromonas hydrophila* were isolated from infected shrimp *Penaeus monodon* (Chandrakala and Ayyavoo, 2006).

### *Antibacterial activity of plant extracts*

The antibacterial activities was carried out by disc diffusion technique (Bonjar, 2004). The aeromonas agar plants were prepared. The bacterial test organisms *Vibrio cholera, Vibrio parahaemolytics*_and *Aeromonas* spp. were swapped over the prepared aeromonas agar plates by using separate sterile cotton buds. The prepared extracts of plan disc were placed on the organisms inoculated plates with equal distance. Control discs were also placed (ciprotoxin). All bacterial plates were incubated at 37°C for 24 hrs. The diameter of the minimum zone of inhibition was measured in mm for each test organism.

### *Polyacrylamide sodium dodecyl sulphate slab gel electrohoresis (SDS-PAGE) or proteins (Fig.3)*

Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis is an excellent method for rapidly assessing the purity of proteins and is routinely used in the development and validation of a purification strategy. It is the most widely used method for analyzing protein samples in a qualitative manner.

The electrohporetic mobility of a polypeptide chain can function solely of its molecular weight, only if,

1. The charge per unit mas (e/m) is approximately constant.
2. The hydrodynamic properties are a function only of molecular weights (length) of the poplypeptide chain.

Both of these criteria are met under the conditions of SDS-PAGE. SDS binding sites of a variety of proteins indicate that above a SDS-monomer concentration of 8X (10 M), 1.4 gm of SDS is bound per gram of protein. This means that the number of bound SDS molecules is approximately equal to half the number of amino-acid residues in the polypeptide chain. This high level of binding and the constant binding ratio will in general "Swamp out" the native (intrinsic) charge contribution of most proteins and an approximately constant negative net charger per unit mass will be obtained

## RESULT

The use of herbal preparations in the treatment of diseases is very common in the rural communities of world. *Andrographis paniculata* or Kalmegh is one of the most widely used plants in ayurvedic formulations. *Andrographis paniculata* is frequently used for the treatment of skin infection, jaundice, colic dysentery and dyspepsia, respiratory tract infection, fever, disorders of liver, colic pain, loss of appetite, irregular stools and diarrhoea, liver protection, anticancer activity, anti-diabetic activity and anti-malarial activity. *Andrographis paniculata* is one of the widely used medicinal herb. The importance of the whole plant in folk medicine as well as its promising pharmacological properties was verified in our laboratories.

**Table 1** Preliminary phytochemical study of *Andrographis paniculata*

| S. No. | Phytochemicals | *Andrographis paniculata* |
|---|---|---|
| 1. | Cardiac glycosides | Negative |
| 2. | Terpenoids | Negative |
| 3. | Steroids | Positive |
| 4. | Flavonoids | Negative |
| 5. | Tannins | Positive |
| 6. | Phlobatannins | Positive |
| 7. | Saponins | Positive |
| 8. | Carbonyl | Positive |
| 9. | Reducing sugar | Positive |
| 10. | Alkaloids | Positive |

The powder of *Andrographis paniculata* was taken in an apparatus and refluxed serially using distilled water and solvent system depending upon the polarity (Fig.1). The extracts of acetone solvent system were transferred separately in previous weighed beaker: the weight of the sample was calculated, weight and character of the sample was found to be more in acetone leaching out of the compounds. *Andrographis paniculata* tested against the test organisms revealed that only acetone extract showed the maximum zone of inhibition against *Vibrio cholera* (15 ± 0.4) and *Vibrio parahaemolyticus* (17 ± 0.62) and *Aeromonas hydrophila* (14 ± 0.86) (Table 2 and Plate II).

The plant extraction samples showed to SDS-PAGE to estimate the molecular weight of proteins present in it. Different standard were used to determine the molecular weight of plant extract proteins. The stained gel revealed the plant extract contained a two population of proteins. There is different molecular weight marker proteins were used only two clear band were detected in the gel that represented peptide of 14.4 kDa and 45 kDa shown in Fig.2.

**Table 2** Assay of Antibacterial activity

| S. No. | Name of the organisms | Zone of inhibition (mm in diameter (M ï SD) | |
|---|---|---|---|
| 1. | *Vibrio Cholerae* | 22 | 15 ± 0.44 |
| 2. | *Vibrio parahaemolyticus* | 14 | 17 ± 0.62 |
| 3. | *Aeromonas hydrophila* | 16 | 14 ± 0.86 |

## DISCUSSION

The plant extract of *Andrographis paniculata* used for the present study was choosing on the basis of their medicinal values. The natural whole plants are having a wide range of medicinal properties like antitumour, antileukemia, antibacterial, antiviral, antifungal, anti-inflammatory, anti-diabetics, anti-diarrhoeal, anti-malarial, hepatoprotective, anti-human immune deficiency virus (HIV), immune stimulatory and antisnakdbite activity. The *Andrographis paniculata* since resveratrol possess several pharmacological actions, which suggests involvements of several signaling pathways (Hostettmann and Marston, 1995; Chun *et al*., 1999; LeCorne *et al*., 2006; Park *et al*., 2007). In traditional Chinese medicine, it is widely used to get rid of body heat, as in fevers and to dispel toxins from the body. In Scandinavian countries, it is commonly used to prevent and treat common cold (Caceras *et al*., 1997). Previous studies have explicitly revealed that *A. paniculata* has a wide range of pharmacological effects and some of them extremely beneficial such as anti-inflammatory (Shen *et al*., 2002), anti-diabetes (Syahin *et al*., 2006), antidiarrhoeal (Gupta *et al*., 1990), antiviral (Wiart *et al*., 2050), antimalarial (Rahman *et al*., 1999), hepatoprotective (Trivedi and Rawal, 2005), anticancer (Zhou *et al*., 2006), antihuman immune deficiency virus (HIV) (Calabrese *et al*., 2000), immune stimulatory (Iruretagoyena *et al*., 2005) and anti snakebite activity (Samy *et al*., 2008). Diterpenoids and flavonoids are the main chemical constituents of parts of the plant are used for jaundice, colic dysentery and dyspepsia, as a bitter tonic, stomachic, anthelmintic and antiplasmodial. It is widely used in traditional medicine as an antidote against poisons of snakes and insect, and as an antimalarial agents. It is benefitial in general debility, asthma, bronchitis, filariasis and in hepatitis during clinical studies. It has also shown immunomodulating/immunostimultory and anticancerous activity. One of the active compound

neoandrographolide has been reported to show anti HIV activity. In the present study, the results of phytochemical screening of acetone extract and water fractions of the plant revealed the presence of steroids, tannins, phlobatannins, saponins, carbomyl, reducing sugar and alkaloids .Presence of phytochemical flavonoids (Roa *et al*., 2004) and diterpenoid lactones (Reddy *et al*., 2003) in *Andrographis paniculata* have been reported. To identify the active constituents of plant extract involved in corrosion inhibition, explicit knowledge of the chemical structures of its photochemical is required (Poonam Kulyam *et al*., 2010; Aliahmadi *et al*., 2011). Although a significant number of studies have been used to obtain purified phytochemcials very few screening program have been initiated on crude plant material. It has been widely observed and accepted the medicinal value of plants lies in the bioactive phytocomponent present in the plants (Veeramuthu *et al*., 2008).This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavonoids, quinines, coumarins, tannins and their glycoside, alkaloids, essential oil etc, the importance of these substance as microbial agents against the pathogen has been emphasized by several workers (Mahadevan, 1979). The production of secondary metabolites by the plant cells growing in culture have been confirm by Ramamurthy *et al*. (2009). In particular, the flavonoids were reported to be responsible for antimicrobial activity associated with some ethnomedicinal plants (Singh and Bhat, 2003). The antimicrobial activity of aqueous extract, andrographolides and arabinogalactan proteins from *Andrographis paniculata* were evaluated. The aqueous extract showed significant antimicrobial activity, which may be due to the combined effect of the isolated arabinogalactan proteins and andrographolides (Singha *et al*., 2003).

Plants contain numerous biologically active compounds. Many of which have been shown to have been shown to have antimicrobial properties (Cowan, 1999). Plant derived medicines have been part of traditional health care in most part of the world for thousands of years and there is increasing interest in plants as sources of agents of fight microbial diseases (Chairandy *et al*., 1999). Brul and Coote (1999) have previously discussed the mechanisms of how natural compounds in herbs exert their functions.Many compounds are responsible for plant flavor, and serve as useful medicinal compounds use some of the same herbs and spices some studies claim that the phenolic compounds present in spices and herbs play a major role in their antimicrobial effects (Hara-Kudo *et al*., 2004).

According to Boominathan and Ramamurthy (2009) medicinal plants and microorganisms are the proper candidates and receive continuous research attention. Growth curves were similar in media adjusted to identical pH values, regardless of whether citric, ascorbic or malic acid was used to attain these values. No viable *V. parahaemolyticus* were detected. The antimicrobial effects of species and herbs from 18 plant species were examined as an food borne pathogen, *V. parahaemolyticus*. These results suggest that the spices and herbs can be practiced for protecting seafood from the risk of contamination by *V. parahaemolyticus* (Yano *et al*., 2006). Antibacterial activity of phytochemical andrographolide, a labdane diterpenoid (Xu *et al*., 2006) isolated from plant materials has been studied. The antibacterial activity of the polar and non-polar extracts of the whole plant *Andrographis paniculata* suggests that every extract contains the effective active phytochemicals responsible for the elimination of microorganisms responsible for skin diseases. In the present study, *in vitro* antibacterial activity of acetone leaf extract of *A. paniculata* and andrographolide against the pathogenic bacteria showed the inhibitory action against *Vibrio cholorae* and *Vibrio parahaemolyticus* and *Aeromonas hydrophila* In the present investigation, plant leaf extraction that showed antimicrobial activity subjected to SDS-PAGE to estimate the number and molecular weight of proteins present. After electrophoresis clear bands were detectedinthegel electrophroses.

## References


Abubacker, M.N., and Vasantha, S., 2010. Antibacterial activity of Ethnolic leaf extract of *Andrographis paniculata* Nees (Acanthaceae) and its bioactive compound andrographolide. *Drug Invention Today*, 2(10): 440-442.

Ammon, H.P.T., and Wahl, M.A., 1991. *Pharmacology of Curcuma longa. J. Planta Medica, 57*: 1-7.

Arora, D.S., and Kaur, J., 1999. Antimicrobial activity of species. *Int. J. Antimicrob. Agent*, 12: 257-262.

Chandrakala, N., and Ayyavoo, M., 2006. Study on the existence of microbial pathogens in the tiger shrimp *Penaeus monodon* (Fab.).

Chandran, B., Rameshkumar, G., and Ravichandran, S., 2009. Antimicrobial activity from the Gill extraction of *Perna viridis* (Linnaeus, 1758). *Global J. Biotechnol. Biochem.*, 4(2): 88-92.

Chariandy, C.M., Seaforth, C.E., Phelps, R.H., Pollard, G.V., and Khambay, B.P., 1999. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol.*, 64: 265-270.

Cowan, M.M., 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, 12: 564-582.

Gopal, S., Otta, S., Kumar, S., Kannasagar, L., Nishibuchi, M., and Karunasagar, I., 2005. The occurrence of *Vibrio* spp. in tropical shrimp culture environment implication for food safety. *Int. J. Food Microbiol.*, 102: 151-159.

Kelmanson, J.E., Jager, A.K., and Van Staden, J., 2000. Zulu medicinal plants with antibacterial activity. *J. Ethnopharmacol.*, 69: 241-246.

Lightner, D.V., and Lewis, D.H., 1975. A Septicemic bacterial disease syndrome of penaeid shrimp. *Mar. Fish. Rev.*, 37(5-6): 25-28.

Mahadevan, Y.D., 1979. Evaluation of *Trichoderma viride* and calbendazin for integrated management of root diseases in cotton. *Indian J. Microbial.*, 37: 107-108.

Negi, P.S., Jayaprakasha, G.K., Jagan Mohan Rao, L., and Sakariah, K.K., 1999. Antimicrobial activity of turmeric oil: A by-product from Curcumin manufacture. *J. Agric. Food. Chem.*, 47: 4297-4300.

Park, C.E., Kim, M.J., Lee, J.H., Min, B.I., Bae, H., Choe, W., Kim, S.S., and Ha, J., 2007. Resveratrol stimulates glucose transport in $C_2C_{12}$ myotubes by activating AMP activated protein kinase. *Exp. Mol. Med.*, 39: 222-229.

Shen, Y.C., Chen, C.F. and Chiou, W.F., 2002. Andrographolide prevents oxygen radical production by human neutrophils: possible mechanism(s) involved in its anti-inflammatory effect. *Br. J. Pharmacol.*, 135: 399-406.

Singh, B., and Bhat, T.K., 2003. Potential therapeutic applications of some antinutritional plant secondary metabolites. *J. Agric. Food Chem.*, 51: 5579-5597.

Singha, P.K., Roy, S., and Dey, S., 2003. Antimicrobial activity of *Andrographis paniculata*. *Fitoterapia*, 74(7-8): 692-4.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G., 1997. The clustal x windows interface, flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucl. Acids Res.*, 24: 4876-4882.

Xu, Y., Marshall, R.L., and Mukkur, T.K.S., 2006. An investigation on the antimicrobial activity of *Andrographis paniculata* extracts and Andrographolide *in vitro*. *Asian J. Plant Sci.*, 5: 527-530.

Yono, S., Terai, M., Shimizu, K.L., Sekine, T., Yamamoto, Y., Takamoto, K., Saito, K., Veno, K., and Watanabe, K., 2000. Antiallergic activity of *Curcuma longa* L. Effectiveness of extracts containing curcuminoids. *Nat. Med.*, 54: 318-324.

Yutakayano, Masataka Satomi, and Hiroshi Oikawa, 2006. Antimicrobial effect of species and herbs on *Vibrio parahaemolyticus*. *Int.J. Food*

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