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Antibacterial activity of *Cymodocea serrulata* root extract against chosen poultry pathogens

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Abstract

The study of marine organisms for their bioactive potential, being an important part of marine ecosystem has picked up the rhythm in recent years with the growth recognition of their importance in human life as well as animal. The *in vitro* antibacterial activity of column chromatographic fractions of root extract of *Cymodocea serrulata* L. were determined for antibacterial activity against 4 poultry pathogens. The results suggest that 6 fractions from acetone extract and one fraction from hexane extract exhibited broad spectrum of antibacterial activity. It is concluded that the bioactive compounds from the root extract of *C. serrulata* can be effectively used as an alternative poultry medicine to replace the conventional antibiotics of having adverse side effects.

Keywords: Bioactive compounds, *Cymodocea serrulata*, Poultry pathogen, Seagrass.

Introduction

Poultry is one of the fastest growing segments of the agricultural sector in India. Production of eggs and broilers has been rising at a rate of 8 to 10% per annum (Sharpley, 1999). India is now the world's 5th largest egg producer and the 18th largest producer of broilers (Debnam & Jackson, 2005). Eggs and chicken meat are important rich sources of protein, vitamins and minerals. Poultry provides rich organic manure and is an important source of income and employment to millions of farmers and other persons engaged in allied activities in the poultry industry (Branckaert & Gueye, 1999). Nowadays enteric diseases in poultry industry cause low productivity, increased mortality and associated contamination of poultry products for human consumption. With increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage in Europe and the potential for a ban in US, there is an increasing interest in finding alternatives to antibiotics for poultry production. A public health concern associated with pathogenic bacteria is the increased incidence of strains that are resistant to antimicrobial agents. Those resistant microorganisms can be disseminated via animal feces to other animals. Resistance to antimicrobials is connected with genetic mechanisms. New trends in drug discovery from natural source emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities for the treatment of many disease such as cancer, inflammatory condition (Margret *et al*., 2009) arthritis, malaria and large variety of viral, bacterial, fungal disease (Ravikumar *et al*., 2005, 2009,2009a, 2010a, 2010b, 2010c, 2010d). Seagrass are the marine flowering plants that successfully growth in tidal and sub tidal marine environment. A variety of medicines and chemical are also prepared from seagrass and their associates (Ravikumar *et al*., 2005). Hence, the present investigation has been undertaken to study the antibacterial activity against chosen poultry pathogens from the root of *Cymodocea serrulata*.

Materials and methods

Collection & extraction of bioactive compounds

Fresh root of *Cymodocea serrulata,* was collected along Palk Strait Coast of Thondi region (Lat 79^0 44'10" N & Long 79°10'45"E) and authenticated by Dr. K. Eswaran, Scientist, CSMCRI, Mandapam, TN. All the collected samples were washed thrice with tape water, twice with distilled water to remove the adhering salts and sands and then shade dried. The chopped air-dried roots of *C. serrulata* (1 kg) were taken separately in an air tight glass jars and required quantity of ethanol and water mixture (3:1 ratio) was added and kept under dark (percolation method). After 7 d, the contents were stirred well and then filtered by using muslin cloth. The plant extracts were concentrated to two third of the volume by distillation. The colloidal form of the plant extract from *C. serrulata* (186 g) was stored in a sterile glass container for further use. The percentage of extraction was calculated using the following formula: Percentage of extraction (%) = Weight of the extract (g) / Weight of the plant material (g) x 100.

Bioassay-Guided fractionation by silica column chromatography:

The colloidal form of extract obtained through percolation were separately suspended in water and defatted with diethyl ether. Individual aqueous layer of extracts were subjected to column chromatography packed with 500 g of silica gel (230-400 mesh) (MERCK) with the maximum height of 50 cm and eluted successively with 30 ml of n-hexane, benzene, chloroform, acetone, ethanol (MERCK AR grade) and water. The obtained fractions were labelled and stored at -80^{0}C (Sanyo-Japan) for further use. The fraction of extract were screened for the presence of phytochemical constituents by the following the method of Sofowora (1982).

Collection of the poultry pathogens

Poultry pathogen *viz*., *Klebsiella, E. coli, Staphylococcus* sp. and *Salmonella* sp. were collected

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Table 1. Antibacterial sensitivity of column chromatographic fractions of root extract of C. serrulata against chosen poultry pathogens.

Name of the poultry pathogens	Zone of inhibition (mm diameter)																
	Acetone fractions (CH_3COCH_3)							Hexane fractions (n-C_6H_{14})							Benzene	Chloroform	Ethanol
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	No zone of inhibition		
Klebsiella sp.	-	-	-	-	-	8±0.57	-	15±0.92	-	-	-	-	-	-			
E. coli	-	-	7±0.26	-	-	7±0.26	8±0.57	12±0.62	-	-	-	-	-	-			
Staphyloccus sp.	12±0.62	-	-	-	-	8±0.57	-	7±0.29	-	-	-	-	-	-			
Salmonella sp.	10±0.6	10±0.6	8±0.76	10±0.6	-	-	-	11±0.85	-	-	-	-	-	-			
Average	11±0.71	10±0.6	7.5±0.32	10±0.6	-	7.6±0.41	8±0.57	11.25±0.71	-	-	-	-	-	-			

± Standard deviation

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Antibacterial assay

Filter paper disc method was used for screening the root extract against chosen poultry pathogens *Klebsiella, E. coli, Staphylococcus* sp. and *Salmonella* sp. Whatmann No. 1 filter paper disc (5 mm dia.) impregnated with different fractions (1mg $disc^{-1}$) were placed on Muller Hinton agar which was previously inoculated with test organisms. Control disc was maintained without the fractions. All the plates were incubated overnight at 37°C under static conditions. After 24 h, the zone of inhibition appearing around the discs were measured and recorded in millimeter in diameter. Triplicate samples were maintained for each bacterial strain (Bauer *et al*., 1966).

Results

The percentage of the extract was identified as 18.6%. The root extract of *Cymodocea serrulata* was tested for the antimicrobial sensitivity against the poultry pathogens *viz*. *Klebsiella, E. coli, Staphylococcus* sp. and *Salmonella* sp. by disc diffusion assay. Seven acetone fractions and 6 hexane fractions were used. The result showed that, 6th fraction of acetone extract showed maximum zone of inhibition against *Klebsiella* sp. (8±0.57) followed by *Staphyloccus* sp. (8±0.57) and *E. coli* (7±0.26). Moreover, the average inhibition was 11.07±0.71. However, 1st fraction of acetone from *C. serrulata* root extract showed maximum zone of inhibition of (12±0.62), (10±0.76) against *Staphylpococus* and *Salmonella*. Likewise 3rd fraction of acetone extract showed sensitivity against *E. coli* and *Salmonella* (7±0.26 & 8±0.52). In addition, 2nd and 4th fractions of acetone extracts showed zone of inhibition of 10±0.6 and 10±0.6 against *Salmonella* sp. 7th fraction showed sensitivity of 8±0.57 against *E. coli*. 1st fraction of hexane extract showed sensitivity 15±0.62 against *Klebsilla* sp. followed by *E. coli* (12±0.62), *Salmonella* sp. (11±0.69) and *Staphylococcus* sp. (7±0.26) respectively (Table 1). The preliminary phytochemical studies reveal that the active fraction extracts of root from *C. serrulata* have variety of phytochemical constituents, namely alkaloids, carboxylic acid, coumarins, flavonoids, phenols, saponins, xanthoprotein, protein, steroids, tannins and sugar (Table 2).

Discussion

In recent years, development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases (Ravikumar *et al*., 2010a). Developed countries have used for a long time systems of surveillance of food safety problems. Keeping this in mind the anti bacterial activity of root extract of *C. serrlata* is applied to develop disease control measures. Of these 7 fractions, acetone fractions showed broad spectrum of activity against poultry pathogens. Previously it was reported that,

Table 2. Photochemical constituents in fraction root extraction of C. serrulata.

Fraction of extract	Alkaloids	Carboxylic acid	Coumarins	Flavanoids	Quinones	Phenol	Saponins	Xanthopriteins	Protein	Resins	Steroids	Tannins	Sugars	Amino aid
Fraction	Acetone fractions													
A1	+	-	-	+		+	-	-	-	-	+	+	-	-
A2	+	-	-	-	-	-	-	-	-	-	+	+	-	-
A3	+	-	-	-	-	+	-	-	-	-	+	+	-	-
A4	+	-	-	-	-	-	-	-	-	-	+	+	-	-
A5	-	-	-	-	-	-	-	-	-	-	-	+	-	-
A6	+	-	-	-	-	+	-	-	-	-	+	-	-	-
A7	+	-	-	+		+	-	-	-	-	+	+	-	-
	Hexane fraction													
H1	+	-	-	-	-	+	-	-	-	-	+	+	-	-
H2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H4	-	-	-	-	-	+	-	-	-	-	-		-	-
H5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H7	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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hexane and acetone leaf extracts were found to show strong antimicrobial and anti-inflammatory activities (Kunle *et al*., 2003). The antibacterial activity of the root extract of *C. serrulata* against the poultry pathogen might be due to the presence of major classes such as alkaloid and tannins. Likewise, mixtures of active constituents showed a broad spectrum of biological and pharmacological activities (Robinson, 1967; Atindehou *et al*., 2002). Tannins (Ravikumar *et al*., 1993) form irreversible complexes with proline rich proteins, resulting in the inhibition of cell protein synthesis of bacteria (Scalbert, 1991). Flavonoids are phenolic structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial cell wall (Cowan, 1999), thus exhibits antibacterial activity through these complexes. From the present findings it can be concluded that, the active fractions of acetone and hexane root extracts from *Cymodocea serrulata* could be effectively used as alternative bioactive agents to rectify the problem of unknown diseases disseminated from the poultry borne food products.

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