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Anti-microbial Effect of *Terminalia arjuna* Bark Extract against *Salmonella* Isolated from the Poultry litter of Rural Area of Chittagong, Bangladesh

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Abstract

Seventy eight samples of poultry litter were collected from different farm of rural area of Chittagong, Bangladesh. Among 78 samples, 43 samples were *Salmonella* positive. Twelve organisms those showing resistance against at least two antibiotics were selected (Table I). Antimicrobial activities of the bark extract of *T. arjuna* against twelve selected isolates of *Salmonella* were then determined (Table II). Eleven of those selected isolates were shown zone of inhibition against crude bark extract of *T. arjuna* and one *Salmonella* (CL-10F) isolates showed no zone of inhibition.

Key words: *T. arjuna, Salmonella,* Poultry, Antibiotic, Resistant, Anti-microbial.

Introduction

Food borne diseases caused by *Salmonella* serotypes occur at high frequently in industrialized and developing countries and represent an important public health problem worldwide. *Salmonella* are among the major bacterial pathogens of poultry in the world and most *Salmonella* infection in humans result from the ingestion of contaminated poultry. (Carli *et al,* 2001)

Salmonella is one of the most important pathogens responsible for human food poisoning in the developed world and chicken products are widely acknowledged to be a significant reservoir for *Salmonella*. They have frequently been incriminated as a source of Salmonella contamination and consequently thought to be major sources of the pathogen in humans (Ozbey and Ertas, 2006). Nearly 1.4 million cases of *Salmonellosis* occur each year in the United States. Such types of data are not available in case of Bangladesh. But we can assume that as a developing country the situation is worse in Bangladesh.

The use of anti-microbial agents in any environment creates selection pressures that favor the survival of antibiotic resistant pathogens. According to the Infectious Diseases Report released by the World Health Organization (WHO) in 2000, such organism has become prevalent worldwide (Anonymous, 2000). The routine practice of giving antimicrobial agents to domestic livestock as a means of preventing and treating diseases, as well as promoting growth is an important factor in the emergence of antibiotic resistant bacteria that are subsequently transferred to human through the food chain (Salehi *et al,* 2005). Most infection with antibiotic resistant *Salmonella* is acquired by eating contaminated foods of animal origin (Fey *et al*, 2000).

Our main objective of this research was to find out a biological tool to resolve the antibiotic resistant *Salmonella* problem. And later, we will proceed to develop a herbal drugs for poultry industry. For this purpose we have determined the antimicrobial activity of bark extract of *T. arjuna* against selected isolates of *Salmonella* .The bark of the *Terminalia arjuna* constitutes an important crude drug, which contains tannins, triterpenoids, saponins, flavonoids, sterols, calcium salts, alkaloidal and glycosidal substances, arjunine and arjunglyciside etc. The bark esteemed as a cardiac tonic. The bark also acts as astringent and febrifuge and is used in the treatment of red and swollen mouth, tongue and gums. It stops bleeding and pus formation in the gums and is useful in asthma, dysentery, menstrual problems, pains, leucorrhoea, wounds and skin eruptions (Ghani, 2003). Due to increased awareness of the importance of traditional medicine in human and animal health care, research into the efficacy of some of the herbs used in the treatment of some illness would be worthwhile (Sanil *et al*. 2009).

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Table I: Antibiotic resistant pattern of 12 selected isolates of *Salmonella*

Name of the organism	Name of the antibiotics													
	Tetracycline	Amoxyclav	Streptomycin	Erythromycin	Cotrimoxazole	Nalidixic acid	Cephalexin	Ceftriaxone	Gentamycin	Cephotaxime	Amoxycillin	Cotrimoxazole	Cefepime	Ciprofloxacin
1. *Salmonella* (CL-2H)	-	-	-	-	-	-	+	+	+	+	+	+	+	+
2. *Salmonella* (CL-5l L)	-	+	+	+	+	+	-	-	+	+	+	+	+	+
3. *Salmonella* (CL-6C)	-	-	+	-	-	+	+	+	+	+	+	+	+	+
4. *Salmonella* (CL-10F)	+	+	+	+	+	+	+	+	+	+	-	+	-	+
5. *Salmonella* (CL-11H)	+	+	+	+	+	+	+	-	+	+	+	+	-	+
6. *Salmonella* (CL-18H)	-	+	+	+	+	+	+	+	+	+	-	+	+	+
7. *Salmonella* (CL-19 H)	-	+	+	+	+	-	+	+	+	+	+	-	+	+
8. *Salmonella* (CL-21H)	+	+	+	+	+	+	-	+	-	+	-	+	+	+
9. *Salmonella* (CL-24 M)	+	+	+	+	+	+	+	+	+	-	+	-	-	+
10. *Salmonella* (CL-30 C)	-	+	+	-	+	+	+	+	+	+	-	+	+	+
11. *Salmonella* (CL-34 C)	-	-	+	-	-	-	+	+	+	+	+	-	+	+
12. *Salmonella* (CL-58 F)	-	+	-	-	+	+	+	+	+	+	+	+	+	+

'+' Indicates organism given zone of inhibition.

'-' Indicates organism given no zone of inhibition.

*CL-stands for Chittagong Lab. and 6, 10, 30, 34 etc. for numbers during sampling.

F, H, M, C, L for name of the sampling area (first alphabet).

Our research findings will also disclose information about the prevalence of antibiotic resistant *Salmonella* in the poultry of rural area of Chittagong region. Resistant *Salmonella* is a serious public health concern and research findings will play vital role to create awareness among mass people about antibiotic resistant *Salmonella*.

Material and Methods

Enrichment of sample and isolation of *Salmonella*

Seventy eight samples of poultry litter were collected from different farm of rural area of Chittagong, Bangladesh. Samples were aseptically cultured into Selenite Cystein Broth (Hi media, India) and incubated at 37^{o}C for 18-24 hours. After incubation a loopful of each broth was streaked onto Salmonella-Shigella agar (SS agar). Xylose Lysine Deoxycholate agar (XLD agar) and Brilliant Green agar were also used as selective media for primary isolation of *Salmonella.* Non-fermenting lactose and negative urease bacteria were selected and transferred to nutrient agar slant (Salehi *et al*, 2005). Biochemical reactions including Triple Sugar Iron test was also performed.

Collection and extraction of plant material

The Bark of *T. arjuna* was collected in fresh condition from Chittagong Hill tracts. The cleaned samples were cut into small pieces (1-2 cm) and dried in air. The samples were ground to fine powder mechanically and the dried powder was kept steeped 72 hours in ethanol. The extract thus obtained was filtered and subjected to rotary vacuum evaporator at 50^{o}C and concentrated to gummy materials under reduced pressure. The gummy materials were then collected in a small vial and then dried. Thus crude extracts were obtained

Antibiotic sensitivity test against standard antibiotics

For *in vitro* susceptibility test, the isolated organisms were subjected to fourteen standard antibiotics (Table IV). The

disc diffusion method was followed to determine susceptibility of the *Salmonella* isolates (Coyle, 2005). The bacterial suspension turbidity adjusted to McFarland standard number 0.5, in Mueller Hinton Broth (Hi-media, India). With a sterile cotton swab bacterial culture was streaked on Mueller Hinton Agar plate (Hi-media, India). Commercial antibiotic discs containing single concentration of each antibiotic was` then placed on the inoculated plate surface. The zone of inhibition of growth around each disc after incubation at 37°C was measured in millimeters.

Determination of antibacterial activity of *Terminalia arjuna* crude extracts

In vitro sensitivity of the 12 selected *Salmonella* isolates to the crude extract of *T arjuna* was determined by disc diffusion method. Dried and sterilized paper discs were treated separately with desired concentration of previously prepared ethanolic solution of the crude extract using a micropipette and dried in air under aseptic condition and placed at equidistance in a circle on the seeded plate. The concentrations of crude extract used were 3mg/disc and 5mg/disc. These plates were kept for 4-6 hours at low temperature, so that the test materials can diffused from disc to the surrounding medium by this time. The plates were then incubated at 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentrations of crude extracts of *Treminalia arjuna* were performed by macrodilution method (Rahman, 2008). Crude extract was dissolved in 30% dimethyl sulfoxide (DMSO) to obtain 10% (w/v) solution. For MIC test of the selected bacteria, the extract was first diluted in sterilized Mueller-Hinton Broth to the concentration of 10,000 µg/ml, 5000 µg/ml, 4000 µg/ml, 3500 µg/ml, 2500 µg/ml, 2000 µg/ml, 1500 µg/ml, 1000 µg/ml, 750 µg/ml, 500 µg/ml and 250 µg/ml in screw capped tubes. Bacterial suspensions of the test organism were prepared in sterilized Mueller- Hinton Broth. Then 1 ml of the culture suspension was added to each sterilized screw capped tube containing 1 ml of compound suitably diluted in the sterilized broth medium to give final volume of 2 ml. Culture medium without samples and others without microorganisms were used in the tests as sample control and microorganism control. Tubes were incubated at 35°C for 20-24 hours and growth was indicated by turbidity.

Results and Discussion

To know the antibiotic resistance pattern of *salmonella* present in different poultry industries, 78 samples of poultry litter were collected from different poultry farm of rural area in Chittagong. Forty three *Salmonella* isolates were isolated from the collected sample. Antibiotic sensitivity test of the isolates against fourteen different standard antibiotics were then performed (Table IV). Twelve organisms were selected on the basis of resistance against at least two antibiotics. Bark extract of *T. arjuna* showed antimicrobial activity against selected isolates of *Salmonella* (Table II). Six antibiotics were found to be resistant against *Salmonella* (CL-2H). But it was found sensitive against *T. arjuna* bark extract. Extract concentration 5 mg/ disc have given highest zone of inhibition (14 mm). *Salmonella* (CL-34 C) was resistant against five antibiotics but 3mg/disc and 5mg/disc concentration of *T. arjuna* bark extract produced zones of inhibition of diameter 8mm and 9mm respectively, against the isolates.

Table II: Antimicrobial activity of bark extract of *T. arjuna* against various antibiotic resistance isolates of *Salmonella* compared with standard antibiotic Ciprofloxacin

Name of the isolates	Diameter of zone of inhibition (mm)		
	3mg/disc	5 mg/disc	Cipro floxacin (5µg/disc)
1. *Salmonella* (CL-2H)	12	14	29
2. *Salmonella* (CL-5l L)	7	8	38
3. *Salmonella* (CL-6 C)	7	10	26
4. *Salmonella* (CL-10F)	-	-	33
5. *Salmonella* (CL-11H)	8	9	25
6. *Salmonella* (CL-18H)	10	13	25
7. *Salmonella* (CL-19H)	7	10	29
8. *Salmonella* (CL-21H	7	9	23
9. *Salmonella* (CL-24 M)	8	8	31
10. *Salmonella* (CL-30 C)	7	9	30
11. *Salmonella* (CL-34C)	8	9	24
12. *Salmonella* (CL-58 F)	8	11	22

The only exception was *Salmonella* isolates (CL-10F) which was not only resistant to Cefepime and Amoxycillin but also resistant to the bark extract. *Salmonella* (CL-24 M) showed same zone (8mm) of inhibition against both extract concentration. Ciprofloxacin was the only antibiotic which was found sensitive against every selected organism. During the course of the anti-microbial screening it was found that among the 12 selected isolates *Salmonella* (CL-2H), *Salmonella* (CL-18H), *Salmonella* (CL-19 H), *Salmonella* (CL-58 F) were showed good sensitivity to crude extract of *T. arjuna.* Therefore MIC of crude extract of *Terminalia arjuna* against of those organism were determined (Table III).

Table III: MIC value of crude extract of *T. arjuna* against selected four *Salmonella* isolates

Name of the organism	Concentration of the sample (µ g /ml)										
	10,000	5000	4000	3500	2500	2000	1500	1000	750	500	250
Salmonella (CL-2H)	-	-	-		- (MIC)	+	+	+	+	+	+
Salmonella (CL-18H)	-	-	-	- (MIC)	+	+	+	+	+	+	+
Salmonella (CL-19 H)	-	-	-	- (MIC)	+	+	+	+	+	+	+
Salmonella (CL-58 F)	-	-	-	- (MIC)	+	+	+	+	+	+	+

'+' Indicates growth of organism.
'-' Indicates no growth of organism

Salmonella are among those known to carry plasmids, which encode for drug resistance. This implies that widespread use of anti-microbial in animals or humans may cause an increase in the frequency of occurrence of bacterial resistance to other anti-microbial as R plasmids may encode resistance to additional anti-microbial agents (Salehi *et al* 2005). Most of these antibiotics are added in poultry feed, as supplement and obvious lack of control on the antibiotic usage may be the provable cause for their high resistance (Murugkar *et al,* 2005).

Though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has developed. Medicinal plants are natural resources, yielding valuable products, which are often used in the treatment of various ailments. Plant materials remain as an important resource for combating illnesses, including infectious diseases, and many of the plants have been investigated for novel drugs or templates

Table IV: Name of the standard antibiotic disc used

Name of the antibiotics	Concentration/disc
Gentamicin	10µg /disc
Chloramphenicol	30µg/disc
Erythromycin	15µg/disc
Cefepime	30µg /disc
Streptomycin	10µg/ disc
Amoxyclav	30µg /disc
Ceftriaxone	30µg /disc
Ciprofloxacin	5µg/disc
Cephotaxime	30µg /disc
Cephalexin	30µg /disc
Nalidixic acid	30µg /disc
Cotrimoxazole	25µg /disc
Tetracycline	30µg/disc
Amoxycillin	10µg/disc

for the development of new therapeutic agents (Karthy, 2009).

Poultry is essential to the national economy of Bangladesh and the welfare of human beings as well. Several constraints- the diseases, poor husbandry, low productivity and shortage of food affect the optimal performance of this industry in Bangladesh (Haque *et al.,* 1991). Infections with bacteria of the genus *Salmonella* are responsible for a variety of acute and chronic disease of poultry in Bangladesh (Bhattacharjee *et al.,* 1996). It is appeared from the results that the crude extract was effective against most *Salmonella* isolates. Its potential application in the treatment of bacterial infection in poultry industry of Bangladesh would therefore be promising. This work will lead to develop herbal drugs from the plant *T. arjuna* for the antibacterial activity against *Salmonella.*

Conclusion

Despite a pressing need for new antibiotics to meet the threat of resistant bacteria, industrial research in this area is declining. New products have faced the inevitable emergence of resistance and the potentially short durability of antibiotics is one of the reasons why the development of new products is decelerating. It is hoped that this study would lead to the establishment of some compounds that could be used to formulate new and more potent anti-microbial drugs of natural origin for poultry industry.

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