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COMPARATIVE ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF COMMERCIALLY AVAILABLE ANTIBIOTICS AND *CINNAMOMUM VERUM* (CINNAMON) AGAINST *SALMONELLA TYPHI* AND *PARA TYPHI* INFECTIONS

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ABSTRACT

Typhoid fever is a life threating disease occurs due to ingestion of contaminated food products. *Salmonella* spp. are highly pathogenic in nature because they have the ability to produce endotoxins within a body. The present research study was designed to carry out comparative assessment of antibacterial activity of commercially available antibiotics and *Cinnamomum verum* extracts prepared in different solvents against *S. typhi* and *paratyphi* infections. In the current study, a total of 500 samples of blood were collected from typhoid patients in pre-sterilized bottles. After that serological tests were performed. Widal test was used for typhoid diagnosis and gave highest efficacy (52.6%) in the diagnosis of salmonellosis. Furthermore, Widal positive samples were investigated for the presence of pathogenic bacteria through conventional culturing technique and two major bacterial species i.e. *S. typhi* (n = 85) and *S. paratyphi* (n = 25) were identified. In the current study, Furthermore, antibiotic sensitivity assay was performed according to Kirby-Bauer disc diffusion method and it was observed that all *S. typhi* isolates were sensitive to Sulbactam (38±2.23 mm) followed by Ceftazidime (37±2.8 mm), Tazobactam (36±2.5 mm), Amoxicillin (35±3.33 mm), Amikacin (29±1.7 mm), Gentamycin (28±1.8 mm), Cephradine (26±2.9 mm) and Meropenem (18±3.1 mm). On the other hand, *S. typhi* showed resistance to Ciprofloxacin (1.5±0.8 mm) and Ofloxacin (1±0.77 mm). Additionally, it was also observed that all *S. paratyphi* showed highest sensitivity to Ciprofloxacin (34±0.87 mm), Ofloxacin (30±1.67 mm), Ceftazidime (28±3.1 mm), Tazobactam (28±2.4 mm) and Sulbactam (28±1.14 mm) while showed resistance to Gentamycin (12±2.8 mm). Furthermore, it was suggested that proper care and preventive measures should be taken for patients with bacterial infections and also antibiotics selection must be after performing culture sensitivity tests. Beside this, different plant extracts should be recommended as alternative for the treatment of typhoid infection and by doing this, chances of antibiotic resistance might be reduced up to greater extent.

1. INTRODUCTION

Salmonella spp is the main causative agent for gastroenteritis in humans and other mammals. When the bacterial cells enter epithelial cells lining within intestinal tract, they cause wrinkles on the host cell surface which resulting in the transiently breakage of the microvilli, present on the cell surface (Macfarlane *et al.*, 2006). After this, the white blood cells (WBCs) enter into the mucosal layer which alters digestion process as a result diarrhea may take place (Zogaj *et al.*, 2001). It has been reported that the prevalence of salmonellosis is increasing day by day throughout the world, especially Africa, Asia and South Asia resulting in millions of deaths. In developing countries like Pakistan, the prevalence of *Salmonella* spp., are almost 61% and most of the causalities occurred due to typhoid fever (Levin *et al.*, 2000).

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Typhoid fever is a life threating disease occurs due to maintaining unhygienic environment in daily life and also due to ingestion of *Salmonella* spp., contaminated food products. *Salmonella* spp. are Gram negative rod shaped bacteria that belong to *Enterobacterace* family. They are highly pathogenic in nature because they have the ability to produce endotoxins within a body and also has ability to produce lipopolysaccharide membrane coat around itself which protect them from unfavorable conditions (Tuin *et al.,* 2006). The lipopolysaccharide membrane coat of *Salmonella* spp. also plays a vital role in their pathogenesis as it is made up of polysaccharide phosphorylated glucosamine and fatty acids (Slauch *et al.,* 1995).

Typhoid or enteric fever is a major health issue throughout the world as more than 15 million cases in poorly developing countries due to lack of providing basic health facilities and also due to unhygienic condition. *Salmonella enterica* subspecies I serovars *Typhi* (*S. typhi)* are also responsible for typhoid fever among health care with a history of prolonged fever, headache, abdominal discomfort and general lethargy (Groisman *et al*., 2001). In addition, Serovars of *S. enterica* subspecies I also cause infections in other warm-blooded mammalians displaying different host specificity. On the other hand, most of the typhoid fever investigations were based on laboratory diagnosis and it has been reported that *S. typhi* did not produce infection in laboratory rodents whereas *S. typhimurium* had ability to produce infection in mice (Slauch *et al.*, 1995). Furthermore, it was also reported by Plano *et al.,* (2001) that the pathogenicity of the non-*typhi Salmonella* was due to host bacterial cell interaction.

The herbs which are used as a medicine are called phytomedicines, and these phytomedicines are isolated from the different parts of the plants e.g. seeds, berries, roots and leaves. Phytomedicines are used for a long time instead of antibiotics because they have no side effects and also cheap as compared to commercially available antibiotics. For example, peppermint in combination of other herbs in liquid is used to treat the stomach problems such as anxiety, burning and stomach pain (Singh *et al.,* 2008). Different type of herbs such as honey activated charcoal, green tea and chilly paper are used in previous studies for the treatment of *Salmonella* infection (Sobel *et al.,* 2000). All of these natural herbs showed tremendous activity against Gram negative bacteria such as *E. coli*, *Salmonella typhi* and *paratyphi* etc. *Cinnamomum verum*, also termed as true cinnamon tree or Ceylon cinnamon tree is a short size tree that belongs to the family *Lauraceae*, native to Sri Lanka. Bark of cinnamon tree is important from medical point of view as their extract have strong antimicrobial activities. Among other species, its inner bark is used to make cinnamon. It was reported that Sri Lanka is the main producer of cinnamon that annually produce and supply 85–92% of *C. verum* throughout the world. The tree of *C. verum* is 10-15 m tall while leaves are oval and extend up to 7-18 cm in length. Furthermore, flowers are arranged and have a greenish color with pleasant smell. In hospitals and community level, antibiotics are used extensively for the management of typhoid fever. This extensive use of antibiotics may lead to antibiotics resistance phenomena. Therefore, the only option is to maintain hygienic environment at community level instead of using antibiotics, this will reduce the risk of *Salmonella* infection (Levin *et al.,* 2000). Antibiotics on other hand are designed to act upon specific target sites within bacteria, therefore safe use of antibiotics is recommended at hospital level for the management of typhoid fever (Gill *et al.,* 2006). The present research study was designed to perform comparative assessment of antibacterial activity of commercially available antibiotics and *Cinnamomum verum* against *S. typhi* and *paratyphi* infections of district Peshawar. This study would help to find out alternative for the treatment of typhoid fever which would further reduce economic burden of the patients by purchasing expensive antibiotics from the market.

2. MATERIALS AND METHODS

The present research study was designed to carry out comparative assessment of antibacterial activity of commercially available antibiotics and *Cinnamomum verum* extracts prepared in different solvents against *S. typhi* and *paratyphi* infections of district Peshawar, Khyber Pakhtunkhwa. All the experiments were carried out in Microbiology Research Laboratory (MRL), Abasyn University Peshawar using standard microbiological protocols.

Samples Collection

In the current study, total 500 samples of blood were collected from typhoid patients admitted in Hayatabad Medical Complex (HMC) in pre-sterilized bottles. After collection, serological tests were performed in HMC diagnostic laboratory while for activity analysis and microbiological assessment, samples were transferred in an ice box to the MRL, Abasyn University Peshawar and preserved at 4ºC in refrigerator till further assessment.

Serological Tests

Immuno-Chromatographic Technique (ICT) was used for the assessment of IgG and IgM antibodies in patient serum. This test gave idea about typhoid

infection using principle of ICT test i.e. high titer of IgG and IgM confirm the presence of infection within body. In addition to this, Widal test was performed to confirm somatic and flagella infection by observing serum agglutinin reaction. Furthermore, alanine transaminase (ALT) and C-reactive protein (CRP) tests were used according to standard protocols in order to determine the level of infection.

Isolation and Identification of Bacteria

Pathogenic bacteria from blood sample were isolated using conventional culturing technique. In this technique, different culture media such as nutrient agar, blood agar, *Salmonella-Shigella* agar media and chocolate agar media were used for culturing purpose. The pathogenic bacteria form different colonies over the surface of culture media and from these diverse colonies, different bacteria species were identified according to their morphology (size, shape, color, opacity and margin), Gram`s reaction and biochemical tests (triple sugar iron, indole, citrate and motility test).

Preparation of *Cinnamomum verum* Extract

About 200 g of fresh *Cinnamon* (*Cinnamomum verum***)** were purchased from the local grain market and then grinded into small pieces or powder form which was then kept in airtight bottles for preparation of different solvent extracts. For the preparation of methanolic and aqueous extracts, about 20 g of powder *Cinnamon* was dissolved separately in 100 ml of methanol solvent. After dissolution, the mixture was left overnight at room temperature and later it was filtered. At the end of this procedure, a dark colour solution (primary solution) having concentration of 200 mg/ml will be obtained. Furthermore, different dilutions such as 1, 3, 5 and 10 mg/ml were prepared from the primary solution. The sample extracts were kept refrigerated at 4°C for bioassay and further well diffusion method was used for the assessment of antibacterial activity of *Cinnamomum verum* against isolated bacteria (Ahmad *et al.*, 2005).

Evaluation of Antibacterial Activity of *Cinnamomum verum* Extract against Test Organisms

Antibacterial activity of aqueous and methanolic extract of *Cinnamomum verum* was evaluated by agar well diffusion method. A lawn of each test organism was prepared and then four wells were prepared with the help of cork borer. Out of these four wells, two wells were used for control (water and DMSO) while the remaining two wells were used for aqueous and methanolic extract. After adding extract within wells, the plates were incubated at 37°C for 24 hrs and after incubation, zones of inhibition were measured in millimetre.

Antibiotic Sensitivity Assay

Antibiotic sensitivity assay was performed according to Kirby-Bauer disc diffusion method using CLSI (2017) guidelines. Different antibiotic discs (Amoxicillin, Ceftazidime, Meropenem, Cephradine, Gentamycin, Amikacin, Tazobactam, Ciprofloxacin, Ofloxacin and Sulbactam) used against isolated bacteria. These antibiotics discs were placed on a plate on which bacteria grow and the antibiotics, which were more effective against the test bacteria, made a clear zone of inhibition around the disc which was measured in millimeters.

Statistical Analysis

The mean values of zone of inhibition produced by antibiotics, methanolic and aqueous extracts of *C. verum* were calculated using Microsoft Excel program. Furthermore, *t*-test were performed using Microsoft Excel program and *p* value less than 0.05 was the limit for significant results.

3. RESULTS AND DISCUSSION

Screening of Blood Samples for Possible Infection by Serological Tests

In the current study, a total of 500 typhoid suspected blood samples were proceeded through serological tests (ICT and Widal) in order to confirm the level of infection. It was observed that 380 blood samples out of 500 samples showed high level of infection through ICT test. Furthermore, these 380 positive ICT samples were processed through Widal test for *Salmonella* infection, and it was observed that 180 samples displayed positive Widal test. In addition to this, ALT and CRP assessment was also carried out and it was observed that the level of ALT and CRP was a little bit high in Widal positive samples. Fig. 1 Screening of blood samples for possible infection by serological tests.

Bacteriological Assessment of Widal Positive Samples

In the current research study, Widal positive samples (n = 180) were further investigated for the presence of pathogenic bacteria through conventional culturing technique. It was observed that out of 180 Widal positive samples, 110 samples showed positive result for cultural isolation and presence of pathogenic bacteria. Further, isolated bacterial colonies were examined morphologically and for complete identification, different biochemical tests i.e. triple sugar iron, indole, citrate and motility tests were performed. Two major typhoid responsible bacterial species were identified, and these include *S. typhi* (n = 85) and *S. paratyphi* (n = 25). Moreover, the frequency

distribution of *S. typhi* (77.3%) was high as compared to *S. paratyphi* (22.7%). In addition to this, it was also observed that both *S. typhi* and *S. paratyphi* were Gram negative rods and showed scattered arrangement under microscope and hence showed positive response to triple sugar iron test and negative response to citrate and indole tests respectively. While in case of motility test *S. typhi* displayed positive response and *S. paratyphi* showed negative result as shown in Table 4.1.

Antibacterial Activity of *Cinnamomum verum* Extract against Test Organisms

In the current study, it was determined that methanolic extract of *C. verum* at concentration of 1 and 3 mg/ml displayed very poor activity against both *S. typhi* and *S. paratyphi*. At concentration of 1 mg/ml, weak zone of inhibition of 3.03±1.05 and 3.7±1.5 mm were observed against *S. typhi* and *S. paratyphi* respectively while at concentration of 3 mg/ml, 7±2.1 mm zone of inhibition was observed against *S. typhi* and 10.4±1.25 mm zone was found against *S. paratyphi*. On the other hand, at concentration of 5 and 10 mg/ml, significant activity of methanolic extract of *C. verum* was found against *S. typhi* and *S. paratyphi* ($p = 0.009$)

On the other hand, no significant activity was observed in case of aqueous extract of *C. verum* at all working concentration except 10 mg/ml. Zone of inhibition of about 15.53±1.25 mm and 15.44±1.4 mm was measured at concentration of 10 mg/ml against *S. typhi* and *S. paratyphi* respectively. Whereas, at concentration of 1, 3 and 5 mg/ml both organisms (*S. typhi* and *S. paratyphi*) showed no response towards the extract.

Antibacterial Activity of Commercially Available Antibiotics against Test Organisms

Antibacterial activity of test antibiotics against test bacteria isolated from blood samples of typhoid patients are shown in Fig. 4.4 and Fig. 4.5. It was observed that all the *S. typhi* isolates were sensitive to Sulbactam (38±2.23 mm) followed by Ceftazidime (37±2.8 mm), Tazobactam (36±2.5 mm), Amoxicillin (35±3.33 mm), Amikacin (29±1.7 mm), Gentamycin (28±1.8 mm), Cephradine (26±2.9 mm) and Meropenem (18±3.1 mm). On the other hand, *S. typhi* showed resistance to Ciprofloxacin (1.5±0.8 mm) and Ofloxacin (1±0.77 mm). Furthermore, it was observed that all the *S. paratyphi* isolates showed highest sensitivity to Ciprofloxacin (34±0.87 mm), Ofloxacin (30±1.67 mm), Ceftazidime (28±3.1 mm), Tazobactam (28±2.4 mm), Sulbactam (28±1.14 mm), Amoxicillin (26±1.7 mm), Amikacin (19±1.8 mm), Meropenem (17±3.3 mm) and Cephradine (14±2.23 mm) while showed resistance to Gentamycin (12±2.8 mm).

Typhoid fever is a life threating disease occurred due to maintaining unhygienic environment in daily life and due to ingestion of *Salmonella* spp., contaminated food products. Typhoid or enteric fever is a major health issue throughout the world as more than 15 million cases in poorly developing countries were due to lack of providing basic health facilities and also due to unhygienic condition (Tuin *et al.*, 2006). *Salmonella* spp., are Gram negative rod shaped bacteria that belong to *Enterobacterace* family. The *Salmonella* are pathogenic in nature because it has ability to produce endotoxins within a body and also has ability to produce lipopolysaccharide membrane coat around itself which protect them from unfavorable conditions like active immune system, antibiotics etc. When the bacterial cells enter epithelial cells lining within intestinal tract, they cause wrinkles on the host cell surface which resulting in the transient breakage of the microvilli, present on the cell surface (Macfarlane *et al.*, 2006). After this, the white blood cells (WBCs) enter into the mucosal layer which alter digestion process as a result diarrhea may take place (Zogaj *et al*., 2001).

It has been reported that the prevalence of salmonellosis is increasing day by day throughout the world resulting in millions of deaths (Levin *et al.*, 2000). In developing countries like Pakistan, the prevalence of *Salmonella* spp., are almost 61% and most of the causalities occurred due to typhoid fever. House *et al*. (2001) described the prevalence of typhoid fever throughout the world and found that the incidence of typhoid fever was high in resource poor population. However, very small data was available upon the pathogenesis of the *Salmonella typhi* and typhoid fever among developed countries. Furthermore, they reviewed three main points regarding *Salmonella* infection, and these include, adherence and invasion, dissemination and multiplication within host cells and concluded to eradicate chances of infection, take step to develop proper policy to control any of these points and by these mean chances of infection would be reduced. The present research study was designed to perform comparative assessment of antibacterial activity of commercially available antibiotics and *Cinnamomum verum* against *S. typhi* and *paratyphi* infections of district Peshawar. This study would help to find out alternative for the treatment of typhoid fever which would further reduce economic burden of the patients by purchasing expensive antibiotics from the market.

In the current study, total 500 samples of blood were collected from typhoid patients admitted in Hayatabad Medical Complex (HMC) in pre-sterilized bottles. After collection, serological tests (ICT, Widal, ALT and CRP) were performed in order to confirm the level of infection and also evaluate appropriate diagnostic techniques for typhoid patients. Comparatively to culture techniques, it was observed ICT gave 24% efficacy while diagnosing typhoid, Widal test was a specific test for typhoid diagnosis and gave highest efficacy (52.6%) while ALT and CRP tests were shown to have non-significant response in the diagnosis of salmonellosis. Furthermore, Widal positive samples were further investigated for the presence of pathogenic bacteria through conventional culturing technique and two major bacterial species i.e. *S. typhi* (n = 85) and *S. paratyphi* (n = 25) were isolated from infected blood samples. Widal test was most commonly used in diagnostic laboratory for the diagnosis of salmonellosis infection as it played role in the detection of antibody level for agglutinins (H and O) level in patient serum with typhoid and paratyphoid fever. Patient antibodies are detected to agglutinins both somatic as well as flagella on surface of *salmonella.* Therefore, widal test was considered a reliable technique while diagnosing *Salmonella* infection. Parry *et al.* (1999) also recommend Widal test for diagnosis of typhoid patients by conducting a series of experiments in their study and concluded Widal test as the best choice for acute infection. Olsen *et al.* (2004) also conducted a study to evaluate rapid diagnostic technique for the diagnosis of typhoid infection and found that molecular immunology provided a base for sensitive and specific diagnostic tools for typhoid fever and technology to manufacture practical and inexpensive kits for their rapid detection. They also used different serological tests such as Widal, typhi-dot and TUBEX tests and found the among these tests Widal gave 53% specificity and sensitivity towards salmonellosis while other tests gave slightly higher sensitivity and concluded that all these tests were recommended for the diagnosis of typhoid patients.

In the current study, it was also demonstrated that methanolic extract of *C. verum* at concentration of 1 and 3 mg/ml displayed very poor activity while at concentration of 5 and 10 mg/ml, significant activity of methanolic extract of *C. verum* was found against *S. typhi* and *S. paratyphi* (p = 0.009). On the other hand, no significant activity was observed in case of aqueous extract of *C. verum* at all concentration except 10 mg/ml. Basic reason of higher antibacterial activity of methanolic extract might be due to absorption of bioactive compounds in methanol solvent. Goñi *et al*. (2007) also conducted similar study to evaluate antibacterial activity of cinnamon extract against different microorganisms i.e. *L. monocytogenes, C. albicans* and *A. Flavis* which showed significant results and concluded that methanolic extract of cinnamon had great potential to show activities against test organisms as compared to aqueous and ethanolic extract. Voss-Rech *et al*. (2011) confirmed that extracts of different plants had ability to show strong antibacterial activity against *Salmonella* spp. Moreover, they stated that plants extracts were effective, safe and cheap source for the control of salmonellosis. Their study showed that the plant extracts had potential antimicrobial activity with effective properties in the inhibition of *Salmonella*, especially those of the *Mytraceae* family. Sen *et al.* (2012) also evaluated different medicinal plant extracts against Gram-negative and Gram-positive bacteria and found strong antibacterial effect. Furthermore, they concluded that natural extracts could be used as alternative therapy for the treatment of highly pathogenic diseases. This may reduce the chances of antibiotic resistance by irrational use of antibiotics and also make treatment cost effective.

Furthermore, antibiotic sensitivity assay was performed in the current study according to Kirby-Bauer disc diffusion method using CLSI (2017) guidelines and different antibiotic discs such as Amoxicillin, Ceftazidime, Meropenem, Cephradine, Gentamycin, Amikacin, Tazobactam, Ciprofloxacin, Ofloxacin and Sulbactam were used against isolated bacteria. It was observed that all *S. typhi* isolates were most sensitive to Sulbactam (38±2.23 mm) followed by Ceftazidime (37±2.8 mm), Tazobactam (36±2.5 mm), Amoxicillin (35±3.33 mm), Amikacin (29±1.7 mm), Gentamycin (28±1.8 mm), Cephradine (26±2.9 mm) and Meropenem (18±3.1 mm). On the other hand, *S. typhi* showed resistance to Ciprofloxacin (1.5±0.8 mm) and Ofloxacin (1±0.77 mm). Additionally, it was also observed that all *S. paratyphi* showed highest sensitivity to Ciprofloxacin (34±0.87 mm), Ofloxacin (30±1.67 mm), Ceftazidime (28±3.1 mm), Tazobactam (28±2.4 mm) and Sulbactam (28±1.14 mm) while showed resistance to Gentamycin (12±2.8 mm). A basic reason for antibiotic resistance might be irrational use of antibiotics and also might be due to genetic exchange among organisms due to which they showed resistance behavior in order to minimize the effect of antibiotics. Metchock *et al.* (1990) also conducted similar study and isolate 60 different clinical isolates of *S. typhi* using serial dilution method and after that they use a number of antibiotics which belongs to macrolide group i.e. ampicillin, amoxicillin, cefaclor, trimethoprim, chloramphenicol, tetracycline, and ciprofloxacin and compare the activity of all these antibiotics against

bacterial isolates. They found that azithromycin was more potent against test organisms as compared to erythromycin. Among other macrolides, only rosaramkan showed maximum activity against *Salmonella* spp., while 22 isolates displayed resistance to one or more of the antibiotic used in their study i.e. ampicillin, amoxicillin, cefaclor, tetracycline, chloramphenicol and trimethoprim. Crump and Mintz (2010) also conducted a research study to describe the severity of typhoid fever especially in infants and evaluate the activity of different antibiotics and found that antimicrobial resistance was reported against drugs considered as the first-line of drugs and these include fluoroquinolones, and third generation cephalosporins, makes hurdles in patient treatment. In addition to this, they also concluded that azithromycin was the best substitute and effective drug for the treatment of simple typhoid fever.

4. CONCLUSION

It was concluded from the current study that a total of 500 blood samples suspected of typhoid were collected from patients admitted in Hayatabad Medical Complex (HMC) in pre-sterilized bottles. After collection, serological tests were performed in order to confirm the level of infection and it was concluded that ICT gave 24% efficacy, Widal test gave highest efficacy (52.6%) while ALT and CRP tests were showed no significant response in the diagnosis of salmonellosis. Widal positive samples were further investigated for the presence of pathogenic bacteria through conventional culturing technique and two bacteria species i.e. *S. typhi* (n = 85) and *S. paratyphi* (n = 25) were isolated from infected blood samples. Furthermore, it was also concluded that methanolic extract of *C. verum* displayed significant activity at higher concentrations (5 and 10 mg/ml) against *S. typhi* and *S. paratyphi* (p = 0.009) while aqueous extract showed no significant activity at all concentration except 10 mg/ml. In addition to this, antibiotic sensitivity assay was performed in the current study according to Kirby-Bauer disc diffusion and it was concluded that all *S. typhi* isolates were sensitive to all antibiotics except Ciprofloxacin and Ofloxacin antibiotics where it showed resistance behavior. Additionally, it was also concluded that all *S. paratyphi* isolates showed highest sensitivity to Ciprofloxacin followed by Ofloxacin, Ceftazidime, Tazobactam and Sulbactam while showed resistance to Gentamycin.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interests regarding the publication of this article.

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Table 1 Bacteriological assessment of Widal positive samples.

Identified specie	Gram Staining	Biochemical tests under consideration			
		TSI	Motility	Citrate	Indole
S. typhi	**(-) Rods**	+	+	-	-
S. paratyphi	**(-) Rods**	+	-	-	-