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PREVALENCE OF *SALMONELLA* FROM OUTPATIENTS AT BASHY HEALTH CENTER AND EVALUATION FOR EFFECTIVENESS OF PLANT EXTRACT AGAINST THE PATHOGEN

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Jimma University
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A Thesis Submitted to Department of Biology, College of Natural Science, Jimma University in partial fulfillment of the requirement for the Degree of Master of Sciences in General Biology

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Abstract

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries. *Salmonella* has been identified as an important food and water-borne pathogen. The aim of this study was to evaluate the prevalence of *Salmonella* among outpatients of Bashy health center and determine associated risk factors that contributed to the observed prevalence. For this research, a community based cross-sectional study design method was used. Accordingly, both quantitative and qualitative data were collected through direct observation; interview, questionnaire and practical work in the laboratory (detection of *Salmonella* from patients' sample and to determine in-vitro antimicrobial activities of extracts of selected medicinal plants) were used. A total of 108 outpatients seeking medication at Bashy Health Center during the study period were recruited for the study. Status of antimicrobial resistance of *Salmonella* isolates were evaluated using gallery of ten antibiotics including: Ampicillin (10µg), Streptomycin (10µg), Tetracycline (30µg), Gentamycin (10µg), Erythromycin (5µg), Chlorampenicol (30µg), Ciprofloxacin (5µg), Kanmycin (30µg), Amikacin (30µg) and Nalidixic acid (30µg). Antimicrobial activity of the crude extracts of *Ocimium lamiifolium* leaves was evaluated by disk diffusion method where activities measured in terms of diameter of zones of inhibition (mm). The result showed different annual frequencies of typhoid fever, the highest being 29.5% and the lowest being 11.9%. The highest cases of typhoid fever (40.7%) were recorded in patients aged between 11 to 20 years old. Poor personal and environmental sanitation and low level of awareness towards typhoid fever were among the major associated risk factors for infection with *Salmonella.* The above associated risk factors had not significant difference between the two sexes ($p>0.05$). Resistance rates were 53.85 for Tetracycline and streptomycin, 38.5% for Ampicillin while all isolated strains were 100% susceptible to Erythromycin and Chlorampenicol. The maximum number of antibiotics resisted by *Salmonella* was six. The high percent of resistance to the 3 antibiotics studied could be attributed to their prevailing usage in the area under study. The studies revealed that antibacterial activity of the crude extracts from *Ocimum lamiifolium* was variable when extracted by different solvents; however, it possesses good anti-*Salmonella* activity.

Keywords: Antimicrobial activities, crude extract, drug resistance, hygienic practice, Salmonellosis

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1. Introduction

1.1. Background of the study

Infectious microbial diseases constitute a major cause of death in many parts of the world, particularly in developing countries (Adil *et al*., 2012). Food borne diseases are a public health problem in developed and developing countries. Evidences indicated that 85.6% were estimated to be food borne, and infection was associated with different food types (Majowicz *et al*., 2011).

Salmonella results in 16 million cases of typhoid fever and 1.3 billion gastroenteritis cases annually, with over 3 million perishing from them globally (Pui *et al*., 2011). As this incurs in high economic burden and remains a grave global public health concern till today (WHO, 2005).

Salmonellosis is a bacterial infection to the blood stream and intestines caused by the bacterial group, *Salmonella* (Harish and Menezes, 2011). It is a type of zoonosis, diseases which are transmittable from animals to human beings under natural circumstances (Netsanet *et al*., 2012). Salmonellosis is one of the commonest and most widely disseminated diseases transmitted via food (WHO, 2005).

Salmonella has been identified as an important food and water-borne pathogen that can infect human and animals by causing the disease known as Salmonellosis resulting in significant morbidity and mortality (Akkina *et al*., 1999). There are many types of *Salmonella,* but they can be divided into two broad categories: Those that cause typhoid and those that do not. The typhoidal *Salmonella*, such as *Salmonella Typhi* and *Salmonella Paratyphi*, only colonize humans and are usually acquired by the consumption of food or water contaminated with human faecal material. Enteric fever is most commonly caused by *Salmonella enterica subsp.enterica se rovarsTyphi* (*Salmonella Typhi*) and *Paratyphi A*. *Salmonella Typhi* has been a major human pathogen for thousands of years, thriving in conditions of poor sanitation, crowding, and social chaos.(Harish and Menezes, 2011)

The outbreak of Salmonellosis is more rampant (uncontrolled) during the hot weather, especially summer, making the disease more common in tropical countries.

The diagnosis of Salmonellosis is more frequent among children, the immune-compromised and geriatrics (aged persons) (Harish and Menezes, 2011). In Malaysia, from 1978 to 1997, a

prevalence of 57% of diarrheal cases among children due to Salmonella was reported (Berger, 2010). Sero prevalence varying from 10.44% to 64.2% is on record from different parts of Ethiopia like Shola, Addis Abeba, Denbi, eastern and central Ethiopia and Mekelle (Melese, 1991 and Yang *et al*; 1996; Ashenafi *et al*.2003; Netsanet *et al*. 2012). In Ethiopia, several factors including under and malnutrition, HIV-AIDS, the unhygienic living circumstances and the close relations between humans' and animals may substantially contribute to the occurrence of Salmonellosis. However, this disease can infect any human being (Harish and Menezes, 2011).

Salmonella is found massively in unpasteurized milk, contaminated water, raw or undercooked eggs and meat. Infected livestock contributes to the severity of the disease just as much (Harish and Menezes, 2011). The signs and symptoms in a *Salmonella*-infected human being usually manifest within one to three days. These include diarrhoea, vomiting and abdominal cramps. Salmonellosis brings about a string of diseases such as: typhoid fever, paratyphoid fever, food poisoning and gastroenteritis. In essence, *Salmonella* exists in various serotypes. Different serotypes result in different diseases and thus, some *Salmonella* serotypes are more hazardous than others, like *Salmonella enteric serovar typhi*, which causes the notorious typhoid fever (Netsanet *et al*. 2012). On the other hand, non-typhoidal *Salmonella* serovars such as *Salmonella Typhimurium* brings about localized gastroenteritis. However, a general consensus has been made that *Salmonella* comprises two species: *Salmonella enterica* and *Salmonella bongori.* In addition, the Kauffmann and White scheme classified 2,600 different serovars under the genus *Salmonella* (Cooke *et al*., 2007) as opposed to only 2501 serovars in 2004 (WHO, 2005).

Nonetheless, an infected person can harbour the bacteria for up to a few months, especially younger people and those who are administered with oral antibiotics (Harish and Menezes, 2011). Apart from direct contact with contaminated food and water sources, typhoid and paratyphoid fever can be transmitted indirectly by travelling to high infection risk developing countries (Mayer *et al*., 2010).

While antibiotics are usually unnecessary, severe diarrheal dehydration and the spread of the infection to the intestines will result in dire consequences, including death. Less severe cases are often resolved within a week without medication (Harish and Menezes, 2011). Unfortunately, most of the antibiotics utilized to treat Salmonellosis, with time, face the issue of resistance.

A wide variety of indigenous and minor crops have been utilized for daily consumption since ancient times. The family Labiateae is one of the largest families, which comprises the larger proportion of medicinal plant species. *Ocimum* is one of the important genera of family Labiateae (Nakamura et al., 1999). Ocimum species often referred to as the "king of the herb. *Ocimum lamifolium* is an important medicinal herb that belonging to family Lamiaceae.

The extract of *Ocimum lamiifolium* leaves have potent antimicrobial activity which uses in traditional system of medicines. Hence, *Ocimum lamiifolium* can be employed as a source of natural antimicrobials that can serve as an alternative to conventional medicines. The antimicrobial activity of the extract of fresh leaves of *Ocimum lamifolium* is found sensitive to *Escherichia Coli, Staphylococcus aureus, Pseudomonas aeroginosa, Proteus vulgaris* and *Bacillus subtilus (*Amabye and Mussa, 2015).

In Bashy town there is no research that has been conducted in well-organized manner regarding on prevalence and associated risk factors of *Salmonella.* Therefore; this research was used to fill the knowledge gap and expand our understanding of the possible factors that contribute to the high prevalence of typhoid fever in the study area; also to identify the resistivity of *Salmonella* for different commercial drugs. In addition to this many peoples in our locality were dependent on traditional medicines. As a result this research proved the effectiveness of traditional medicinal plants on *Salmonella*.

1.2. Statement of the problem

Salmonella is phenotypically one of the multi-drug resistant organisms. Outbreaks of typhoid fever caused by multidrug-resistant strains pose therapeutic challenges for physicians. While resistance to single antibiotic occurs, development of multi-drug resistance by this bacterium has complicated the health problem. Typhoid fever, caused by *Salmonella*, has become a significant cause of morbidity and mortality over recent years. Due to the occurrence of drug resistant *Salmonella*, which is mostly due to the misuse of antibiotics by unqualified practitioners, often without laboratory support in antibiotic sensitivity test of organisms, an efficient and reliable identification and sensitivity testing is essential for proper therapy (Mohammad, 2015).

This study was therefore, undertaken to determine the antimicrobial susceptibility patterns of local isolates of *Salmonella* by blood culture in clinically suspected cases of typhoid fever with special reference to their drug resistance. Since there is no any research that had been conducted in organized manner in Bashy town, so that the research was filled the gap in information in the study area.

In addition to this, many peoples in Keffa zone did not have access to commercial drugs. As a result they were dependent on traditional medicines. Therefore; this research was to evaluate the effectiveness of traditional medicinal plant on typhoid fever.

1.3. Objectives of the study

1.3.1. General objective

The general objective of this study was to evaluate prevalence of *Salmonella* among outpatients of Bashy health center and determine associated contributing risk factors.

1.3.2. Specific objective

The specific objectives of this study are:

- To assess the prevalence of *Salmonella* in Bashy town.
- To identify the risk factors associated with prevalence of *Salmonella* in the study area.
- To assess drug resistance pattern of *Salmonella* in the study area.
- To determine in-vitro antimicrobial activities of crude extracts of *Ocimum lamifolium* plants against the isolated *Salmonella*.

1.4. Significance of the study

This study was given insight for the clinic workers about the prevalence and the risk factors of typhoid to give training for the people in different meetings about the causes and prevention methods of this disease. It serves as a secondary source of information for those who intended to carry out further survey on typhoid.Similarly in the study area there was no evidence data on the prevalence and risk factors related to typhoid fever. Therefore; this study has helped to fulfill the knowledge gap and improved their knowledge and perception level related to typhoid fever through the district health office and also it serves as a literature for other researcher.

They were many people's that did not have access to commercial drugs. Therefore; this study evaluated the effectiveness of *Ocimum lamiifolium* against *Salmonella* in the study area, although phytotochemical analysis and toxicity test need to be carried out.

2. Literature Review

2.1. *Salmonella*

Typhoid fever is caused by *Salmonella typhi,* a Gram negative bacterium. A very similar but often less severe disease is caused by *Salmonella serotype para typhiA, para typhi B, para typhi C*. The ratio of disease caused by *Salmonella typhi* to that caused by *Salmonella para typhi* is about 10 to 1 in most of the countries where this matter has been studied. But in Sub Saharan Africa, where the burden is the least characterized, hospital based studies indicates that non-typhi serotype of *Salmonella* particularly *S.enterica serotype entritides* and *S. enerica serotype typphimurium,* greatly outnumber *S.typhi* and *S. paratyphi* as a cause of blood stream infection (Gizachew, 2011).

Humans are the sole reservoirs for typhoid. Family contacts may be transient or permanent carriers and in advertently spread infection (Heymann, 2008). The incubation period is usually 8–14 days but can range from 3–60 days depending on the size of infecting dose and host factors (Heymann, 2008)

2.2. Epidemiology of Typhoid Fever

Typhoid fever is endemic throughout Africa and Asia and persists in the Middle East, a few southern and eastern European countries and central and South America. In the US and most of Europe, apart from occasional point source epidemics, typhoid is predominantly a disease of the returning traveller (Ackers *et al*., 2000). A recent study estimated there to be approximately 22 million cases of typhoid each year with at least 200 000 deaths (Crump *et al*., 2004). However, the true magnitude is difficult to quantify because the clinical picture is confused with many other febrile illnesses and most typhoid endemic areas lack facilities to confirm the diagnosis. (Pietro and Duncan, 2005).

Human Salmonellosis is one of the major diseases in Ethiopia and several factors including under and mal-nutrition and HIV-AIDS may substantially contribute to its occurrence. Despite its importance, surveillance and monitoring systems are not in place and a comprehensive picture of its epidemiology is not available (Tadesse, 2014).

2.3. Sources of infection

Foods of animal origin, particularly meat, poultry, and, in some instances, unpasteurized egg products are considered to be the primary sources of human salmonellosis. It has been reported that livestock and their products can contribute to as much as 96% of the total *Salmonella* infection in humans (Dahal, 2007). Most of these food products, e.g. beef, mutton and poultry, become contaminated during slaughter and processing, from the gut contents of healthy excreting animals. In the same way, all food that is produced or processed in a contaminated environment may become contaminated with *Salmonella* and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation (Dahal, 2007).

2.4. Mode of transmission of *Salmonella*

A report by Washington State Department of Health (2011) estimated that transmission of *Salmonella* is faecal-oral and vehicle-borne. Infection may result from ingesting food or water that has been contaminated with human or animal faeces, from direct exposure to animals or their waste and foods from handled in ways that permit multiplication of organisms (Molbak *et al.*, 2006).

Persons with typhoid fever carry the bacteria in their bloodstream and intestinal tract. In addition, a small number of persons, called carriers, recover from typhoid fever but continue to carry the bacteria. Both ill persons and carriers shed *Salmonella* in their faeces (stool). You can get typhoid fever if you eat food or drink beverages that have been handled by a person who is shedding *Salmonella* or if sewage contaminated with *Salmonella* bacteria gets into the water you use for drinking or washing food. Therefore, typhoid fever is more common in areas of the world where hand washing is less frequent and water is likely to be contaminated with sewage. The infection is rarely spread by casual contact. Shellfish (particularly oysters) taken from sewage-contaminated beds, raw fruits, vegetables fertilized by human excrement and eaten raw, contaminated milk and milk products (usually contaminated by hands of carriers), are important sources of infection to consider (Alberta Health, 2014).

Once *Salmonella* bacteria are eaten or drunk, they multiply and spread into the bloodstream. The body reacts with fever and other signs and symptoms (Alberta Health, 2014).

2.5. Period of Communicability

The period of communicability lasts as long as the bacilli are present in the excreta. This usually begins from the first week after onset of illness and continues through convalescence and for a variable period thereafter. It is estimated that approximately 10% of untreated typhoid cases can continue to discharge bacilli for three months after onset of symptoms (Heymann, 2008)

The carrier state may follow acute illness, mild or even sub clinical infections. Between 2–5% of cases infected with *Salmonella* may become chronic carriers and can be more commonly seen in individuals (especially women) infected during middle age, and those who have biliary tract abnormalities, including gallstones. Chronic urinary carriers may occur with schistosome infections or kidney stones (Heymann, 2008)

2.6. Pathogenesis and Clinical diagnosis

2.6.1 Pathogenesis

Salmonella organisms penetrate the mucosa of both small and large bowel, coming to lie intracellular where they proliferate. There is not the same tendency to mucosal damage as occurs with Shigella infections but ulceration of lymphoid follicles may occur (Sarman, 2001). The evolution of typhoid is fascinating. Initially *Salmonella* proliferates in the second part of the Payer's patches of the lower small intestine from where systemic dissemination occurs, to the liver, spleen, and reticuloendothelial system. For a period varying from 1 to 3 weeks the organism multiplies within these organs. Rupture of infected cell occurs, liberating organisms into the bile and for a second time cause infection of the lymphoid tissue of the small intestine particularly in the ileum (Sarmanh, 2001).

Susceptibility is general and is increased in persons with achlorhydria (a condition in which production of gastric acid in the stomach is absent or low or more commonly, treatment with acid suppression agents). Relative specific immunity follows clinical disease, asymptomatic infection and immunization (Heymann, 2008).

2.6.2 Antimicrobial resistance

In the absence of adequate access to safe drinking water, antimicrobial chemotherapy is the only major option available under such conditions for the control of typhoid fever. Apart from curing the individual of the disease, it has the potential to decrease the risk of faecal carriage and

thereby preventing onward transmission of infection. Effective antimicrobial chemotherapy is available for the past 50 years (Tamilarasu, 2013).

Extensive and unregulated use of antibiotics for the empirical treatment of fever in the community setting has resulted in a general decline in complications and mortality due to typhoid fever. However, this practice has unwittingly resulted in the emergence of antibiotic resistant strains of *Salmonella* causing localized outbreaks and epidemics. With continued antibiotic selection pressure, these resistant strains have become endemic in many parts of the world (Tamilarasu, 2013).

2.6.3 Emergence and Spread of Drug-resistant

Chloromycetin (chloramphenicol) was found to be effective in the treatment of typhoid fever in 1948, and soon it became the standard antibiotic for treating typhoid fever. Several years later, ampicillin and then co-trimoxazole were added to the therapeutic armamentarium. However, resistance to chloramphenicol emerged rapidly within two years after its introduction, and by 1972 chloramphenicol-resistant typhoid fever had become a major problem. Outbreaks occurred in Mexico, India, Vietnam, Thailand, Korea and Peru. But ampicillin and co-trimoxazole were still effective against these chloramphenicol-resistant strains, making them effective alternatives (Rupali *et al*., 2004).

In 1990s, isolates of *Salmonella* resistant to all the three first-line drugs then in use (chloramphenicol, ampicillin, and co-trimoxazole) started emerging and sooner outbreaks of infections with these strains (designated as multidrug-resistant *Salmonella* [MDR-ST]) occurred in India, Pakistan, Bangladesh, Vietnam, the middle-east and Africa. Slowly MDR-ST became the predominant strain in many parts of Asia, including India. This change in pattern of susceptibility was reflected even in faraway places such as the United States and the United Kingdom. Fortunately, more or less over the same period, fluoroquinolones were discovered and found clinical use in the treatment of typhoid fever. Unparalleled in clinical efficacy even today, fluoroquinolones achieved rapid defervescence of fever in less than four days' time with markedly reduced rates of relapse. These features together with the widespread emergence of MDR-ST strains made ciprofloxacin the drug of choice for typhoid fever (Tamilarasu, 2013).

2.6.4 Ciprofloxacin-Failure – A Clinical Paradox

Towards the end of 20th century, it was observed that fever took longer time than before to clear and sometimes did not at all respond to ciprofloxacin therapy (Kadhiravan *et al*., 2005). These isolates had reduced susceptibility to ciprofloxacin as evidenced by comparatively higher minimal inhibitory concentrations (MICs), although they were reported susceptible to ciprofloxacin by conventional disk-diffusion testing using recommended breakpoints (Kadhiravan *et al*., 2005).

The MICs of ciprofloxacin for these isolates are about 10 times that of fully-susceptible strains, and these isolates are usually resistant to the first generation quinolone nalidixic acid. Subtly reduced susceptibility of this magnitude has been amply documented to result in poor clinical response to treatment with ciprofloxacin and often in treatment-failure (Rupali et al., 2004). Nalidixic acid-resistance serves as a surrogate marker of decreased susceptibility to ciprofloxacin, and the clinical response to ciprofloxacin in patients infected with nalidixic acid-resistant *Salmonella* is far inferior to the response in those infected with nalidixic acid-sensitive *Salmonella* (NASS). (Madhulika *et al*., 2004).Unregulated access and indiscriminate use of fluoroquinolones have obviously promoted this debacle. Isolates with reduced susceptibility to ciprofloxacin are fast becoming a major problem in South and South-East Asia. In India, currently, as high as 70 to 80% of isolates in hospital-based studies are NARS and 20 to 50% of isolates are MDR-ST (Ray *et al*., 2006).

2.6.5 Clinical features

Salmonella enteric serovar Typhi infections result in a clinical syndrome that varies widely in severity (Stuart and Pullen, 1946). After ingestion of the bacteria, an incubation period follows usually lasting 8 to 14 days (range 3–60 days). Fever and malaise mark the onset of bacteraemia but patients do not usually present to hospital until towards the end of the first week of symptoms (Osler and McCrae, 1926).

Fever flu-like symptoms with chills (although rigors are rare) and a dull frontal headache are common. The fever, initially low grade, rises progressively, and by the second week is often high and sustained (39–40 ◦C) (Stuart and Pullen, 1946). Other symptoms include malaise, anorexia, poorly localized abdominal discomfort, a dry cough and myalgia. Physical signs are few, but a coated tongue, tender abdomen, hepatomegaly and/or splenomegaly may be found. The

abdominal pain is usually diffuse and poorly localized, but occasionally sufficiently intense in the rightiliac fossa to suggest appendicitis. Dilated loops of bowel may be palpatedindicating an ileus. Nausea and vomiting are infrequent in uncomplicated typhoid but are seen with abdominal distension in severe cases (Christopher, 2005).

Relative bradycardia is not a consistent feature. Rose spots are reported in 5–30%of cases but are easily missed in dark-skinned patients. These rose spots are small blanching erythematous maculopapular lesions typically on the abdomen and chest. Melanesian typhoid patients may develop purpuric macules that do not blanch. Constipation is generally more common in adults,but in young children and adults with HIV infection diarrhoea predominates. *S. Enteric* serovar Paratyphi causes a similar, although less severe syndrome (Christopher, 2005).

Untreated, the fever persists for two weeks or more and defervescence occurs slowly over the following 2–3 weeks. Convalescence may last for 3–4months. If an appropriate antibiotic is given the fever gradually falls over3–4 days. The duration of untreated illness prior to the initiation of therapy influences the severity of the disease. Those individuals infected with multi-drug-resistant (MDR) isolates of *S. enteric serovar Typhi* may also suffer more severe disease. Patients with typhoid fever in the second to fourth week present with accelerating weight loss, weakness, an alteration of mental state and the development of complications that occur in 10–15% of hospitalized patients and occasionally dominate the clinical picture deflecting attention from the underlying diagnosis of typhoid(Christopher, 2005).

Typhoid fever in pregnancy may be complicated by miscarriage, although antimicrobial treatment has made this less common. Vertical intra-uterine transmission from a typhoid-infected mother may lead to neonatal typhoid, a rare but severe and life-threatening complication. Relapse occurs in 5–10% of patients, usually 2 to 3 weeks after defervescence. The illness is usually, but not invariably, milder than the original attack and the relapse *S. Enteric serovarTyphi* isolate has the same susceptibility pattern as in the original episode. Reinfection may also occur. Up to 10% of untreated convalescent typhoid cases will excrete *S. Enteric serovarTyphi* in faeces for 1–3 months and between 1 and 4% become chronic carriers excreting the organism for more than one year (Christopher, 2005). Chronic carriers give no prior history of typhoid fever in up to 25% of cases. Faecal carriage is more frequent in individuals with gallbladder disease and is most common in women over 40; in the Far East there is an

association with opisthorchiasis. Chronic carriage carries an increased risk of carcinoma of the gallbladder, pancreas and large bowel (Caygill *et al*., 1994).

2.6.6. Carrier state

Around 1-5% of patients, depending on age, become chronic carrier are harbouring *Salmonella* in the gallbladder (WHO, 2011).Cases of typhoid fever can be classified into three i.e. Confirmed case, Probable case and Chronic Carrier. When a patient with persistent fever (38 °C or more) lasting 3 or more days, with laboratory-confirmed *Salmonella* organisms (blood, bone marrow, bowel fluid) it is Confirmed case while a patient with persistent fever (38 °C or more) lasting 3 or more days, with a positive sero-diagnosis or antigen detection test but no *Salmonella* isolation it is Probable case however; when an individual excreting *Salmonella* in the stool or urine for longer than one year after the onset of acute typhoid fever is Chronic Carrier(WHO, 2011).

2.7. Prevention and Treatment

2.7.1 Prevention of Salmonellosis

Quite recently, a longitudinal study in Bangalore, India, investigated the morbidity rate due to water-borne diseases and the bacteriological quality of water (Darwis, 2011).

It was discovered that the majority were diarrheal cases and thus, it was concluded that in developing countries, diarrheal diseases can be prevented by having sufficient safe and clean drinking water (Jadhav, 2011).

A research from the Food Science and Human Nutrition Department, University of Florida, reported that an understanding on the outbreaks and factors mediating plant produce contamination allows the development of intervention strategies to minimize the risk of such contamination by *Salmonella* (Darwis, 2011). Therefore, Salmonellosis is not only confined to animal-based products. This is supported by the findings of Romling et al. that the increase in food-borne outbreaks by *Salmonella* involving fruits and vegetables is due to plants being vital vectors for transmission of *Salmonell*a between hosts (Romling *et al*., 2007).

2.7.2 Antimicrobial therapy

Efficacy, availability and cost are important criteria for the selection of first-line antibiotics to be used in developing countries. It should be noted, however, that therapeutic strategies for children, e.g. the choice of antibiotics, the dosage regimen and the duration of therapy, may differ from those for adults (WHO, 2003).

The fluoroquinolones are widely regarded as optimal for the treatment of typhoid fever in adults (Chinh *et al*, 2000). They are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs, viz. chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole (Arnold *et al*., 1993). The majority of isolates are still sensitive. The fluoroquinolones attain excellent tissue penetration, kill *Salmonella* in its intracellular stationary stage in monocytes/macrophages and achieve higher active drug levels in the gall bladder than other drugs (WHO, 2003). They produce a rapid therapeutic response, i.e. clearance of fever and symptoms in three to five days, and very low rates of post-treatment carriage (Cristiano *et al*., 1995).

2.7.3. Treatment of Drug-resistant Typhoid Fever

Fluoroquinolones (ciprofloxacin or ofloxacin 15 mg/ kg/day) are the current treatment of choice for typhoid fever in patients of all age groups, including pregnant women, infected or likely to be infected with fully drug susceptible *Salmonella* or MDR-ST (Parry, 2002).

Short courses of treatment for 5 to 7 days would suffice in such settings. Third-generation cephalosporins (cefixime 20 mg/kg/ day for 7 to 14 days or ceftriaxone 60-75 mg/kg/day for 10 to 14 days) and azithromycin (8-10 mg/kg/day for 7 days) are suitable alternatives for the treatment of MDRST infections (Parry, 2002).

The optimal treatment for NARST infections is not known yet. Azithromycin and third-generation cephalosporins are effective for the treatment of these infections, though their cost is prohibitive (Parry, 2002). High-dose fluoroquinolones (20 mg/kg/day) given for at least 10 to 14 days still achieve cure in up to 75% of patients infected with NARST. However, the fever clearance time is prolonged (about 7 days), and the rate of convalescent faecal carriage is high (Parry, 2004).

Indirect evidence suggests that the use of fluoroquinolones as first-line treatment in settings where NARST is highly prevalent may result in an excess of complications. The role of newer fluoroquinolones such as levofloxacin and gatifloxacin and combination chemotherapy is currently being evaluated in clinical trials (Parry, 2004).

2.8 VACCINATION

Vaccination is indicated for the following groups:

Travelers to areas in which there is a recognized risk of exposure to *Salmonella.* Risk is greatest for travelers to developing countries (e.g., countries in Latin America, Asia, and Africa) who have prolonged exposure to potentially contaminated food and drink (Edelman *et al*., 1986).

Multidrug-resistant strains of *Salmonella* have become common in some areas of the world e.g., the Indian subcontinent (Rao *et al*., 1993) and the Arabian Peninsula (Elshafie S., Rafay A. *et al*., 1992), and cases of typhoid fever that are treated with ineffective drugs can be fatal. Travellers should be cautioned that typhoid vaccination is not a substitute for careful selection of food and drink. Typhoid vaccines are not 100% effective. A fourth vaccine, an acetone-inactivated parenteral vaccine, is currently available only to the armed forces (CDC, 1994).

2.9 Medicinal values of *Ocimum lamiifolium*

According to WHO (2008), 80% of the population in developing countries depend on herbal medicine for primary health care. The situation is the same in Ethiopia where traditional medicine has been in use since time immemorial and found to be culturally entrenched in all communities. About 95% of traditional medicine preparations in the country are of plant origin (Kibebew, 2001) as evidenced by the wealth of indigenous knowledge on the utilization of medicinal plants for treating human and livestock ailments.

Runyoro et al. (2010) applied an agar-dilution technique and reported antimicrobial role of extracts from *Ocimum lamiifolium* against *S. aureus* and *S. epidermidis*. In addition, the investigated strong growth inhibitory activity of extracts from *Ocimum lamiifolium* and *R. steudneri* was also found to support recorded ethnobotanical uses of the species to treat common bacterial diseases, such as diarrhoea, in the District. Previous studies have described anti-inflammatory, anti-pyretic, and analgesic properties of aqueous and ethanol extracts of leaves from *O. lamiifolium* (Makonnen et al., 2003).

The extreacvts of fresh leaves of *Ocimum lamiifolium* have antibacterial activity against *Escherichia Coli, Staphylococcus aureus, Proteus vulgaris, Bacillus subtilus, Staphylococcus epidermidis* (Ambaye and Musa, 2015).

3. Materials and Methods

3.1. Brief Description of the Study Area

This study was conducted in Bashy town. Bashy town is located in South Nations Nationalities Peoples Regional State, Keffa Zone, Cheta Woreda. The town is situated 499km Southwest of Addis Ababa (the capital city of Ethiopia). Its Geographical location is 7^{o} 16' north latitude and $36^{o}14'$ east longitude. According to the data of Bashy Health Center gained in 2019 during the vaccination of Guinea worm, the peoples in the town were 3054. The town had one governmental clinic, one private clinic, one high school and three primary schools. It has 9 months rain fall per year and the mean annual temperature of 29^{o}C (a minimum of 26°C and a maximum of 32°C (Getahun, 2012).

3.2 Research Design

A community based cross-sectional study design method was used.This study was used both quantitative data and qualitative data collected through direct observation, interview, questionnaire and laboratory based practical work (particularly for the detection of *Salmonella* in patients sample, to determine in-vitro antimicrobial activities of extracts of selected medicinal plants, and to assess drug resistance pattern of *Salmonella*).

3.3 Inclusion and exclusion criteria

3.3.1 Inclusion criteria

All outpatients seeking medication for characteristic symptoms of *Salmonella* at clinic during the study period were included.

3.3.2 Exclusion Criteria

Those that were physically and mentally incapable to be interviewed and taking medication during the study period were excluded from the current study.

3.4 Sample Size

The sample sizes of this study were the total *Salmonella* out patients seeking medication in Bashy health centre during the study period. During the study period the total of 108 *Salmonella* outpatients were seeking medication in Bashy Health Centre. Therefore, the sample size of this study was 108 *Salmonella* outpatients seeking medication during November 12, 2019 up to Decemember15, 2019.

3.5. Sampling Techniques

Purposive sampling methods were used for both traditional medicinal healers and *Salmonella* outpatients in the study area during the study period.

3.6. Ethical Considerations

Approval and permission was sought from the Research and Ethical Review Board of College of Natural Sciences, Jimma University. Permission was also obtained from the Bashy health center administration. To ensure confidentiality the names of patients were replaced with the code. The confidentiality of both written and verbal responses was made known to the participants. All blood samples were taken by physicians or health professionals.

3.7. Data Collection Method

3.7.1 Socio-demographic data and risk factors for Prevalence of Salmonella

Socio-demographic data and risk factors for Prevalence of *Salmonella* (knowledge, attitudes and practices) were collected through direct observation, as well as using a pretested questionnaire for interview of outpatients seeking medication in the clinic, pharmacists (1), other clinic workers(2) and other outpatients (105). Different patients were visited for data collection (patient profile, patient history, etc.)

3.7.2 Detection of *Salmonella*

Blood samples were collected in EDTA (Ethyle diamine tetra acetic acid) tubes from patients assisted by physicians. About 1ml blood samples were added to 9ml buffered peptone water at 37°C for 24h to recover damaged cells. After incubation, 1ml mix of blood sample and peptone water was added to 10ml selenate F broth and incubated at 37°C for 24h. About 1ml sample was inoculated onto XLD agar by using sterile swabs. The inoculated plates were incubated at 37°C

for 24h. The presumptive *Salmonella* colonies were then sub-cultured by streaking onto the fresh XLD agar using a sterile inoculating loop and incubated for 24 hours at 37°C. The presumptive *Salmonella* isolates were identified following by triple-sugarIron (TSI) agar test The presumptive *Salmonella* colonies were directly stabbed into the TSI agar slant. The inoculated samples were incubated with loosened caps for 24 h at 37°C. The inoculated tubes were shaken gently and incubated with loosened caps for 48 h at 37 °C in an incubator. The TSI agar was checked for the production of hydrogen Sulphide (H_2S) gas and the alkalinity. The *Salmonella* colonies that were hydrogen sulphide gas positive on TSI agar was sub-cultured onto fresh XLD agar plates and incubated for 24 hours at 37°C. After incubation a single colony of *Salmonella* was transferred into 10mL of nutrient broth and incubated for 24 h at 37°C for further studies

3.7.3 Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing of the *Salmonella* isolates to various routinely used antibiotics were determined by the disc diffusion method on Muller Hinton agar using commercially available discs.

About1ml sample from isolated *Salmonella* and added to 10ml nutrient broth. Then, it was incubated at 37^{0c} for 24h overnight. This was for the aim of activation of isolated *Salmonella*. About 9 ml saline solutions which contain 0.85% Nacl were prepared in some test tubes side by side. About 1ml was taken from the original culture and added into the first test tube which contain saline solution and mixed it well until the colonies distributed evenly in a test tube. Again 1ml from test tube one were taken and added to the second test tube. This procedure was continued until the density of inoculums suspension was equal to 0.5MacCFarLand standard. The density of inoculums suspension will be compared visually to 0.5MacCFarLand standard. When the densities of inoculums suspension were too low, more *Salmonella* were added, while the density of inoculums suspension were too high more saline were added.

Then, sterile cotton swab and broth culture of test *Salmonella* was taken. The tube was opened and rim of the tube was briefly flamed. Swab was dipped into the broth and sterile MHA plate was taken. The plate was swabbed with the test culture. Swabbing was done to cover the entire plate and swabbing was continued after changing plate direction. Swab was discarded safely in a disinfectant. With sterile forceps antibiotic disks were taken and placed on the swabbed agar

surface and the disks were gently pressed to ensure contact. Plate lid and disk containers were closed. Plates were inverted. Test culture details were noted. Plates were incubated over night at 37^{0c} for 24h. Following incubation, plates were taken and examined. Diameter of growth inhibition zone was measured. The results of the antimicrobial susceptibility were interpreted based on the guidance of National Committee for Clinical Laboratory Standards (NCCLS, 2007).

In this study; the following antibiotics were tested based on the above procedure: Ampicillin (10µg), streptomycin (10µg), Tetracycline (30µg), Gentamycin (10µg), Erythromycin (5µg), Chlorampenicol (30µg), Ciprofloxacin (5µg), Kanmycin (30µg), Amikacin (30µg) and Nalidixic acid (30µg).

3.7.4 Evaluation of Medicinal plants extract against *Salmonella* Isolates

Those traditional medicinal healers were selected for interview and questioner about traditional medicinal plants which can cure typhoid fever. The respondents were selected by using purposive sampling technique, because they should be traditional medicine experts to get useful information.

3.7.4.1 Plant collection and processing

The leaves of *Ocimium lamiifolium* (ዳማ ከሰይ) were collected from Bashy town and washed with tap water to remove dirt and soil. The plants were identified by prof. ketesa Hundera (Jimma University, Biology department) and deposited at herbarium. The collected plant materials were dried at room temperature away from sun light to prevent loss of volatile photochemical that might be the target constituents. The dried plant materials were also grinded to uniform fine powder using an electric grinder and were stored in closed glass bottles in the dark at room temperature for 10 days.

3.7.4.2 Extraction with different solvent

The powder (1 kg) of *Ocimium lamiifolium* leaves were successively extracted by maceration with ethanol, distilled water, petroleum ether, and methanol for three consecutive days each at room temperature. The extracts were filtered using whatman filter paper No.1 (150mm) and evaporated under vacuum (Rotavapor) to obtain the respective crude extracts.

3.7.4.3 Testing the activity of crude extracts against *Salmonella* isolates

In order to check the anti-*Salmonella* activity of the crude extracts of *Ocimium lamiifolium* leaves disk diffusion method on Muller Hinton Agar was used and inhibitory activity was detected in terms of diameter zones of inhibition (mm). For this study different minimum inhibitory concentrations (50mg/ml, 100mg/ml, 200mg/ml and 500mg/ml) were used.

The isolates were tested for its susceptibility towards the plant extracts using the disc diffusion method on the Muller Hinton agar. *Salmonella* isolates were grown in nutrient broth at 37°C for 12 h (cultures were adjusted to 0.5 McFarland units), and were spread evenly on Muller-Hinton agar using sterile cotton swabs. Sterile 6 mm Whatman filter paper discs were impregnated with few of the plant extracts, which was dissolved in dimethyl sulfoxide (DMSO). The possible inhibitory effect on DMSO was also be evaluated by placing sterile discs impregnated with the solvent. The plates were kept at room temperature to facilitate the diffusion of the extracts into the agar containing the isolate. Subsequently, the plates were incubated at 37°C for 24 h. The plates were observed for the inhibition zones after the incubation and the zone of inhibition was recorded in mm (Steenkamp et al., 2007).

3.8 Methods of Data Analysis

Analyses were performed by using SPSS statistical package window version 20. Pearson chi-square was used to evaluate the statistical significance difference of associated risk factors of *Salmonella by sexes*. A probability value <0.05 was considered statistically significant. The data were presented in graphs, and tables. For each assay, the percentage of prevalence was estimated by comparing with various parameters like gender, age, and study sites.

4. Result

4.1 Socio-demographic of Characteristics of the study participants

A total of 108 *Salmonella* outpatients in Bashy health center were involved in study with 100% response rate. All the respondents aged greater than or equal to one year were included in this study. Majorities (32.4%) were aged 11-20 years. Around 68.5% were married and the remain 31.5% were single, separated or divorced. Majorities (42.6%) were illiterate while 73.1% lived in rural areas and 26.95 were lived in urban areas. females constituted 57.4% while males were 42.6% (Table 1).

Table 1. Socio-demographic characteristic of the study participants (outpatients), Bashy health Center, 2019

Variables	Category	No of respondents	Percent (%)
Sex	Male	46	42.6
	Female	62	57.4
Age	1-10	10	9.3
	11-20	35	32.4
	21-30	26	24.1
	31-40	16	14.8
	41-50	13	12
	>50	8	7.4
Educational level	Illiterate	46	42.6
	Grades 1-4	21	19.4
	5-8	14	13
	9-12	15	13.9
	>12	12	11.1
Residence	Urban	29	26.9
	Rural	79	73.1

4.2 Prevalence of *Salmonella* in the study area

This study found a lowest annual frequency of 11.9% in 2015 and a highest frequency of 29.5% in 2014 (Table 2). As the result showed there were fluctuations of typhoid fever within the

consecutive five years. A total of 511, 206, 423, 304, and 286 cases of typhoid were recorded in the 2014, 2015, 2016, 2017, and 2018, respectively.

Table 2. Frequency of typhoid fever from 2014-2018 in the study area, Bashy health Centre, 2019

Year	Case of typhoid	Frequency (%)
2014	511	29.5
2015	206	11.9
2016	423	24.5
2017	304	17.6
2018	286	16.5
Total	1730	100

The highest cases of typhoid fever (40.7%) were recorded in patients aged between 11 to 20 and the lowest case of typhoid fever were recorded in the age group above 50 and 0-10 age groups at with frequency of 3.4% and 3.8%, respectively (Table 3). While in the age group 21-30 36.4%, 41-50 5.7% cases of typhoid fever were recorded.

Table 3. Age related frequency distribution of typhoid in the study area, Bashy health Center, 2019

Age (Years)	Cases of typhoid	Percentage (%)
0-10	66	3.8
11-20	704	40.7
21-30	629	36.4
31-40	175	10.1
41-50	98	5.7
>50	58	3.4
Total	1730	100

The result showed a highest typhoid fever in Boba and Shollay kebele and low incidence was recorded in Kolla and Keyi kebele respectively (Table 4). In Boba kebele the frequency of typhoid fever was 29.6% and 27.2% in Shollay kebele while in Keyi and Kolla kebele the frequency was 23.2% and 19.9% respectively.

Table 4. Variation in frequency distribution of typhoid among different sampling sites, Bashy health Center, 2019

Place/kebele	Case of typhoid	Population	Frequency (%)
Kolla	345	6,212	19.9
Keyi	402	3,421	23.2
Boba	512	4,028	29.6
Shollay	471	5,575	27.2
Total	1730	19,236	100

The result showed that women were more affected by typhoid fever than male. The frequencies of women affected by this disease were 62% while the remaining 38%of the patients were males (Fig.1).

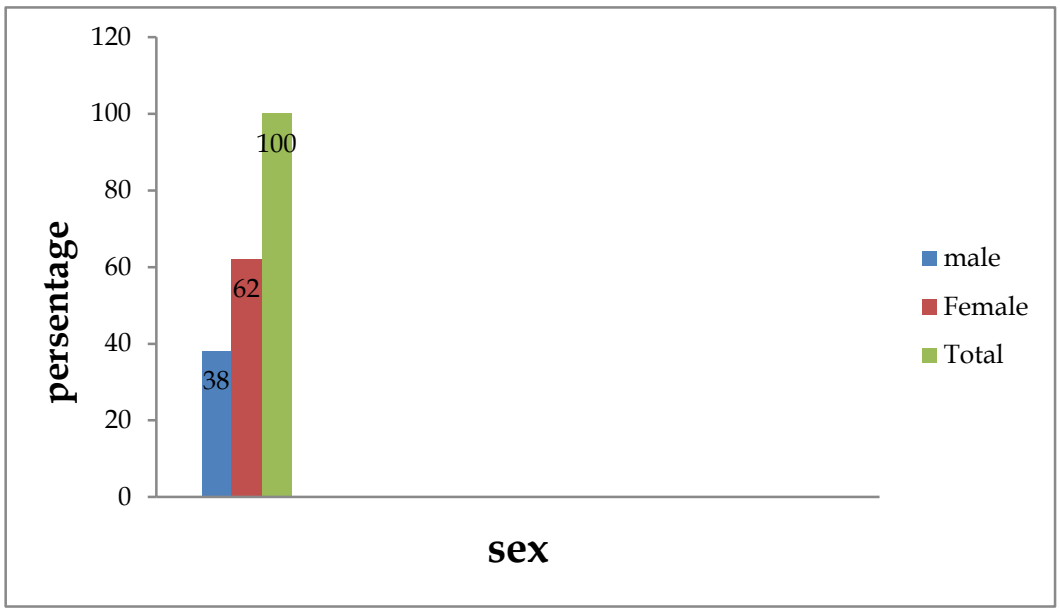

Figure 1 . Sex related frequency Distribution of typhoid fever

Risk factors of *Salmonella*

The total of 108 patients was interviewed from November 12, 2019 to December 15, 2019. Among 108 patients, 46 were males while 62 were females. As shown in Table 6; 89 of the patients did not treat drinking water before use and 79 of the respondents did not know about the causes, prevention and treatment of typhoid.

Table 5. The data of the respondents about associated risk factors of *Salmonella* by sexes.

Variables	Number of respondents				Pearson chi-square value($x^{2)}$	p-value
		Yes	Sometimes/partially	No		
Habit of washing hand before preparing and eating food	M	1	45	-	0.108	0.742
	F	2	60	-		
	T	3	105	-		
Habit of washing hand after using toile with soap	M	-	11	35	0.169	0.681
	F	-	17	45		
	T	-	28	80		
Treating drinking water before use	M	-	7	39	108.000	0.000
	F	-	12	50		
	T	-	19	89		
Washing vegetables and fruits before use	M	4	30	12	0.481	0.784
	F	8	39	15		
	T	12	69	27		
Habit of disposing liquid and solid wastes in well constructed containers	M	-	19	27	0.380	0.538
	F	-	22	40		
	T	-	41	67		
Keeping their personal and environmental sanitation	M	-	33	13	0.000	1.00
	F	-	45	17		
	T	-	78	30		

Having well constructed toilet	M	4	24	18		
	F	3	38	21	57.279	0.000
	T	7	62	39		
Knowing the causes, transmission, prevention and treatments of typhoid	M	2	14	30		
	F	1	12	49	2.747	0.253
	T	3	26	79		

Table 6. Antibiotic resistance patterns of *Salmonella* isolated from outpatients seeking Medication at Bashy Health Center, 2019

Salmonella isolates	Inhibition zone diameters (mm) of antibiotics against *Salmonella* (coded Sal 1-Sal 13) isolated from different outpatients										No of susceptible (S),Intermediate (I) and Resistant (R)		
	Nalidixic acid	Gentamycin	Streptomycin	Chloramphenicol	Erytromycin	Kanamycin	Ciprofloxacin	Amikacin	Ampicillin	Tetracycline	S	I	R
Sal 1	19	24	14	28	33	20	17	18	12	11	6	3	1
Sal 2	20	13	14	27	34	17	27	15	8	12	4	5	1
Sal 3	30	25	10	24	34	23	24	21	10	14	7	1	2
Sal 4	32	19	20	35	36	27	21	21	15	10	9	-	1
Sal 5	19	31	19	30	37	26	23	10	19	19	9	-	1
Sal 6	8	28	11	19	34	12	18	24	8	9	4	1	5
Sal 7	18	19	9	26	35	25	27	18	14	21	8	1	1
Sal 8	9	29	10	30	37	31	28	17	19	8	7	-	3
Sal 9	18	12	19	27	36	23	13	13	18	10	5	1	4
Sal 10	16	19	21	21	35	10	28	21	9	11	6	1	3
Sal 11	20	14	10	32	35	19	24	24	13	19	6	2	2
Sal 12	10	28	11	33	34	26	24	19	18	9	7	-	3
Sal 13	12	10	9	26	33	30	22	15	10	12	4	2	4

Where, S= Susceptible; I= Intermediate; R= Resistant

4.3 Multi drug resistance pattern of isolated *Salmonella*

The multi drug resistance profile of *Salmonella* indicated that 23.1% of the isolates were resistance to three, four and six antibiotics followed by two and five (7.7 %) antibiotics (Table 7). The maximum number of antibiotics resisted by *Salmonella* was six.

Table 7. Multiple Drug Resistance (MDR) patterns of *Salmonella* isolated from outpatient seeking medication at Bashy health centre, 201

Salmonella Isolate	Multiple Drug Resistance pattern
Sal 1	S/C/AMP/TET
Sal 2	GM/S/K/AMK/AMP/TET
Sal 3	S/AMP/TET
Sal 4	TET
Sal 5	AMK
Sal 6	NAL/S/K/C/AMP/TET
Sal 7	S/AMP
Sal 8	NAL/S/TET
Sal 9	NAL/GM/C/AMK/TET
Sal 10	NAL/K/AMP/TET
Sat 11	GM/S/K/AMP
Sat 12	NAL/S/TET
Sat 13	NAL/GM/S/AMK/AMP/TET

Where; NAL, Nalidixic acid; GM, Gentamycin ; K , Kanamycin ;C , Ciprofloxacin ; AMK, Amikacin ; AMP, Ampicillin; TET, Tetracycline; S, Streptomycin

Table 8: Overall MDR patterns of *Salmonella* isolated from outpatients seeking medication at Bashy health centre, 2019

No of MDR	MDR pattern	Frequency	%
2	S/AMP	1	7.7
3	S/AMP/TET	1	23.1
	NAL/S/TET	1	
	NAL/S/TET	1	
4	S/C/AMP/TET	1	23.1
	NAL/K/AMP/TET	1	
	GM/S/K/AMP	1	
5	NAL/GM/C/AMK/TET	1	7.7
6	GM/S/K/AMK/AMP/TET	1	23.1
	NAL/S/K/C/AMP/TET	1	
	NAL/GM/S/AMK/AMP/TET	1	

All isolates of *Salmonella* were 100% susceptible to Erytromycin and Chloramphenicol. However; high rate of antimicrobial resistance was observed mainly to streptomycin, tetracycline, and ampicillin.

Table 9. Drug susceptibility of *Salmonella*

Antibiotics	Susceptible	intermediate	Resistance
Chloramphenicol	13(100%)	0	0
Gentamycin	9(69.2%)	2(15.4%)	2(15.4%)
Ciprofloxacin	10(76.9%)	2(15.4%)	1(7.7%)
Erytromycin	13(100%)	0	0
Nalidicic acid	7(53.8%)	2(15.4%)	4(30.8%)
Tetracycline	4(30.8%)	2(15.4%)	7(53.8%)
Ampicillin	5(38.5%)	3(23%)	5(38.5%)
Amikacin	9(69.2%)	2(15.4%)	2(15.4%)
Streptomycin	4(30.8%)	2(15.4%)	7(53.8%)
Kanamycin	9(69.2%)	1(7.7%)	3(23%)

4.4 Phytochemical components of *Ocimum lamiifolium* analysed for antimicrobial activity against Salmonella isolates

Alkaloid, flavonoid and phenol were detected in all the solvents used for extraction (methanol, ethanol, petroleum ether and distilled water). However; terpnoid was found only in petroleum ether extract. Accordingly, ethanol and methanol had better extraction ability for leaves of *Ocimum lamiifolium* than petroleum ether and distilled water. Among the four extracts, distilled water had less extracting ability than ethanol, methanol and petroleum ether (Table 10).

Table 10. Phytochemical test result of leaves of *Ocimum Lamiifolium* in four different solvents

Phytochemicals tested for	Solvents used for extraction			
	Ethanol	Methanol	Petroleum ether	Distilled water
Alkaloid	++	++	+++	+++
Flavonoid	+	+++	+	+
Phenol	+++	+++	++	+++
Terpenoid	_	-	+	_
Steroid	+	+	+	_
Tannin	+	+++	-	-
Saponin	+++	+++	+/-	+++

Where; +++, highly present; ++, moderately present; + slightly present; +/-, present or absent; -, absent

4.5 Antimicrobial activities of the plant

The leaves of *Ocimum lamiifolium* with methanol extract showed antibacterial activity against isolated *Salmonella* at the minimum inhibitory concentration of 100mg/ml while ethanol and petroleum ether extracts showed anti-bacterial activity at the minimum inhibitory concentration of 200mg/ml. However; the crude extracts of distilled water were not active at any of the four

minimum inhibitory concentrations. In this study none of the four crude extracts of *Ocimum lamiifolium* leaves was able to inhibit the growth of isolated *Salmonella* with the minimum inhibitory concentration of 50mg/ml (Table 11).

Table 11. Inhibition Zone diameters (mm) of different concentration of extracts of leaves of *Ocimium lamiifolium a*gainst *Salmonella* isolates

Isolates	Inhibition zone diameters (mm) of different isolates against extracts of different solvents																	
	Ethanol				Methanol				Petroleum ether				Distilled water				Chloramp	DMSO
	50mg/ml	100mg/ml	200mg/ml	500mg/ml	50mg/ml	100mg/ml	200mg/ml	500mg/ml	50mg/ml	100mg/ml	200mg/ml	500mg/ml	50mg/ml	100mg/ml	200mg/ml	500mg/ml		
Sal 1	0	0	9	13	0	8	12	14	0	0	8	10	0	0	0	0	28	0
Sal 2	0	0	8	12	0	7	11	14	0	0	8	10	0	0	0	0	27	0
Sal 3	0	0	8	11	0	8	10	13	0	0	7	9	0	0	0	0	24	0
Sal 4	0	0	10	13	0	9	13	16	0	0	9	11	0	0	0	0	35	0
Sal 5	0	0	9	13	0	9	12	15	0	0	9	10	0	0	0	0	30	0
Sal 6	0	0	7	9	0	8	9	12	0	0	7	8	0	0	0	0	19	0
Sal 7	0	0	8	11	0	8	10	15	0	0	8	9	0	0	0	0	26	0
Sal 8	0	0	9	13	0	9	12	15	0	0	8	10	0	0	0	0	30	0
Sal 9	0	0	8	11	0	8	11	14	0	0	8	10	0	0	0	0	27	0
Sal 10	0	0	8	12	0	8	10	13	0	0	7	9	0	0	0	0	21	0
Sal 11	0	0	9	13	0	8	12	15	0	0	9	11	0	0	0	0	32	0
Sal 12	0	0	9	13	0	8	12	15	0	0	9	11	0	0	0	0	33	0
Sal 13	0	0	8	12	0	8	10	13	0	0	8	10	0	0	0	0	26	0

5. Discussion

This study showed a lowest annual frequencyof 11.9% in 2015 and a highest annual frequency of 29.5% in 2014. The observed result differs from results reported earlier (Mbula *et al*., 1993). According into Mbula *et al*. (1993) the frequency of isolation of *Salmonella* was 1%. It must be said that the fluctuation of this rate over time depends on the efforts of both the national and health departments in controlling the impacts of this pathogen.

According to Mbula *et al* (1993), more males were affected by typhoid fever than females. However; in a study carried out in Bamako, Sako (2004) showed that women were more affected by typhoid fever than males. The result of Sako is in agreement with the current observation.

There was high incidence of *Salmonella* among outpatients in Boba and Shollay kebele. Several factors may accounts for the observed high prevalence, namely lack of safe drinking water, poor personal and environmental sanitation, people's way of life, low awareness of safe food handling and low awareness of the peoples towards the causes, transmit ion, prevention, and treatments of typhoid fever (Salerno-Gonçalves *et al.*, 2017).

In relation to educational status and frequency of isolation, this study results revealed that those with low level of education suffered from typhoid more than those who had attained a higher level of education. Accordingly, 42.6% of the *Salmonella* outpatients were illiterates while 11.1% were above grade 12.This study is comparable with earlier study (Aziz *et a.,* 1990) where education was considered vital to create awareness in the community with regard to the mechanism of management of infectious diarrhea and control of other factors that lead to this disease. Poor environmental sanitation, malnutrition, inadequate water supply, poor food handling and limited education are the major factors implicated in the occurrence, spread and severity of this disease (Nath *et al*., 2006).

Typhoid fever may occur at any age. In this study, adults were more affected (84.6%) by *Salmonella*. The result of the present study showed 15.4% of *Salmonella* isolates were encountered in children less than 10 years of age. This finding differs from the observation made by Saha *et al* (2003) and found that children between 2-3 years of age were most susceptible age group (35.6%). Almost similar studies done by Saha *et al* (2003) previously showed that 44% of children were positive for *Salmonella* were aged less than 5 years. Accordingly, relatively high isolation rate was observed in the age group between11-20 (n=4, 30.8%) and in the age group between21-30 (n=3, 23.1%). According to Mengistu *et al* (2014) and Mache

(2002), the highest isolation rate of *Salmonella* was observed in the age group between 20 and 24 9 (26.3%) and 5 (26.3%) respectively.

There are reports on widespread occurrence and distribution of *Salmonella* in Ethiopia (Reda *et al*., 2011). Recently, the number of *Salmonella* related outbreaks in humans has still increased considerably in the same country (Mengistu *et al*., 2014). Accurate estimates of the burden of diarrheal diseases caused by *Salmonella* species and other food borne pathogens are needed to effectively set public health goals and allocate resources to reduce disease burden (Awole *et al*., 2002).

This study revealed 13(12.04%) isolates of *Salmonella* after Investigating 108 blood samples from clinically suspected Patients of typhoid fever. This is at variance with an earlier report who observed a higher incidence of 33 (31.7%) stool samples after examining 104 samples (Ogunbiyi and Onabowale, 1997). These findings support previous studies undertaken in the current study area, reporting 13.3% prevalence of *Salmonella* in meat samples (Anbessa and Ketema, 2012). Reports from Addis Ababa indicated 9.8% isolation rate of *Salmonella* from abdominal and 11.9% from diaphragmatic muscles (Nyeleti *et al*., 2000). The prevalence reported in the current study is higher than other reports including 4.8% prevalence (Sefinew and Bayleyegn, 2012) in Bahir Dar Ethiopia from slaughtered cattle. The prevalence rate of *Salmonella* in this study is in agreement with the earlier studies reported 13.6% (Addis *et al*., 2011) and 10.5% (Mengistu *et al*., 2014) from lactating cows and stool samples, respectively, but lower than that reported as 15.4% (Mache, 2002) and higher than the 7.2% prevalence reported by Awole et al. (Awole *et al*., 2002) from stool samples. This difference possibly arises from the source of animals, types of samples, and sampling technique.

This study was also conducted to know the antibiotic resistant patterns of *Salmonella.* Accordingly, all the *Salmonella* isolates showed a significant decrease in susceptibility to streptomycin and tetracycline. Both of antibiotics had a common sensitivity rate of only (30.8%). Since 1989 outbreaks caused by strains of *Salmonella typhi* resistant to tetracycline have been reported in many developing countries, especially in India (Threlfall *et al*., 1992).The resistance of *Salmonella* towards ampicillin (38.5%) and tetracycline (53.8%) was in agreement with earlier report made by Beyene and Tasew(2014) where most of the *Salmonella* isolates were resistant to ampicillin and tetracycline. It may be due to the overuse of ampicillin and tetracycline in the treatment of typhoid and in other unrelated infections. It can be purchased over the counter of private clinics and pharmacies. Ampicillin is widely used in Bashy town to treat many infections without prescription and is likely to result in high prevalence of resistance, limiting its utility. Incomplete treatment due to many reasons in a developing country like ours may also be the factors contributing to the development of resistance.

Chloramphenicol and erythromycin had a sensitivity rate of 100% for all *Salmonella* isolates. This value is showed an increase in susceptibility of *Salmonella* to the above two antibiotics. This result is in agreement with the earlier reports that *Salmonella typhi* is highly susceptible to Chloramphenicol (Dhanashree B., 2007). This increase can be explained by the fact that Chloramphenicol and erythromycin may not be regularly used; strains that were resistant to Chloramphenicol and erythromycin are slowly losing their resistance factors and therefore become susceptible to chloramphenicol and erythromycin.

In the present study, only 7.7% isolates showed resistance to Ciprofloxacin. This result is in parallel with the previous reports from other Studies. In Bangladesh, 74% of *Salomonella typhi* strains isolated with decreased ciprofloxacin susceptibility, whereas it was 50% in the United Kingdom (Threlfall E., 2001). The difference in *Salmonella* antimicrobial resistance levels in different areas may be related to agent risk factors such as virulence, pathogenecity, infectiousness, and host specificity associated with the genetic composition of *Salmonella* strains. The high level of antibiotic susceptibility of *Salmonella* to ciprofloxacin (76.9%) is in agreement with earlier studies reported from Ethiopia (Beyene and Tasew, 2014).

However, only53.8% *Salmonella* isolates were susceptible to nalidixic acid in this study. This result is in agreement with the previous results that 52.6% of *Salmonella* isolates were susceptible to nalidixic acid (Tesfahun *et al*., 2016).

Due to selective pressure created by the use of antimicrobials in food processing, the risk of antimicrobial resistance among food borne pathogens has increased. Mobile elements such as plasmids and transposons may facilitate the rapid spread of antibiotic resistant genes among bacteria (Sunde M., 2005). In addition, high rates of antibiotic resistant bacteria may possibly result from inappropriate or uncontrolled use of antibiotics. Therefore, it is necessary to pay attention to hygienic food handling practice as well as avoiding uncontrolled use of antibiotics (Van *et al*., 2007). An increase in the antimicrobial resistance in *Salmonella* makes the treatment of infection more challenging. Therefore, epidemiological information and monitoring system are necessary to control *Salmonella* infection in public health sectors.

Of the 13 isolates of *Salmonella*, only two antibiotics have completely susceptibility to the organism. However; eight antibiotics have not 100% effective to the *Salmonella* isolates. We therefore face the imminent prospect of encountering untreatable typhoid fever in the near future. A national guideline on the proper usage of antibiotics is required for urgent implementation in Ethiopia.

The output of the present investigation substantiates the potential therapeutic role of *Ocimum lamiifolium* against isolated *Salmonella*. The antimicrobial activity of extracts *from Ocimum lamiifolium* against the pathogen with the inhibition zone ranging from 100mg/ml to 500mg/ml to methanol extract and 200mg/ml to 500mg/ml to ethanol and petroleum ether extract supports wide traditional use reports of these species in Ethiopia and neighbouring countries (Mesfin *et al*., 2009).

These results are in parallel with the previous report on *Ocimum* species (Wiart C., 2006). The result of Wiart C. (2006) showed *Salmonella typhi* was found to be resistant to all the extracts of *Ocimum* speies tested. However; the present result showed the three extracts of *Ocimum lamiifolium* have antibacterial activity against isolated *Salmonella.* In addition, the investigated growth inhibitory activity of extracts of *Ocimum lamiifolium* was also found to support ethno botanical uses of the species to treat common bacterial diseases such as *Salmonella* in Bashy town. Previous studies have described anti-inflammatory, ant-pyretic, and analgesic properties of

aqueous and ethanol extracts of the leaves from *Ocimum lamiifolium* (Makonnen *et al*., 2003). The discrepancy may be attributed to difference in methods, followed for activity difference testing, and difference in *Salmonella* strains. A difference in geographical location, season, and developmental stage of samples collected could also be mentioned as a factor for difference in outputs of activity tests (Runyoro *et al*., 2010).

The crude leaf extracts of methanol showed the highest antibacterial activity followed by ethanol and petroleum ether extracts. However; the extracts of distilled water did not have antibacterial activity against the pathogen. The study revealed that antibacterial activity of the crude extract from the leaves of *Ocimum lamiifolium* were variable when extracted by different solvents, however; possesses good antimicrobial activity which supports the traditional uses of *Ocimum lamiifolium* in the treatment of *Salmonella* infections under study. These may be due to its inability to extract bioactive components from the leaves of *Ocimum lamiifolium*. However; all the crude extracts have less inhibitory activity compared to commercial drug Chloramphenicol (positive control).

The result of the present study support the traditional usage of *Ocimum lamiifolium* plant extracts which possesses secondary phytochemical with antimicrobial property that can be used as antibacterial agent in new drugs from the therapy of infectious diseases caused by pathogens and further work may be carried out for pharmacological evaluation.

6. Conclusion and Recommendations

6.1 Conclusion

The highest prevalence of typhoid fever was recorded during the period of 2014(29.5%) and the lowest prevalence was recorded in the year 2015 (11.9%). The age range 11 to 20 years was the most affected age groups that were 40.7% from the whole cases of typhoid fever. There were more women than men with typhoid disease. The highest prevalence was recorded in Boba and Shollay kebeles while the lowest prevalence was recorded in kolla and key kebele respectively.

According to the responses of *Salmonella* in outpatients, majorities of the patients had no hand washing habit before preparing and eating food. There were no well constructed toilet and waste containers. Therefore; both liquid and solid wastes were directly thrown to the field. They did not keep their personal and environmental sanitations because they had low awareness on the transmit ion and prevention of typhoid fever. Due to the above reasons the prevalence of typhoid was very high in the study area. Therefore; the possible factors were poor environmental sanitation, inadequate water supply, and limited educational levels of the people towards typhoid fever.

This study revealed that *Salmonella* isolates are present in our clinical specimens in this area and are seriously becoming a concern due to their drug resistance pattern observed in this and documented analysis of trends of occurrence of this resistant isolates to help guide in study and urgent steps should be taken to have anEvaluation of less commonly resistant antimicrobials when occur. The high percentage of resistance to the three antibiotics studied could be attributed to their prevailing usage and abuse in the area under study. The implication of the high percentage recorded for the antibiotics is that erythromycin, chloromapenicol, and ciprofloxacin will effectively treat *Salmonella* infections, though one strain was resistant to ciprofloxacin.

Findingsof the current study revealed higher prevalence (12.04%, N = 108) of *Salmonella* species, dominantly among outpatients aged between11-30 (53.9%). years. Furthermore, most *Salmonella* spp. was resistant to streptomycin and tetracycline although more than 65% of the isolates were susceptible to ciprofloxacin, kanamycin, gentamycin, and amikacin. This calls for designing of strategies for better awareness development among the community on hygienic food and water handling practices besides appropriate control measures.

The results of present study supports, the traditional uses of *Ocimum lamiifolium* to treat typhoid in the study area as the crude extracts of methanol, ethanol and petroleum ether showed good antimicrobial activity against isolated *Salmonella*. However; the four extracts have different antimicrobial activity with different minimum inhibitory concentration. At 500mg/ml methanol extracts have the maximum inhibition zone of 16mm, ethanol extract 13mm, and petroleum ether extracts 11mm while distilled water extract did not have inhibition zone to all the *Salmonella* isolates. The sources of different antimicrobial activity for the extracts of different solvents may be due to its ability to extract bioactive phytochemical.

6.2. Recommendation

Findings of the current study revealed higher prevalence (12.04%, $N = 108$) of *Salmonella* species. These calls for designing of strengthen for better awareness development among the community on hygienic food and water handling practices based appropriate control measure. Thus, result of the present study will strengthen the knowledge in the field of epidemiology of *Salmonella* to generate further trials which may help policy makers in planning interventions for the at risk population in the field of water sanitation and hygienic food handling practice.

A high rate of antimicrobial resistance was observed mainly to streptomycin, tetracycline, and ampicillin which are of special concern in Ethiopia due to antimicrobial resistance problems. Regulatory controls of antibiotic usage in humans are recommended ways to minimize resistance problems. Furthermore, the observed drug resistance in *Salmonella* can be used as an input by the health institutes for appropriate drug subscription. Further studies with large number of specimens are highly recommended to validate the present study and to monitor microbial trends and antimicrobial resistance patterns in other parts of Ethiopia. A national guideline on the proper usage of antibiotics is required for urgent implementation in Ethiopia.

The studies revealed *Ocimum lamiifolium* possesses good antibacterial activities which support the traditional uses of *Ocimum lamiifolium* in the treatment of *Salmonella* in the study area. To support the traditional medicinal plant users, scientific verification on phytochemical analysis and toxicity test should be carried out to confirm user's safety.

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APPENDXES

Table 12 APPENDEX-1 QUESTIONERS

The objective of this study is to collect some information about the prevalence of *Salmonella* and efficiency of available medicinal plants being used for treatment of typhoid. Therefore; the researcher kindly requests you to give true and valuable information for the study.

Format for collecting information about the prevalence of *Salmonella* and effect of *Ocimium lamiifolium* on *Salmonella* (checklist questioners prepared). The whole checklist questioners prepared below will be translated to Kaffinoono language during interview.

I. Demographic information

1. Name of the respondent----------sex----------

   Kebele----------Occupation----------Age--

Research Questions

The current study will address many research questions with regards to prevalence of *Salmonella* and efficiency of available medicinal plants being used for treatment of typhoid. The research questions are:

For outpatients seeking medication

1. Do you wash your hand properly before preparing and eating food?
   A/ yes B/ Sometimes C/ no
2. Have you well constructed toilet?
   A/ yes B/ Partially C/ no
3. What is the source of your drinking water?
   A/ pipe line B/ spring C/ river
4. Have you treat drinking water well?
   A/ yes B/ Sometimes C/ no
5. If you say yes for question No 3, mainly how do you treat drinking water?

A/ by boiling B/ by chlorinating C/ by adding bishangari D/ simply drink without treatment

6. Do you wash vegetables and fruits well before eat?
   A/ yes B/ Sometimes C/ no
7. Do you have separate container for different types of wastes (liquid and solid wastes)?
   A/ yes B/ no
8. Where do you dispose solid wastes at the end?
   A/ open field B/ local pit C/ municipality D/ compost
9. Where do you dispose liquid wastes at the end?
   A/ open field B/ local pit C/ link to sewage system
10. What are the main causes of typhoid in Bashy town?
11. How typhoid is transmitted from one to another?
12. How you dispose liquid and solid wastes in your locality?

For physicians and extension workers

1. Why the prevalence of typhoid is high in Bashy town?
2. What are the risk factors contributing to high prevalence of typhoid in Bashy town?
3. How peoples keep their environmental sanitation?
4. How is the awareness of peoples towards the causes, transmition, prevention and treatments of typhoid fever?

For traditional healers

1. What are the anti-typhoid plants being used in Bashy town?
2. Which part of the plant is used as a remedy?
3. What are the methods of preparation of the anti-typhoid plants?
4. What are the routes of administration of anti-typhoid plants?

APPENDEX-2 Consent Form

My name is MastewalAsaye and I'm a postgraduate student at Jimma University, College of Natural Sciences Department of Biology.

I'm conducting a research to the prevalence of *Salmonella* in outpatient seeking medication at Bashy health center and associated risk factors including its drug resistance in Bashy town.

If you agree to participate in the study, with your permission, your card number, age, sex, marital status, family member in the house and medical history will be obtained from the history that you gave to the clinician and then about 10 ml of blood will be taken from you or you will allow us to use the sample that you give for your laboratory examination.

- During the collection of blood, you may feel some discomfort, but this does not produce serious pain.
- All the data obtained will be kept in secret, using only code numbers and only study personnel will have access to the files.
- There is no cost to you for participating in this study and you are not asked to pay
- There is no benefit in participating in this study.
- Your participation is purely voluntarily, and even you can withdraw any time after you get involved in the study.
- Participants before and after or in the middle of the process he/she can refuse to be involved in this study,
    - Participant's unique code: ________
    - Participant's card number_____

I clearly understand the objective of the study and decide to participate; the signature below indicates that I agree to participate.

- Study participant's signature _____

  Date of signature_____
- Signature of Person Obtaining Consent_____

  Date of signature _____

APPENDEX-3 Images of Antibiotic Susceptibility test

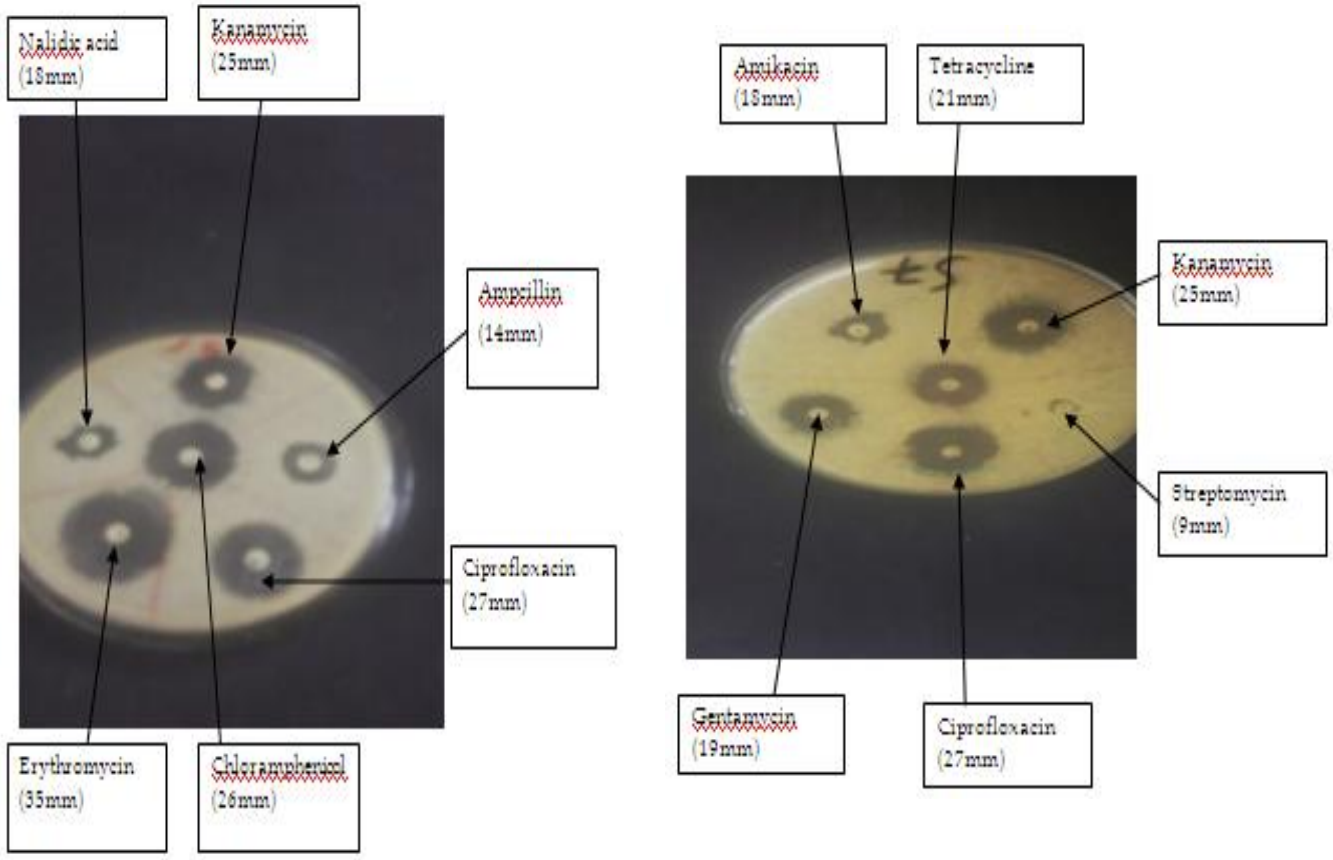

Figure. 2. Shows examples of the antimicrobial susceptibility of isolated *Salmonella* 7 for different antibiotics

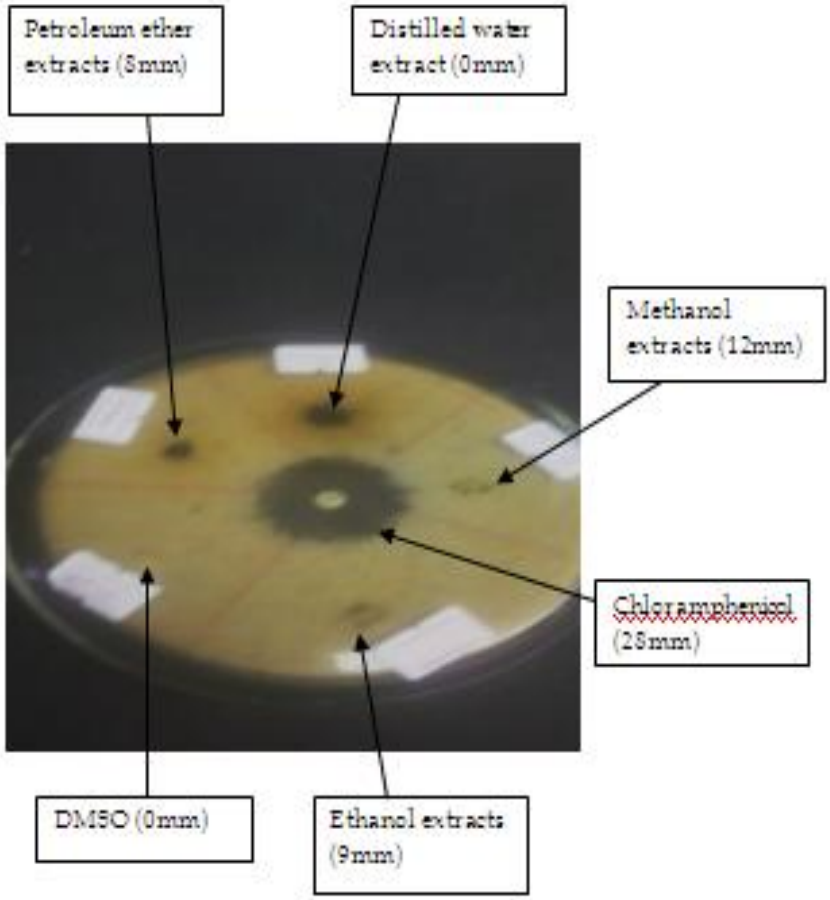

Figure.3. shows the effect of crude extract of *Ocimum lamiifolium* leaves against isolated *Salmonella* 1 with MIC of 200mg/ml.

Appendix 4:- Image of *Ocimum lamiifolium*