**2.2 Test microorganism**

**2.2.1 *Escherichia coli***

*Escherichia coli* are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic (Kaper *et al.,* 2004). When pathogenic they usually cause urinary tract infections, systematic infections and enteric infections (Mandeli *et al.,* 2005). The development of resistance by *Escherichia coli* due to increase in the use of antimicrobial agents has led to the use of medicinal plants extracts against it (Akram *et al.,* 2007). Medicinal plant extracts have shown to have antimicrobial activity against enteropathogenic *Escherichia coli* found in food material (Fullerton *et al.,* 2011). Traditional products used in food preservation (spices) have antimicrobial activity against multiple antibiotic resistant *Escherichia coli* isolated from water (Rahman *et al., 2011*). Other studies carried out on plants with a medical value such as *Allium sativum* has shown antimicrobial activity against *Escherichia coli* (Ziarlarimi *et al., 2011*).

**2.2.2 *Salmonella typhi***

*Salmonella typhi* is a Gram-negative bacterial pathogen that causes gastroenteritis in humans (Ibarra and Steele, 2009). In developing countries, it is mainly associated with causing typhoid fever (Watson and Holden, 2010). Typhoid fever is a major cause of death around the world in a limited setting and globally remains as one of the most infectious diseases (Buckle *et al.,* 2012). The disease is estimated to be responsible for about 26.9 million infections and 269,000 deaths in 2010 (Buckle *et al., 2012*). Studies carried out have shown that herbal extracts and dietary spices from medicinal plants have antimicrobial activity against *Salmonella typhi* (Shan *et al., 2007*). Other studies have shown that herbal extracts from medicinal plants not have antimicrobial activity on *Salmonella typhi* found in vegetables but also against other disease-causing pathogens such as enteropathogenic *Escherichia coli* and *Listeria monocytogenes* (Cutter, 2000).

**2.2.3 *Staphylococcus aureus***

*Staphylococcus aureus* is a Gram-positive bacteria that causes skin and soft tissues infections as well as food poisoning and toxic shocks (Perez et al., 2009). The rate of mortality associated with *Staphylococcus aureus* in developing world exceeds one of the developed countries (Nickerson *et al.,* 2009). The increasing use of antimicrobials against *Staphylococcus aureus* has led to the development of resistance hence need to develop new antimicrobial agent (Kwon *et al.,* 2007). Medicinal plant extracts have shown a wide range of antimicrobial activity against both bacterial and fungal pathogens (Manvi *et al.,* 2010). Studies carried out have shown that some edible plants extracts also have antimicrobial activity against *Staphylococcus aureus* (Alzoreky *et al.,* 2003). Other studies carried out have shown a great synergistic activity of plant extracts and spices when used against not only pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus*, *Escherichia coli* and other bacteria organisms, both Gram positive and Gram negative (Das *et al.,* 2012).

**2.2.4**

**2.2.5**

**Preparation of EETI seed**

The dried seed of *T. indica* were collected, washed, dried (oven 60°C), crushed by employing blender and convert in to powder after sieving through sieve no.80 then subjected to successive solvent extraction using ethanol at room temperature in a soxhlet apparatus. The extract was vacuum dried and kept in desicator for further studies.[13,14]

13. Gupta R, Gupta MK, Bhandari A, Gupta J. Preliminary pharmacognostical and physicochemical analysis: A poly herbomineral formulation. Int J Drug Dev Res 2014;6:85-92.

14. Gupta R, Gupta MK, Bhandari A, Gupta J, Pathan IK. Preparation and standardization of polyherbomineral formulation. Int J Drug Dev Res 2014;6:211-9.