**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.

4. The most common uses of tamarind in Africa

Aligning the available information on the species ethnopharma- cology in Africa, we find that tamarind is most commonly used as a laxative and in the treatment of wounds and abdominal pains, followed by diarrhoea, helminth infections, fever, malaria, aphro- disiac, respiratory problems and dysentery

Tamarind is used in herbal medicine in many parts of the world (Siddhuraju, 2007) (Table XII), and medicinal uses of tamarind are uncountable (Morton, 1987). Medicinal uses of tamarind can be found in many cultures and for a wide array of applications (Morton, 1987). The medicinal value of tamarind has been mentioned already in tradi- tional Sanskrit literature (El-Siddig *et al.*, 2006).

Taxonomical classification

Kingdom Plantae

Phylum Spermatophyte

Class Angiosperm

Sub class Dicotyledone

Family Leguminosae

Subfamily Caesalpiniaceae

Genus *Tamarindus*

Species *indica*

Abstract

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Diarrhoeal diseases continue to be a major cause of morbidity and mortality throughout the world and there is renewed interest in the discovery of novel compounds that can be used to fight these diseases. Numerous studies have validated the traditional use of antidiarrhoeal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption or reduce electrolyte secretion. Of the numerous phytochemicals (such as alkaloids, tannins, flavonoids and terpenes) present in active extracts, tannins and flavonoids are thought to be responsible for antidiarrhoeal activity by increasing colonic water and electrolyte reabsorption. Others act by inhibiting intestinal motility. As some of the active ingredients are potentially toxic, there is a need to evaluate the safety of plant preparations. A few clinical trials have evaluated the safety and tolerability of traditional and herbal medicine preparations used to treat diarrhoea and generally indicate that minimal side effects are observed. However, with the increased popularity of plant-derived and herbal medicines in Western society, the benefits and potential dangers of these medicines must be considered.

Yusha’u M., Gabari D. A., Dabo N. T., Hassan A. and Dahiru M. (2014). Biological activity and phytochemical constituents of Tamarindus indica stem bark extracts. *Sky Journal of Microbiology Research*, Vol. 2(9), pp. 067 – 071.

**3.1.3 Preparation of Reagents**

**3.1.3.1 Preparation of Dragendorff's Reagent**

Dragendorff’s reagent is a solution of potassium bismuth iodide K[BiI4] prepared from basic bismuth nitrate (Bi(NO3)3) , potassium iodide, (KI) and acidic solution.

Dragendorff’s reagent will be prepared by dissolving 0.88 g of Bi(NO3)3 in a mixture of distilled water (40 cm3) and acetic acid (10cm3). An 8.0 g KI will also be weighed separately and dissolved in 20 cm3 of distilled water. The two solutions will then be mixed together in a 250 cm3 volumetric flask and made up to volume with distilled water (Wikipedia, 2019).

**3****.****1.3.2 Preparation of Meyer’s Reagent**

In the preparation of this reagent, a 1.36g of mercury (ii) chloride (HgCl2) will be weighed and dissolved in about 40 cm3 of distilled water, a 5.0 g of potassium iodide will also be weighed and dissolved in about 20 cm3 of distilled water. These two solutions will then be mixed in a 100 cm3 volumetric flask and the volume made up to the mark with distilled water.

**3****.1.3.3 Preparation of Wagner’s Reagent**

In the preparation of this reagent, a 30.0 g of potassium iodide will be weighed and dissolved in about 40 cm3 of distilled water .To the resulting solution of potassium iodide, a 20 g of iodine crystal will be added and stirred properly to homogenize into solution this will then be transferred quantitatively into 100 cm3 volumetric flask and filled up to the mark with distilled water.

**3.1.3.4 Preparation of 1 %, 2 % and 10% w/v Ferric chloride solution**

A 1 % ferric chloride solution will be prepared by weighing 1.0 g of ferric chloride , FeCl3 and dissolving in a small quantity of water transferred quantitatively into 100 cm3 volumetric flask and will be made up to volume with distilled water.

A 2 % Ferric chloride solution will be prepared by weighing 2.0 g of FeCl3 and dissolving in a small amount of water, transferred quantitatively into 100 cm3  volumetric flask and will be made up to volume with distilled water.

A 10 % ferric chloride solution will be prepared by weighing 10.0 g of FeCl3 and dissolved in about 40 cm3 of water, the resulting solution will be transferred quantitatively into 100 cm3  volumetric flask and will be made up to mark with distilled water.

**3****.1.3.5 Preparation of 10% v/v Ammonia solution**

In the preparation of this reagent, a 10 cm3 of concentrated ammonia solution will be measured and introduced into 100 cm3 volumetric flask containing 40 cm3 of distilled water. This will then be agitated to achieve homogeneity and then made up to mark with distilled water.

**3****.****1.3.6 Preparation of 10% w/v Lead acetate**

A 10 g of lead acetate will be accurately weighed and dissolved in about 40cm3 of distilled water; this will then be transferred quantitatively into 100 cm3 volumetric flask and filled to the mark with distilled water.

**3****.****1.3.7 Preparation of 1% v/v Hydrochloric acid**

A 1.0 cm3 of concentration HCl will be measured and dissolved in about 100 cm3 volumetric flask and made up to mark with distilled water.

**3****.****1.3.8 Preparation of 1% w/v Barium chloride**

A 1.0 g of BaCl3 will be weighed using triple beam balance and dissolved in 100 cm3 volumetric flask with distilled water.

**3****.1.3.9 Preparation of 1% v/v Sulphuric acid**

A 1.0 cm3 H2SO4 will be measured using posture pipette into 100 cm3 volumetric flask containing 99.0 cm3 of distilled water.