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**EVALUATION OF ANTIDIARRHEAL ACTIVITY OF THE ROOT BARK OF**

Tamaridus indica

**BY**

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**ABSTRACT**

*Tamarindus indica*

**CHAPTER ONE**

**1.0 INTRODUCTION**

**1.1 Background of the study**

Frequency of passage of three or more loose or watery stools and unformed feces per day indicates diarrhea. Diarrhea is not a disease, but it may be associated with several diseases like abdominal pain and irritation within the lining of the small or large intestine leads to diarrhea. Decrease in water absorption and increase in loss of water with stools also leads to diarrhea. Loss of fluid in the form of diarrhea causes electrolyte imbalance and dehydration. Food tolerance, food poisoning, intestinal disease, infection(parasitic, bacterial and viral), malnutrition, and sometimes medication factors are responsible for diarrhea (Victora *et al.,* 2000). Plants having medicinal activity are the source of antidiarrheal drugs (Longanga *et al.,* 2000). Medicinal plants are used by almost 80 % of the world’s population for their basic health care because of their low cost and ease in availability (Shahzadi *et al.,* 2010). From the dawn of civilization, people have developed a great interest in plant-based drugs and pharmaceutical products (Shahzadi *et al.,* 2010). In the last few decades, many bacterial organisms have continued to show increasing resistance against current antimicrobial agents (Nascimento *et al.,* 2000). Herbal drugs made from medicinal plants have been used from ancient times to treat various diseases and their antimicrobial properties make them a rich source of many potent drugs (Srivastava *et al.,* 2005). The World Health Organization encourages the scientific study of traditional medicine pertaining prevention and treatment of diarrheal disease. Diarrhea is a major cause of ill health, especially for children because particularly rotavirus responsible for it (Chitme *et al.,* 2004).

*Tamarindus indica* Linn is belonging to the family *Fabaceae*, commonly known as tamarind. It is indigenous to tropical Africa and exotic to Asia and Central America. India and Thailand are the major tamarind world producers and generating 300,000 and 140,000 tons annually, respectively. There are two main types of tamarind: sour (the most common) and sweet (mostly comes from Thailand). Tamarind can be eaten fresh (ripe or unripe) and it can be consumed processed into different products. It grows as a large tree and is found in all medicinal system for a number of diseases, these includes its usefulness in jaundice, in liver, complains, as an acid refrigerant, as a gentle laxative, in yellow fever, as a blood tonic, and as a skin cleanser. It contains invert sugar, citric acid, oleic acid, linoleic acid, volatile oils (geraniol, limonene), pipecolic acid, lupeol, orientin, vitamin B3, vitamin C, vitexin, phenylalanine, leucine, potassium, Campesterol, β-amyrin, β-sitosterol, Tannins, saponins, glycosides. It has various pharmacological activity like hypolipidemic, weight reducing, antidiarrheal, antimicrobial, hepatoprotective, anthelmintic, antioxidant, analgesic & anti-inflammatory etc. This will be helpful to create interest towards Tamarind and in developing new formulations with more therapeutic and economical value (Zohrameena *et al.,* 2017).

**1.2 Statement of the problem**

The worldwide escalation in both community and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, prudent infection control, and new treatment alternatives. Therefore, the need to develop efficient and safe drugs from plant sources is of great importance, because continued investigation of plants secondary metabolites has led to important breakthrough in pharmacology and has helped tremendously in the development of modern pharmacotherapeutics in Africa and other parts of the world. Therefore the increasing rise in demand for the development of new and safe drugs from plants to combat resistance of bacteria towards antibiotics, high mortality and infections caused by disease causing bacteria necessitate this research work.

**1.3 Significance of the study**

The result of the determination will serve as the source of information on the validity usage of the extracts of the root bark of *Tamarindus indica* in the treatment of diarrhea and may also serve as a source of new drugs.

**1.4 Justification of the study**

This research work will be carried out to justify the claim that the root bark of *Tamarindus indica* contains secondary metabolites and spectroscopic profile that can be used in treatment and confirmation respectively, of its medicinal value towards diseases associated with diarrhea causing bacteria.

**1.5 Aim and Objectives**

**1.5.1 Aim of the study**

The aim of this research is to evaluate the antidiarrhea activity of the root bark of *Tamarindus indica* in order to establish the scientific basis for its therapeutic properties in folkloric use.

**1.5.2 Objective of the study**

The objectives include:

* To extract the root bark of the plant with different solvent systems.
* To study the antidiarrheal activity of the extracts.
* To study the phytochemical activity of the extracts.
* Purification of the extracts.

**1.6 Scope of the study**

The scope of this research work is to carry out phytochemical screening and antidiarrheal activity of the root bark of *Tamarindus indica* within Bauchi metropolice.

**CHAPTER TWO**

**2.1 Medicinal Plants**

Medicinal plants have been identified and used throughout human history (Lichterman, 2004). The use of medicinal plants to treat diseases is almost universal amongst non-industrialized societies and is often more affordable than purchasing expensive conventional drugs (Fabricant and Farnsworth, 2001). The world Health Organization (WHO) estimates that 80% of the world population especially Asian and African countries use herbal medicine for some aspect of primary health care (<http://www.traffic.org/medicinalplants>, 30th march, 2014). Over 120 active compounds currently *isolated* from the higher plants are widely used in modern medicine and 80% of these show a positive correlation between their modern day therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth, 2001).

**2.1.1 *Tamarindus indica***

**2.2 Extractions**

**2.3 Secondary metabolites**

Secondary plant metabolites (Phytochemicals) have been extensively investigated as a source of medicinal agents (Krishnaraju, 2005). Plants can synthesize and accumulate a great variety of phytochemicals in their cells including saponins, tannins, flavonoids, cyanogenic, phenolic compounds, lignins, lignans, alkaloids and glycosides (Okwu, 2004). Plants also have a great potency of antimicrobial activity due to the presence of phenolic compounds and essential oils (Aboaba and Efuwape, 2001). Medicinal plants have been known to produce an array of phytochemicals with recognized antibacterial activity belonging to chemical structural classes: phenolics, terpenoids, alkaloids, lectins, polypeptides, and polyacetylenes but the most bioactive constituents are alkaloids, tannins, flavonoids, and phenolic compounds (Hill, 1995). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Afolayan, 2003). Some of the commonly used traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone *et al., 2004*).

**2.3.1 Tannins**

Tannin is astringeny vegetable product found in a wide range of plants parts ranging from the barks, roots, fruits, leaves, galls and roots (Ramakrshnan, 2006). They occur naturally In plants and are water soluble phenolic compounds of the higher molecular weight of about 500 – 3000 containing phenolic

Hydroxyl groups that make them to effectively cross-link with proteins and other macromolecules (Ramkrishnan, 2006).

Tannins are generally found in plants and they are thought to function as chemical defenses against pathogens and herbivores (Gedir *et al., 2005*). They have been commercially used primarily in the preservation of leather, making glue stains and mordant (Kanth *et al., 2009*). It has also been used in the vegetable industry in different concentration in picking process to provide protection against bacteria, mold, and yeasts (Andrade *et al., 2005*). Antimicrobial activity of tannins has been tested in various fields of medicine providing positive results such as antioxidant activities, anticarcinogenic activities and antimutagenic properties (Lopes *et al., 1999*). Tannins have been used in inhibiting the growth of many fungi, yeasts, bacteria and viruses (Chung *et al.,* 1998). Studies carried out have shown that tannins such as catechin and pyrogallol found in vegetable tannins have been found to be toxic to microorganisms (Cowan, 1999). Tannins have been found not only effective against pathogenic microbes but also have a significant value as a cytotoxic and an antitumor agent (Josh *et al.,* 2013).

**2.3.2 Flavonoids**

Flavonoids or bioflavonoids are secondary metabolites of plants that chemically have a general structure of 15 carbon skeleton consisting of two phenyl rings and a heterocyclic ring (Mc Naught, 1997). There are over 500 groups of flavonoids that have been characterized from various plants according to their chemical structure (Ververidis *et al.,* 2007). They are usually subdivided into anthoxanthins, flavanones, flavanols, flavans, and anthocyanidin (Zhao *et al.,* 2012). In plants they are responsible for floral pigmentation, ultraviolet ray’s filtration in higher plants and symbiotic nitrogen fixation (Galoetti *et al.,* 2008). They are also known to have inhibitory activities against organisms that cause plant diseases for example *Fusarium oxysporum* (Galoetti *et al.,* 2008). Flavonoids have been known to posses antimicrobial activity against bacterial, fungal and viral microorganisms (Cowan, 1999). They are usually known for their antimicrobial activity of inhibiting the synthesis of the nucleic acids, tampering with the integrity of the cytoplasmic membrane function and the energy metabolism process (Cushnie and Lamb, 2005). Flavonoids from some medicinal plants have been found to inhibit the synthesis of the nucleic acids, cause permeability of the inner bacterial membrane and a dissipation of the membrane potential of Gram negative and Gram positive bacteria (Cushnie and Lamb, 2005). Some of the bioactive components have been isolated from flavonoids have been found to contain antifungal, antibacterial and insecticidal activities (Abdel *et al., 2013*). Previous studies carried out have shown that when mixed with antibiotics they have synergistic activity and suppress many pathogenic microorganisms in numerous in vitro and in vivo studies (Cushnie and Lamb, 2011; Manner et al., 2013). Additional in vivo studies have shown that flavonoids can be used as pharmaceutical drugs for bacterial infections or through the dietary intake to offer protection against infection (Zamora *et al.,* 2012).

**2.3.3 Alkaloids**

They are a group of naturally occurring compounds that contain nitrogen and can be neutral or have weakly acidic properties (Mc Naught, 1997). They may also sometimes contain oxygen, Sulphur, more rarely other elements such as chlorine, bromine, and phosphorus (Schardl *et al.,* 2007). They are mainly secondary metabolites of plants but can also be produced by a variety of organisms including bacteria, fungi, and animals (Kittakoop *et al., 2014*). They dissolve in water poorly but readily dissolve in organic solvents (Shi *et al.,* 2014). They are divided into five major groups namely: true alkaloids (contain nitrogen in heterocyclic and originate from amino acids), proto alkaloids, polyamine alkaloids, peptide and cyclopeptides alkaloids and pseudoalkaloids (Faulkner *et al.,* 2006). They have a wide range of pharmacological activities such as antiasthma, antimalarial, anticancer, cholinomimetic, vasodilatory, antiamyhyrithic, analgesic, antibacterial and antihyperglycemic activities (Cushnie and Lamb, 2014). Some alkaloids have been known to possess psychotropic and stimulant activities and have been used as recreational drugs and entheogenic rituals (Blankenship *et al., 2005*). Alkaloids have great antimicrobial activity against bacterial pathogens such as *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aureginosa* (Maatalah *et al., 2012*).

Some of the bioactive components of alkaloids such as morphine and cordine have been found to be active not only against bacterial and fungal pathogens but also trypanosomes and plasmodia (Feiburghaus *et al.,* 1996; Omulokoli *et al., 1997*). Some of the Alkaloids found in dietary food materials have also been found to contain microbiocidal and antidiarrheal effect in the small intestine where they show the ability to intercalate with the microbial genetic material (Ghoshal *et al.,* 1996; Phillipson and Niell, 1997). Other studies carried out on alkaloids extracted from a variety of medicinal plants in Nigeria showed a great antifungal activity (Garba and Okeniyi, 2012).

**2.3.4 Saponins**

They are a class of chemical compounds found in various plant species and they are amphipathic glycoside grouped structurally by having one or more hydrophilic glycosides moieties combined with liphophilic triterpene (Hostettmann and Martson, 1995). In plants, saponins are known to provide protection against microbes and fungi (Riguera, 1997). Saponins have been used by a wide range of commercial therapeutic claims for natural products whereby in organismal or human benefit are often based on preliminary biochemical and cell biology studies (Skene and Phillip, 2006). Saponins are also considered as one of the natural antimicrobial products that make up the defense system of the plants and some can be beneficial rather than harmful to animals (Rupasinghe *et al.,* 2003; Hubert *et al., 2005*).

There has been evidence of the presence of saponins in traditional medicine preparations where the administration is through oral means that is expected to lead to the hydrolysis of glycosides from terponoids (Asl *et al., 2008*). Studies carried out have shown medicinal plant extracts fractions rich in saponins are effective against microorganisms such as *Escherichia coli, Salmonella typhi, Aeromonas hydrophilia* and other fungal pathogens such as *Candida albicans* (Deshpande *et al., 2013*). Saponins antimicrobial activity is attributed mainly to its capability of lysing microorganism’s membranes rather than the surface tension of the extracellular medium (Asl, 2008). Apart from antimicrobial activity, saponins have shown other biological properties with its cytotoxic activity on cancer or tumor cells being considered the most important one (Yokosuka and Mimaki, 2009). Other plants are known to produce steroidal saponins for example cholestane glycosides which are known to have a broad spectrum of biological activities such as cytotoxic activity, antifungal, antibacterial and in vivo antitumor activities (Li *et al.,* 2012).

**2.4 Current trend in Phytochemistry and Medicinal Plant**

Synthesis of secondary metabolites by plants is often with highly complex structures. Most of these important secondary metabolites are obtained from wild or cultivated plants because their chemical synthesis is not economically feasible. Various biotechnological methods have been employed in producing some of the secondary metabolites of plants through plant cell cultures. However, this has had limited success because of lack of understanding of how these metabolites are synthesized. State-of-the art genomic tools, however, can be used to enhance the production of known target metabolites or to synthesis entire novel compounds by so-called combinatory biochemistry in cultivated plant cells (Oksman-Caldenteya and Inzé 2004). Some plant cells have been used as factories to produce some secondary metabolites. Examples of these are paclitaxel, an anti-cancer drug originally extracted from the bark of 50-60-year-old Pacific yew trees (*Texus brevifolia*); shikonin, produced by cell suspension cultures of *Lithospermumerythrorhizon;* berberine, produced by cell cultures of *Coptis japonica;* rosmarinic acid, produced by cell cultures of *Coleus blumeli,* which has been achieved on a large scale, and sanguinarine, produced by cell cultures of *Papaversomniferum,* which has market potential in oral hygiene products (Oksman-Caldenteya and Inzé 2004) .

**2.5 Chromatographic analysis**

Chromatography is a laboratory technique for the separation of a mixture. The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases. Subtle differences in a compound's partition coefficient result in differential retention on the stationary phase and thus affect the separation (Mcmurry and John, 2011). Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for later use, and is thus a form of purification . Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive (Hostettmann *et al.,* 1998).

**2.6 Biological assays**

**2.6.1 Anti-fungal activity**

Tamarind fruits are reported to have anti-fungal as well as anti-bacterial proper- ties (Ray & Majumdar (1976), Guerin and Reveillere (1984), Bibitha *et al.* (2002), Metwali (2003) and John *et al.* (2004) all cited in El-Siddig *et al.*, 2006). Extracts from the fruit appear promising as a potential fungicidal agent against cultures of *Aspergillus niger* and *Candida albicans* (El-Siddig *et al.*, 1999; El-Siddig *et al.*, 2006).

**2.6.2 Antidiarrhea activity**

**2.6.3 Anti-viral activity**

Plant extracts of tamarind were reported to have antiviral activity on watermelon mosaic viruses (Chapman (1984) cited in El-Siddig *et al.*, 1999), cow pea mosaic viruses (Singh *et al.* (1989) cited in El-Siddig *et al.*, 1999) and tobacco mosaic viruses (Stovakova *et al.* (1994) cited in El-Siddig *et al.*, 1999).

**2.6.4 Antioxidant activity**

Tamarind seed kernels have a relatively high antioxidant activity and phenolic con- tent (Soong *et al.*, 2004). Four anti-oxidative compounds were isolated and identified from the seed coats: phenolic antioxidants, such as 2-hydroxy-3’, 4’-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin (Tsuda *et al.*, 2004). These antioxidants may be used for increasing shelf life of food products and improving the stability of lipids and lipid-containing foods by preventing loss of sen- sory and nutritional quality by preventing lipid peroxidation. These compounds may also find a place as food additives though studies are needed to evaluate their effectiveness within food matrices. Extracts exhibit antioxidant potential by reducing lipid peroxida- tion *in vitro* (Tsuda *et al.*, 2004; Tsuda *et al.* (1993) cited in Sudjaroen *et al.*, 2005). Raw and dry heated tamarind seed coats exhibit good antioxidant activity against the linoleic acid emulsion system and the values were lower and higher than the synthetic antioxidant, butylated-hydroxy-anisole (BHA), and ascorbic acid, respectively (Siddhuraju, 2007).

Phenolic plant compounds may have many biologic effects in terms of health pro- motion. An important protective effect is reduction of oxidative damage, mediated by lipid peroxidation, which in living systems is strongly associated with mutagenesis, car- cinogenesis, ageing, and atherosclerosis (Tsuda *et al.*, 2004; Yagi (1987) and Cultar (1984 and 1992) all cited in Sudjaroen *et al.*, 2005). Pumthong (1999), cited in Sudjaroen *et al.* (2005), described the antioxidant activity of extracts of tamarind pericarp, and reported the presence of mainly polymeric tannins and oligomeric procyanidins but the latter were not yet identified or quantified.

The anti-oxidative activity of tamarind seed was also investigated by Osawa *et al.* (1994, cited in El-Siddig *et al.*, 2006). They found that ethanol and ethyl acetate extracts prepared from the seed coat exhibited anti-oxidative activity. This suggests that tamarind seed coats, a by-product of the tamarind gum industry, may have potential as a low cost *source* of antioxidants (Tsuda *et al.*, 2004), but we note that so many plants and plant extracts show anti-oxidative activity (Ramos *et al.* (2003) cited in El-Siddig *et al.*, 2006).

**2.6.5 Cytotoxicity assay**

Al-Fatimi et al., reported that methanolic extracts of *Tamarindus indica* showed remarkable cytotoxic activity against FL-cells, a human amniotic epithelial cell line, with IC50 values below (Al-Fatimi *et al.,* 2007). Sano M et al., examined the carcinogenic potential of tamarind seed polysaccharide in both sexes of B6C3F1 mice. The results demonstrated that its polysaccharide is not carcinogenic in B6C3F1 mice of either sex. Bioassay-guided fractionation of methanolic extract of tamarind seeds led to the isolation of L- di-n-butyl maleate which is having pronounced cytotoxic activity against sea urchin embryo cells (Sano M *et al*., 1996). In order to study structure-activity relationships of its analogs, L-di-n-pentyl maleate was the most effective inhibitor to the development of the fertilized sea urchin eggs, and significant inhibitory activity was not in the esters of D-isomer (Kobayashi *et al.,* 1996).

**2.7 Spectroscopic techniques**

**CHAPTER THREE**

**3.0 MATERIAL AND METHODS**

**3.1.1 Equipment / instruments**

**3.1.2 Reagents and solvents**

**3.2 Sample collection**

**3.3 Methods**

**3.3.1 Collection of Plant and identification of Plant meterial**

**3.3.2 Preparation of plant extract**

**3.3.2.1 Qualitative phytochemical analysis**

The presence of saponins, tannins, flavonoids and alkaloids in the crude extract will be determined according to the method defined by Congesta *et al* (2005).

**3.3.2.2 Tannins**

Each of the extracts will be weighed to 0.5 mg and dissolved in 1 ml of distilled water. Filtration will be carried out after 2 ml of FeCl3 will be added. If there is presence of a blue or black precipitate then it indicate the presence of tannins.

**3.3.2.3 Flavonoids**

Each of the extracts will be weighed to 0.5 mg and dissolved in 1 ml of ethanol and filtered. 2 ml of 1% HCl and magnesium ribbon will be added to the filtrate. If there is formation of a pink or red colour it indicates the presence of flavonoids.

**3.3.2.4 Alkaloids**

Each of the extracts will be weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. 1 % HCl will be added to the filtrate and the solution heated. Mayor’s reagent will be added dropwise and if there is formation of any colored precipitate it indicate the presence of alkaloids.

**3.3.2.5 Saponins**

Each of the extracts will be weighed to 0.5 mg and dissolved in 1 ml of methanol and filtered. Distilled water will be added and shaking done for a few minutes. If there is persistence frothing then it indicates the presence of saponins.

**3.3.2.6 Phenols**

A fraction of the extract will be treated with aqueous 5 % ferric chloride solution. The formation of deep blue or black color indicates the presence of phenols (Solomon *et al.,* 2013).

**3.3.3 Microorganisms**

**3.3.4 Analysis of antidiarrheal activity**

**3.3.4.1 Preparation of sample extract for microbiological assay**

**3.3.4.2 Disc diffusion technique**

**3.3.4.3 Determination of Minimum Inhibitory Concentration (MIC)**

**3.3.4.4 Determination of Minimum Bactericidal Concentration (MBC)**

**CHAPTER FOUR**

**4.0 Expected Result and Conclusion**

At the end of this research work, the phytochemical screening should reveal the presence of bioactive components of the plant extract such as flavonoids, alkaloids, saponins, tannins, and the antidiarrheal activity should indicate that the plant contains medicinal and therapeutic properties and can be used as medicine for combating diseases causes by diarrhea causing bacteria.

**4.1 Expected Result**

**4.2 Conclusion**

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