*ANTIBACTERIAL* ACTIVITY OF *TAMARINDUS INDICA* ON *SATAPHYLOCOCCUS AUREUS*

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*ANTIBACTERIAL* ACTIVITY OF *TAMARINDUS INDICA* ON *STAPHYLOCOCCUS AUREUS*

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SUBMITTED TO

THE FACULTY OF SCIENCE DEPARTMENT OF BIOLOGICAL SCIENCE, MICROBIOLOGY, ABUBAKAR TAFAWA BALEWA UNIVESITY BAUCHI, BAUCHI STATE.

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DECLARATION

I declare that this project on ‘’The antibacterial Activity of *Tamarindus indica* on *Staphylococcus aureus* is an original work done by me carried out in the laboratory of microbiology, Abubakar Tafawa Balewa University Bauchi under the supervision of Mrs Agbo, Veronica Markus

AHMED AISHA UMAIMA ………………. …………………

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The above declaration is confirmed by

MRS AGBO VERONICA MARKUS ……………… ………………..

(Project Supervisor) Signature Date

CERTIFICATION

This is to certify the project titled: - “Antibacterial activity of tamaridus indica “*Staphylococcus aureus”* is an original work undertaken by Ahmed Aisha umaima with Registration number 13/33958/U/1 and has been in accordance with the regulation governing the award of B. Tech Degree in Applied Microbiology of the Abubakar Tafawa Balewa University, Bauchi.

…………………………...

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(Student)

**APPROVAL PAGE**

This work is thoroughly checked and was found to meet the necessary requirements of a B.Tech in Applied Microbiology. The work is approved for its valuable contribution to knowledge and opens up to subsequent studies.

**By**

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……………………………… …………………. ………………

External Examiner Signature Date

DEDICATION

This research project is dedicated firstly to ALMIGHTY ALLAH for the enabling strength he bestowed on me in completing this work.

Secondly to my beloved parent Malam Ahmed Shehu and Hajiya Aisha Saad and my entire family members.

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**ABSTRACT**

Tamarindus indica is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser. To evaluate the scientific basis for the use of the plant, the antimicrobial activities of extracts of the leaves were evaluated against some common gram positive bacteria. The study also investigated the chemical constituents of the plant .

The phytochemical constituents of the dried powdered plant parts were extracted using aqueous and organic solvents ( ethanol). The antimicrobial activity of the concentrated extracts was evaluated by determination of the diameter of zone of inhibition against gram positive bacteria*(Staphylococcus auureus*) using the agar well diffusion method. Results of the phytochemical studies revealed the presence of tannins, saponins, alkaloids, flavonoid, phenol, molish and the extracts were active against the gram positive bacteria *(Staphylococcus auureus*) Studies on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts on the test organisms showed that the lowest MIC and the MBC against Staphylococcus aureus.

Tamarindus indica has broad spectrum antibacterial activity and a potential source of new classes of antibiotics that could be useful for infectious disease chemotherapy and control**.**

**CHAPTHER 1**

**1.0 INTRODUCTION**

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources (Bibitha*et al.,* 2007). The bacterial effect of plant extract have been reported and several attempt have been made to destroy bacteria by the application of these extract (ugbogu, 2010) and (kotzekidon *et al.,2008)*

Microorganisms exist everywhere in the earth, which cause various infectious diseases to human being. Man used the antimicrobial drugs against microbes, cause various side effects. The employment and development of these drugs against microbes continued throughout civilization until the modern era, Sometimes microbes constantly developing resistant to these drugs (Abdallah, 2011). Plants are prospective source of antimicrobial agents in different countries. Traditionally crude plant extracts are used as herbal medicine for the treatment of infectious diseases because of the presence of phytochemical. The phytochemicals work in the human system and due to their therapeutic properties cure many ailments which cannot be cured by the modern drugs (Rahman *et al*., 2007). In recent years attempts have been made to investigate the new drug against infectious diseases. This may help to develop safer antimicrobial drugs (Khanzada *et al*., 2008).

.  *Tamarindus indica* fruit is very rich in minerals, potassium, phosphorus, calcium and magnesium. It has one of the highest levels of protein and carbohydrate of any fruit, though it contains smaller amounts of iron and vitamin A (Khanzada *et al*., 2008). *Tamarindus indica* fruit pulp is a dessert fruit which is often eaten directly from thepod and also used for the preparation of beverages, jam, syrup, candy, curries, chutneys, sauces and ice cream in different regions of the world (Gunasena and Hughes, 2007). Many parts of tamarind plant have long been used in traditional medicines for the treatment of a wide variety of ailments and diseases such as jaundice, gonococci and gastrointestinal disorders. Extract from the pulp is used as a therapeutic drink in febrile conditions, convalescence, bowel complaints, bilious disorders, dysentery and rheumatism (Souza and Aka, 2007). T. *indica* pulp extract is also administered to alleviate sunstroke, Datura poisoning and alcoholic intoxication (Morton, 2007). Tamarind preparations are used as aid in the restorationof sensation in cases of paralysis, reduction of body temperature in fevers, and as laxatives, expectorant and blood tonic (Komutarin *et al*., 2009). Other parts of the plant possess antibacterial, antifungal, hypoglycaemic, cholesterolemic (Khanzada *et al*., 2008). Ingestion of T. *indica* fruit has been reported to have an additional beneficial effect on the mobilisation of deposited fluoride from bone, by enhancing urinary excretion of fluoride (Khandare *et al*., 2007).

In a work done on the phytochemical and antimicrobial studies of extracts from the tamarind pulp by M.Abdul Kapur and S.Ahmed John (2014) shows the Phytochemical constituents present in the extract were found to include Saponins, Quinine, Alkaloids, Lignin, Glycosides. Large zone of inhibition was observed (16 mm and 15.25 mm*) Staphylococcus aureus* and *Bacillus cereus* respectively.

**1.1 STATEMENT OF THE PROBLEM**

Microorganisms have developed resistance to many antibiotics and has created immense clinical problem in the treatment of infectious diseases. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has also increased.

**1.2 JUSTIFICATION**

The use of antibiotics to control diseases is producing adverse toxicity to the host organs, tissues and cells. The toxicity produced by the antibacterial agents can be prevented by using herbs, *Tamarindus indica*is used in traditional medicine for treatment of different infections such as impetigo and has been proven effective.

**1.3 Aim and objectives**

The general aim of the work is to evaluate the antibacterial activity of aqueous extract of *Tamarindus Indica* against *Staphyloccus specie,* with the following objectives:

1. To prepare the cold aqueous extract of tamarind
2. To document the phytochemical constituent of tamarind
3. To evaluate the antibacterial activities of the extract of tamarind using zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)
4. To make appropriate recommendations base on the findings.

**CHAPTER TWO**

**2.0 LITERATURE REVIEW**

**2.1 TAMARIND**

Tamarind (*Tamarindus indicus*) is a semi-evergreen tree with large [alternatelyarranged](http://www.britannica.com/EBchecked/media/374/Common-leaf-morphologies) and [pinnately compound](http://www.britannica.com/EBchecked/media/374/Common-leaf-morphologies) leaves. It may reach a height of 10 to 20m. The flowers are pale yellow and streaked with red. When the tree is in full bloom, the flowers give a yellowish color to the tree. The fruits are thick, rough pods that are 4 to 13 cm long and usually curved. Each pod contains 1 to 10 seeds embedded in a brown, sticky, fibrous edible (but sour) pulp surrounding the seeds. Tamarind is widely planted in the tropics and subtropics not only for its fruits, but also as an ornamental shade tree. Tamarind trees are sometimes clipped into gnarled bonsai in Thailand (Mabberley, 2008).

***2.1.1 Origin and geographical distribution of tamarind***

Various geographical areas have been proposed for the origin of tamarind: India (Morton, 2007) or the far east or Africa but the consensus is that it is Africa, placed it in Ethiopia but others considered it indigenous to the drier savannahs of the tropical Africa, from sudan, Ethiopia,Kenya and Tanzania, westward through sub-sahelian Africa to Senegal (El-Siddig *et al*., 2008). It is thought to have been introduced to south and southeast Asia a very long time ago and neutralized in many areas where it was introduced (coronel, 2007)

According to (simons *et al*., 2007) the native distribution of tamarind is as follows: Burkina faso, Camaroon, Niger, Nigeria, Senegal, Sudan, Tanzania, Uganda, Kenya, Guinea, Chad, Mali, Madagascar, Ethiopia, Gambia.

***2.1.2 Tamarindus leaves and pod***

Tamarind is harvested by pulling the pod from its stalk. A mature tree may be capable of producing up to 175 kg (350 lb) of fruit per year. Veneer grafting, shield (T or inverted T) budding, and air layering may be used to propagate desirable selections. Such trees will usually fruit within three to four years if provided optimum growing conditions.

PLATE 1: *Tamarindus indica* **(**Rudd, 1991)

****

PLATE 2: Tamarind Friuts **(**Rudd, 1991)

***2.1.3 Scientific classification***

**TABLE 1: BOTANICAL CLASIFICATION OF TAMARIND (**Rudd, 1991)

|  |  |
| --- | --- |
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Superdivision | Spermatophyta |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Subclass | Rosidae |
| Order | Fabales |
| Family | Fabaceae |
| Genus | *Tamarindus L.* |
| Species | *T indica.* |
| Binomial name | *Tamarindus indica L.* |

**2.2 CHEMICAL CONSTITUENT OF TAMARIND**

The chemical composition of amino acids, fatty acids, and minerals of tamarind plant parts have been reported. Differences in values found in the literature are likely to be due to differences in genetic strains, stages of maturity at which the plant parts were harvested, growing conditions (Glew *et al*., 2008), harvesting and handling techniques as well as to differences in analytical methodologies. Nevertheless, a review of the phytochemistry will provide insight into the relative value that this species provides when used.

Fruits Nutritional composition of tamarind fruit varies considerably. However, a typical fruit contains 40% pulp (El-Siddig *et al.,* 2007). According to other authors, the fruit contains about 55% pulp, 34% seeds, and 11% shell (pod) and fibres (Kumar & Bhattacharya, 2008).

Chemical Composition Tamarind pulp typically contains 20.6% water, 3.1% protein, 0.4% fat, 70.8% carbohydrates, 3.0%fibre and 2.1% ash (El-Siddig *et al*., 2007), thus the pulp has a low water content and a high level of protein, carbohydrates and minerals. Nevertheless, the proximate composition of the tamarind fruit depends on locality (El-Siddig *et al.,* 2007).

***2.2.1 Use sof tamarind in traditional medicine***

Tamarind is used in herbal medicine in many parts of the world (Siddhuraju, 2007) and medicinal uses of tamarind are uncountable (Morton, 2007). Medicinal uses of tamarind can be found in many cultures and for a wide array of applications (Morton, 2007). The medicinal value of tamarind has been mentioned already in traditional Sanskrit literature (El-Siddig *et al.,* 2008). Traditionally, tamarind products, leaves, fruits and seeds have been extensively used in traditional Indian and African medicine. A number of recent surveys have listed local folk uses of tamarind as remedies for a number of ailments (Rimbau *et al*., 2007). There is medical interest in the use of purified xyloglucan from tamarind in eye surgery for conjunctival cell adhesion and corneal wound healing (El-Siddig *et al*., 2007).

Tamarind preparations are universally recognized as refrigerants for fevers, and as laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the pulp is considered to be effective as a digestive as a remedy for biliousness and bile disorders, and as an antiscorbutic (Morton, 2007). The laxative properties of the pulp and the diuretic properties of the leaf sap have been confirmed by modern medical science (El-Siddig *et al*., 2008). In traditional practice, the pulp is applied on inflammations, is used in a gargle for sore throat and, mixed with salt, as a cream for rheumatism. It is, further, administered to alleviate sunstroke, Datura poisoning, and alcoholic intoxication. In south-east Asia, the fruit is prescribed to counteract the ill effects of overdoses of false chaulmoogra, Hydnocarpus anthelmintica Pierre, given in leprosy. The pulp is said to aid the restoration of sensation in cases of paralysis. In Colombia, an ointment made of tamarind pulp, butter, and other ingredients is used to rid domestic animals of vermin (Morton, 2007). Tamarind fruits were well-known in Europe for their medicinal properties, having been introduced by Arab traders from India. The pulp has been reported in several pharmacopoeias, such as the British and American. Some 90,000 kg of shelled fruits are annually imported into the United States for the drug trade, primarily from the Lesser Antilles and Mexico. The European supply largely come from Calcutta, Egypt and the Greater Antilles (Morton, 2007).

The powdered seeds are made into a paste for drawing boils and, with or without cumin seeds and palm sugar, are prescribed for chronic diarrhea and dysentery. The seed coat, too, is astringent, and is also specified for the latter disorders. An infusion of the roots is believed to have curative value in chest complaints and is an ingredient in prescriptions for leprosy (Morton, 2007).

Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. The latter are usually applied after grinding leaves and flowers into powder whereby they are used in lotions or infusions. Lotions and extracts made from them are used in treating conjunctivitis, as antiseptics, as vermifuges, treatments for jaundice, erysipelas and haemorrhoids, and various other ailments (Morton, 2007). The leaves, mixed with salt and water, are used to treat throat infections, coughs, fever, intestinal worms, urinary troubles and liver ailments. Leaves and pulp act as a cholagogue, laxative and are often used in treating liver ‘congestion’, constipation and haemorrhoids (El-Siddig *et al*., 2008).

The bark of the tamarind tree is regarded as an effective astringent, tonic and febrifuge (El-Siddig *et al*., 2007). It is used as a tonic and in lotions or poultices to relieve sores, ulcers, boils and rashes (El-Siddig *et al*., 2007). Fried with salt and pulverized to an ash, it is given as a remedy for indigestion and colic. A decoction is used in cases of gingivitis, asthma and eye inflammations. Lotions and poultices made from the bark are applied on open sores and caterpillar rashes (Morton, 2007). The bark of the tree should be peeled off if needed for medicinal purposes during the time when the tree is not flowering or when the flowering season ends (El-Siddig *et al.,* 2007).

***2.2.2 Other health benefits of tamarind***

Tamarind fruit contains certain health benefiting essential volatile chemical compounds, minerals, vitamins and dietary fiber. Its sticky pulp is a rich source of non-starch polysaccharides (NSP) or dietary-fiber such as gums, hemicelluloses, mucilage, pectin and tannins. 100 g of fruit pulp provides 5.1 or over 13% of dietary fiber. NSP or dietary fiber in the food increases its bulk and augments bowel movements thereby help prevent constipation. The fiber also binds to toxins in the food thereby help protect the colon mucus membrane from cancer-causing chemicals(Rahman *et al*., 2008).

In addition, dietary fibers in the pulp bind to bile salts (produced from cholesterol) and decrease their re-absorption in the colon; thereby help in expulsion of “bad” or LDL cholesterol levels from the body.While [lemon](http://www.nutrition-and-you.com/lemon.html) compose ofcitric acid, tamarind is rich in tartaric acid**.** Tartaric acid gives sour taste to food besides its inherent activity as a powerful antioxidant. (Anti-oxidant E-number is E334). It, thus, helps human body protect from harmful free radicals.Tamarind fruit contains many volatile phytochemicals such as limonene, geraniol, safrole, cinnamic acid, methyl salicylate, pyrazine and alkyl­thiazoles. Together, these compounds account for the medicinal properties of tamarind.This prized condiment spice is a good source of minerals like copper, potassium, calcium,iron, selenium, zinc and magnesium. Potassium is an important component of cell and body fluids that helps control heart rate and blood pressure. Iron is essential for red blood cell production and as a co-factor for *cytochrome oxidases* enzymes(Nordeide *et al*., 2007).

In addition, it is also rich in many vital vitamins, including thiamin (36% of daily required levels), vitamin-A, folic acid, riboflavin, niacin, and vitamin-C. Much of these vitamins plays antioxidant as well as co-factor functions for enzyme metabolism inside the body.

***2.2.3 Pharmacological activities of tamarind***

1. **Peptic ulcer**

Peptic ulcer (mucosal damage deeper than 0.5 centimeters) is painful gastrointestinal damage in stomach and duodenum. It has been shown that *Tamarindus indica* seed extract has dose dependent protective effect on ulcer models induced by ibuprofen, alcohol and pylorus ligation. It is a possible new ulcer treatment (Kalra*et al.,* 2007). The protective effect of *Tamarindus indica* seed comes from its polyphenolic compounds, mainly procyanidin, epicatechin and polymeric tannins. These compounds have anti oxidant effect and protective role against free radicals. Tannins also prevent the ulcer development.

1. **Antioxidant properties**

Antioxidant properties of *T. indica* seed and leaves has been shown in many studies (De Caluw*et al*., 2010). Not only phenolic properties (tannins) of raw seeds but also heat dried seeds has antioxidant properties (Siddhuraju, 2007). Phenol rich food and beverages like red wine, grape seed, green tea and tamarind have hypolipidemic, antiatherosclerotic, antioxidant, anti-inflammatory and immunomodulatory effect. *Tamarindus indica* fruit is rich in organic acid, pectin, vitamin, mineral content, polyphenol and flavonoid content. Rich polyphenol content exists in seed and fruit and they show regulatory effect on neutrophils (Paula*et al*., 2009).

1. **Toxicity, side effects and drug interactions**

The 2-year follow-up study done by (Iida *et al*., 2009) reported that there were no side effects in animals fed with *T. indica* seed extract in different doses. (Heimbach *et al*., 2013) also reported that there were no change in blood biochemistry, urine analysis, liver function test, body weight in animals fed with *T.indica* seed polysaccharide for 28 d. In another study toxic effect is not reported, but increase in white blood cells and thrombocyte is observed (Bhadoriya*et al*., 2011). *T. indica* seed contain tannin and the other compounds that make the digestion difficult so it is suggested that to consume it after boiling or waiting inside water. And also in long term use because of its acidic content it can cause dental erosion (Nayak*et al*., 2012). Ibuprofen and acetyLlsalicylic acid when consumed with *T. indica* can increase their bioavailability and increase the blood levels of them (Garba*et al*., 2007) .

***2.2.4 BIOLOGICAL ACTIVITY***

**Anti-Microbial Activity**

Tamarind fruits are reported to have anti-fungal and anti-bacterial properties. According to Al-Fatimi *et al*., (2007), in an agar diffusion assay, extracts from *T. indica* flowers showed antibacterial activity against three bacteria tested (*Staphylococcus aureus, Bacillus subtilis, streptococcus species*). Tamarind leaves possess a strong in vitro antibacterial activity against more then 13 (81%) common gram positive and gram negative bacteria that were tested (Meléndez and Capriles, 2007). They latter also reports that tamarind leaf extract was very effective against *E. coli*. Not much is known, however, about the antibacterial compounds present in the tamarind leaves (Meléndez and Capriles, 2007) nor the specific compounds responsible for such activity. Tamarind plant extracts have been used to purify drinking water in Burkina Faso and Vie

**CHAPTER THREE**

1. **RESEARCH METHODOLOGY**

**3.1 Collection of plant materials**

*Tamarindus indica* fresh leaves were obtained from a tree at Abubakar Tafawa Balewa University yelwa campus. It was then air dried for some days after which it was pounded using mortar and pestle in the microbiology laboratory and kept in an air tied container.

**3.2 Preparaton of extract using cold acqueos**

The fresh tamarind pulps were air dried in shade so as to prevent the decomposition of chemical constituents for five days.. The powdered shade dried material was extracted with water and ethanol. The tamarind pulps were weighed using a weighing balance. 25g of the portion *Tamarindus Indica* was placed in a 500ml beaker of conical flask and 250ml of cold distilled water was added and stirred vigorously using a stirrer. It was covered with aluminum foil paper and labeled then another 25g of portion *Tamarindus indica* was placed in a 500ml beakerof conical flask and 250ml of ethanol was added and stirred vigorously using a stirrer. They were covered with aluminum foil paper, labeled, and were allowed for 24hrs under anaerobic condition after which they were filtered using a muslin cloth. The extracts were then evaporated using rotary water bath. It was then kept in the refrigerator in a beaker sealed with aluminum to prevent contamination (Khan *et al*., 2008)

**3.3 Test organism**

*Staphylococcus aureus* was collected from specialist hospital bauchi. The bacteria were first sub cultured in a nutrient agar and incubated for 24hours at 370C before storing at 40C.

**3.4 Antibacterial Sensitivity Screening**

***3.4.1 Preparation of aqueous and ethanoic extract concentration of tamarindus indica***

**0**.2g of each*tamarindus indica*extract were dissolved in 1ml of dimethyl sulphoxide (DMSO) which make up 200mg/ml, it was then serial diluted to obtain 100, 50, and 25mg/ml concentrations*(Ibekwe et al.,2011)*

***3.4.2 Agar Diffusion Method***

Mueller Hinton media was prepared according to the manufacturer’s instruction. The media was poured into plates aseptically before inoculation. The plates were allowed to stand for 10minutes for even diffusion of the test organisms. After which a sterile cork borer of 5mm diameter was used to prepare a holes also called wells in the middle of each plates. The wells are then filled with 0.1ml of the extracts and left on the bench for 10minutes for adequate diffusion of the extracts; the plates are then incubated at 370C for 24hours. The diameter of the zone of inhibition round of the well, it is then measured to the nearest millimeter (Agwa *et al*., 2007).

***3.4.3 Preparation of Antibiotic Disc***

Mueller hinton media was prepared according to the manufacturers instruction. The media was poured into plates aseptically before inoculation. The test organisms was streaked on the media and allowed to stand for 10minutes for even diffusion. After which antibiotics discs (gram +ve) were aseptically using a sterile scalpel. The plates were then incubated at 370C for 24hours, clear zone was checked and recorded on the plates (Agwa *et al*., 2007).

**3.5 Broth Dilution Method**

Different concentrations of the extract were prepared at 200, 100, 50, and 25mg/ml respectively.having obtained the different concentrations of the extract, 0.5ml of nutrient broth was added to 0.5ml of each concentration. Each concentration was inoculated with 0.1ml of standardization inoculums of test organism and incubated at 370c for 24hrs. The tubes were observed for growth as indicated by the turbidity. The minimum concentration of the tamarind extracts that inhibited growth was taken as the minimum inhibitory concentration (MIC). The determination of the value of minimum bactericidal concentration (MBC) follows the determination of MIC using broth dilution technique.

The minimum bactericidal concentration (MBC) is the lowest concentration of the antibacterial agent that kills at least 99.9% of the test organism, to determine this value about 0.5ml of sample are removed from the tubes used in determination of MIC. In which there is no designable growth and spread over the surface of nutrient agar plate inoculated at 370C for 24hours (Doughari, 2007)

**3.6 Phytochemical Screening**

The freshly prepared extract was subjected to standard phytochemical analysis to test for the presence of the phytoconsituents, tannins, saponins, alkaloids, flavonoids, phenol and molish using the standard methods of Abba *et al,* (2009) and Tiwari, (2011).

**1. Test for Tannins**

A 0.2g sample of extract was stirred with water and filtered. A dirty-green precipitate, or blur-black, or blue-green precipitates**,** on addition of few drops of 5% ferric chloride to the test extract was taken as an indication of the presence of tannins.

**2. Test For Saponins**

A 0.2g sample of extract was dissolved in 5ml of distilled water. 2ml of the resulted solution was taken into a test tube and was shaken vigorously for a few minutes. Frothing which persists on warming was taken as an evidence of the presence of saponins.

**3. Test for Flavonoids**

A small quantity of the extracts was dissolved in dilute 2% NaOH. A yellow solution that turns colorless on addition of 1% HCL acid indicates the presence of flavonoids.

**4. Test For Phenols**

Test extracts was dissolved in ferric chloride solution. Blue-black or brown coloration indicates the presence of phenols.

**5. Test For Molish**

Few drops of molish reagents were added to 2ml of test extracts in a test tube. 1ml of H2SO4was allowed to flow down the side of the inclined test tubes so that the acid forms a layer beneath the aqueous solution without mixing it. A reddish brown solution indicates a positivetest.

**CHAPTER FOUR**

1. **RESULTS**

**4.1** Table 1 shows reaction in tubes anddifferences that occurred in the color intensity of the cold aqueous extracts of tamarind in test tubes at varying dilutions

**Table 1**:Reaction In Tubes Before And After Incubation Of Cold Aqueous extract of tamarind.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  | | --- | --- | --- | | CONCENTRATION | COLOR | COLOR CHANGES AFTER 24HRS | | 200mg | Brown Color | Cloudy Brown | | 100mg | Brown Color | Cloudy Brown | | 50mg | Light Brown | Cloudy light Brown | | 25mg | Pale brown | Cloudy pale brown | |

**.** KEY: (+) = present (-) = Not detected

**4.2** Table 2 shows the results of the antibacterial screening of the plant extracts by the ditch plate method. The results indicated that the extracts are potent antimicrobials against *Staphyloccus aureus.* The antibacterial activity was screened for the zone of inhibition, the inhibitory effect of the extract were compared with standard antibiotic.

**Table 2:** Antibacterial Activity of ethanolic and aqueous extract of *Tamarindus indica on Staphylococcus aureus* showing diameter of zones of inhibition used at different concentrations.

|  |
| --- |
| **(Zone of inhibition mm)**  **Conc(mg/ml) EE AE**  200 18 6  100 12 4  50 8 0  25 0 0 |

KEY:

EE= Ethanolic Extract

AE= Aqueous Extract

0= No zone of inhibition

**4.3** Table 3 shows the result of antibiotic sensitivity test using disc

**TABLE 3:** Antibiotic Sensitivity Test Using Disc

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| |  |  |  |  |  | | --- | --- | --- | --- | --- | | S/N | DISC | ZONE OF INHIBITION | DIAMETER OF ZONE OF INHIBITION | INTERPRETATION | | 1 | Pefloxacin | Present | 30mm | Sensitive | | 2 | Erythromycin | Present | 24mm | Sensitive | | 3 | Septrin | Present | 18mm | Sensitive | | 4 | Streptomycin | Present | 14mm | Intermediate | | 5 | Ciproflaxacin | Present | 20mm | Sensitive | | 6 | Rocephin | Absent | 0mm | Resistant | | 7 | Amoxacillin | Present | 24mm | Sensitive | | 8 | Zinnacef | Present | 24mm | Sensitive | | 9 | Ampiclox | Present | 20mm | Sensitive | | 10 | Gentamycin | Present | 28mm | Sensitive | |

**KEY**

**≤** 12**=**Resistant

12-15 = Intermediate

≥ 15 = Sensitive

**4.4** Table 4 shows the minimum inhibitory concentration and minimum bactericidal concentation of cold aqueos in mg/ml

**Extract sensityivity of organisms**

MIC cold aqueos 0.9mg/ml

MBC cold aqueos 0.3mg/ml

**4.5** Phytochemical analysis of ethanoic and aqueous extract of *Tamarindus indica* leaves

**Table 6:** Phytochemical Screening of Aqueous Extract of Tamarind

|  |
| --- |
| **Phytochemicals**  **Ethanol Water**  **Tanins + +**  **Phenol - +**  **Saponins + +**  **Alkanoid + +**  **Molish + +**  **Flavonoids - -** |

**S**

**CHAPTER FIVE**

**5.1 DISCUSSION**

The antibacterial activities of medicinal plants are attributed due to the presence of tannins, flavonoids, saponins, steroids and glycoside (Burapedjo *et al.,* 2007). These reports the presence of tannins, saponins, flavonoids, steroids, in the extract confirm it as potential against the test organism. Tannins have been rSSeported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them (Sadipo *et al*., 2008). The study suggest that the tamarind pulp extract has antibacterial activity although the degree of susceptibility differs. The results of this study showed that the plant extract exhibited varied antibacterial activities against the test organisms which support their usage by traditional system of medicine as effective antibacterial agent against microbes. This is in conformity of findings of (*Uzama et al*., 2011).

Antibacterial agents currently available in the market are limited due to the toxicity, low effectiveness and prove costly in case of prolonged treatment, therefore there is need to develop new antibacterial agents which can satisfy the present demand. The basic quantitative measures of the in-vitro activities of antibiotics and plant extracts with antibacterial potentials are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The MIC is the lowest concentration of the antibiotics that results in the inhibition of visible growth (that is the colonies on a plate) under standard conditions while the MBC is the lowest concentration of the antibiotics that kills 99% of the original inoculums in a given time. The lowest values of MIC in this study are indications that the organism is not usually resistant to the plant extracts.

**5.2 CONCLUSION**

The activity of these extracts with standard antibiotic suggest that the cold aqueous extract can yield a high potent of antibacterial violence due to the presence of tannins, flavonoids, saponins, steroids and glycoside, it is documented that flavonoids, tannins and steroids show a wide spectrum of biological activities.

**5.3 RECOMMENDATIONS**

I therefore, recommend that

* Hospitals should also encourage the use of medicinal plants as an antimicrobial agent.
* Government should allocate revenue on refining medical plants in Nigeria so that money will not be wasted on the importation of drugs from western countries.
* In addition, the pharmaceutical companies, should exploit the medical values of Nigeria plant so as to contribute to the growth of the Nigerian economy.
* Further research should be carried out on the antibacterial activity of this plant