Phytochemical Screening and Antibacterial Activity of the Fruit and Leaf Extracts of *Tamarindus Indica* (Linn.)

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Abstract: The phytochemical screening and the antibacterial activity of *Tamarindus indica* Linn was carried out. The result of the phytochemical screening revealed the presence of alkaloids, anthraquinones, glycosides, flavonoids, phlobatannins, reducing sugars, saponins and tannins.The extracts of the fruits and the leaves of *Tamarindus indica* were tested on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* as test organisms. Agar well diffusion method and macro broth dilution method were employed in determining the zone diameter of inhibition (ZDI) and minimum inhibitory concentration (MIC) of the extracts respectively. Different concentrations of 50, 100, 250 and 500 mg/ml of the extracts were used for the antibacterial activity against the test organisms. The result revealed that the fruit extracts exhibited a higher antibacterial activity than the leaf extracts. The methanol fruit extract gave the highest zone diameter of inhibition of 41±1.0 mm for *E.coli* and 37±0.4 mm for *S. typhi* at 500 mg/ml while the methanol leaf extract gave 0.0 mm for *E.coli* and 25±0.4 mm for *S. typhi* at 500 mg/ml. The aqueous and methanol fruit extracts were effective against *E. coli, S. typhi* and *P. aeruginosa*. The ethanol extract was also effective against *E.coli, S. typhi, P.aeruginosa* and *S.aureus*. The leaf ethanol and methanol extracts were effective against *S.typhi* and *P. aeruginosa.* Generally, the fruit extracts exhibited a higher antibacterial activity than the leaf extracts but both are effective against the test organisms The result also shows that the extracts can inhibit and as well kill the test organisms indicated by their respective MIC and MBC values. These findings were compared with those produced by ampiclox, a reference antibiotic. The results were analyzed statistically using Chi-square at p = 0.05 level of significance *T. indica* can be harnessed to produce broad-spectrum antibiotics and can be a potential source of new classes of antibiotics that could be useful for infectious diseases caused by the test organisms.

[Ugoh, Sylvanus Chukwudi and Haruna, Isa Mohammed. **Phytochemical Screening and Antibacterial Activity of The Fruit and Leaf Extracts of *Tamarindus Indica* (Linn.)** *Rep Opinion* 2013;5(8):18-27]. (ISSN: 1553-9873). http://www.sciencepub.net/report. 3

Keywords: *Tamarindus indica,* Phytochemical screening, antibacterial activity, zone diameter of inhibition

Introduction

Plants have not only provided humankind with food, clothing, and flavors, cosmetic,

Ornamental, fumigants, insect deterrents and fragrance, but also served humanity in the treatment of ailments (Mbatchou *et al.,* 2011). Herbal medicine involves the use of plants for medicinal purposes. The term "herb" includes leaves, stems, flowers, fruits, seeds, rhizomes and bark (Palwinder *et al.,* 2011).

The frequency of life-threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries. All through history, irrespective of culture, plants have been a dependable source of medicine (Stockwell, 1988; Thomson, 1978).

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 (Bibitha *et al.,* 2002; Maghrani *et al.,* 2005).

Progress over the centuries towards a better understanding of a plant-derived medicine has depended on two factors that have gone hand-in-hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound(s) in the plant (Chhetri *et al.,* 2008). Enormous number of potential enemies such as bacteria, viruses, fungi, wild fires and sometimes flood, always surrounds plants. Plants protect themselves through a chemical defense system (VanWyk and Gericke, 2000). Therefore, it is expected that plants as chemical defense measures against their enemies produce biological active compounds. The search for biologically active agents is a part of a wider renaissance of scientific significance to bring into being new chemotherapeutics (Moundipa *et al.,* 2001). Plants synthesize very complex molecules with specific stereochemistry and can show biological activity with new modes of action (Houghton, 1996). Several useful drugs have been developed from medicinal plants use in the traditional medicine in the

treatment of variety of illnesses. According to Gilani and Atta-ur-Rahman (2005), the use of plant extracts or plant pure–derived chemicals to treat disease is a therapeutic modality, which has stood the test of time.

Many studies indicated that medicinal plants contain substances like peptide, unsaturated long chain fatty acids, aldehydes, alkaloids, essential oils, phenols and water or ethanol soluble compounds hence, most of the clinical drugs that are currently in use were derived from plants and developed because of their use in the traditional medicine (Mbatchou *et al.,* 2011).

African traditional medicine is the oldest and perhaps the most diverse of all medicine systems. Africa is considered the cradle of humankind with a rich biological and cultural diversity, and there are marked differences between different regions of this continent when it comes to healing practices (Gurib-Fakim, 2006). Medicinal and poisonous plants, including a diverse array of woody plants, have always played an important role in African life. The traditions of collecting plants, processing herbal remedies and applying them have been handed down from generation to generation (Mbatchou *et al.,* 2011).

Tamarindus indica (Tamarind) is derived from Arabic romanized *tamar hind* (Indian date) is a tree in the family Fabaceae indigenous to tropical Africa. The genus *Tamarindus* is a monotypic taxon, having only a single species. The tamarind tree produces edible, pod-like fruit which are used extensively in cuisines around the world (Propenoe, 1974). The common names giving to tamarindus include Tamarind (English); In Colombia, the Dominican Republic, Mexico, Puerto Rico, Venezuela and throughout the Lusosphere, it is called *tamarindo*. In the Caribbean, tamarind is sometimes called *tamón* (Morton, 1987). In Arabic, it is called *tamr hindi*; In Ghana, it is called *dawadawa*. In Malawi, it is called *bwemba*. In Zambia (nyanja) it is called *viwawasha*. In the Kiswahili language of east and central Africa, it is called *kwaju* while it common names in Nigeria include tsamiya (Hausa), jatami (Fulani), ajagbon (Yoruba), ichekun oyibo (Igbo), tamsugu (Kanuri), and the Nupes called it darachi (Keay *et al.,* 1989).

Tamarindus indica has been used traditionally to treat many diseases in different part of the world (Doughari, 2006). In northern Nigeria, fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorders, general body pain, jaundice, and yellow fever, and as blood tonic and skin cleanser. Particularly in Kaduna State against trypanosomiasis in domestic animals and in Bauchi State against guinea worms (Atawodi *et al.,* 2002; Fabiyi *et al.,* 1993). In Indonesia, Malaysia and the Philippines and Javanese traditional medicine, *asem* leaves are used as herbal infusion for malarial fever, the fruit juice as an antiseptic, and for scurvy and even cough cure. Throughout Southeast Asia, fruit of the tamarind is used a poultice applied to foreheads of fever sufferers (Doughari, 2006). The pulp has been documented in both the British and American pharmacopoeias as anti-pyretic, antiscorbutic, laxative, carminative and remedy for biliousness and bile disorder and the leaves have antihelmintic and vermifuge properties, destroying intestinal parasites (Pamploma-Roger, 1999).

It has been widely reported that the medicinal value of plants lies in the chemical (bioactive) substances present in the plants (Chhetri *et al.,* 2008). For instance, tannins are widely known for their astringent properties, which hasten wound healing and ameliorate inflamed mucous membrane (Mota *et al.,* 1985; Tyler *et al.,* 1988). Alkaloids, according to Zee-Cheng (1997), a very good antihypertensive and detoxifying agents. Saponins may have antidiarrhoeal properties according to Al-Rehaily *et al.* (2001). Also, flavanoids have antimicrobial (Narayana *et al.,* 2001), anti-inflammatory (Middleton *et al.,* 2000), antioxidant (Parker *et al.,* 1999) and anti-tumor (Inoue and Jackson, 1999) activities. Moreover, steroids have anti-nociceptive properties (Miguel *et al.,* 1996) while the presence of terpenoids in a plant could confer purgative properties on the plant (Bosland, 2006). The presence of anthraquinone in a plant may, however, not confer any advantage or disadvantage in the therapeutic value of the plant as its therapeutic applications and effects are vaguely understood (Sofowara, 1993; Awoyinka *et al.,* 2007).

Materials and Methods

The research work was carried out in the Microbiology Laboratory of the Department of Biological Sciences, University of Abuja, and Abuja-Nigeria.

Collection and Identification of the Sample Plant: Fresh leaves of *T. indica* was collected from University of Abuja Permanent Site while the fresh fruits of the plants was bought from a herbalist in Gwagwalada market, Gwagwalada, Federal Capital Territory (FCT), Abuja, Nigeria in September, 2012. The plants materials were authenticated by Mr. Segun O. of the department of Biological Science, University of Abuja, Gwagwalada-Abuja.

Preparation of the Plant Materials: This was carried out according to the method described by Predrag *et al.* (2005) and Mann *et al.* (2011). The fresh leaves and fruits of the plant were shade-dried at room temperature till they become properly dried to make micronization easy. The dried samples were crushed (for the fruit, the seeds were removed first) to smaller granules using mortar and pestle. The

granules were further reduced to powder with the use of laboratory blender. This was done to allow for maximum penetration of extracting solvents (ethanol, methanol and water) into the micronized samples allowing for the release of active components in the plant materials.

Extraction Procedure: This was done according to the methods describe by Kubmarawa *et al.* (2007) and Ngulde *et al.* (2010). Approximately 50g of the powdered leaves and fruit was weighed with weighing balance and dissolved in 500ml of water, ethanol and methanol respectively (that is, in the ratio 1:10). The mixtures were agitated at 30 minutes interval for 3 hours and then soaked for 3 days. The soaked materials were filtered into clean containers using Whatman's No. 1 filter paper. The resulting filtrates were evaporated to dryness using a boiling water bath. The yields were separately weighed and kept in a refrigerator for further use.

Extract and Drug concentration Preparation: Four different concentrations (500, 250, 100 and 50mg/ml) of the six extracts; Aqueous Leaves (AL), Aqueous Fruit (AF), Ethanol Leaves (EL), Ethanol Fruit (EF), Methanol Leaves (ML) and Methanol Fruit (MF) were prepared in sterile sample bottles by dissolving approximately 500mg, 250mg, 100mg and 50mg respectively of each extract in 1ml of Dimethyl sulfoxide (DMSO).

Also, the same four different concentrations (500, 250, 100 and 50mg/ml) of Ampiclox were at the same time prepared by dissolving 500mg, 250mg, 100mg and 50mg of the powdered ampiclox in 1ml of DMSO each respectively in a sterile bottle containers. The ampiclox is to serve as controls for test bacteria (Mallikharjuna *et al.,* 2010; Nascimento *et al.,* 2000).

Phytochemical Screening: Phytochemical screening was conducted to determine the presence of natural products (alkaloids, anthraquinones, glycosides, flavonoids, phlobatannins, reducing sugars, saponins, steroids and tannins) in the extracts obtained from the leaf and fruit of *T. indica* (Trease and Evans 1989; Harbone, 1998; sofowora, 1993).

Evaluation of Antimicrobial Activity of the Plant Extracts: Antimicrobial activity was assessed by the agar well diffusion method (Kinsbury and Wagner, 1990). Approximately 20ml of prepared molten Mueller Hinton agar was poured into a set of well labeled sterile Petri dishes under aseptic conditions and was allowed to solidify. Each plate was inoculated with about 200µl of pure cultures of the test organisms and was evenly spread using a sterile bent glass rod to ensure proper seeding of the organisms on the plates. After allowing the agar surface to dry, a 4mm (in diameter) sterile cork borer was used to make 4 wells on the agar plates at fairly equidistant positions. Then, 0.2ml of the 100, 200, 250 and 500mg/ml of the extracts and the control (ampliclox) were respectively dispensed in their corresponding wells for each microorganism (Karou *et al.,* 2006).

The same procedure was respectively repeated for the aqueous, ethanol and methanol leaf and fruit extracts of *T. indica*. The plates were allowed to stay for about 30 minutes for proper diffusion of the extracts and the control before being placed in the incubator at 37^0C for 24hours. After the incubation period, the plates were observed for zones of inhibition (indicated by clear zones) which were measured and recorded in millimeters using a transparent measuring ruler (Cheesbrough, 2000).

Minimum Inhibitory Concentration (MIC) Determination: The least concentration of the extract that is able to inhibit the growth of the microorganisms was determined by the macro broth dilution method as described by Spencer and Spencer (2004). To approximately 2ml of prepared nutrient broth in series of sterile and well labeled test tubes, 2 drops of the test organisms (previously diluted to 0.5 McFarland turbidity standard) and 2 drops of different concentrations (100, 200, 250 and 500mg/ml) were added. The same procedure was repeated on the test organisms using the different concentrations (100, 200, 250 and 500mg/ml) of the antibiotic (ampliclox), a test tube containing nutrient broth only was seeded with the test organisms as described above to serve as control. The tubes were then properly corked and incubated at 37^0C for 24hours. The MIC was read and recorded as the least concentration of the extract that shows no visible bacterial growth (Isu and Onyeagba, 1998).

Minimum Bactericidal Concentration (MBC) Determination: The MBC of potent extracts were determined by plating out the tubes that showed no growth (inhibited visible growth) during the MIC determination. Using a sterile wire loop, a loopful from each tube was subculture onto freshly prepared nutrient agar plates. Another sets nutrient agar plates were only streaked with the test organisms respectively to serve as control. All the plates were incubated at 37^0C for 24hours. The MBC was recorded as the least concentration at which no growth was observed (indicating 99.5% killing of the tested organisms).

Statistical Analysis: Chi-square Test was used (using the statistical package SPSS version 18) at $p = 0.05$ to analyze the results obtained. $P > 0.05$ was considered significant and $p < 0.05$ was not significant.

Results

Table 1: Phytochemical Constituents of Fruit and Leaf Extracts of *T. indica*

Bioactive Agent	AF	EF	MF	AL	EL	ML
Alkaloids	+	+	+	+	+	+
Anthraquinines	+	+	+	+	+	-
Glycosides	+	+	+	+	+	+
Flavonoids	+	+	+	-	+	+
Phlobatannins	-	+	+	-	+	+
Reducing Sugars	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Steroids & Terpenoids	-	-	-	-	-	-
Tannins	+	+	+	+	+	+

Key: + = Present, MF = Methanol fruit extract, - = Absent (not detected)
AL = Aqueous leaf extract, AF = Aqueous fruit extract, EL = Ethanol leaf extract
EF = Ethanol fruit extract, ML = Methanol leaf extract

Table 2: Zone Diameter of Inhibition (mm) of the Aqueous Fruit Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	0±0.0	5±0.8	11±0.6	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	0±0.0	12±0.7	23±1.0	39±0.5	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	0±0.0	10±0.4	20±1.1	35±0.6	8±0.6	18±0.5	31±0.8	43±0.7
P. aeruginosa	0±0.0	6±0.8	18±0.3	18±0.7	12±0.2	25±0.4	36±0.3	47±0.9

Table 3: Zone Diameter of Inhibition (mm) of the Ethanol Fruit Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	0±0.0	11±0.8	23±0.5	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	10±0.6	16±0.1	30±0.4	40±0.5	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	9±0.7	13±0.4	27±0.9	36±1.1	8±0.6	18±0.5	31±0.8	43±0.7
P. aeruginosa	0±0.0	8±0.8	22±0.6	30±0.3	12±0.2	25±0.4	36±0.3	47±0.9

Table 4: Zone Diameter of Inhibition (mm) of the Methanol Fruit Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	6±0.3	11±0.6	19±0.5	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	8±0.7	15±0.7	28±0.8	41±1.0	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	9±0.6	13±0.4	25±0.7	37±0.4	8±0.6	18±0.5	31±0.8	43±0.7
P. aeruginosa	0±0.0	6±0.9	20±0.6	30±0.5	12±0.2	25±0.4	36±0.3	47±0.9

Table 5: Zone Diameter of Inhibition (mm) of the Aqueous Leaf Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	0±0.0	0±0.0	8±0.8	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	0±0.0	0±0.0	0±0.0	0±0.0	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	0±0.0	6±0.8	10±1.0	12±0.7	8±0.6	18±0.5	31±0.8	43±0.7
P. aeruginosa	0±0.0	0±0.0	0±0.0	0±0.0	12±0.2	25±0.4	36±0.3	47±0.9

Table 6: Zone Diameter of Inhibition (mm) of the Ethanol Leaf Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	0±0.0	6±0.8	9±1.2	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	0±0.0	0±0.0	0±0.0	0±0.0	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	0±0.0	8±0.6	12±0.5	27±1.0	8±0.6	18±0.1	31±0.8	43±0.7
P. aeruginosa	0±0.0	8±0.9	11±0.4	21±0.8	12±0.2	25±0.4	36±0.3	47±0.9

Table 7: Zone Diameter of Inhibition (mm) of the Methanol Leaf Extracts of *T. indica.*

Test Organism	Conc. of Extracts (mg/ml)				Conc. of Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	0±0.0	0±0.0	0±0.0	0±0.0	10±0.3	21±0.5	34±0.1	45±0.4
E. coli	0±0.0	0±0.0	0±0.0	0±0.0	8±0.5	16±0.3	30±1.0	42±0.7
S. typhi	0±0.0	13±0.7	25±0.9	25±0.4	8±0.6	18±0.5	31±0.8	43±0.7
P. aeruginosa	0±0.0	0±0.0	10±1.1	21±0.6	12±0.2	25±0.4	36±0.3	47±0.9

Key: Control = Ampiclox

Table 8: Minimum Inhibitory Concentration (MIC) of Aqueous Fruit Extract of *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	-	-	-	-	-
E.coli	+	-	-	-	+	-	-	-
S. typhi	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	-	-	+	-	-	-

Table 9: Minimum Inhibitory Concentration (MIC) of Ethanol Fruit Extract of *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	-	-	-	-	-	-
E.coli	-	-	-	-	+	-	-	-
S. typhi	+	-	-	-	+	-	-	-
P. aeruginosa	+	+	-	-	+	-	-	-

Table 10: Minimum Inhibitory Concentration (MIC) of Methanol Fruit Extract of *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	-	-	-	-	-	-
E.coli	+	-	-	-	+	-	-	-
S. typhi	+	-	-	-	+	-	-	-
P. aeruginosa	+	+	-	-	+	-	-	-

Table 11: Minimum Inhibitory Concentration (MIC) of Aqueous Leaf Extract 0f *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	+	-	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	+	-	+	-	-	-
P. aeruginosa	+	+	+	+	+	-	-	-

Table 12: Minimum Inhibitory Concentration (MIC) of Ethanol Leaf Extract 0f *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	-	-	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	+	-	+	-	-	-

Table 13: Minimum Inhibitory Concentration (MIC) of Methanol Leaf Extract of *T. indica.*

Test Organism	MIC Value (mg/ml)				MIC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	+	-	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	+	-	+	-	-	-

Table 14: Minimum Bactericidal Concentration (MBC) of Aqueous Fruit Extract of *T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	+	+	-	-	-
E.coli	+	+	-	-	+	-	-	-
S. typhi	+	+	-	-	+	-	-	-
P. aeruginosa	+	+	+	-	+	+	-	-

Table 15: Minimum Bactericidal Concentration (MBC) of Ethanol Fruit Extract of *T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	-	+	-	-	-
E.coli	+	-	-	-	+	-	-	-
S. typhi	+	-	-	-	+	-	-	-
P. aeruginosa	+	+	-	-	+	+	-	-

Table 16: Minimum Bactericidal Concentration (MBC) of Methanol Fruit Extract of *T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	-	+	-	-	-
E.coli	+	+	-	-	+	-	-	-
S. typhi	+	-	-	-	+	-	-	-
P. aeruginosa	+	+	+	-	+	+	-	-

Table 17: Minimum Bactericidal Concentration (MBC) of Aqueous Leaf Extract of *T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	+	+	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	+	+	+	-	-	-
P. aeruginosa	+	+	+	+	+	+	-	-

Table 18: Minimum Bactericidal Concentration (MBC) of Ethanol Leaf Extract *of T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	-	+	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	+	-	+	-	-	-
P. aeruginosa	+	+	+	-	+	+	-	-

Table 19: Minimum Bactericidal Concentration (MBC) of Methanol Leaf Extract *of T. indica.*

Test Organism	MBC Value (mg/ml)				MBC Value for Control (mg/ml)			
	50	100	250	500	50	100	250	500
S. aureus	+	+	+	+	+	-	-	-
E.coli	+	+	+	+	+	-	-	-
S. typhi	+	+	+	+	+	-	-	-
P. aeruginosa	+	+	+	+	+	+	-	-

Key:
+ = Bacterial growth observed
- = No bacterial growth observed
Control = Ampiclox

DISCUSSION

Medicinal plants are the backbone of traditional medicine (Fransworth, 1994) and represent a rich source of potentially useful materials from which new chemotherapeutic agents can be developed (Srivastava *et al*., 1996; Chhetri *et al*., 2008; Mallikharjuna *et al*., 2010).

The results obtained from the antimicrobial activity of the fruit and leaf extracts of *T. indica* (clearly shown in table 2-7) suggest that both the fruit and the leaf extracts shows antimicrobial activity on the tested microorganisms which is in line with the research conducted by Doughari (2006); Srinivasan *et al.* (2001); Mohamedain *et al.* (1996). Though the fruit extract was found to be more effective than the leaf which may be due to the chemical constituents and properties of the fruit and which serves as storage material for the plant (Uchechukwu *et al*; 2011). Of all the solvents used for the extraction of the plant materials (aqueous, ethanol and methanol), the ethanol extract proved to have higher activity on the tested organisms. The reason for this may be that the active components were principally ethanol soluble and were stabilized by ethanol (Uchechukwu *et al.,* 2011) or the compounds extracted by ethanol interfered with the antimicrobial activity (Kuljanabhagavad *et al.,* 2010). However, the fruit was found to have the highest activity against *E. coli* (table 2-4) for all the extracts with the diameter of zones of inhibition ranging from 0-39mm for aqueous, 10-40mm for ethanol and 8-41mm for methanol extracts. But interestingly, the same *E. coli* was resistance to all the extracts of the leaf at different experimented concentrations contrary to the research conducted by Doughari (2006) and Uchechukwu *et al.* (2011) who reported the antimicrobial activity of the leaf extract against *E. coli*. Also, *S. typhi* is susceptible to the extracts of *T. indica* (table 2-7) though higher activities were found in the fruit extracts with zones of inhibition ranging from 0-37mm for methanol fruit extract (with the highest activity) and 0-27mm for the ethanol leaf extract (with the highest activity) and this correlate with the research by Uchechukwu *et al,* (2011). The aqueous, ethanol and methanol extracts of the plant show average antimicrobial activity against *P. aeruginosa* and just like with the other organisms, higher activity is shown with the fruit extracts. In fact, *P. aeruginosa* is resistant to only the leaf aqueous extract at all experimented concentrations which may be because water was not able to extract much of the active components of the leaf (Kuljanabhagavad *et al.,* 2010) but may have effect at higher concentrations. On all the tested organisms, lesser activity is shown against *S. aureus* (table 2-7) though the fruit extracts shows higher effect (0-23mm for ethanol extract) compare with the leaf extracts (0-9mm for ethanol extract) and this is in agreement with the research by Doughari (2006) but in disagreement with the research of Uchechukwu *et al.* (2011) who reported that *S. aureus* is resistant to the leaf extracts of the plant. The contradiction may be as the result of the difference in location from where the plants materials were gotten or the differences in extraction procedures.

From the overall antimicrobial activity of the plant (table 2-7), it has been seen that the effects of all the extracts except those resistance, are concentration dependent and this shows that with higher concentrations, more effect (antimicrobial activity) is expected even with the extracts resistant to.

From the assay of the MIC and MBC of the extracts of *T.indca* (table 8-19) the results revealed that the plants extracts can be both bacteriostatic and bactericidal (Palwinder *et al.,* 2011; Doughari, 2006; Warda *et al.,* 2007; Uchechukwu *et al.,* 2011). Although other extracts of the leaf were not able to inhibit the growth of the tested microorganisms at the highest experimented concentration (500mg/ml) like the aqueous and methanol leaf against *S. aureus* and *P. aeruginosa* in fact, *E. coli* is completely resistant to all the extracts of the leaf at the tested concentrations (table 12-14). Interestingly, lower MIC values are seen with fruit extracts where the 50mg/ml of the ethanol fruit extract inhibited the growth of *E. coli* and higher values are seen with *S. aureus* where the 500mg/ml of the aqueous extracts inhibited it growth (Doughari, 2006). From table 7-9, the results shows that the leaf extracts were more bacteriostatic rather than bactericidal. However, some leaf extracts killed the

microorganisms like the ethanol extracts but at rather higher concentrations (500mg/ml).

It has been widely observed and accepted that the antimicrobial activity of plants lies in the bioactive phytocomponents present in the plants (Veermuthu *et al.,* 2006; Rojas *et al.,* 1992) and the components are the products of secondary metabolites of plants that serves as defense mechanisms against predation by microorganisms, insects and herbivores (Nascimento *et al.,* 2000; El-mahmood, 2009). The presence of such phytochemicals in the different extracts of *T. indica* may accounted for it antimicrobial activity (Marjorie, 1999; Mahajan and Badgujar, 2008; Srinivasan *et al.,* 2001; Doughar, 2006). The quantitative analysis of these phytochemicals in *T. indica* revealed the presence of alkaloids, Anthraquinones, glycosides, flavonoids, phlobatannins, reducing sugars, saponins and tannins in the experimented plant parts (table 1). This finding is in line with the report of Uchechukwu *et al.* (2011) but differ in the detection of steroids and terpenoids (in the ethanolic fruit extract) which was found to be absent in all the extracts during the experiment. Also, Doughari (2006) reported the presence of phlobatannins in the aqueous leaf extract but in contradiction, it was absent in this study. These differences may be due to the difference in environmental conditions and geographical locations of the place where the plant materials were obtained or the use of different methods and/or procedures (Kubmarawa *et al.,* 2007).

The general antimicrobial activity of *T. indica* can be compared with that of ampiclox (standard antibiotic), especially the fruit extracts. From the results in table 2-7 the activity of ethanolic fruit extract against *E. coli* is the same with that of the ampiclox at 250mg/ml with zone of inhibition of 30mm both. So also, for the MIC results in table 8-10, most of the values are the same or almost the same with that of the ampiclox. In fact, at 50mg/ml (of ethanolic fruit extract) growth of *E. coli* was inhibited while it was the 100mg/ml of the ampiclox that inhibited same organisms (and this shows that the extract is more active than the ampiclox). For MBC results, most of the values are the same while in other cases, that of the plant extracts are higher.

Statistically, the analysis shows that there was significant difference ($p<0.05$) in the activities of the extracts on the test organisms. Although, some results however, showed no significant difference ($p>0.05$). And this means that the responses of the organisms to the extract concentrations were markedly different, and usually, higher concentrations will produce greater susceptibility of organism while for others, the responses of the organisms were similar independent on the concentration of the extracts.

CONCLUSION

T. indica is a medicinal herb that could be considered for integration into orthodox health care given that it is also consumed as food or beverage and, therefore, generally regarded as safe. The antibacterial activity exhibited by the ethanolic extracts is significant but however, the fruit which is the part commonly used by herbal practitioners, showed better activity than the leaf. The bacterial strains used for this work were those involved in enteric disturbance, food borne diseases, skin and soft tissue infections which are amongst the most common diseases of concern in the tropics. Medicinal plants like *T. indica* however, may represent new sources of antimicrobials with suitable, biologically active components that can establish a scientific base for the use of plants in modern medicine. These local ethno-medical preparations and prescriptions of plant sources should be scientifically evaluated and then disseminated properly and the knowledge about the botanical preparartion of traditional sources of medicinal plants can be extended for future investigation into the field of pharmacology, phytochemistry, ethnobotany and other biological actions for drug discovery.

REFERENCE

1 Al-Rehaily, A.J., El-Tahir, K.E.H., Mossa, J.S. and Rafatullah, S. (2001). Pharmacological studies of various extracts from hexane extract of *Ticlea nobilis* in rodents. *Natural Products & Science,* 7: 76-82.

2 Atawodi, S.E., Ameh, D.A. and Ibrahim, S. (2002). Indigenous knowledge system for treatment of trypanosomiasis in Kaduna state of Nigeria. *Journal of Ethnopharmacology,* 79(2): 279-282.

3 Awoyinka, O.A., Balogun, I.O. and Ogunnowo, A.A. (2007). Phytochemical screening and *in vitro* bioactivity of *Cnidoscolus aconitifolius* (*Euphorbiaceae*). *Journal of Medical plants Research,* 1(3): 63-65.

4 Bibitha, B., Jisha, V.K., Salitha, C.V., Mohan, S. and Valsa, A.K. (2002). Antibacterial activity of different plant extracts. *Indian Journal of Microbiology,* 42: 361-363.

5 Bosland, P.W. (2006). Chiles: History, cultivation and uses. *Spices, Herb and Edible fungi.* InG Charalambus (ed.), Elsevier, New York: 347-366pp.

6 Cheesbrough, M. (2000). *District laboratory Practice in Tropical countries* (part 2). Cambridge University press, United Kingdom. Pp 235-238.

7 Chhetri, H.P., Yogol, N.S., Shernan, J., Anupa, K.C., Mansoor, S. and Thapa, P. (2008). Phytochemical and antimicrobial evaluations of

some medicinal plants of Nepal. *Kathmandu University Journal of Science, Engineering and Technology,* 1(5): 49-54.

8 Doughari, J.H. (2006). Antimicrobial activity of *Tamarindus indica* Linn. *Tropical Journal of Pharmaceutical Research,* 5(2): 597-603.

9 El-Mahmood, A.M. (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacteria associated with enteric infections. *Journal of Medicinal Plants Resources,* 3(7): 498-505.

10 Fabiyi, J.P., Kela, S.L., Tal, K.M. and Istifanus, W,A. (1993). Traditional therapyin the State of Bauchi-Nigeria. *Journal of Medicinal Plants,* 38(2): 193-195.

11 Fransworth, N.R. (1994). Ethnopharmacology and drug discovery. Ciba Foundation Symposium 185. *Wiley, Chichester,* Pp 42-59.

12 Gilani, A.H. and Atta-ur-Rahman, B. (2005). Trends in Ethnopharmacology. *Journal of Ethnopharmacology,* 100(2): 43-49.

13 Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Journal of Molecular of Medicine,* 27(1): 1.

14 Harbone. J.B. (1998). Phytochemical methods: *A Guide in Modern Techniques of Plants Analysis*. Chapman and Hall Ltd, London. Pp 182-190.

15 Houghton, P.J. (1996). Compound with anti-HIV activity from plants. *Transactions Royal Society Tropical Medicine Hygiene,* 90: 601-604.

16 Inoue, T. and Jackson, E.K. (1999). Strong antiproliferation effects of baicalcin in cultured rat hepatic stellate cells. *European Journal of Pharmacology,* 378: 129-135.

17 Isu, N.R. and Onyeagba, R.A. (1998). *Basic Practicals in Microbiology.* Fasmen Communications, Nigeria. Pp 140-141.

18 Karou, D., Aly, S., Antonella, C.,Saydou, Y., Carla, M.J., Vittorio, S., Alfred, C. and Traore, S. (2006). Antibacterial activity of alkaloids from *sida acuta. African Journal of Biotechnology,* 5(2): 195-200.

19 Keay, R.W., Onochie, C.F. and Stanfield, D.P. (1989). *Trees of Nigeria.* Clarendon press, Oxford. Pp 169-188.

20 Kingsbury, D.T. and Wagner, G.E. (1990). *Microbiology.* Williams and Wilkins, Maryland. Pp 77-82.

21 Kubmarawa, D., Ajoku, G.A., Enwerem, N.M. and Okorie, D. (2007). Preliminary phytochemical and antimicrobial screening of 50 medicinal plants from Nigeria. *African Journal of Biotechnology,* 6(14): 1690-1696.

22 Kuljanabhagavad, T., Sriubolmas, N. and Ruangrungsi, N. (2010). Chemical composition and antimicrobial activity of the essential oil from *Heracleum siamicum. Journal of Health Resources,* 24(2): 55-60.

23 Landi, A.P., Chrysostomo, T.N. and Azzolini, A.E. (2007). Effect of extract of tamarind fruitof the complement system: studies *in vitro* and in hamsters submitted to a cholesterol-enriched diet. *Journal of Food chemistry and Toxicology,* 45(8): 1487-1495.

24 Maghrani, M., Zeggwah, N., Michel, J. and Eddoukas, M. (2005). Antihypertensive effect of *Lepidium sativum* in spontaneous hypertensive rats. *Journal of Ethnopharmacology,* 102(1): 193-197.

25 Mahajan, R.T. and Badgujar, S.B. (2008). Pytochemical investigations of some Laticiferous plants belonging to Khandesh regionof Maharashtra. *Journal of Ethnobotany,* 12: 1145-1152.

26 Mallikharjuna, P.B., Seetharam, Y.N. and Radhamma, M.N. (2010). Phytochemical and antimicrobial studies of *Strycnos wallichiana steud Ex Dc. Journal o Phytology,* 2(3): 22-27.

27 Mann, A., Ifarajimi, O.R., Adewoye, A.T., Ukam, C., Udeme, E.E., Okorie, I.I., Sakpe, M.S., Ibrahim, D.R., Yahaya, Y.A., Kabir, A.Y. and Ogbadoyi, E.O. (2011). *In vitro* antitrypanosomal effects of some ethnomedicinal plants from Nupeland of North Central Nigeria. *Journal of traditional, complementary and Alternative Medicine,* 8(1): 15-21.

28 Marjorie, MC. (1999). Plant products as antimicrobial agents. *Journal of Clinical Microbiology (Rev.),* 12(4): 564-582.

29 Mbatchou, V.C., Ayebila, A.J. and Apea, O.B. (2011). Antibacterial activity of phytochemicals from *Acacia nilotica, Entada Africana and Mimosa pigra* Linn on *Salmonella typh. Journal of Animal and plants Sciences,* 10(1): 1248-1258.

30 Middleton, E.J.R., Kandawami, C. and Theoharides, T.C. (2000). The effects of plant flavonoids on mammalian cells. Implications for inflammation, heart disease and cancer. *Pharmacology Review,* 52: 673-751.

31 Miguel, O.G., Calixto, J.B., Santos, A.R., Messana, I., Ferrari, F., Filho, V.C., Pizzolatti, M.G. and Yunes, R.A. (1996). Chemical and preliminary analgesic evaluation of geranin and furosin isolated from *Phylanthus sellowianus, Planta. Med,.* 62(2): 146-149.

32 Mohamedain, K.M., Mohamed, O.S.A., Elbadwi, S.M.A. and Adam, S.E.I. (1996). Effect of feeding *Tamarindus indica* ripe fruit in Brown Hisex chicks. *Phytother. Res.,* 10: 631-634.

33 Morton, J.F. (1987). *Tamarindus indica: Fruit of Warm Climates*. Creative Resources Systems, Inc., Santa Ana, CA, USA. Pp 115-121.

34 Mota, M.L., Thomas, G. and Barbasa-Filhu, J.M. (1985). Antiinflamatory actions of tannins isolated from the bark of *Anarcadium occcidentale*. *Journal of Ethnopharmacology,* 13(3): 289-295.
35 Moundipa, F.P., Njayou, F.N., Yanditoum, S., Sonke, B. and Mbiapo, T.F. (2001). Medicinal plants used in the Bamoun region (West Cameroon) against jaundice and other liver disorders. *Cameroon Journal of Biological and Biochemical Sciences,* 2: 39-46.
36 Narayana, K.R., Reddy, M.S., Chaluvadi, M.R. and Krishna, D.R. (2001). Bioflavovoids: Classification, pharmacology, biochemical effects and therapeutic potentias. *Journal of Pharmacology,* 33: 2-16.
37 Nascimento, G.G.F., Locatelli, J., Freitus, P.C. and Silva, G.L. (2000). Antibacterial activity of plant extracts and phytochemical on antibiotic-resistant bacteria. *Brazillian Journal of Microbiology,* 31: 247-256.
38 Ngulde, S.T., Sanni, S., Sandabe, U.K. and Sani, D. (2010). Phytochemical and antimicrobial screening of the aqueous extract of *Cassia arereh Del*. Stem bark. *African Journal of Pharmacy and Pharmacology,* 4(8): 530-534.
39 Palwinder, K., Nilesh, K., Shivananda, T.N. and Gagandeep, K. (2011). Phytochemical screening and antimicrobial activity of the plant extracts of *Mimosa pudica* L. against selected microbes. *Journal of Medicinal Plants Research,* 5(22): 5356-5359.
40 Pamploma-Roger, G.D. (1999). *Encyclopedia of Medicinal Plants*. Education and health library. Madrid, Spain. Pp 536.s
41 Parker, L., Rimbach, G. and Vigil, F. (1999). Antioxidant activity and biological properties of pyrocynidin-rich extract from pine (*Pinus martona)* bark. *Biology and Medicine,* 27: 704-724.
42 Predrag, L., Hui, S., Uri, C., Hasswan, A. and Arieh, B. (2005). The effects of extracts prepared from leaves of *Pistacia lentiscus* in experimental liver disease. *Journal of Ethnopharmacology,* 100(1-2): 198-204.
43 Propenoe, W. (1974). *Manual of Tropical and Subtropical Fruits*. Hafner press. Pp 432-436.
44 Rojas, A., Hernandez, l., Pered-Miranda, R. and Mata, R. (1992). Screening for antimicrobial activity of crude drug extract and natural products from Mexican medicinal plants. *Journal of Ethnopharmacology,* 35: 111-115.
45 Sofowora, E.A. (1993). *Medicinal Plants and Traditional Medicines in Africa.* Chichester John Wiley & Sons, New York. Pp 97-145.
46 Spencer, A.L.R. and Spencer, J.F.T. (2004). *Public Health microbiology: Methods and Protocols*. Human press Inc. New Jersey, USA. Pp 325-327.
47 Srinivasan, D., Perumalsamy, L.P, Nathan, S. and Sures, T. (2001). Antimicrobial activity of certain Indian medicinal plants used in fokloric medicine. *Journal of Ethnopharmacology,* 94: 271-222.
48 Srivastava, J., Lambert, J. and Vietmeyer, N. (1996). Medicinal plants: An expanding role in development. *World Bank Technical Paper,* No. 320.
49 Stockwell, C. (1988). *Nature's Pharmacy*. Century Hutchinson Ltd., London, UK. Pp 27-32.
50 Thomson, W.A.R. (1978). *Medicines from the Earth*. McGraw-Hill Book Co. Maidenhead, UK. Pp 632-640.
51 Trease, G.E. and Evans, W.C. (1989). *Text Book of Pharmacognosy* (11th Ed.). Balliere Trinadal Can. Macmillan Publishers, London. Pp 239-241.
52 Tyler, V.E., Braddy, L.R. and Roberts, J.E. (1988). *Pharmacology*. Lea and Ferbiger, Philadelphia. Pp 85-90.
53 Uchechukwu, U.N., Obiiyeke, G.E., Vincent, N.C. and Anthony I.O. (2011). Assessment of *Tamarindus indica* extracts for antibacterial activity. *International Journal of Molecular Sciences,* 12: 6385-6396.
54 Van-Wyk, B. and Gerricke, N. (2000). *Peoples Plants:* A guide to useful plants of Southern Africa, Briza Publications, Pretoria. Pp 48-53.
55 Veermuthu, D., Muniappan, A. and Savarimuthu, I. (2006). Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamilnadu, India. BMC Compliment. *Journal of Alternative Medicine,* 6: 35
56 Warda, S., Abdel, G., Fathia, M. and Amel, O.B. (2007). Antibacterial activity of *Tamarindus indica* fruit and *Piper nigrum* seed. *Research Journal of Microbiology,* 2(11): 824-830.
57 Zee-cheng, R.K. (1997). Anticancer research on *Loranthaceae* plants. *Drug Future,* 22(5): 515-523.

7/29/2013