Phytochemical Screening and *Invitro* Antibacterial Effect of *Tamarindus Indica* L. Stem and Leaves On *E. Coli, S. Aureus and P. Mirabilis.*

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Abstract- This study was carried out to determine the phytochemical composition and antibacterial effect of *Tamarindus indica* on *E. coli, S. aureus* and *P. mirabilis*. The plant materials of *Tamarindus indica* stem bark and leaves were obtained from Ahmadu Bello University, Zaria Kaduna State. One hundred (100g) of the plant powder each, was extracted with methanol and chloroform by Soxhlet extraction method. All the extracts were subjected to standard phytochemical screening for the presence or absence of various secondary metabolites. The susceptibility test of the plant extracts on *E. coli*,*S. aureus* and *P. mirabilis* were done using agar well diffusion method. Ciprofloxacin (10 µg) was used as positive control. The phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, saponins, cardiac glycoside, steroids and carbohydrates. The antibacterial activity of stem and leaves showed that, the plant extract was effective in all the bacterial isolates, and the activity increased with increase in concentration of the plant extracts. Highest activity was showed by leaves methanol extract (LME) at 100mg/ml with zone of 16mm, and it has higher activity than ciprofloxacin which is the positive control with zone of 15mm.

Index Terms- Phytochemicals, Antibacterial effect, *Tamarindus indica*

I. INTRODUCTION

Medicinal plant is any plant in which one or more of its organs contain substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs (WHO, 2007). Medicinal plants are abundant source of antimicrobial molecules. A wide range of medicinal plants extracts are used to treat several infections as they have potential antimicrobial activity. Some of these bioactive molecules are screened and traded in market as raw materials for many herbal industries (Renisheya *et al*., 2011). In Pakistan, 80% of the population belonging to the rural areas depends on the traditional medicines (Munir *et al*., 2013). Tamarind is leguminous tree of genus *Tamarindus* which is monotypic with only species *indicum* (Bently and Trimen, 2004). *Tamarindus indica* belongs to the family Fabaceae and sub-family Caesalpinaceae and is a tropical evergreen tree native to Africa and Southern Asia. Its various parts such as seeds, root, leaves, bark and fruits have been extremely used in traditional India and African medication (Gunasena, 2000). *Tamarindus indica* is the third largest family of flowering plants with a total of 727 genera and 19,327 species. Tamarind grows well in full sun, clay, loamy, sandy, and acidic soil types and is drought resistance (Doughari, 2006). India is the world largest producer of tamarind, it is estimated that 300,000 tons are produced annually (ElSiddiq, 2006). One of the most known health benefits of tamarind is its use as medicines since the ancient times. It has been known to be useful for treating constipation and liver problems among others (Aida *et al*., 2001). For years tamarind has proven to be particularly useful for treating liver and gall disorders and has been studied severally on the role it plays in treating bile problems. Tamarind is particularly useful for managing pain and inflammation on joints. It has been seen that leaves and pulp crushed and applied on swollen joints provides great relief and reduces inflammation. Tamarind is also used for treating sore throats (Vyas *et al*., 2009).

II. MATERIALS AND METHODS

Study area.

Tamarindus indica stem bark and leaves were collected from Ahmadu Bello University, Zaria Kaduna State. The plant was identified at herbarium unit Department of Botany, Ahmadu Bello University, Zaria.

Authentication of plant materials

The stem bark and leaves of *Tamarindus indica* were carefully washed with tap water and rinsed with distilled water. All the plant materials were spread in a clean stainless-steel and air dried under shade at room temperature. Dried leaves and bark were crush and ground into coarse and powder using mortar and pestle. The powdered materials were kept in a nylon bag.

3.2.1 Preparation of the extracts

This was carried out according to the procedure describe by Sigaroodi *et al*., (2008). One hundred (100g) each of dried powdered plant materials were soaked in chloroform and methanol at room temperature for 36hours. The extraction was carried out with solvent under shaking conditions. The respective extracts were then filtered through Whatmann filter paper and aqueous extract was obtained and solvent was removed completely under reduced pressure. Filtered extract was collected in conical flask. The chloroform and methanol extracts were evaporated by rotary

evaporator at 45ºC and the crude extracts were obtained for the determination of antibacterial activity.

Phytochemical screening of *Tamarindus indica*

All the extracts were subjected to standard phytochemical qualitative screening for secondary metabolites as described by Sofowora (2006).

Preparation of different concentrations of the extract

Ten 10mg each of the plants (stem bark and leaf) extracts were dissolved in 10ml of distilled water to form two stock solutions of each extract. From the first stock solutions of each extracts, serial dilutions was carried out to prepare 4 different concentrations as,100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml respectively.

Standardization of inoculum

The bacterial isolates of *Escherichia coli*, *Staphylococcus aureus* and *Proteus mirabilis* were collected from microbiology laboratory, Department of microbiology, Ahmadu Bello University, Zaria. An overnight culture of the bacterial isolates were prepared in nutrient broth 0.1 ml of the nutrient broth was emulsified into 20ml of physiological saline, until the turbidity of the suspension of test organism matches with 0.5 Mcfarland turbidity standards (Deeni and Hussain, 1994)

Determination of inhibitory activity (susceptibility test) of the extract using agar well diffusion:

The standardized inocula of the bacterial isolate were streaked on sterilized Mueller hinton agar plates respectively with the aid of sterile wire loop. Four wells were bored on each inoculated agar plate with a sterile cork borer. The well was properly labeled according to different concentrations of the extract prepared which were 100, 50, 25 and 12.5mg/ml respectively. Each well was filled up with approximately 0.2ml of the extract.

The inoculated plates with the extract were allowed to stay on the bench for about one hour; this is to enable the extract to diffuse on the agar. The plates were then incubated at 37ºc for 24 hours. At the end of incubation period, the plates were observed for any evidence of inhibition which will appear as a clear zone that was completely devoid of growth around the wells (zone of inhibition). The diameter of zones was measured using transparent ruler calibrater in millimeter and the result was recorded.

III. STATISTICAL ANALYSIS

Data obtained were subjected to one way analysis of varience (ANOVA) using SPSS version 20.0 to determine the mean zones of inhibition of the plant extract on *E. coli*, *S. aureus* and *P. mirabilis*.

IV. RESULTS AND DICUSION

A total of three bacterial isolates have been used in the present study to assess the antibacterial effect of methanolic and chloroform extracts of *Tamarindus indica* The antibacterial activity of the extracts were determined by agar well diffusion method. Table 2 showed the antibacterial effect of *Tamarindus indica*, the result showed that, Stem bark methanol extract (SME) has higher activity at 100mg/ml concentration with mean zone of 13mm. The least activity was showed in stem bark chloroform extract (SCE) and leaves chloroform extract (LCE) at 25mg/ml concentrations with zone of 4mm.The result also showed that, *S. aureus* was susceptible to all the concentrations of stem bark methanol extract (SME). The activity of the extract against *E. coli* showed that there was no significant difference P>0.05 in the activity of *Tamarindus indica* stem bark methanol extract between 100 and 50mg/ml concentrations. Highest activity was showed by leaves methanol extract (LME) at 100mg/ml with zone of 16mm, and it has higher activity than ciprofloxacin which is the positive control with zone of 15mm. Ciprofloxacin has the higher activity on *P. mirabilis* than the plant extracts with zone of 19mm. *Proteus mirabilis* was susceptible to all the concentrations of stem bark methanol extract (SME) and leaves methanol extract (LME).

The result of *Tamarindus indica* extract (Table 1),showed that, stem bark and leaf chloroform extracts revealed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, saponin and carbohydrate. The result of phytoconstituents of *T. indica* is similar to what have been reported by (Subramanya *et al*., 2012).

Table 1: Phytochemical screening of *Tamarindus indica* stem bark and leaves extracts.

S/N	Phytochemicals	SME	SCE	LME	LCE
1	Alkaloids	+	+	_	+
2	Flavonoids	-	+	+	+
3	Tannins	-	+	+	+
4	Saponins	-	+	+	+
5	Cardiac glycoside	+	+	+	+
6	Streriods	+	+	+	+
7	Anthraquinon	-	-	-	-
8	Carbohydrates	+	+	+	+

Key: SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, +=Present, - =Not present.

Table 2: Mean zones of inhibition of *Tamarindus indica* extract

Bacteria		Extract			
	Concentration	SME	SCE	LME	LCE
S. aureus	DMSO (-)	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{e}
	C (+)	21.00±0.29^{a}	21.00±0.29^{a}	21.00±0.29^{a}	21.00±0.29^{a}
	100	13.00±0.29^{b}	8.00±0.58^{b}	5.00±0.29^{b}	8.00±0.58^{b}
	50	9.00±0.28^{c}	6.00±0.29^{c}	2.00±0.29^{c}	6.00±1.16^{c}
	25	8.00±0.58^{c}	4.00±0.29^{d}	0.00±0.00^{d}	4.00±0.29^{d}
	12.5	5.00±0.86^{d}	0.00±0.00^{e}	0.00±0.00^{d}	0.00±0.00^{e}
	P-value	0.000	0.000	0.000	0.000
E. coli					
	Concentration	SME	SCE	LME	LCE
	DMSO (-)	0.00±0.00^{c}	0.00±0.00^{e}	0.00±0.00^{e}	0.00±0.00^{e}
	C (+)	15.00±0.58^{a}	15.00±0.58^{a}	15.00±0.58^{a}	15.00±0.58^{a}
	100	13.00±0.58^{a}	6.00±0.29^{b}	16.00±0.58^{a}	10.00±0.48^{b}
	50	13.33±3.58^{a}	4.00±0.12^{c}	12.00±0.57^{b}	8.00±0.23^{c}
	25	8.00±0.57^{b}	2.00±0.29^{d}	7.00±0.35^{c}	4.97±0.26^{d}
	12.5	6.00±0.12^{b}	0.00±0.00^{e}	4.00±0.17^{d}	0.00±0.00^{e}
	P-value	0.000	0.000	0.000	0.000
P. mirabilis					
	Concentration	SME	SCE	LME	LCE
	DMSO (-)	0.00±0.00^{f}	0.00±0.00^{d}	0.00±0.00^{f}	0.00±0.00^{e}
	C (+)	19.33±0.73^{a}	19.33±0.73^{a}	19.33±0.73^{a}	19.33±0.73^{a}
	100	12.00±0.12^{b}	7.00±0.17^{b}	14.00±0.58^{b}	9.00±0.29^{b}
	50	9.00±0.52^{c}	5.00±0.58^{c}	11.00±1.15^{c}	5.00±0.58^{c}
	25	7.00±0.06^{d}	4.00±0.17^{c}	6.00±0.12^{d}	3.00±0.06^{d}
	12.5	4.00±0.40^{e}	0.00±0.00^{d}	4.00±0.35^{e}	0.00±0.00^{e}
	P-value	0.000	0.000	0.000	0.000

Mean values with different superscripts in the same column are significantly different.
SME=Stem bark methanol extract, SCE=Stem bark chloroform extract, LME=Leaf methanol extract, LCE=Leaf chloroform extract, DMSO=Negative control, C=Ciprofloxacin positive control

V. CONCLUSION

Tamarindus indica contained significant phytochemicals (secondary metabolites). *Tamarindus indica* extract revealed antibacterial activity against the bacterial isolates, *E. coli*, *S. aureus* and *P. mirabilis*. The activity of the extracts on the test organisms increased with increase in concentration.

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