Sky Journal of Microbiology Research Vol. 2(9), pp. 067 - 071, December, 2014
Available online http://www.skyjournals.org/SJMR
ISSN 2315-876X ©2014 Sky Journals

Full Length Research Paper

Biological activity and phytochemical constituents of *Tamarindus indica* stem bark extracts

Yusha'u M.[1]*, Gabari D. A.[1], Dabo N. T[1]., Hassan A.[2] and Dahiru M.[2]

[1]Department of Microbiology, Bayero University, P. M. B., 3011, Kano, Nigeria.
[2]Department of Biological Sciences, Federal University, Kashere, P. M. B., 0182, Gombe State, Nigeria.

Accepted 21 November, 2014

Tamarindus indica **(L.), Leguminosae, is a plant mostly found and used medicinally in West Africa including Nigeria. The stem-bark powder of the plant was extracted with ethanol using percolation method and the extract was further fractionated in 50 ml each of chloroform and distilled water (1:1). The extract and fractions were tested for the presence of secondary metabolites using standard procedures. They were further tested for antibacterial activity against clinical isolates of *Salmonella typhi, Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Vibrio cholerae and Vibrio parahaemolyticus* using disc diffusion and broth dilution techniques. The results of phytochemical screening indicated the presence of secondary metabolites including alkaloids, glycosides, reducing sugars, tannins and saponins in ethanol extract and water fraction of the extract. Bioassay test results showed that *K. pneumoniae, P. mirabilis, V. cholerae and V. parahaemolyticus* were sensitive to ethanol extract and water fraction of the plant with highest sensitivity to water fraction against *E. coli* (19 mm) at 125 µg/disc using disc diffusion test and having MIC and MBC values of 500 and 1000 µg/ml respectively. The plant extract and fraction were found to show inhibitory activity against the test isolates which may be related to the presence secondary metabolites, some of which are reported to be responsible for antimicrobial properties of medicinal plants. The results suggest that *T. indica* used in this study has the potential for the production of drugs against bacterial infections.**

Key words: *Tamarindus indica,* secondary metabolites, antibacterial activity, bacteria.

INTRODUCTION

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most common in Asia, Latin America and Africa and is reported to have minimal side effects (Bibitha et al., 2002; Maghrani et al., 2005). In recent years, pharmaceutical companies have spent a lot of money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. *Tamarindus indica* Linn (commonly called Tamarind), family Fabaceae, subfamily *caesalpiniaceae* is a tropical evergreen tree native to fertile areas throughout Africa and Southern Asia. It is widely cultivated as an ornamental tree and for its acidic fruits used in making drinks and a popular component of many decoctions used as health remedies. *T. indica* is used as a traditional medicine in India, Sudan, Nigeria, Bangladash and most of the tropical countries. *T. indica* is rich in nutrients and plays an important role in human nutrition, mainly in the developing countries (Mohamed and Rangappa, 1992; Yanez et al., 1995). It contains high level of crude protein with many essential amino acids, which help to build strong and efficient muscles. It is also high in carbohydrate which provides energy and also rich in minerals such as potassium, phosphorus, calcium and magnesium. It can also provide smaller amount of iron and vitamin A. Phytochemical investigations carried revealed the presence of many active constituents, such as phenolic compounds, cardiac glycosides (Rasu et al., 1989), malic acid (Kobayashi et al., 1996), tartaric acid, mucilage, pectin, arabinose, xylose, galactose glucose and uronic acid (Ibrahim and Abbas 1995; Coutino-Rodriguez et al., 2001).

***Corresponding author. E-mail: myushau.mcb@buk.edu.ng. Tel.: +2348034589499.

In Northern Nigeria, the fresh stem bark and leaves are used as decoction mixed with potash for the treatment of stomach disorder, general body pain, jaundice, yellow fever and as blood tonic and skin cleanser. Tamarind preparations are used as aid in the restoration of sensation in cases of paralysis, reduction of body temperature in fevers, and as laxatives, expectorant (Komutarin et al., 2004). The plant parts have been extensively studied in terms of pharmacological activity of its major compounds and results indicate potent antibacterial, antifungal, hypoglycaemic, cholesterolemic (Khanzada et al., 2008), hypolipomic, antioxidant (Tsuda et al., 1994; Martinello et al., 2006), antihepatotoxic (Joyeux et al., 1995), anti-inflammatory (Rimbau et al., 1999), antimutagenic (Ramos et al., 2003) and antidiabetic (Maiti et al., 2004) properties. Shehla et al. (2007) isolated two triterpenes, lupanone and lupeol, from methanolic extract of the leaves of *T. indica*. Ingestion of *T. indica* fruit has been reported to have an additional beneficial effect on the mobilization of deposited fluoride from bone, by enhancing urinary excretion of fluoride (Khandare et al., 2004). 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., 2001). 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). Because of its wide usage and availability, this study was aimed to investigate the antimicrobial activity of stem back of the plant against some clinical isolates.

MATERIALS AND METHODS

Collection of plant material

Fresh stem bark of the plant was collected from the Department of Biological Sciences and was identified at the Department of Biological Sciences Department, Bayero University, Kano. It was air dried and grounded into powder using mortar and pestle separately in the laboratory in accordance with Mukhtar and Tukur (1999).

Extraction procedures

The protocol described by Fatope and Hamisu (1993) was followed. 100 g of powdered plant material was soaked in 1 l of 95% ethanol at room temperature for two weeks. The filtrate was evaporated using the rotary evaporator. Part of the residue was retained as crude fraction while the other part was further fractionated between chloroform and distilled water in a ratio of 1:1 (50 ml). Both fractions were evaporated at room temperature and stored in a freezer prior to use.

Phytochemical screening

The extracts were subjected to phytochemical tests after filtration before they were concentrated to determine the groups of secondary metabolites present in the plant material according to Ciulci (1994), Oyeleke and Manga (2008) and Brain and Turner (1975).

Biochemical identification for the test organisms

The test organisms were clinical isolates obtained from Murtala Muhammad Specialist Hospital Kano and further subjected to biochemical tests for re-identification (Cheesbrough, 2005; Oyeleke and Manga, 2008). In addition, the following biochemical tests were carried out. indole, motility, citrate utilization, urease production, ornithine decarboxylase, methyl red, hydrogen sulfide production as well as acid and gas production according to standard procedures (Cheesbrough, 2005).

Disc preparation

The extracts were dissolved using di-methyl sulphoxide (DMSO). Aqueous extract was dissolved using sterile distilled water. The extract (0.002 g) was dissolved in 1 ml of DMSO as the stock solution. 0.5 ml of the stock solution was taken and placed into 50 sterile improvised Whatman No. 1 filter paper discs that take up 0.01 ml to make the required disc potency and was labeled 20 µg. Half ml of DMSO was added into the remaining stock solution making 1ml. 0.5 ml was taken and placed into another bottle containing 50 filter paper discs and labeled 10 µg/disc, 0.5 ml of DMSO was added, another 0.5 ml was taking and placed into another 50 filter paper discs and labeled 5 µg/disc. The same process of serial doubling dilution as explained above was employed in the preparation of organic solvent extract discs.

Standardization of inoculums

Few colonies of the overnight growth of confirmed isolates to be tested were dispensed in sterile normal saline to match the 0.5 McFarland standards for sensitivity tests as described by NCCLS (2008).

Bioassay

Disc diffusion test

The sensitivity testing was achieved by disc diffusion method (NCCLS, 2008). Standardized inocula of the isolates were swabbed onto the surface of prepared and solidified Mueller Hinton Agar in separate petri dishes. This was followed by placing the prepared discs onto the surface of inoculated media at intervals in a clockwise

Table 1. Properties of extracts obtained from the stem bark of *Tamarindus indica* using different solvents.

Physical Parameters	Ethanol extract	Aqueous extract	Chloroform extract
Weight of extract	25.50g	3.50g	0.26g
Percentage yield (%)	25.5	3.5	0.25
Color	Brown	Brown	Brown
Texture	Sticky	Crystalline	Soft

Table 2. Phytochemical constituents of *Tamarindus indica* stem back extracts.

Test	Ethanol extract	Aqueous extract	Chloroform extract
Alkaloids	+	+	-
Flavonoids	-	-	-
Glycosides	+	+	-
Reducing sugars	+	+	-
Tannins	+	+	-
Saponins	+	+	-

direction using ciprofloxacin as a control. The plates were incubated at 37°C for 24 h before observation and measurement of zones of inhibition.

Minimum inhibitory concentration

MIC was determined by preparing various concentrations of the extracts by serial doubling dilution and incorporated into test tubes containing 2 ml nutrient broth. 0.1 ml of standardized inocula of each isolate was introduced. Tubes containing broth and plant extracts without inocula served as positive control while tubes containing broth and inocula served as negative control. The set of tubes were incubated aerobically at 35°C for 24 h after which the lowest concentration that showed no evidence of growth was recorded as the MIC (NCCLS, 2008).

Minimum bactericidal concentration

Nutrient agar plates were inoculated with sample from each of the tubes that show no turbidity and the plates were incubated at 37°C for 24 h. The highest dilution that yielded no bacterial colony was taken as the MBC (NCCLS, 2008).

RESULTS AND DISCUSSION

The results of extraction yield higher extract from the plant material using methanol as solvent for extraction as shown in Table 1. This may be due to its stronger extraction capacity. However, both water and ethanol extracts contain more secondary metabolites than chloroform extract which may be related to the polarity of both solvent and the constituents of the extracts. The results of this study showed that methanol extracted more components than water and chloroform (having the least percent extraction) from *T. indica* which may be associated with the polarity of the components making them more soluble in more polar (methanol and water) than least polar solvent (chloroform) that may be responsible for the variation in physical properties of the extracts respectively.

Results of phytochemical screening of the plant material indicated presence of some secondary metabolites including alkaloids, reducing sugars, glycosides, tannins and saponins as shown in Table 2. The plant was found to contain some secondary metabolites such as alkaloids, reducing sugars, glycosides, saponins and tannins in one or more of the extracts (Table 2). This may be due to the fact that some solvents used for the extraction were unable to dissolve appreciable amount of the metabolite to be detected by phytochemical screening procedure employed. The presence of some secondary metabolites like alkaloids, reducing sugars, glycosides and saponins was in agreement with the works of Doughari, (2006) and Ahmad and Abdul (2014). However, the finding of tannins was not in line with that of Ahmad and Abdul (2014). Some of these metabolites were reported to be responsible for antimicrobial activity associated with some ethno-medicinal plants (Singh and Bhat, 2003).

In-vitro sensitivity tests using disc diffusion method indicated, the extracts were slightly active against the test organisms as follows; *E. coli* (12 mm), *Klebsiella* spp. (10 mm), *Proteus* spp. (9 mm), *Salmonella* spp. (11 mm). The observed 12 mm zone of inhibition for *E. coli* correlates with the work of Ahmad and Abdul (2014), while that of *Klebsiella* spp was slightly lower. The zones of inhibition for *E. coli*, *Proteus* spp. and *Salmonella* spp. reported in this work, did not conform with that of

Table 3. Antibacterial activity of *Tamarindus indica* stem bark extracts on some clinical isolates.

Isolates	EE (µg/ml)				AE (µg/ml)				CE (µg/ml)				CPX (µg/ml)			
	125	250	500	1000	125	250	500	1000	125	250	500	1000	125	250	500	1000
Escherichia coli	6	6	6	6	18	19	20	22	6	6	6	6	40	41	42	43
Proteus mirabilis	6	8	11	12	6	6	10	11	6	6	6	6	28	30	34	36
Vibrio cholerae	6	6	12	15	8	9	10	11	6	6	6	6	26	30	33	35
V. parahaemolyticus	6	6	8	9	6	6	8	10	6	6	6	6	28	29	30	34
Klebsiella pneumonia	6	8	11	12	6	6	10	11	6	6	6	6	28	30	34	36
Salmonella typhi	6	6	6	6	6	6	6	6	6	6	6	6	20	22	23	24

Key. EE - Ethanol extract, AE - Aqueous extract, CE - Chloroform extract, CPX – Ciprofloxacin.

Table 4. Antibacterial activity of *Tamarindus indica* stem back extracts on some clinical isolates.

Isolates	EE (µg/ml)		AE (µg/ml)		CE (µg/ml)	
	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	**	**	500	1000	**	**
Proteus mirabilis	500	1000	1000	1000	**	**
Vibrio cholerae	500	1000	500	1000	**	**
Vibrio parahaemolyticus	500	1000	500	1000	**	**
Klebsiella pneumoniae	500	1000	500	1000	**	**

Key. EE - Ethanol extract, AE - Aqueous extract, CE - Chloroform extract, MIC – Minimum inhibitory concentration, MBC – Minimum bacteriocidal concentration, ** - MIC or MBC greater than 2000µg/m

Doughari (2006), the zones of inhibition reported in his research on these organisms, doubled those of each reported in this study. *E. coli* and *P. mirabilis* were sensitive to aqueous extracts with inhibition zone diameters of 20 mm and 10 mm in 500 µg/disc respectively. *V. cholera* and *V. paraheamolyticus* were also sensitive to 500 µg/disc concentration at 12 mm and 8 mm respectively of the ethanolic extract. Aqueous extract was equally sensitive to *Vibrio* spp at 500 µg/disc. However, chloform extract was least sensitive to the tested organisms at all concentrations (Table 3). *E. coli* was only inhibited at a concentration of more than 2000 µg/ml of both ethanolic and chloroform extracts, while *P.* mirabilis and other species of bacteria tested were found to be inhibited at 500 µg/ml of the ethanolic extract but vary in their MBC values.

The MBC values for all the extracts ranged from 1000 µg/ml to more than 2000 µg/ml, with highest MBC values of chloroform extract on all the isolates. This indicated chloroform extract was more active than other extracts (Table 4).

The results of bioassay showed that most of the tested isolates were less sensitive to chloroform extract and more sensitive to aqueous and ethanolic extracts of *T. indica* respectively. Although flavonoids were reported to be responsible for antimicrobial properties of some medicinal plants (Singh and Bhat, 2003), but however was not detected in this work. The activity of the plant extracts reported in this research may be related to the presence of other metabolites like alkaloids and tannins whose antimicrobial properties were well documented (Tschehe, 1971). The results of this study had

demonstrated some antimicrobial properties of *T. indica* stem back that may served in further ethno medicinal and pharmacological candidate for future research.

Conclusion

The results obtained in this research showed that *T. indica* has the potential for consideration as candidate for drugs production on treatments of diseases caused by these organisms.

Recommendations

Further research need to be carried out to determine the toxicity/safety level of the plant extracts, administration as well as to isolate and identify the active compound responsible for the activity.

Acknowledgement

The authors wish to acknowledge the managements of Bayero University, Kano for funding this study.

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