

Full Length Research Paper

Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamarindus indica* against some diseases causing bacteria

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Crude aqueous and ethanol extracts of *Tamarindus indica* were investigated for antibacterial activity. The susceptibility of five clinical bacterial isolates against these two crude extracts was determined using the disk diffusion method. The ethanol extracts produce strong antibacterial activity against *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi A* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* was resistant to the extracts. The aqueous extracts have the least antibacterial activity compared to ethanol extract except against *P. aeruginosa*. The phytochemical analysis revealed the presence of alkaloids, flavonoids, saponins and tannins. The antibacterial activity of the extracts against the test bacteria suggest that there is a scientific basis for their utilization in traditional medicine for the treatment of some bacterial infections as claimed by traditional medical practitioners.

Key words: Antimicrobial activity, *Tamarindus indica*, crude extract.

INTRODUCTION

The use of plants for medical purposes dates back to antiquity (Sofowora, 1982). Recent research has focused on natural plants product alternative for disease control in developing countries. The majority of rural dwellers do not have access to modern health care, so they mostly depend on medicinal plant to prevent or eliminate diseases. Medicinal plants are cheaper, more accessible to most of the population in the world. Thus, there is need to encourage the use of medicinal plants as potential sources of new drugs. There has therefore been an upsurge in the interest in herbal remedies in several parts of the world with many of the herbal remedial being incorporated into orthodox medical practice. Industrial interest in exploiting plants for medical purpose is exclusively found in China and Japan. Some African countries have also made advances in the area of the use of plants for the production of new drugs. These countries are Egypt, Burkina Faso, Ghana, Nigeria, Zimbabwe, Zambia and South Africa (Sofowora, 1984).

Tamarindus indica, is a native to tropical Africa and grows wild through out the Sudan. It is extensively culti-

vated in tropical areas of the world. It was long ago introduced into India and it reached the Persians and the Arabs called it 'Tamar Hindi' (Indian date, from the date-like appearance of the dried pulp.) giving rise to both its common and generic names. *T. indica*, is commonly called tamarind, the botanical name is *T. indica* and it is variously called Tsamiya (Hausa), Ajagbon (Yoruba), lekeku-oyibo (Igbo) and Dara (Nupe). *T. indica* belongs to the family Ceasalpiniaceae (Fabaceae). It is a large, beautiful evergreen tropical tree that can grow up to 80 feet high with a spread of 20 to 35 feet, it is highly wind-resistant, with strong, supple branches. The leaves are normally evergreen but may be shed briefly in very dry areas during the hot season. The brown woody pods contain the seeds; black, shining squares surrounded by pulp. *Tamarindus* is a slow grower but can live and still remains productive for 150 years or longer. The pulp of Tamarind is light brownish-red, sweetish acidic and edible. The fruit pulp is rich in tartaric and citric acid, high amount of vitamin C and sugar.

The fruit has so many uses; it is sold in the market in the form of cakes or balls of the pulp pressed together with the seed. The laxative properties of the fruit-pulp are recognized; it is use as food seasoning in various ways, boiled with cereal pap, and it is drunk for constipation. A

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pleasantry acid drink is made by a cold infusion of the pulp with sugar or honey and water, left to mature for several days. The pulp is pleasant to relieve thirst on a journey. In Senegal, meal called Bengal is made from the unripe fruit and given to febrile patients. It is also furnished as drinks for patients with fever and dysentery. The fruit pulp is use in syrup, juice concentrates and exotic food-specialties like chutney, curries, pickles and meat sauces.

following changes occurred in the property of urine after consuming the diet containing tamarind extract; complete disappearance of calcium oxalate aggregate, fall in density of crystalluria, reduction in crystal size, decreased excretion of oxalic acid, increased inhibitory growth (Anasuya and Sasikala, 1990). *T. indica* has numerous applications in traditional medicine and all parts of the plant have therapeutic uses. The study was undertaken to determine the antibacterial activities of the dry fruit-pulp of *T. indica* on *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and Identification of the fruit pulp

The dry fruit-pulp Tamarind was purchased at the Minna central market and identified by Prof. M. S. Ezenwa of the Crop Production Department, Federal University of Technology, Minna, Nigeria.

Preparation of Crude extracts

50 g of the dry fruit pulp was weighed and extracted with 95% ethanol and distilled water in the ratio of 1:6 (50 g of fruit pulp to 300 ml each of distilled water and ethanol). Each was blended using electric blender (National Mx 391N, Matsuhita electric). The use of water for extraction was to stimulate the condition in which the medical practitioner generally uses the herbs, and ethanol was used because of its broad spectrum and relative non-selective property of extraction (Iyamabo, 1991). The water extraction was for 24 h at 4°C with occasional shaking, while ethanol extraction was for two weeks at room (28±2°C) temperature. Each mixture was filtered and the filter and the filtrate was evaporated to dryness in an evaporating dish on a steam bath at a temperature of 70°C to obtain a brown semi-solid substance. This extract was stored in a screw-capped bottle and kept in the laboratory refrigerator for further research.

Phytochemical screening of the plant extract

The preliminary phytochemical analysis to screen the sample for the presence of bioactive components was performed following the method of Sofowora (1984).

Test bacteria

The test organisms were clinical isolates from the stock culture of Microbiology Department of the Federal University of Technology, Minna and Microbiology Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria.

They include: *E. coli*, *K. pneumoniae*, *S. paratyphi*, *P. aeruginosa* and *S. aureus*. Each of these organisms was subcultured unto nutrient broth to test for viability and subsequently on nutrient agar slants and kept at 4°C prior to susceptibility testing.

Culture medium

Nutrient broth/agar was used as culture medium. The media was prepared and sterilized as instructed by the manufacturers. About 25 ml of molten agar was poured into 90 mm diameter sterile petri dish to give a depth of 4 mm.

Inoculum preparation and disk diffusion tests

To standardize the inoculum density for a susceptibility test; a BaSO₄ turbidity standard, equivalent to a 0.5 McFarland standard was used (Mathew et al., 2006). The direct colony suspension method was use for inoculum preparation (Mathew et al., 2006). Chloramphenicol, Penicillin and Ampicillin was used as the standard drug for comparative purposes with the extracts. Stock concentrations of the standard drug were prepared in sterile distilled water to give a concentration of 100 µg/ml.

Standard procedure for performing the disk diffusion test of Mathew (2006) was followed for inoculation of test plates and application of disks to inoculated agar plates. After 24 h of incubation, each plate was examined for zones of inhibition. The diameter of the disk of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk was measured to the nearest whole millimeter (mm).

Each of the extracts was autoclaved to determine the stability of the crude extract at the temperature of 121 °C for 15 min.

RESULTS

Phytochemical screening of dry fruit pulp of *T. indica*

The phytochemical screening of *T. indica* in Table 1 shows that the ethanol extract had higher concentration of bioactive components except for antraquinones and sesquiterpenes which were absent. Alkaloids and tannins were concentrated in the ethanol and aqueous extracts, respectively. The concentration of tannin and alkaloids were moderate for ethanol and aqueous extracts. Flavonoids and saponins were slightly present in both extracts (Table 1).

Action of extracts on microorganisms

From the results of the bioassay conducted with the crude ethanolic extract there was a strong activity against *E. coli* followed by *K. pneumoniae* and *S. paratyphi* A. There was no activity against *P. aeruginosa*, *S. aureus*, *S. typhi*, *S. paratyphi* B and C (Table 2). The water-soluble extract showed strong activity against *P. aeruginosa* but moderate against *E. coli* and *K. pneumoniae*. There was no activity against *S. aureus*, *S. typhi* and *S. paratyphi* A, B, C.

After autoclaving of the extracts, ethanol extract had activity with *E. coli* followed by *S. typhi*, *S. paratyphi* A, B,

Table 1. Phytochemical component of *Tamarindus indica*.

Component	Test	Ethanol extract	Aqueous extract
Alkaloids	Dradrendoff's test	+++	++
Anthraquinones	Borntrager's test	–	–
Flavoids	General test	+	+
Saponins	Frothing test	+	+
Sesquiterpenes	General test	–	–
Tannins	General test	++	+++

+ = Slightly present; ++ = moderately present; +++ = highly present; and – = absent.

Table 2. Zone of inhibition (mm) of extract of *Tamarindus indica*.

Organism	Ethanol extracts	Aqueous extracts
<i>P. aeruginosa</i>	–	19 mm
<i>S. aureus</i>	–	–
<i>E. coli</i>	20 mm	13 mm
<i>K. pneumoniae</i>	17 mm	10 mm
<i>S. typhi</i>	–	–
<i>S. paratyphi A</i>	15 mm	–
<i>S. paratyphi B</i>	–	–
<i>S. paratyphi C</i>	–	–

C but moderate against *S. aureus*. The aqueous extract had activity against *S. typhi*, *E. coli*, *S. paratyphi* and *P. aeruginosa* between (8 to 15 mm) (Table 3).

The presence of alkaloids, flavonoids, tannins and saponins may be responsible for the antimicrobial activity in the dry fruit pulp of *T. indica* and indicates that the species is medically important.

DISCUSSION

The preliminary phytochemical analysis shows that *T. indica* contain alkaloids, flavonoids, saponins and tannins (Table 1). These compounds have been reported to inhibit bacteria growth and are capable of protecting certain plants against bacterial infection (Clark, 1981; Mather and Gonzalez, 1982). *T. indica* was known to have been used by local people for fever, dysentery and other ailments caused by bacteria. Our results revealed activity against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. paratyphi A*. These microorganisms are aetiological agents in urinary tract infections (UTI), wounds, pneumonia and paratyphoid fever. Thus, further work on extracts of *T. indica* may be of value in these and other medical conditions. With the increasing menace of typhoid fever in places such as Nigeria, the activity against *S. paratyphi* requires confirmation and elucidation. Similarly, *P. aeruginosa* is known to be resistant to majority of antibiotics; the confirmation of the extracts against these bacteria will be of medical importance.

We suggest that *T. indica* extracts, showing antimicro-

Table 3. Zone of inhibition (mm) extracts of *Tamarindus indica* after autoclaving.

Organism	Ethanol extracts	Aqueous extracts
<i>E. coli</i>	21	14
<i>P. aeruginosa</i>	–	08
<i>K. pneumoniae</i>	–	–
<i>S. aureus</i>	14	–
<i>S. typhi</i>	–	–
<i>S. paratyphi A</i>	20	15
<i>S. paratyphi B</i>	19	–
<i>S. paratyphi C</i>	19	12

bial activities *in vitro*, be subjected to further *in vivo* and clinical trials after isolation and characterization of the bioactive components.

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