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Antibacterial Activity of *Tamarindus indica* Fruit and *Piper nigrum* Seed

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Abstract: A study is described in which petroleum ether, ethanol and water extracts from *Tamarindus indica* ripe fruit and *Piper nigrum* seed in different concentrations (10-100%) were evaluated for their possible antibacterial activity against four standard pathogenic microorganisms, *Staphylococcus aureus* (gram-positive bacterium), *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* (gram-negative bacteria). The ethanol extract from *T. indica* fruit in different concentrations exhibited higher activity against all test bacteria than that from *P. nigrum* seed and that the activity is concentration-dependent. Petroleum ether extract from *P. nigrum* seed in different concentrations had no activity against *E. coli*, *Ps. aeruginosa* or *S. typhi* but had activity against *S. aureus* (inhibition zones 12-15 mm). Petroleum ether from *T. indica* fruit in concentrations of 10-100% had no antibacterial activity against *E. coli* and *Ps. aeruginosa* but the growth of *S. aureus* and *S. typhi* was only inhibited by 50% concentration of petroleum ether extract (inhibition zones, 5 mm). Water extract from *T. indica* fruit at 100% concentration produced inhibition zones at 14-15 mm for *E. coli* and *Ps. aeruginosa*, respectively, but had no activity against *S. aureus* or *S. typhi*. Water extract in 100% concentration from *P. nigrum* seed caused inhibition zones at 15 and 12 mm against *S. aureus* and *S. typhi* but had no activity against *E. coli* or *Ps. aeruginosa*. These findings were compared with those produced by gentamicin (10 μg), a reference antibiotic.

Key words: *Tamarindus indica*, *Piper nigrum*, antibacterial activity

INTRODUCTION

Medicinal plants play an important role in the health care system of the Third World population (Akerele *et al*., 1991). In Sudan, plants are used in local traditional medicine in form of a decoction, poultice, cream, or other preparations in rural areas for alleviation of suffering and disease.

Tamarindus indica L. a member of the family Caesalpiniaceae and locally known as Aradaib, is widely distributed in Sudan and other Afro-Asian countries, is used in traditional medicine as laxative, emollient, anthelmintic and antipyretic and for the treatment of a variety of ailments including dysentery, cough, sore throat, rheumatism, hemorrhoids, fruncles, malaria and aphthous stomatitis (Mohamedain *et al*., 1996). Phytochemical investigations of the aerial parts of this plant have demonstrated the presence of tartaric, acetic, citric and succinic acids, gum, pectin, sugar, tannins, alkaloids, flavonoids, sesquiterpenes and glycosides (Chopra *et al*., 1958; Algohary *et al*., 1994; Mohamedain *et al*., 1996; Aida *et al*, 2001). *T. indica* extracts were found to have anti-meracidial and

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anti-cercarial activities (El Sheikh *et al*., 1990). Rajkumar *et al.* (2005) reported that *T. indica* seed aqueous extract had antidiabetic potency and that the leaf methanolic extract of this plant had anti-*Burkholderia pseudomallei* (*Pseudomonas pseudomallei*) activity.

Piper nigrum belongs to the Piperaceae family and is known locally as Felfel Aswad. The plant fruit is used in folk medicine as aphrodisiac, carminative, stomachic, antiseptic, diuretic, galactagogic and emmenagogic and for the treatment of acne, cough, rheumatoid arthritis, peripheral neuropathy, melanoderma and leprosy due to the presence of volatile compounds, tannins, phenol and other unknown substances (Chiranjib *et al*., 1990; Algohary *et al*., 1994; Ali, 1995; Park *et al*., 2001). It has been found that *P. nigrum* leaf extract inhibits the growth of *Pseudomonas aeruginosa* (Larhsini *et al*., 2001).

Because of the common use of *T. indica* fruit and *P. nigrum* seed in the treatment of various disorders as well as the paucity of information on their comparative antibacterial activity, we investigated the possible growth inhibition of these plant extracts against a gram-positive bacterium (*Staphylococcus aureus*) and three gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*).

MATERIALS AND METHODS

Plant Materials

Tamarindus indica ripe fruits and *Piper nigrum* seeds were purchased from herbalist in Khartoum local market, Sudan, cleaned and separately ground by a mechanical grinder.

Standard Microorganisms

The test microorganisms utilized in the present study were kindly provided by scientists, Khartoum National Health Institute and designated as follows:

Staphylococcus aureus NCTC 25953, Gram +ve cocci.
Escherichia coli NCTC 25922, Gram-ve rod.
Salmonella typhi NCTC 25936, Gram-ve rod.
Pseudomonas aeruginosa NCTC 27853, Gram-ve rod.
NCTC = National Collection Type Culture, London, UK.

Antibiotic

Gentamicin (Oxoid Ltd. England) was used in concentration of 10 μg (microgram).

Plant Material and Preparation of Crude Extracts

Twenty grams of the powdered *Tamarindus indica* fruits and *Piper nigrum* seeds were exhaustively extracted in a soxhlet apparatus with petroleum ether 90% at 37°C for 3 h and the extract was evaporated under reduced pressure and dried. The material was then exhaustively extracted with ethanol 95% at 37°C for 3 h and the extract was again evaporated under reduced pressure, air dried and yields were recorded. The aqueous extract was dried by freeze dryer and weighed. The extracts from each plant were reconstituted at the time of testing in concentration of 100, 50 and 10%.

Preparation of Stock Extract Solutions

One gram of each extract was dissolved in 1 mL of the same solvent used for extraction.

Preparation of the Test Organisms

The properties of the standard bacteria are summarized in Table 1.

Table 1: Differential characteristics of test bacteria

Characteristics	*S. aureus*	*E. coli*	*S. typhi*	*Ps. aeruginosa*
Gram stain	+ve	-ve	-ve	-ve
Motility	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	-	+ve
Nitrate	+ve	+ve	-	+ve
Arginine	+ve	+ve	-	+ve
Indole	-ve	+ve	-ve	-ve
Methyl red	-ve	+ve	+ve	+ve
Vagues proskures	+ve	-ve	-ve	-ve
Gas production from glucose	-ve	+ve	-ve	+ve
Acid production from glucose	+ve	+ve	+ve	-ve
Acid production from sucrose	+ve	+ve	+ve	+ve
Acid production from lactose	+ve	+ve	-ve	-ve
Oxidase	-ve	+ve	-ve	+ve
Urease	+ve	-ve	-ve	+ve

+ve = 90% or more strains had positive reactions, -ve = 90% or more strains had negative reactions, - : Non-determined

Staphylococcus aureus

Gram-positive organism, 0.5 mm in diameter, occurring singly, in pairs or irregular clusters, non-motile and non-spore forming, many strains form a golden yellow pigment on colony of good growth on ordinary media, aerobic, facultatively anaerobic, catalase and urease positive, oxidase and indole negative.

Escherichia coli

Gram-negative rod, 1.1-1.5 mm wide and 2.0-6.0 mm long with rounded ends and shape varying from coccoid to rod, motile, aerobic, facultatively anaerobic, oxidase and urease negative, citrate can not be used as a sole carbon source, most strains are fermenters of methyl red positive, catalase and indole positive and VP negative.

Pseudomonas aeruginosa

Gram-negative bacillus, non-sporing, non-capsulated, motile by one or two polar flagella aerobic, facultatively anaerobic, grows on a wide variety of culture media, catalase and oxidase positive.

Salmonella typhi

Gram-negative, straight rods, aerobic, facultatively anaerobic, motile with peritrichus flagella, non acid and gas formation from glucose and mannitol, formation of H_2S, indole, urease and VP negative.

Antibiotic

Gentamicin (Oxoid, England) was used in concentration of 10 μg for comparative evaluation of antibacterial activity of plant extract.

Preparation of Culture Media

Nutrient broth (Peptone 5 g, sodium chloride 5 g, beef extract 1 g and yeast extract 2 g at pH 7.4) forms the bases of most bacteria used in microbiological studies. Nutrient agar (Oxoid Ltd., London, UK) was used to prepare enriched culture media. Thirteen grams of nutrient broth powder were dissolved in a liter of distilled water and autoclaved at 121°C for 15 min. The nutrient agar was prepared by adding 12 g of the agar (Oxoid) to 5 g of peptone, 8 g sodium chloride and 3 g of beef extract and the pH was adjusted to 7.6. Nutrient agar was used to obtain excellent colonies for evaluation of antibacterial activity of plant extracts.

Evaluation of Antibacterial Activity of Plant Extracts

Antibacterial activity was assessed by the agar well diffusion method (Kinsbury and Wagner, 1990). The nutrient agar medium was properly inoculated with the standard organisms separately at

10^6 cfu mL^{-1} to achieve confluent growth and allowed to dry at room temperature. On each inoculated plate, 10 mm-diameter wells (4 wells at equal distances in one plate) were bored in the agar using sterile cork borer. Concentration at 100, 50 and 10% of each extract was added to each well by a sterile Pasteur pipette and allowed to diffuse for 1 h before incubating the plates for 18 h at 37°C.

The diameter of the inhibition zone resulting from the activity of the extracts was measured in mm, two replicates were made from each concentration and comparative activity was recorded. The antibacterial activity of the plant extract against the standard microorganisms was evaluated and compared with that of the antibiotic, gentamicin (Oxoid Ltd., London).

RESULTS AND DISCUSSION

The results reported in Table 2 and 3 indicate that ethanol extract from *T. indica* fruit in different concentrations (10-100%) exhibited higher activity against all test bacteria, *S. aureus*, *E. coli*, *Ps. aeruginosa* and *S. typhi* than that from *P. nigrum* seed. It has been clearly shown that the antibacterial activity against these test microorganisms is concentration-dependent as depicted in Fig. 1 and 2. However, petroleum ether extract from *P.nigrum* seed, in different concentrations, (10-100%) had no antibacterial activity against *E. coli*, *Ps.aeruginosa*, or *S. typhi* but it possessed antibacterial activity against *S. aureus* (inhibition zone 12-15 mm). The petroleum ether extract from *T. indica* fruit, in different concentrations (10-100%), had no antibacterial activity against *E. coli* and *Ps. aeruginosa*. However, the growth of *S. aureus* and *S. typhi* was only inhibited by 50% concentration of petroleum ether extract (inhibition zone 5 mm).

Table 2: Evaluation of antibacterial activity of *T. indica* fruit and *P. nigrum* seed petroleum ether extract

Microorganisms	Concentration of *T. indica* (%)	Inhibition zone of *T. indica* (mm)	Concentration of *P. nigrum* (%)	Inhibition zone of *P. nigrum* (mm)
S. aureus	100	-	100	14
	50	5	50	12
	10	-	10	15
E. coli	100	-	100	-
	50	-	50	-
	10	-	10	-
Ps. aeruginosa	100	-	100	-
	50	-	50	-
	10	-	10	-
S. typhi	100	-	100	-
	50	5	50	-
	10	-	10	-

- = No inhibition zone observed

Table 3: Evaluation of antibacterial activity of *T. indica* fruit and *P. nigrum* seed ethanol extract

Microorganisms	Concentration of *T. indica* (%)	Inhibition zone of *T. indica* (mm)	Concentration of *P. nigrum* (%)	Inhibition zone of *P. nigrum* (mm)
S. aureus	100	30	100	-
	50	24	50	7
	10	15	10	11
E. coli	100	27	100	-
	50	22	50	14
	10	12	10	7
Ps. aeruginosa	100	27	100	17
	50	18	50	6
	10	15	10	7
S. typhi	100	31	100	18
	50	20	50	7
	10	12	10	13

- = No inhibition zone observed

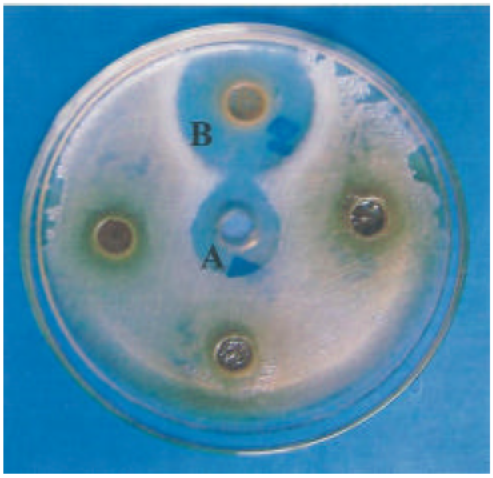

Fig. 1: *T. indica* ethanol extract showing *S. typhi* growth inhibition, A = 50% conc.; B = 100% conc

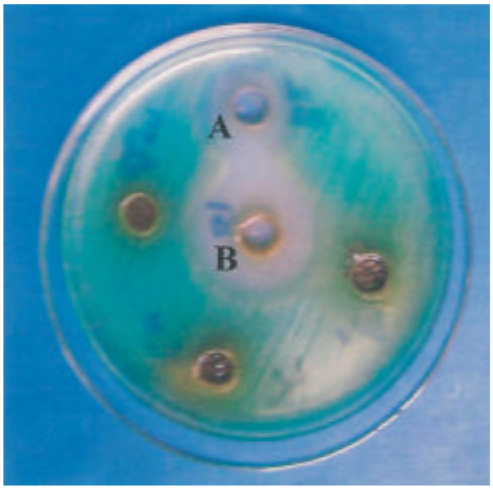

Fig. 2: *T. indica* ethanol extract showing *Ps. aeruginosa* growth inhibition, A = 50% conc., B = 100% conc

The antibacterial activity of *T. indica* fruit water extract at 100% concentration produced inhibition zones at 14 and 15 mm when examined for *E. coli* and *Ps. aeruginosa*, respectively, but had no antibacterial activity against *S. aureus* or *S. typhi*. The antibacterial activity of *P. nigrum* seed aqueous extract at 100% concentration produced inhibition zones at 15 and 12 mm against *S. aureus* and *S. typhi* but had no activity against *E. coli* or *Ps. aeruginosa*, respectively (Table 4).

Phytochemical screening of Sudanese *T. indica* fruit or *P. nigrum* seed has not so far been properly performed. Previous investigations have demonstrated the presence of flavonoids, acids, pectins, sugar, tannin, saponins, alkaloids, sesquiterpenes and glycosides in *T. indica* (Chopra *et al*., 1958; Mohamedain *et al*., 1996; Aida *et al*, 2001) and of volatile compounds, tannins, phenol and probably other substances in *P. nigrum* (Chiranjib *et al*., 1990; Algohary *et al*., 1994; Ali, 1995; Park *et al*., 2001).

Table 4: Evaluation of antibacterial activity of *T. indica* fruit and *P. nigrum* seed water extract (100%)

Microorganisms	Inhibition zone of *T.indica*	Inhibition zone of *P.nigrum*
S. aureus	-	15
E. coli	14	-
Ps. aeruginosa	15	-
S. typhi	-	12

- : No inhibition zone observe

Table 5: Evaluation of antibacterial activity of gentamicin (10 μg)

Microorganisms	Inhibition zone (mm)
S. aureus	22
E. coli	22
Ps. aeruginosa	13
S. typhi	23

In this study, the antibacterial activity of *T. indica* fruit and *P. nigrum* seed petroleum ether, ethanol and water extracts has been compared with that of gentamicin, a well known aminoglycoside antibiotic (Table 5). The antibacterial activity of *T. indica* fruit ethanol extract at 100% concentration against *S. aureus*, *E. coli*, *Ps. aeruginosa* and *S. typhi* excelled that of gentamicin at 10 μg or was equivalent at 50% concentration to gentamicin particularly against *S. aureus* and *E. coli*. This may justify the traditional uses of the plant as a remedy for the treatment of bacterial infections.

Since the activity of *T. indica* fruit water extract at 100% concentration produced inhibition zone at 15 mm when examined for *Ps. aeruginosa*, this activity is considered almost equivalent to that of gentamicin.

In Nigeria, Doughari (2006) investigated the antimicrobial activity of *T. indica* dried powdered plant aqueous an organic solvents (acetone an ethanol) extracts against *S. paratyphi*, *S. typhi*, *B. subtilis* and *S. aureus* an found that the lowest Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were demonstrated against *S. paratyphi*, *S. typhi* and *B. subtilis* an the highest MIC and MBC were exhibited against *S. aureus*. This author also found that the main constituents in *T. indica* are tannins, saponins, sesquiterpenes, alkaloid an phlbatamins.

Oliver-Bever (1986) and Omer (1997) summarized the chemical constituents possessing antibacterial activity in several medicinal plants. The main compounds in some of the plants are alkaloids from *Argemone mexicana* (Papaveraceae), phenols from *Anacardium occidentale* (Anacardiaceae), quinones from *Drosera indica* (Droseraceae), acids from *Acacia Arabica* (Leguminosae), flavonoids from *Camellia sinensis* (Theaceae), volatile oils from *Carum copticum* (Umbelliferae), aldelydes from *Teucrium polium* (Labiatae), terpenes and eugenol from *Commiphora myrrha* (Burseraceae) and proteolytic enzymes from *Calotropis procera* (Asclepiadaceae).

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