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Anti-Microbial Activity of *Tamarindus indica* and *Adansonia digitata* Extracts Against *E. coli* Isolated from Urine and Water Specimens

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Abstract: The petroleum ether, ethanol and aqueous extracts of the *Tamarindus indica* and *Adansonia digitata* were screened for the presence of possible anti-microbial activity using the cup plate agar diffusion method. They were tested against *Escherichia coli* that isolated from urine and water sources. Each extract was used in concentration of 100, 75, 50 and 25%. *E. coli* isolated from clinical urine samples showed more susceptibility toward both plant extracts. The ethanol extract of both plants was more effective than petroleum ether and water extracts. Ethanol extract showed variation in the antimicrobial activity toward *E. coli* that isolated from water and clinical sources, the zones of their inhibition ranged between (15-60 mm). On the other hand, the petroleum ether extract of *Adansonia digitata* was found to be inactive against all tested organisms. The susceptibility of the microorganisms to the extracts of these plants was compared with each other and with selected antibiotics. Ethanol extract of *Tamaridus indica* have more powerful antibacterial activity compared with all antibiotics. The antimicrobial activities of these plants were discussed according to their phytochemical components.

Key words: Medicinal plants, extracts, susceptibility, resistance, gram-negative bacteria

INTRODUCTION

The international aware of medicinal plants has enormously increased during the last years. The World Health Organization (WHO) estimated that 80% of the population in developing countries rely on traditional medicine, mostly plant based drugs, for primary health care. The rising incidence in multidrug resistance among pathogenic microbes has further necessitated the need to search for newer antibiotic sources *Tamarindus indica* is a plant that is used in traditional medicine for the treatment of cold, fever, stomach disorder, diarrhea and jaundice and as skin cleanser (Doughari, 2006), *Tamarindus indica* were good sources of zinc and used to make *dawwa* (porridge) commonly consumed during pregnancy (Cassius *et al*., 2000). The phytochemical studies revealed the presence of tannins, saponins, sesquiterpenes, alkaloids and phlobatamins and the extracts of *Tamarindus indica* have broad-spectrum antibacterial activity against both gram positive and gram-negative bacteria (Doughari, 2006).

Adansonia digitata was found to be an excellent source of protein. Amino acid analyses revealed high glutamic and aspartic acid contents and the sulfur-containing amino acids as being the most limited amino acid (Osman, 2004). The same authors determine the presence of fatty acid (linoleic and linolene, vitamin E, carotenoid and mineral (iron, magnesium, calcium and zinc). They also mentioned that *Adansonia digitata* leaves were nutritionally superior to the fruit of the tree; however, the fruit contain useful quantities of potassium, phosphorus, zinc and acid. Seed was found to be a good source of energy, protein and fat (Cassius *et al*., 2000). The same author added that *Adansonia digitata* given to infants to increase weight gain, it was high in fat, calcium, copper, iron and zinc. In West Africa the roots of *Adansonia digitata* are cooked and eaten, presumably in times of famine. Malaria patients

take the dried-powdered root prepared as a mash perhaps as a tonic, or used to bath babies in order to promote a smooth skin (Aida *et al*., 2001; Kristensen and Lykke, 2003). The bark, pith and the seeds are certainly used for the treatment of fever, in some countries the bark is used for tanning or as mouthwash for toothache. The ash from the bark and fruit toiled in oil and used as soap. The leaves are used medicinally as a diaphoretic, an expectorant and as a prophylactic against fever, The leaves also have hyposensitizes and antihistamine properties, being used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, inflammations, insect bites, guinea worm, the fibers lining the husk are used as a decoction to treat amenorrhoea, it may be used as a febrifuge and as an anti-dysenteric and in the treatment of smallpox and measles as an eye instillation (Shahat, 2006; Doughari, 2006). As mention by Osman (2004) and Doughari (2006) *Adansonia digitata* contains the alkaloid adansonin, which has a strophanthus like action, the bark is used as an antidote to strophanthus poisoning.

Anani (2000) reported that the extracts of *Adansonia digitata*, were active against viruses (herpes simplex, Sindbis and poliovirus). The ethyl acetate and n-butanol fractions of pericarp were found to be active against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Sterptococcus mutans* and *Pseudomonas aeruginosa* and strongly trypanocidal (Marjorie, 1999; Atawodi *et al*., 2002, 2003; Doughari, 2006). This study was aimed to investigate the presence of antimicrobial agent in *Tamaridus indica* and *Adansonia digitata* against *E. coli* to confirm their traditional usage as medicinal plants, it also aimed to study the activity of those plants at same bacterium that isolated from different sources and to compared their antimicrobial activity with certain antibiotics.

MATERIALS AND METHODS

Plant materials were collected from Omdurman area, Khartoum state. This plants were identified by our taxonomist Dr. Alawia A. El Awaad, Department of Biology, School of Life Science. Faculty of Science and Technology, El Neelain University, where the study conducted at 2006.

Preparation of the Extracts

These was carried out as described by Predrag *et al*. (2005). Fifty gram of each plants were coarsely powdered using a mortar and pestle and were further reduced to powder using an electric blender. The powder was transferred into closed containers. Each of powdered air-dried plant material was extracted with water, ethanol and chloroform. Twenty five grams of each powdered sample was mixed in a conical flask with 100 mL of deionized distilled water or organic solvent, plugged, then shaken at 120 rpm for 30 min and kept for 24 h. After 24 h, each of the extracts was filtered rapidly through gauge and then by filter paper. The filtrates were then concentrated in rotary evaporator.

Test Organisms

E. coli was isolated from urine and water samples. Twenty isolates (10 from urine samples and 10 from Water samples) were subject to test against different extracts. The bacterium was identified according to Cheesbrough (1985).

Determination of Anti Microbial Activity

Antimicrobial activity of organic and aqueous extracts of the plants samples were evaluated by both paper disc diffusion method (Aida *et al*., 2001) and standard diffusion method (Kavanagh, 1972). For determination of antibacterial activity, 1 mL of 10^6 overnight incubated broth were inoculated on MacConkey agar plates, sterile filter paper discs 6 mm diameter impregnated with 100 μL extract dilutions (100, 75, 50 and 25%) or the same amount were directly inoculated in the wells prepared at plates. The plates were incubated at 37°C for 24 h. Three replicates were carried out for each extract

against each of the test organism. Simultaneously, controls involving the addition of the respective solvents instead of extract were carried out. After incubation the diameters of the inhibition zones were measured, averaged and the mean values were tabulated. The same procedure was done using antibiotic for comparison.

RESULTS

The ethanol extracts showed highest inhibition zones; the zone of inhibition was gradually reduced with the reduction of the concentration. *E. coli* isolated from urine samples showed more susceptibility than that isolated from water samples. The chloroform extracts gave inhibition zone only at full concentration (100%), these were 40 mm with urine samples and 20 mm with water samples, the organism showed resistance towards other concentrations (75, 50 and 25%). Water extract gave 30 mm zone of inhibition with urine isolates compared with 20 mm with water isolates. As shown in Table 1 the activity of the plant extracts was more effective than amikirain antibiotic.

The water extract showed highest inhibition zone (30 mm) with urine isolates compared with ethanol extract at concentrations of 100 and 75% (each gave 20 mm), other concentrations (50 and 25%) and similarly all concentrations of chloroform extracts were devoid from any antimicrobial activity towards all isolates. All isolates of *E. coli* from water samples showed resistance towards all extracts of *Adansonia digitata* except ethanol extract at concentrations 100 and 75% (Table 2).

It is clear from Table 3 that ethanol extract of the *Tamaridus indica* was two to 5 times powerful more that the most effective antibiotic gentamicin that gave mean inhibition zone of 12 mm with *E. coli* isolated from urine and 9 mm inhibition zone with *E. coli* isolated from water samples. The chloroform extract gave antibacterial activity only at concentration of 100%, other dilution showed no effect against isolated organisms. The water extract of the plant showed powerful effect comparing with all test antibiotics.

Table 1: Antimicrobial activity of extracts of *Tamaridus indica* against isolated *E. coli* (zone of inhibition by mm)

	Ethanol extract				Chloroform extract				
Source of isolation	100%	75%	50%	25%	100%	75%	50%	25%	Water extract
Urine	60	40	30	25	40	-	-	-	30
Water	40	35	25	15	20	-	-	-	20

-: Absent

Table 2: Antimicrobial activity of extracts of *Adansonia digitata* against isolated *E. coli* (zone of inhibition by mm)

	Ethanol extract				Chloroform extract				
Source of isolation	100%	75%	50%	25%	100%	75%	50%	25%	Water extract
Urine	20	20	-	-	-	-	-	-	30
Water	30	20	-	-	-	-	-	-	-

-: Absent

Table 3: Antibiotic activity against *E. coli* isolated from urine and water samples (zone of inhibition by mm)

Antibiotics	Concentration (mcg)	Urine isolates	Water isolates
Amikacin	30	9	7
Amoxicillin	10	0	0
Nitrofurantoin	300	7	7
Ciprofloxacine	5	5	4
Tetracycline	25	5	3
Cephalexin	30	3	4
Chloramphenicol	10	11	9
Amoxicillin-Clavuline	30	0	0
Naldilic acid	30	8	7
Gentamicin	10	12	9
Norfloxacin	10	8	6
Ofloxacin	5	9	7

The ethanol extract of *Adansonia digitata* at concentration of 100 and 75% gave greatest activity comparing with all test antibiotics toward both urine and water isolates while water extract showed greater powerful activity against urine isolates comparing with all antibiotics.

DISCUSSION

Phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds of the *Tamaridus indica* and *Adansonia digitata* have known suppressive action against many microorganisms. This may therefore explain the demonstration of antibacterial activity of those plants against *E. coli*.

Out of the three solvents used for extraction, the ethanol extract showed the highest activity followed by water extract, different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent (Marjorie, 1999; Doughari, 2006). Ethanol extracts in this study might have had higher solubility for more phytoconstituents, consequently the highest antibacterial activity. The demonstration of antimicrobial activity by water extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine men use water as their solvent in which the decoctions are prepared and these confirm the findings of Aida *et al*. (2001), Osman (2004), Predrag *et al*. (2005) and Shahat (2006). The absence of any antibacterial activity of chloroform extraction of *Adansonia digitata* indicated that the active ingredient (s) of this plant does not extracted with chloroform. This also explain that extractable active ingredient(s) of *Tamaridus indica* is only effective at crude extraction and the dilution interfere with the activity against the bacteria. The demonstration of antibacterial activity of this plant confirm the findings of Marjorie (1999), Anani *et al*. (2000) and Doughari (2006).

In this study *E. coli* isolated from water samples showed less susceptibility toward both plant extractions and these might be due to environmental mutation acquired by the organisms.

The demonstration of antibacterial activity of those two plants may be indicative of the presence of antibiotics compounds in them. This may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation might aid the possibility of use of those plants in drug development for human consumption possibly for treatment of gastrointestinal, urinary tract and wound infection and typhoid fever. The effect of these plants on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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