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Anti-microbial Activity of *Acacia nilotica* Extracts Against Some Bacteria Isolated from Clinical Specimens

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Abstract: The comparative antimicrobial activity of ethanol and chloroform extracts from *Acacia nilotica* fruit was studied. The bacteria isolated from abscesses or wounds of hospitalized patients were *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The susceptibility of isolated bacteria against ethanol extract (13, 6.7, 5, 3 and 1%) was higher than chlorform extract used in similar concentrations especially *S. aureus* (30-4 mm), *E. coli* (25-9 mm), *Kl. pneumonia* (18-0 mm), *P. vulgaris* (10-1 mm) and *Ps. aeruginosa* (20-4 mm). The inhibitory effects of the extracts on bacteria were compared with those of selected antibiotics. The ethanol extract of *A. nilotica* fruit was either equally or more effective than the test antibiotics.

Key words: *Acacia nilotica,* extract, antimicrobial, pathogenic bacteria

Introduction

Acacia nilotica, also known as *Mimosa nilotica,* is a member of the family Mimosaceae and is known in the Sudan as *Garad*. Other Acacia species such as *A. seyal*, *A. arabica* and *A. abyssinica* and are considered the most utilized and widely distributed species in eastern, southern and northern Africa and some Asian countries such as Saudi Arabia (El Shanawany, 1996).

Acacia nilotica and other *Acacia* species are used in folk medicine by people in rural areas as a remedy for tuberculosis, leprosy, small pox, dysentery, cough, ophthalmia, toothache, skin ulcers and cancers and as astringents, antispasmodics, aphrodisiac (Duke, 1983; Van Wyk *et al*., 2000). Phytochemical analysis of the aerial parts of the plant demonstrated the presence of flavonoids and polyphenolic compounds in the flowers, tannins, glycosides, volatile oils, organic acids, coumarins and carbohydrates in the fruits (El-Shanawany, 1996).

Acacia nilotica leaf is very digestible and has high levels of protein, the fruit is higher in glutamic and aspartic acid and lower in most other amino acids, the methionine was absent from the fruit of Australian materials but present in the seed of African material (Fagg, 2001; Spies and March, 2004).

The plant, *A. nilotica* selected for this study, is used in local traditional medicine for the treatment of various disorders but this has not been confirmed by experiments. Trials were undertaken to examine the anti-bacterial activity of the plant fruit ethanol and chloroform extracts against both Gram-negative and Gram-positive bacteria which had been isolated from abscesses and wound infections in patients at Khartoum Hospital. The anti-bacterial activity of the plant fruit extracts was compared with that of different antibiotics.

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Materials and Methods

Collection of Samples

Samples were collected following examination of hospital patients with soft tissue infections (superficial abscesses) and others from the nostrils in Khartoum during 2004. Sterile dry cotton swabs were used to collect specimens from abscesses and nostrils.

The swabs were circled tightly through both nostrils four times consecutively. Swabs were taken from wounds and the wall of the abscesses cavities after evacuation of pus. The bacteria were isolated and identified according to the methods described by Cheesbrough (1984).

Plant Material and Preparation of Extract

Acacia nilotica fruits were finely ground and a weighed quantity of the powder was soxhlet extracted using 75% ethanol or 75% chloroform as the extraction solvent at 65°C for 4 h day^{-1} for three days. The solvent was slowly evaporated at room temperature for 2-4 days. Extracts were stored at 4°C for determination of anti-bacterial activity.

Anti-bacterial Activity

The dilution technique described by Cheesbrough (1984) was used. On Muller and Hinton medium using sterile cutter, 5 circular wells at equal distances were made and cultured by the organisms tested (*Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Klebsiella pneumoniae*) from nutrient broth and under sterile conditions. Fifty microliter of each concentration of *A. nilotica* fruit extract were added by automatic pipettes to labeled wells and incubated at 37°C for 18-24 h. Inhibition zones were measured using a ruler.

Results

Out of hundred abscesses and wounds clinical specimens, 45 (45%) were Gram-positive cocci while 55(55%) were identified as Gram-negative rods. The Gram-positive cocci were further identified as *Staphylococcus aureus* (catalase and coagulase positive). The Gram-negative bacteria were identified further according to biochemical activities as *E. coli* (23), *Proteus vulgaris* (18), *Klebsiella pneumoniae* (12) and *Pseudomonas aeruginosa* (2).

The antibacterial activity of both ethanol and chloroform extracts from *A. nilotica* fruits against all test organisms was reduced with decrease of extracts concentrations. *Staphylococcus aureus* showed the highest inhibition zones (29 mm) with ethanol extract which was reduced gradually with reduction of extract concentration to 23 mm at 6.7%, 11 mm at 5%, 6.5 and 4.5 mm at concentrations 3% and 1.7%, respectively. The chloroform extract showed smaller inhibition zones, 18 mm at concentration 13% and 15 and 5 mm at concentrations 6.7 and 5%, respectively. The organism showed resistance towards chloroform extract at concentrations 3 and 1.7% (Table 1).

Escherichia coli had a marked sensitivity towards both ethanol and chloroform extracts except with 1.7% chloroform extract. This sensitivity was markedly reduced with decrease in extract concentration. On the other hand, *Klebsiella pneumoniae* showed marked resistance for both ethanol and chloroform extracts at concentrations of 5, 3 and 1.7%, it was only affected by 13 and 6.7% of the *A. nilotica* ethanol and chloroform extracts.

Proteus vulgaris tended to show the smallest inhibition zones at all concentrations of both ethanol and chloroform extracts when compared with other organisms. It also showed marked clear resistance against chloroform extracts at 3 and 1.7% concentrations.

Table 1: Anti-microbial activity of ethanol and chloroform extracts from *A. nilotica* fruits against isolated organisms

	Inhibition zone (mm) Ethanol extract (conc.)					Inhibition zone (mm) Chloroform extract (conc.)				
Bacteria	13%	6.7%	5%	3%	1.7%	13%	6.7%	5%	3%	1.7%
Staphylococcus aureus	29	23.5	11	6.5	4.5	18	15	5	0®	0®
E. coli	25	22	18	10	9	18	12	8	5	0®
Klebsiella pneumoniae	18	10	0®	0®	0®	13	5	0®	0®	0®
Proteus vulgaris	10	8	6	2	1®	12	6	4	0®	0®
Pseudomonas aeruginosa	20	14	12	6	4	15	10	4	0®	0®

® = Resistant

Table 2: Anti-microbial activity of some antibiotics against isolated organisms

	Zones of inhibition (mm)				
Antibiotic (conc.)	*Staphylococcus aureus*	*E. coli*	*Klebsiella pneumoniae*	*Proteus vulgaris*	*Pseudomonas aeruginosa*
Tetracycline (30 mcg)	5	5	6	7	7
Cefotaixma (30 mcg)	6	8	9	12	0 ®
Ciporofloxima (5 mcg)	9	11	12	7	7
Pefloxacin (5 mcg)	9	8	8	8	4
Ampcillin (20 mcg)	5	3	4	6	0 ®
Co-Trimoxazole (25 mcg)	9	9	12	12	0 ®
Ceftriazone (30 mcg)	8	9	8	9	11
Gentamicin (10 mcg)	8	6	6	7	7
Amikacin (10 mcg)	5	6	5	6	6
Chloramphenicol (30 mcg)	7	9	8	15	6
Piperamphenicol (100 mcg)	ND	6	9	11	11
Ofloxacin (10 mcg)	10	9	8	9	10

® = Resistant; ND = Not Determined

The antimicrobial activity of ethanol and chloroform extracts from the *A. nilotica* fruits against *Pseudomonas aeruginosa* was more effective than both *Klebsiella pneumoniae* and *Proteus vulgaris.* All concentrations of ethanol and chloroform extracts showed inhibition zones except chloroform extract at concentrations of 3 and 1.7%.

The ethanol extracts from the *A. nilotica* at concentrations of 13, 6.7 and 5% and chloroform extracts at concentrations of 13% and 6.7% gave larger inhibition zones when compared with all test antibiotics against *Staph. aureus* and *E. coli.* Both ethanol and chloroform extracts from the *A. nilotica* fruits at concentration of 13% showed larger inhibition zones with *Klebsiella pneumoniae* when compared with all test antibiotics. The chloroform extract at concentration of 13% gave the largest inhibition zone with *Proteus vulgaris,* which was equal to the inhibition zones produced by both cefolaxima (30 mcg) and Co-Trimoxazole (25 mcg). Chloramphenicol showed inhibition zone (15 mm) with these organisms (Table 2). The ethanol extract at concentrations of 13 and 6.7% and chloroform extract at 13% showed largest inhibition zones when compared with other test antibiotics.

Discussion

The present study showed that *A. nilotica* fruit extracts were effective inhibitors of bacterial growth. The extracts of the plant showed varying degrees of activity against Gram-negative bacteria. The ethanol extract was more effective against all test bacteria than chloroform extract. This may be due to the ability of the ethanol to extract a wide range of chemical constituents of the plant fruit while the chloroform might have extracted less numbers of the ingredients.

The fruit extracts showed higher activities against *Staph. aureus* compared with other test bacteria (Gram negative). As *A. nilotica* fruit contains tannins, flavonoids, polyphenolic compounds, glycosides, volatile oils, organic acids and coumarins (El-Shanawny, 1996) the antimicrobial activity of the plant fruit might be due to polyphenolic compounds. The same author added that it has been found that the polyphenolic compounds are responsible for the antibacterial activity of plants. Both essential and volatile oils in the fruits of *Xylopia aethiopica* were found effective against *Staph. aureus, E. coli* and *Candida albicans* (Boakye-Yiadom *et al*., 1977).

The studies of Cheesbrough (1984) also indicated that polyphenolic compounds and/or volatile oils cause inhibition of a wide range of microorganisms. Phenol is well known as a chemical antiseptic. The presence of tannins may have accelerated wound healing probably due to their astringent effect.

The permeability of the cell wall of the Gram-negative organism is generally less efficient than Gram-positive ones probably because of the presence of the high level of phospholipids in the cell wall compared with Gram-positive bacteria (Cheesbrough, 1984).

Al-Yahya *et al*. (1990) found that both ethanol and chloroform extracts from the *A. nilotica* fruit were equally effective against both *Bacillus subtilis* and *Staph. aureus* and that the ethanolic extract was also active against *Proteus vulgaris*. Sotohy *et al*. (1995) reported the effect of ethanol extracts against *Clostridium perfringens*.

The *A. nilotica* fruit is used for the treatment of sore throat, cold, bronchitis, pneumonia, ophthalmia, diarrhea, dysentery, leprosy and venereal diseases (El Shanawny, 1996). Some of these diseases such as, diarrhea and gonorrhea are bacterial diseases and may possibly confirm the antimicrobial activity of the plant fruit. Indeed, the ethanol extract from *A. nilotica* fruit was more effective against the test organisms than the test antibiotics used in this study.

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