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ANTIBACTERIAL ACTIVITY AND ACUTE TOXICITY STUDY OF *ACACIA NILOTICA*

Mulugeta Guta[1,*], Kelbessa Urga[1], Ashenafi Assefa[1], Hirut Lemma[1], Getachew Addis[1], Negero Gemeda[1], Kidist Yirsaw[1], Kissi Mudi[1] and Daniel Melaku[1]

ABSTRACT: Methanol crude extract and chloroform, ethyl acetate, n-butanol and aqueous fractions of the methanolic extract of fruits and stem bark of *Acacia nilotica* (L.) Willd. ex Del. (Fabaceae) were screened for antibacterial activity of diarrhoea-causing bacterial species (*Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhi*) in Ethiopia using standard agar dilution method. Oral acute toxicity studies were also carried out on mice with the ethyl acetate fruit fraction of *A. nilotica*. Compared with standard antibiotic (chloramphenicol and tetracycline) extracts and fractions, *A. nilotica* had low activity ($P<0.05$). The ethyl acetate fruits fraction showed antibacterial activity with minimum inhibitory concentration (MIC) of 0.25 mg/ml. n-Butanol fraction of fruits and stem bark of *A. nilotica* showed lower activity ($P<0.001$) than ethyl acetate and chloroform fraction. The aqueous fraction of the plant did not exhibit any activity at concentration of 2 mg/ml. LD_{50} value of the ethyl acetate fruit fraction was 7393.4 mg/kg with 95% confidence limit of 6019.5-9207.9 mg/kg which was significantly higher in comparison to the active dose of the fraction ($P<0.001$). Results of this study suggested the potential of this highly active fraction and supported the ethnomedicinal uses of the plant to some extent in Ethiopian traditional medicine to treat diarrhoea.

Key words/phrases: *Acacia nilotica*; Antibacterial activity; Crude extract; Traditional medicine.

INTRODUCTION

Plants are the main medicinal sources to treat infectious diseases in some developing countries. In these countries, crowded living conditions and poor hygiene result in diarrhoea and dysentery which are among the main causes of morbidity and mortality and are caused by bacterial enteropathogens (Bern *et al*., 1992). To alleviate the problem of diarrhoea in developing countries, the World Health Organization (WHO) has launched a holistic disease control program that includes all aspects of traditional medicinal practices, evaluation of health education and preventive approaches (Syder and Merson, 1982; Abdullahi *et al*., 2001).

[1]Ethiopian Health and Nutrition Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia
*Author to whom all correspondence should be addressed. E-mail: mulugetaguta@yahoo.com or ddr@ethionet.et

Diarrhoeal diseases are among the ten top diseases in all regions of Ethiopia. Studies in communities, school children and among cases of health institutions show that bacteria and intestinal protozoa are the predominant causes of diarrhoea in Ethiopia (Chang, 1962; Ministry of Health, 2004). *Shigella* species, *Salmonella* species and some of the commonly recognized diarrhoeagenic *Escherichia coli* strains are among the bacterial pathogens recorded in Ethiopia to be the major causative agents of diarrhoeal diseases, both in adults and children (Aberra Geyid, 2004).

The use of herbal drugs in the treatment of diarrhoea is a common practice in many African countries. In Ethiopia, several medicinal plants are used traditionally for treating diarrhoeal infections. One of these plants is *Acacia nilotica*, commonly known by different vernacular names in different localities of the country; Delbeta /Elbeta (Konso), Girar (Amharic), Keselto (Afar) etc. *Acacia nilotica* (L.) Willd. ex Del., (Fabaceae) is a tree 2.5-14 m high, widely distributed in wood lands around 600 m above sea level. The plant is common in Ethiopia, Sudan, Egypt and west Senegal (Thulin, 1989). Many species of *Acacia* such as *A. nilotica*, *A. nubica* and *A. farnesiana* are used in traditional medicine as anthelmintics, antidysentrics, styptics and for the treatment of nasal catarrh, stomatitis, gingivitis, measles, gonorrhea and pustular dermatitis (Elkamali and Khalid, 1998; Dawit Abebe *et al*., 2003).

In this study, an acute toxicity study in mice and activity of crude extract and fractions of *A. nilotica* against *Escherichia coli*, *Shigella dysentrae* and *Salmonella typhi* is reported.

MATERIALS AND METHODS

Plant material

The stem bark and fruits of *A. nilotica* were collected from South Ethiopia, Konso Wereda (600 Km southwest of Addis Ababa) in September 2005.

The plant was identified and Voucher specimens were deposited (GM3) at the Herbarium of Drug Research Department, Ethiopian Health and Nutrition Research Institute.

Extraction and solvent partition procedure

After collection, the plant tissue was separated into stem bark and fruits, air-dried in the shade and ground to a fine powder. The powdered materials (250 g each) were extracted separately by maceration in conical flask for 48 h with 80% methanol (1000 ml x 2) and continuous shaking. The solvent

was removed under vacuum. The percentage yield of the crude methanol extract of the stem bark and fruits of *A. nilotica* was found to be 4.42% and 9.3%, respectively. Some of the crude methanol extract (10 g) obtained was dissolved in water (250 ml) and fractionated by consecutive liquid/liquid partition with chloroform (100 ml x 4), ethyl acetate (100 ml x 4) and n-butanol (100 ml x 4). The extracts were filtered (Whatman No.1 filter paper), concentrated to dryness under reduced pressure, using a rotary evaporator at 40°C (Buchi R-205, Switzerland) yielding, respectively, chloroform fraction (Stem bark: 0.828 g; Fruits:1.29 g), ethyl acetate fraction (Stem bark: 0.07 g; Fruits: 0.175 g), and butanolic fraction (Stem bark: 0.357 g; Fruits: 0.775 g). The last aqueous fraction was freeze-dried (Stem bark: 7.14 g; Fruit: 4.25 g) using a Freeze dry system (LyoPro 6000, Heto, Denmark).

Bacterial strains

All bacterial strains used in the study were clinical isolates. The activity of plant extracts was tested on three different species: *E. coli*, *S. dysenteriae* and *S. typhi*. The bacterial isolates were kindly provided by the Department of Infectious and Other Diseases Research, Ethiopian Health and Nutrition Research Institute.

Antibacterial test

Standard agar dilution method was used to test the antibacterial activity of the *A. nilotica* plant extracts and the standard drug (Mitscher *et al*., 1987; Rios *et al*., 1988). Prior to bioassay procedures, the dried methanol, n-butanol and water extracts were re-dissolved in methanol (20%), whereas the chloroform and ethyl acetate fractions were re-dissolved in acetone. Each extract was diluted and 2 ml of each solution were incorporated in 18 ml of melted Muller Hinton Agar culture medium and poured into a petri dish. The final concentrations of the extract in the medium ranged from 0.25 mg/ml to 4 mg/ml. Once the adjusted inocula were prepared, the suspension was delivered to the agar surface. Inoculated plates were incubated, in air, at 35°C for 24 h before being read. The lowest concentration that inhibited visible growth was recorded as the MIC (minimum inhibitory concentration). Plates containing inoculated growth medium without test extract and medium with solvents used for dilution were used as controls. Each sample concentration was tested in triplicate. For comparison purposes, chloramphenicol and tetracycline were used as reference standards.

Acute toxicity study

Groups of five male adult albino mice weighing 20-25 g (obtained from the animal house of the Ethiopian Health and Nutrition Research Institute) were housed under the same conditions. The animals were treated orally by means of a stomach tube with graded doses of the ethyl acetate fruit fraction of *A. nilotica* (500-12500 mg/kg). Control groups of mice were treated with the pure solvents. The mortality was determined 24 h later, in each group of animals (Shankar *et al*., 2002). The LD_{50} value was determined using probit analysis of SPSS.

RESULTS AND DISCUSSION

Results for anti-diarrhoeal activity of the extracts and fractions are given in Table 1. Inhibition data of bacterial growth in the presence of chloroform, ethyl acetate, butanol, aqueous fractions and methanol extracts of fruits and stem bark of the *A. nilotica* plant were tested against clinical isolates of *E. coli, Shigella sp.* and *Salmonella sp*. Minimum inhibitory concentration (MIC) of standard drugs (chloramphenicol and tetracycline) was found to be at 0.004 and 0.002 mg/ml, respectively, against the test organisms. All tested clinical isolates showed the same sensitivity to the same *A. nilotica* fractions and standard drugs. This showed that the extract might have the same active ingredient which might be responsible for the observed activity, the mechanism of action of the active ingredient might be the same or milligram scale of measurement might obscure slight differences in activity.

Table 1 Minimum inhibitory concentration (MIC) of methanol extracts and chloroform, ethyl acetate, n-butanol and aqueous fractions of fruits and stem bark of *A. nilotica.*

Bacterial strains	MIC (mg/ml)											
	Methanol extract		Chloroform fraction		Ethyl acetate fraction		n-Butanol fraction		Aqueous fraction		chloram phenicol	tetracy cline
	Stem bark	Fruit	Stem bark	Fruit	Stem bark	Fruit	Stem bark	Fruit	Stem bark	Fruit		
E.coli	1	0.5	1	0.5	0.5	0.25	2.5	2.0	4.0	3.5	0.004	0.002
S. typhi	1	0.5	1	0.5	0.5	0.25	2.5	2.0	4.0	3.5	0.004	0.002
S. dysenteriae	1	0.5	1	0.5	0.5	0.25	2.5	2.0	4.0	3.5	0.004	0.002

As can be seen from the results, the most potent activity was obtained from the ethyl acetate fraction of the fruit of *A. nilotica*, in which case the growth of all the three organisms was inhibited at 0.25 mg/ml (Table 1). Similarly, the ethyl acetate fraction of the stem bark gave better activity ($P<0.001$) when compared to other fractions and total inhibition occurred at 0.5 mg/ml (Table 1). n-Butanol fraction of fruits and stem bark of *A. nilotica* showed a lower activity ($P<0.001$) than ethyl acetate and chloroform fractions. It is interesting to note that the aqueous fraction of the study plant parts, which would be the preferred method of preparing the plants when used in traditional medicines, did not exhibit any activity at concentration of 2 mg/ml. There are two possible explanations for effects observed: (i) the same active substances were present in water extracts but at concentrations at which bioactivity was no longer detectable; (ii) active substances were soluble in organic solvents and, therefore, basically not present in water extracts. Although the MICs of the fruits and the stem bark fractions were high ($P<0.05$), when compared to those of the available antibacterial agents such as chloramphenicol and tetracycline, the present investigation lends a reasonable support to the use of the fruit and stem bark extracts interchangeably especially by the Afar people of Eastern Ethiopia for treatment of diarrhoea. Moreover, the antibacterial activity of the stem bark and the fruit should not be underestimated since further studies aimed at the isolation and identification of the active constituents may result in compounds with better therapeutic value.

As shown in Table 2, the LD_{50} value of ethyl acetate fruit fraction which was selected for its highest antibacterial activity was 7393.4 mg/kg with 95% confidence limit of 6019.5-9207.9 mg/kg. The LD_{50} value was found to be significantly higher ($P<0.001$) in comparison to the active dose of the extracts. The relatively high safety margin observed indicates the prospect of the plant for further development. Toxicity studies of other *Acacia* species have also revealed that the plants are not toxic to a range of animal systems (Redhaiman *et al*., 2003).

Table 2 Acute toxicity response of ethyl acetate fractions of fruits of *A. nilotica* in mice[a].

Dose of extract (mg/kg)	Total no. of mice/group	No. of dead mice	% of dead mice
500	5	0	0
1000	5	0	0
2500	5	0	0
3500	5	0	0
5000	9	2	22.2
7500	10	4	40
10000	4	3	75
12500	5	5	100

[a] LD_{50} value of 7393.4 mg/kg and 95% confidence limit is 6019.5-9207.9 mg/kg

CONCLUSION

It can be concluded from the present study that *A. nilotica* is a plant with a potent activity against diarrhoea-causing microorganisms, particularly *E. coli*, *S. typhi* and *S. dysentrae*, which are among the major causes of diarrhoea in Ethiopia. This study can be a basis for further investigation with detailed and more bacterial isolates and for the isolation of pure compounds from the active fraction.

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