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In vitro antimicrobial activity of *Albizia amara* leaves from Lindi region, Tanzania

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ABSTRACT

Ethnopharmacological relevance: *Aqueous Albizia amara leaf extract is used by traditional healers for treatment of diarrheal diseases.*
Aim of study: *To evaluate the antimicrobial activity of chromatographic fractions obtained from Albizia amara leaf extract on microorganisms.*
Materials and methods: *Chloroform and chloroform-methanol fractions were eluted from silica gel column chromatography, monitored by TLC and evaluated for antimicrobial activity using the disk diffusion method on the following microorganisms; Escherichia coli, Salmonella typhi, Klebsiella pneumoniae, Bacillus cereus, Staphylococcus aureus, Candida albicans and Cryptococcus neoformans. Diameters of zones of inhibition were used to indicate antimicrobial activity.*
Results: *Antimicrobial activity was observed to increase with polarity of eluting solvent system. Fractions eluted by less polar solvent systems did not show antimicrobial activity against any of the tested microorganisms. Fractions eluted with the mobile phase with higher methanol concentration showed the broadest antimicrobial spectrum by inhibiting all the tested microorganisms. The most susceptible bacteria were E.coli and S. typhi, and the least susceptible was K. pneumoniae while the most susceptible of the fungi was C. neoformans.*
Conclusion*: Polar fractions displayed broadest antimicrobial activity; hence, aqueous leaf extracts of the plant reported to be used in traditional medicine is supported by the findings of this study. Importance of fractionating crude extracts to obtain full picture of biological activity is emphasized and cytotoxic studies to determine toxicity of the fractions and isolation of the active compound(s) are recommended.*

Keywords*: Antimicrobial activity, Traditional medicine, Extract, Fractions, Chromatography*

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INTRODUCTION

Many of the antimicrobial drugs currently used to treat bacterial and fungal infections were originally developed from natural sources such as (medicinal) plants, minerals and animal (Coe and Anderson, 1996). Importance of medicinal plants is emphasized by the fact that over 80% of the world population relies on medicinal plants for basic health care. (Sabir et al., 2007).

Albizia amara (Fabaceae) is one the medicinal plants used for alleviating a variety of ailments including microbial infections. It is one of 150 fast growing species of the genus Albizia. Synonymously, the plant is also known as *Acacia amara* (Willd), *Acacia nellyrenza* (Wight & Arn), *Acacia wightii* (Wight & Arn), *Mimosa amara* (Roxb), and *Mimosa pulchella* (Roxb) [1]. In Shinyanga region in Tanzania, the plant is locally known as Igala in Bariadi and Shinyanga rural districts while in Meatu district the plant is known as Mtangala (Mbuya et al., 1994.).

The plant is a perennial, non-climbing tree well distributed in the tropics and subtropics, Tanzania inclusive (Brenan, 1959). In Tanzania, the plant is medicinally used by traditional healers for treatment of various ailments. For example it is used for treatment of epilepsy in Bariadi district, alleviating backache and loin pain by farmers in Meatu district, and for abdominal problems including diarrhea in Shinyanga rural districts (Mbuya et al,. 1994).

Other medicinal uses have also been reported from other parts of the world. In Tamilnadu, India, the plant is ethnomedically used for dandruff whereby the leaves previously dried in the shade are powdered and applied on the scalp. Non medicinal used of the plants include adulteration of tea and fodder for animals, firewood and for making charcoal, timber for making furniture, and the roots for making soap (Farnsworth et al.,1985,)

Biological investigations on the plant have shown the crude leaf extract to exhibit bactericidal activity against *Salmonella typhimurium* strain TM677 (Pezzuto et al., 1991). The plant is also reported to show larvicidal and pupicidal activity on larvae and pupae of *Aedes aegypti* (Woongchon et al., 1991). *Albizia anthelmintica*, a different species in the same genus was also reported to show strong anti-candidal activity (Runyoro et al., 2006)

The leaves contain macrocyclic pithecolobine alkaloids which inhibit the catalytic activity of DNA polymerase, RNA polymerase and HIV-1 reverse transcriptase. The alkaloids also inhibited platelet aggregation, human lymphocyte transformation, phorbol-ester-induced chemiluminescence with human granulocytes, and cyclo-oxygenase activity (Pezzuto et al., 1991 and Dery et al., 1999).

Antimicrobial activities on the plant have performed elsewhere on the crude extract but none on the fractionated extract in Tanzania. Thus, antimicrobial activities of thin layer chromatography (TLC) monitored fractions from fractionated crude leaf extract of *A. amara* are presented in this paper.

MATERIALS AND METHODS

Collection of the plant materials

Leaves of the plant were collected from Lindi region tropical forest (November, 2006) in a previous study to screen for plants with cytotoxic activity against cancer cells. The plant was identified and authenticated at the University of Dar es Salaam, Faculty of Science, Department of Botany where the herbarium specimen is also deposited.

Extraction process

Air dried leaves were ground using a hammer mill to yield 518 g of ground leaf material of which 516 g were exhaustively extracted by cold maceration in 2.5L of methanol at room temperature with occasional shaking for three days. The mixture was then filtered through Whatman filter paper (No 1, England) and the extract was concentrated *in vacuo* under 40°C (Buchi, Essen), followed by freeze drying to obtain 103.5 g of crude dry powder extract.

Chromatographic procedures

Column chromatography

Column chromatography was preceded by TLC analysis which identified several spots with different HR_f values suggesting presence of several different compounds in the extract.

An amount of 84.7 g of the dry powder crude methanolic leaf extract was subjected to column chromatography and eluted with solvent systems of gradually increasing polarity using chloroform and methanol in the ratios of chloroform: methanol; 100:0, 95:5, 90:10, 80:20, 60:40, 50:50 and 0:100. A total of 270 fractions, 50 ml labeled F1 to F270 were obtained. (Table 1)

Thin Layer Chromatography (TLC)

Thin layer silica chromatographic plates were used to monitor the 270 fractions obtained from column chromatography (F1 to F270) using chloroform and methanol at different proportions as a mobile phase.

Staining of TLC plates was conducted using previously prepared anisaldehyde and visualization was done under short (250nm) and long range (375nm) ultra violet (UV). After TLC analysis, fractions were divided into 11 groups depending on the HR_f values which were obtained. These groups (D1 to D11) were differently concentrated *in vacuo* and freeze dried to obtain dry powders. Weights of each group were recorded as shown in Table 1.

Antimicrobial Studies

Test materials and Test Microorganisms

The combined fractions (D1 to D11) were dissolved in acetone and DMSO (for D7 and D8 which were not soluble in acetone) to obtain test solutions (500mg/ml) which were tested against five standard bacteria and two standard fungi strains.

Bacterial strains used were *Escherichia coli* (NCTC 10418), *Salmonella typhi* (NCTC 8385), *Klebsiella pneumoniae* (NCTC 9633), *Bacillus cereus* (NCTC 7464), and *Staphylococcus aureus* while the fungal strains were *Candida albicans* and *Cryptococcus neoformans*, all obtained from the pharmacology laboratory at the Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences (MUHAS).

Susceptibility Tests

The disc diffusion method (Hewitt and Vincent, 1989) was used to screen the antimicrobial activity of the combined fractions.
Paper discs (diameter 6mm) were made from sterile filter paper and impregnated with 10µL of test solution to obtain 5mg/disc. The procedure was repeated to produce eleven sets of impregnated discs for the 11 test samples.

Tryptone soybean agar and Saboraud's dextrose agar (SDA) were used to prepare solid growth media for bacteria and fungi respectively. Plates were made by pouring molten media into sterilized Petri dishes which were left to cool and solidify. Then 0.1% inoculum suspension of microorganism (equivalent to Mc Farland turbidity standard (Cheesbrough, 1984)) was applied uniformly on the surface of the solidified agar using sterile cotton swab. This procedure was repeated to obtain five sets for the bacteria and two for the fungi. The sets were then left to dry.

The differently impregnated discs were placed (in triplicates) on the medium and left for 30 minutes to allow for diffusion of the compounds. Gentamycin and clotrimazole used as standard antibacterial and antifungal agents respectively were incorporated at the center while the pure solvents, acetone and DMSO were used as negative controls. The agar plates were afterward incubated at 37ºC for 24 hours.

Subsequently the diameters of the clear zones around the discs were measured using a ruler and recorded in millimeters. The difference between disc diameter (6mm) and diameter of the clear zone was used to calculate the inhibition zone for each sample as adopted from Jose et al., 2002.
Activity index (AI) of each sample was calculated by using the following formula:

$$Activity\ Index = \frac{Mean\ Inhibition\ Zone\ of\ the\ Sample}{Mean\ Inhibition\ Zone\ of\ the\ S\tan dard}$$

RESULTS AND DISCUSSION

Table 1 presents the fractions (D1 to D11) collected from column chromatography with their respective elution solvent systems.

Table 1: TLC- Monitored fractions from column chromatography

Combined Fraction (D)	Column Fractions (F)	Solvent System (%)		Weight (Gm)
		Chloroform	Methanol	
D1	F1 – F10	100	0	36.9
D2	F11 – F20	95	5	40
D3	F21 – F41	90	10	12.5
D4	F42 – F82	80	20	39.4
D5	F83 – F120	60	40	48.2
D6	F121 – F129	60	40	37.4
D7	F130 – F136	60	40	40.3
D8	F137 – F189	50	50	41.2
D9	F190 – F241	0	100	35
D10	F242 – F262	0	100	39.3
D11	F263 – F270	0	100	36.3

Antimicrobial activities of the fractions are summarized below showing the diameter of the inhibition zones (table 2) and activity indices (Table 3, figure 1)

Table 2: Antimicrobial activity of *A. amara* column chromatography fractionated extract

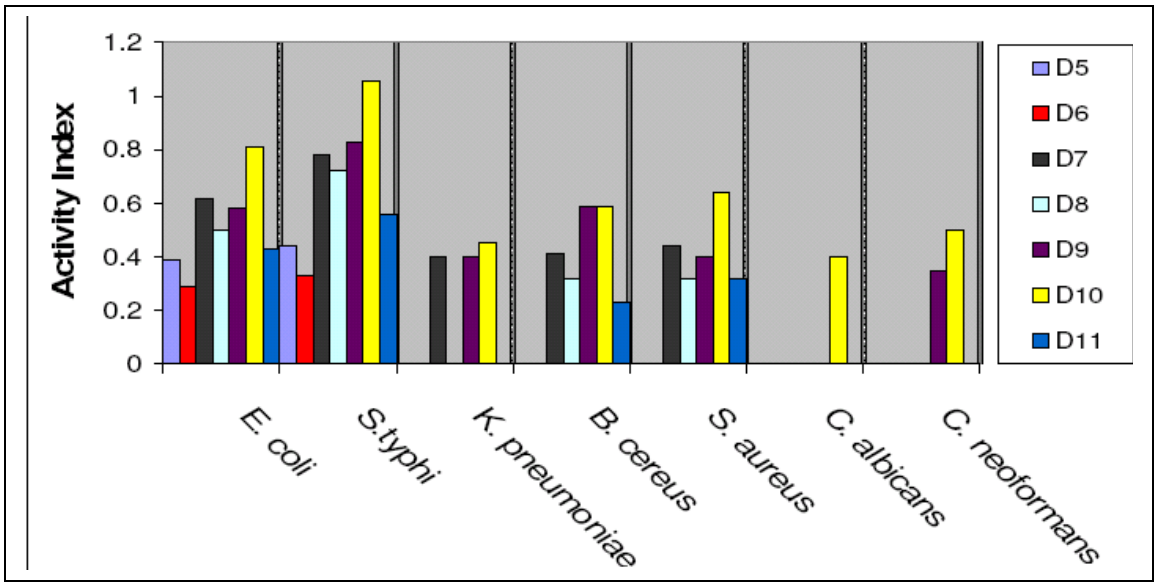
Fraction/ Standard	Microorganism Inhibition zone (mm)*						
	E. coli	***S. typhi***	***K. pneumoniae***	***B. cereus***	***S. aureus***	***C. albicans***	***C. neoformans***
D1	-	-	-	-	10	7.5	16
D2	13	15	21	11	26	NA	-
D3	-	-	-	8	6	14	13
D4	15	19	10	18	NA	-	-
D5	-	-	-	-	8	-	8
D6	9	-	20	NA	-	-	-
D7	-	-	-	9	7	13	13
D8	5	22	NA	-	-	-	-
D9	-	-	11	8	10	16	8
D10	25	NA	-	-	-	-	-
D11	-	-	-	-	8	-	NA
Genta.	20	-	-	-	-	-	-
Clotr.	-	-	7	10	-	NA	20

D1-D11: Combined fractions
Genta: Gentamycin
Clotr: Clotrimazole
NA: Not applicable
*Includes diameter of disc (6mm)

Table 3: Activity indices of active fractions

Microorganism	Active Fractions						
	D5	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**
E. coli	0.39	0.29	0.62	0.50	0.58	0.81	0.43
S. typhi	0.44	0.33	0.78	0.72	0.83	1.06	0.56
K. pneumoniae	-	-	0.40	-	0.40	0.45	-
B. cereus	-	-	0.41	0.32	0.59	0.59	0.23
S. aureus	-	-	0.44	0.32	0.40	0.64	0.32
C. albicans	-	-	-	-	-0.40	-	-
C. neoformans	-	-	-	-	0.35	0.50	-

Figure 1: Activity indices of the active fractions



Seven out of the 11 fractions tested for antimicrobial activity (D5 to D11) showed antimicrobial activity by inhibiting one or more microorganisms.

The active fractions were more active against bacteria than fungi. Only two fractions (D9 and D10) exhibited antifungal activity.

The Gram-negative bacteria *Escherichia coli* and *Salmonella typhi* were the most susceptible while *Klebsiella pneumoniae* was the least susceptible bacterium. Of the two fungi tested, *Cryptococcus neoformans* was the most susceptible.

Fraction D10 showed the highest and widest antimicrobial activity by inhibiting all tested micro organisms with the highest activity exhibited against the bacteria compared to the fungi. The fraction (D10) exhibited an interestingly higher activity against *Salmonella typhi* compared to the standard drug, gentamycin (AI = 1.06; Table 3, figure 1). Since fraction D10 was eluted with the most polar mobile phase system (higher methanol concentration which was 50:50) then, it contains polar compounds from the crude extract.

Antimicrobial activity shown in fraction D11 could be due to tailing effects during chromatographic elution that could have caused some compounds that were supposed to be collected in D10 to appear in D11.

Fraction D7 was active against all tested bacteria but the activity was less than that shown by D10. It showed the highest activity against *E. coli* and the least activity against *K. pneumoniae*.

A similar pattern was observed for fractions D8 and D11, both of which were active against *E. coli*, *S. typhi*, *B. cereus* and *S. aureus* but inactive against *K. pneumoniae*.

Of the seven active fractions tested, D5 and D6 showed the narrowest range of antimicrobial activity, inhibiting only the Gram-negative bacteria, *E. coli* and *S. typhi.*
Antimicrobial study previously conducted on the extract was positive for *Salmonella typhi* only (Pezzuto et al., 1991).

The broad antimicrobial activity observed in this study indicates that fractionation of the extract may have concentrated some compound (s) that could not display or partially display their antimicrobial activity in presence of other compound (s) (antagonism) present in the crude extract.

Antimicrobial results also show an increase in the spectrum of activity with the increase in the polarity of the solvent system used to elute the column as depicted by the trend in chart 1. The first four fractions (D1 to D4) were inactive, D5 and D6 were active against the Gram-negative bacteria, D7 to D11 were active against both Gram negative and Gram positive bacteria.

These findings also support the use of the plant in traditional medicine for treatment of diarrhea and other abdominal problems. Microorganisms (*Escherichia coli, Bacillus cereus*, *Staphylococcus aureus* and *Salmonella typhi*) against which the fractions exhibited activity are among the microorganisms implicated for causing diarrhea and other abdominal problems in humans.

The highest antimicrobial activity was exhibited by the fractions eluted with the most polar solvent system correlating with the use of aqueous extract of the plant used in tradition medicine (and by traditional healers).

CONCLUSION

Wider antimicrobial activity was shown by the fractionated extract (fractions) compared to the narrow antimicrobial spectrum of the crude extract [7]. This suggests low concentration in the crude extract. Fractionation of crude extract is therefore essential as biological activity is increased when the active principles are concentrated by fractionation compared to the crude extract in which active principles may be interacting with other components that may musk their biological activity. Polar fractions have the highest antimicrobial activity; hence use of aqueous *Albizia amara* leaf extract for treatment of diarrhea and other infectious abdominal problems is justifiable. However, antimicrobial activity is significant and of wide spectrum after fractionation. This study presents findings of antimicrobial activity of the fractions obtained after column chromatography. Further studies are recommended to isolate and identify the nature of the antimicrobial (s) present in the extract.

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