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ANTIBACTERIAL INVESTIGATION OF CRUDE EXTRACTS OF THE ROOT BARK OF G*LIRICIDIA SEPIUM*

Abulude, F.O[1] and Adebote V.T[2]
[1]Department of General Studies, Federal College of Agriculture, Akure
[2]Department of Science Laboratory Technology, Rufus Giwa Polytechnic, Owo

ABSTRACT
Antibacterial activity of aqueous and organic root bark extracts of *Gliricidia sepium* were investigated against *S. aureus*, *B. subtilis*, *C.pyogenes*, *S. typhi* and *E. coli* using the disc diffusion method. The water and ethanolic extracts showed inhibitory effects on the microorganisms under study. The n-hexane extract was not active on all the microorganisms. The results showed that the plant extracts of *Gliricidia sepium* would be useful as antifungi, antibacterial and anti-inflammatory.

KEYWORDS: *Gliricidia sepium*, ethanol, antimicrobial agent, inhibitory effects.

INTRODUCTION

Gliricidia is an increasingly used forage crop in cut-and-carry systems in parts of the humid tropics including Southeast Asia and Sri Lanka. In other areas such as West Africa, India and the Philippines, its use is severely limited by palatability problems; it is little used as forage within its native range in Central America. Despite mixed perceptions of Gliricidia as a forage crop, it has been widely promoted by development agencies and researched, due largely to its high productivity and quality. Interest has increased in recent years following the widespread defoliation of *Leucaena* by psyllid. Gliricidia is one of the few forage trees capable of leaf yields comparable to those of Leucaena and will grow on a wider range of soils, tolerating low pH provided that this is not associated with high aluminium saturation. After *Leucaena leucocephala*, Gliricidia is believed to be the most widely cultivated multipurpose tree. It has not been used in commercial livestock production systems. Gliricidia occurs in abundance throughout its native range in Mesoamerica. Its domestication has been in progress for millennia. The Spanish called it 'madre de cacao' to describe its use as a cocoa shade and Yorubas called it ‘agunmaniye’. The toxic properties of its seeds and bark give rise to the generic epithet (*Gliricidia* = mouse killer) as well as some common names. Present day uses (including firewood, hedges, shade, poles and as an ornamental) amongst the Yoruba natives are likely extensions of early utilization. *Gliricidia sepium* has also been used extensively throughout the humid tropics. These landrace populations are remnants of introductions used to shade plantation crops although recently they are being integrated into farming practices for poles, firewood, hedges, forage, green manure and soil stabilization.

Gliricidia sepium has been reported to be expectorant, insecticidal, rodenticidal, sedative and suppurative. Madre de Cacao is a folk remedy for alopecia, boils, bruises, burns, colds, cough, debility, eruptions, erysipelas, fever, fractures, gangrene, head-ache, itch, prickly heat, rheumatism, skin, sore, tumors, ulcers, urticaria, and wounds (Duke and Wain, 1981). This study was carried out to determine the antibacterial activity of crude extracts of the root bark of G*liricidia sepium.*

EXPERIMENTAL

Collection and preparation of plant sample

The root bark of *Gliricidia sepium* was collected from Federal College of Agriculture, Akure, Ondo State, premise. The sample was authenticated at the Department of Horticulture, of the College. The root bark was air-dried, ground, sieved and stored in air tight container in an ambient temperature prior to analyses.

Source of Microorganisms

The antimicrobial activities of prepared solutions were determined using paper disc method. The papers were cut into small sizes and introduced into beakers containing the diluted extracts. They were dried at 50^{o}C and used in determining the minimum inhibitory concentration and zones of inhibition. Standard strains of

Staphylococcus aureus, NCTC 6571, *Bacillus subtilis* ATCC 11779, *Corynebacterium pyogenes*, ATCC 10242, *Escherica coli* NCTC 10418 and *Salmonella typhi* NCTC 52311 were obtained from Department of Microbiology, Lagos State University Teaching Hospital (LUTH), Lagos. All inocula were standardized using the methods described by Bauer *et al*., (1966)

Extraction of phytoconstituents and determination of antimicrobial activity of extracts
Aqueous and organic (ethanol and n-hexane) extraction was carried out by soaking 25 g of the stem bark of plant material in three different conical flasks, and left to stand for 36 h. The extracts were filtered and later concentrated by rotary evaporation and the concentrated extracts were cooled and then stored in the refrigerator prior to analyses.

The antibacterial activities of the extracts were determined using the disc diffusion method (Faruq *et al*., 2004). Serial concentrations of extracts ($1x10^3$ $2x10^3$ $3x10^3$ $4x10^3$ mg/ml) were prepared with solvents (water, ethanol and n-hexane). The bacterial load is 0.5ml of 1.0×10^{-3}. The isolates were separately cultured over each nutrient agar plate. The sterilized discs incorporated with the extract were incubated at 37 ^oC for 24 h.

The zones of inhibition were measured by the use of a transparent plastic ruler and the minimum inhibitory concentration (MIC) was also determined.

RESULTS AND DISCUSSION
Table 1: Zone of inhibition (mm) of bacterial growth of the root bark extract of *Gliricidial sepium*

	Water				Ethanol				n-Hexane			
	Concentration											
Organism	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$	$1x10^3$	$2x10^3$	$3x10^3$	$4x10^3$
S . *aureus*	34	34	40	30	35	36	38	0	0	0	0	0
C. pyogenes	32	35	37	40	33	34	36	37	0	0	0	0
B. subtilis	24	28	30	28	28	30	31	33	0	0	0	0
S. typhi	30	36	36	40	30	32	33	35	0	0	0	0
E. coli	12	20	18	19	17	17	20	20	0	0	0	0

The results of antibacterial test are shown in Table 1. All the microbial organisms were found to be susceptible to various concentrations of the extracts, with zone of inhibition ranging from 12 - 40 mm (water extracts) and 17 - 38 mm (ethanol extracts), but hexane extracts did not exhibit any activity against the test bacteria. The results of the antibacterial test are corroborated by the findings of Akpan and Usoh (2004), who reported on the antibacterial activity of the extracts of *Raphia hookeri* and found that it inhibited the growth of *E. coli, and S. aureus.* Fagbohun *et al*., (2004) reported on the antibacterial activity of the extracts of the peels of *Dioscorea dumentorum* and found that it inhibited the growth of *E. coli and S. aureus* and Hassan *et al*., (2004), who also reported inhibitory role of *Detarium microcarpum* on *S. aureus, C. pyogenes, B. subtili, S. typhi and E. coli*. Antimicrobial substances may affect the synthesis of peptidoglycan around a bacterial cell, the cell will die by osmotic shock (Akpan and Usoh, 2004).

Table 2: Determination of Minimum inhibition concentration (MIC) of *Gliricidia sepium* extracts

Organism	Concentration (mg/ml)					
	$4x10^3$	$3x10^3$	$2x10^3$	$1x10^3$	$9x10^4$	$8x10^4$
Water						
S. aureus	+	+	+	+	+o	-
C. pyogenes	+	+	+	+	+o	-
B. subtilis	+	+	+	+o	-	-
S. typhi	+	+	+	+	+o	-
E. coli	+	+	+	+o	-	-
Ethanol						
S. aureus	+	+	+	+o	-	-
C. pyogenes	+	+	+o	-	-	-
B. subtilis	+	+	+	+o	-	-
S. typhi	+	+	+o	-	-	-
E. coli	+	+o	-	-	-	-
n-Hexane						
S. aureus	-	-	-	-	-	-
C. pyogenes	-	-	-	-	-	-
B. subtilis	-	-	-	-	-	-
S. typhi	-	-	-	-	-	-
E. coli	-	-	-	-	-	-

+ = Inhibition - = No inhibition +o = MIC

Table 2 shows the MIC of the water and ethanol extracts of G. *sepium*. Results showed that the extracts of water and ethanol from this plant have bactericidal activities, over the concentration range of 9.0×10^4 to 4×10^3 mg/ml. Doughari *et al.,* (2007) showed that extracts of P*sidium guajava* was strong antimicrobial agents against enteric pathogens . Tambekar *et al*., (2007) examined triphala: a traditional Indian herbal preparation and recorded potent antibacterial against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus epidermidis, Salmonella typhi, Salmonella typhimurium, Enterobacter aerogenes.* Jombo and enenebeaku (2007) reported that the extract of *Jatropha curcas* inhibited the some pathogens. All the microbial data obtained in the present study are in concord with the finding of these scientists.

The activity against the different category of microbial organisms by the barks is an indication that the plant can be used to source newer group of antibacterial substances that can be used to develop more effective antibacterial agents. the present study also revealed the importance of natural products to control enteric bacterial pathogens which are being a threat to human health.

CONCLUSION

This present study has confirmed that the crude extracts of the root bark of G*liricidia sepium* is a good antimicrobial agent.

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