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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF THE LEAF AND ROOT BARK EXTRACTS OF *CALOTROPIS PROCERA (AIT)*

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The antimicrobial activity of the cold methanolic extracts of the leaf and root bark of *Calotropis procera (Ait)*, (Fam: Asclepiadaceae) was determined using the agar diffusion method. *Staphylococcus aureus, Escherichia coli, Candida albicans, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Enterobacter aerogenes* were used as test organisms. Antimicrobial activity was observed with the leaf extract on four of the organisms tested, while the root bark showed activity on only two of the test organisms. The minimum inhibitory concentration (MIC) for the positively tested organisms lie within the range of 10-50 mg ml^{-1}. Phytochemical analysis conducted on the samples revealed the presence of saponins, glycosides, and simple sugars in the leaves, while the root bark was found to contain tannins in addition to these groups. Fractionation of the crude extract by solvent-solvent extraction procedure indicated that the organic acidic and organic basic fractions were more active. Infra-red (IR) spectroscopy of the two factions indicated the presence of carboxylic acids and carbonyl groups. The findings of this study conforms with the local use of the plant.

Key words: *Calotropis procera*, Antimicrobial activity, Phytochemical analysis.

Introduction

Calotropis procera (Ait) (Fam: Asclepiachaceae) commonly known as "swallow warts" is a shrub or small tree, reaching up to 2.5m in height. The leaves are broad and the stem is usually simple, rarely branched, woody at the base and covered with a fissured corky bark.

The plant is reported (FAO 1986) to be found in areas of abundant cultivation, especially sandy soils in areas of low rainfall. The report also indicated that the compounds of emato-cathetic, digitalic, bactericidal and vermicidal properties are present in the plant. The root bark is an emetic, while the flower is a digestive stomachic and a tonic. Flowers of the plant are used for asthma and cattarrh, while the local fulani traditional herbalist mostly use the root decoction with red potash against vomiting. The latex from the plant contains heteroside such as Calotropin (acardiac poison), a proteolytic enzyme (calotropin) as well a Calactin, Ucharidan and Uscharin (Watt and Breyer-Brandwijk 1962). The plant has been reported by Audu (1989) to be a very useful actiseptic for wounds.

Due to the fact that this plant is very useful, as indicted by these reports and the fact that little information is available on its biological activity, there is a need to find out more about the potential of this plant as antimicrobial agent. The present study is therefore designed to assess the potency of the plant extract on selected microorganisms and to determine the goup of compounds present in the extent.

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Materials and Methods

Sampling and extraction. Fresh samples of the root bark and leaves of the plant were collected. The root bark was dried under shade and ground into powder, while the leaves were used fresh for extraction. Both samples were extracted (cold) in methanol in the ratio of 1:5 w/v for 72 h. The extracts were evaporated to dryness, by first concentrating using the rotary evaporator and further dried in the fume cupboard.

Pure cultures of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, Klebsiella aerogenes* and *Enterobacter aerogenes* were collected from the microbiology laboratory of Abubakar Tafawa Balewa University (ATBU), Bauchi, to be used as test organisms.

Anti-microbial screening. The antimicrobial activities of the two extract were determined using the impregnated paper disc method, described by Casal (1979) and Chung *et al* (1990). Zones of inhibition for each extract on each organisms were measured and recorded in millimeter. The minimum inhibitory concentration (MICs) for each organism tested positive for the extract were also determined by preparing varying concentrations of the extracts and were tested using the procedure already described.

Phytochemical analysis. Extracts of the two parts were subjected to phytochemical analysis for the detection of some groups such as tannins, alkaloids, cardiac glycosides, saponins and reducing sugars, using standard analytical procedures (Pew 1948; Plummer 1948; Wall *et al* 1954; Shoppe 1964; Harborne 1973; Trease and Evans 1983). A general purpose

protocol for the extraction of natural products soluble in organic solvents was used to fractionate the crude extracts. Each fraction obtained was tested for activity using the same procedure for antimicrobial activity and then subjected to IR, using the Parkin Elmer 1310 IR spectrometer.

Results and Discussion

The results for the preliminary antimicrobial activity of the crude extracts are given in Table 1. The leaf extract was found to be more active, with the highest activities recorded with *S. aureus* and *Ent. aerogenes* (12.0 mm and 19.0 mm, respectively). Activity of the root bark was observed, only on *Ps. aeruginosa* and *Ent. aerogenes*. The high activity observed with the leaf extract over the root bark could be attributed to disparity in distribution of the active ingredients among other factors.

The results for the minimum inhibitory concentration (MIC) of both the extracts are presented in Table 2. The MIC refers to the least concentration of extract with some inhibition. It was observed that for the root bark, the MIC for the various organisms tested was between 100 and 150 mg ml^{-1}, while for the leaf it was between 10 and 50 mg ml^{-1}. Different responses exhibited by the test organisms to varying concentrations may be due to their nature, as indicated by the reports of Joda *et al* (1989). In some cases it can also be attributed to the fact that concentration of the active ingredient was not high enough to cause inhibition (Garreft and Brown 1964).

Fractions from solvent-solvent extraction were also observed to have activity on the test micro-organisms. The results of this analysis is shown in Table 3. High activities were observed with these fractions, especially the organic basic fraction. This would probably be due to the fact that the active

Table 1
Preliminary antimicrobial screening of crude extract

Sample	Zones of inhibition (mm), 24 h					
	S.aureus	*E.coli*	*Ps. Aeruginosa*	*K. aerogenes*	*Ent. aerogenes*	*C.albicans*
Leaf extract	12.00	10.00	3.00	7.00	19.00	10.00
Root extract	<1.00	1.50	8.50	<1.00	10.00	<1.00
N.C. (dist. water)	-	-	-	-	-	-
P.C. (Ampiclox)	24.00	13.00	7.00	7.00	27.00	10.00

Key: N.C., Negative control; P.C., Positive control; -, Not active

Table 2
Minimum inhibitory concentration of extracts for different micro-organisms

	Root bark extract		Leaf extract			
	Zones of inhibition (mm), 24 h		Zones of inhibition (mm), 24 h			
Conc. (mg m^{-1})	*Ps. Aeruginosa*	*Ent. aerogenes*	*Ent. aerogenes*	*S.aureus*	*E.coli*	*C.albicans*
500	6.20	8.10	13.20	9.60	8.60	8.00
450	4.50	6.30	11.50	8.70	8.20	8.00
400	4.20	5.00	10.80	8.20	7.80	7.40
350	3.00	5.00	10.20	7.10	7.50	700
300	2.60	4.20	9.50	6.50	6.20	6.20
250	2.00	400	9.00	6.00	5.70	6.10
200	1.70	3.50	7.50	5.00	5.20	5.10
150	1.20	1.70	5.50	4.80	4.20	3.80
100	>1.00	1.20	4.40	2.50	3.10	3.00
50	-	<1.00	2.00	1.30	1.70	1.70
		-	-	<0.5	-	

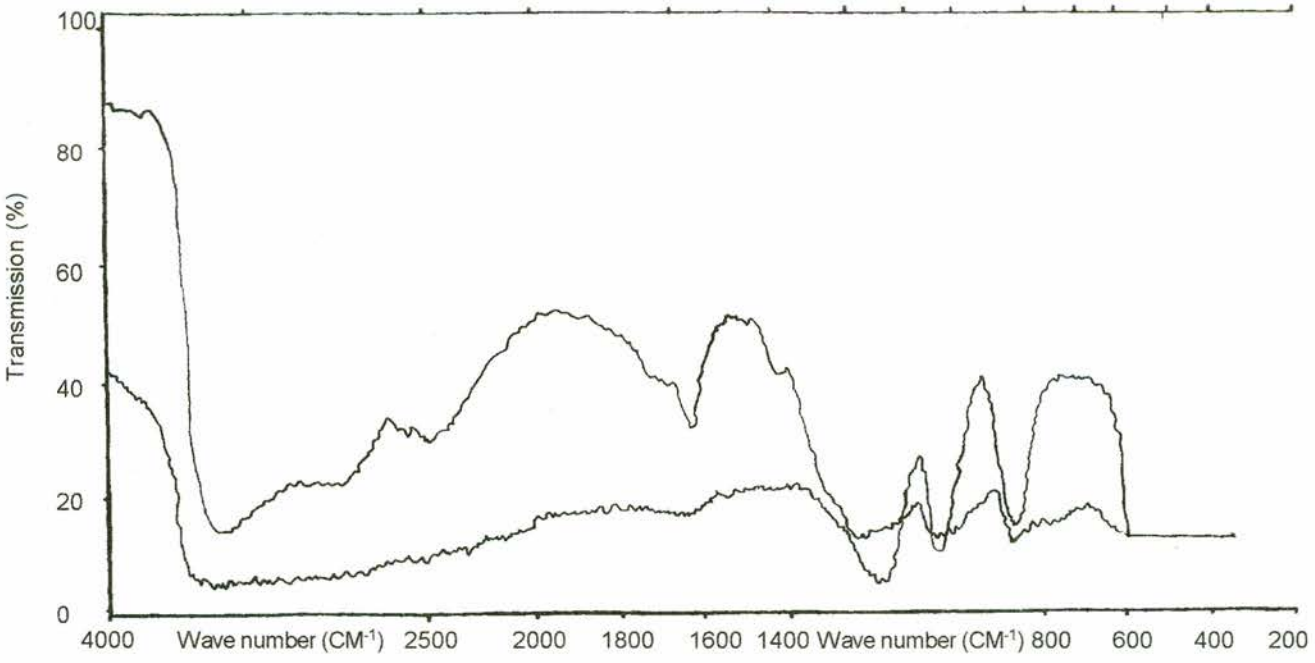

Fig 1. IR absorption spectra of extracts. (Key: A=Root bark extract, B=Leaf extract).

Table 3
Effects of various fractions of extracts on test organisms

		Fraction of extracts		
Sample	Test organisms	Org. acidic	Org. basic	Org. neutral
Leaf	*S. aureus*	+++	+++	-
Leaf	*E. coli*	+	++	-
Leaf	*Ent. Aerogenes*	-	++	-
Leaf	*C. abicans*	-	+	-
Root bark	*Ps. aeruginosa*	+	+	-
Root bark	*Ent. aerogenes*	-	++	-

Key: +++, highly active (zone of inhibition> 14mm); ++, moderately active (zone of inhibition <14mm); +, active (zone of inhibition <7mm); not active (zone of inhibition <1mm)

ingredient(s) were more soluble in basic medium than in either acidic or neutral.

Activity of the basic fraction was also found to be even higher than that of the parent crude extract, suggesting that it may contain nearly pure active compounds with most of the impurities removed in the aqueous layers.

Results for the different goups of compounds analysed indicated the presence of tannins, saponins, cardiac glycosides and reducing sugars in the leaf extracts, while the root bark was found to contain only saponins, cardiac glycosides and reducing sugars. The presence of some of these compounds might be responsible for the activity of the extracts. This is in agreement with the previous claims (Watt and Brandwijk 1962; Scherbanvaskii 1971; Leven *et al* 1979; Hashem *et al* 1980) linking the antimicrobial properties of plants to the presence of tannins, alkoloids, saponins, glycosides, unsaturated sterols and terpenes, most especially tannins. Fig 1 shows the IR absroption spectra of the organic basic fraction of both the root bark and the leaf extracts. The broad absorption at 3000-3600 cm^{-1} suggests the presence of an OH (hydroxyl) group of a caboxylic acid, while the absorption at 1625 cm^{-1} suggests the presence of a carbonyl ($\geq$ C=O) group of aldehydes or ketones.

In summary, the result of this work further justifies the use of this plant as antiseptic for wounds and ulcers, as already claimed by Audu (1989).

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