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ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS AGAINST SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT

Detarium microcarpum, Anogeissusleio carpus and *Carissa edulis* plants are traditionally used for their medicinal value in tropical and subtropical regions in West and North Africa. Therefore, this study was aimed to determine the phytochemical ingredients and the efficacy of aqueous and chloroform extracts of the *Detarium microcarpum, Anogeissusleio carpus* and *Carissa edulis* for the treatment of some bacterial isolates causing opportunistic skin infections. The aqueous and chloroform extracts of *Detarium microcarpum, Anogeissusleio carpus* and *Carissa edulis* were prepared through percolation method. Extracts were evaluated for antibacterial activities against *Streptococcus aureus* and *Streptococcus pyogenes* using disc diffusion method and the standard antibiotic (Ciprofloxacin) at 250 mg/ml was used as positive control. Phytochemical analysis of the extracts revealed the presence of flavonoids, saponins, steroids, terpenes, tannins and glycosides. *In vitro* antibacterial activity of the *Detarium microcarpum, Anogeissus leiocarpus* and *Carissa edulis* extracts ranged from 9-14 mm to 18.00-35.00 mm for the standard antibiotic discs. The range of the minimum inhibitory concentration (MIC) value of the extracts was 2.5-20 mg/ml while the minimum bactericidal concentration (MBC) was 5-10 mg/ml. The extracts of stems of *Detarium microcarpum, Anogeissus leiocarpus* and *Carissa edulis* were found to have bioactive potentiality on *Streptococcus aureus* and *Streptococcus pyogenes.* Therefore, these plant parts may be recommended to be used as herbal medicine for the treatment of skin infections caused by *Streptococcus aureus* and *Streptococcus pyogenes.*

Key words: *Detarium microcarpum*, phytomedicine, antibiotic and bacteriostatic

INTRODUCTION

Ethnomedicine is concerned with the study of medical systems from the native point of view, native categories and explanatory models of illness, including aetiologies, symptoms, causes of sickness and treatments that are investigated (Kleinmann, 2000).

Ethnomedicinal approach proves particularly useful for the study of indigenous therapeutic agents because it allows researchers to understand treatment patterns according to natural explanatory models instead of only through the lens of biomedicine (Kleinmann, 2000). Use of herbal medicine in Nigeria represents a long history of human interaction with the environment; rural communities in particular depend on plant resources mainly for herbal medicine (Veeramuthu, 2006).

Disease is the impairment of health, a condition of irregular functioning and conditions that affect the

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body of an organism. It is often considered as a medical state associated with specific signs and symptoms (Olapade, 2000). The skin, being the outermost and first line of defense, is easily exposed to physical agents and different pathogens leading to various infections and wounds (Aboesede, 1999).Wound, which is the breakage of the skin, results in the loss of continuity of epithelium with or without the loss of underlying connective tissue. Physical, chemical, thermal, microbial and immunological factors may be responsible for causing wounds in humans and animals (Haak, 2012).

Skin infections and topical wounds require special attention as they make human and animal prone to bacterial, fungal and viral contaminations, thereby making them further susceptible to other types of secondary complications (Haak, 2012). The most common pathogens isolated from wounds are *Streptococcus spp., Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus spp., Klebsiella, Enterobacter, Enterococci, Bacteroides, Clostridium, Candida, Peptostreptococcus, Fusobacterium and Aeromonas* (Mann *et al*., 2008). These pathogens can seriously delay wound healing process by disrupting the normal clotting mechanisms and promoting disordered leukocyte function and poor quality granulation tissue formation, reduce tensile strength of connective tissue and impair epithelization (Mann *et al*., 2008).

Secondary metabolites on pigments can have therapeutic actions in humans and can be refined to manufacture drugs. Chemical compounds in plants mediate their consequence on human body through identical processes to those already known for the chemical compound in conventional drugs; thus, herbal drugs do not differ greatly from conventional drugs in terms of their working pattern (Tesfaye and Zemede, 2009). Health is the most precious of all things and the foundation of all happiness. The use of herbal medicine for treatment is one component of balancing the body system and has become part of cultural life and heritage of people (Adebisi, 1998). The objective of this study was to evaluate the phytochemical compositions present in most of the frequently used plants for treating skin infections and to determine the efficacy of aqueous and chloroform extracts of the plants against some clinical bacterial isolates.

MATERIALS AND METHODS

Sample collection and preparation

Three ethnomedicinal plant species were used for the study. These include *Detarium microcarpum* (Fabaceae*)*, locally called ‘‘taura’’, *Anogeissus leiocarpus* (Combretaceae*)* locally called ‘‘marke’’ and *Carissa edulis (*Moraceae*)* locally called ‘‘macen tsada’’.

The plants were collected from Gadau village and Azare town in Katagum Local Government Area of Bauchi State. The stem and bark of the plants were dried under shade at room temperature and then ground into powder using mortar and pestle. The powder was sieved until a fine powder was obtained (Abalaka *et al.,* 2010). The extract was stored until required for further use.

Preparation of extracts

Chloroform extract

The plant material was extracted using percolation extraction method. Fifty (50) g of the powdered plant was suspended in 500 ml of chloroform contained in a 1,000 ml conical flask. The filtrates were evaporated to dryness in water bath at 60ºC and left overnight. The residue was stored at 4ºC for subsequent use.

Aqueous extrac

About 50 g of the powdered plant was suspended in 500 ml of distilled water contained in a 1,000 ml conical flask and shaken using a mechanical shaker. The mixture was filtered using Whatman No.1 filter paper. The filtrates were evaporated to dryness in water bath at 60ºC. The residue was stored at 4ºC for subsequent use.

Phytochemical screening
Aqueous and chloroform extracts of each plant were screened for the presence or absence of different phytochemical constituents to relate the secondary metabolites with antibacterial activity. Tests for alkaloids, flavonoids, glycosides, saponins, steroids and tannins were carried out following standard procedures described by Adebiyi and Ramstad (2001).

Preparation of sensitivity disc
Disc diffusion method described by Adebiyi and Ramstad (2001) was adopted. Whatman No. 1 filter paper was punched using paper perforating machine from which discs of 6.0 mm in diameter were obtained. Fifty (50) discs were placed in each of the sterilised Bijou bottles using sterilised forceps. These were placed in sterile capped Bijou bottles and then sterilised in a hot oven at 60^{o}C for one hour. About 2 ml of dimethylsulphoric acid (DMSO) was measured using a syringe and then dispensed into the bottles to enable impregnation of discs aseptically by soaking (Prescott, 2002).

Preparation of concentration for sensitivity test
This was done according to the method described by Adebiyi and Ramstad (2001). The serial broth dilution method was used to obtain different concentrations (20 mg\ml, 40 mg\ml, 60 mg\ml and 80 mg\ml) of the extracts.

Stock solution was prepared using dimethylsulphoxide (DMSO) as above; 0.020 g, 0.040 g, 0.060 g and 0.080 g of the chloroform and aqueous extracts of plant materials were weighed using weighing balance. The extracts were then dissolved in sterile screw capped bottles containing 2 ml of DMSO and the discs. A standard antibacterial agent (Ciprofloxacin) at a concentration of 250 mg\ml was used on the gram-positive and negative bacteria and its zone of inhibition was compared with those of the plant extract.

Test organisms
The test organisms used in the study were isolates of *Staphylococcus aureus* and *Streptococcus pyogenes* obtained from Federal Medical Centre, Azare, Bauchi state, in screw-capped Bijou bottles containing nutrient agar. The Bijou bottles containing nutrient agar were stored at 4^{o}C in a refrigerator as described by Hussein and Deeni (1991).

Standardisation of inoculum
Each culture of the isolates was standardised by culturing on nutrient agar for 48 hours at 37^{o}C. The cultures were diluted in normal saline (0.5 w/v) until turbidity marched with 0.5 Macfarland standard to give a mean of $3.3x10^{6}$ CFU/ML (Hussain and Deeni, 1991).

Antimicrobial Susceptibility
A disc diffusion method was used to test the antimicrobial activity of the plant extracts. Mueller Hilton agar plates were used in the inoculation of the organism. The organism was streaked evenly on the surface of the agar plates with the use of sterilised wire loop. With the aid of a sterile forcep, the impregnated paper discs were arranged radially and pressed slightly and firmly to the inoculated agar surface to ensure even contact. The plates were inoculated at 37^{o}C for 24 hrs. The degree of sensitivity was determined by measuring the diameter of the visible zones of inhibition (Abalaka *et al.,* 2010).

Minimum Inhibitory Concentration (MIC)
The MIC was determined by using a modification of the serial broth dilution method of Abalaka *et al. (*2010). About 2 ml of sterilised nutrient broth was dispensed into five tubes with the 5^{th} tube serving as the control; 40 mg/ml, 20 mg/ml, 10 mg/ml, 5 mg/ml and 0.025 ml of the extracts were aseptically transferred into all the test tubes and mixed thoroughly on a vortex mixer. Similarly, the five test tubes were made for the standard antibiotic and inoculated with the bacterial isolates (*Staphylococcus aureus* and *Streptococcus pyogens*).

The test tubes were covered and incubated for 24 hours at 37ºC and then examined for turbidity. The least concentration of the extracts that inhibited the growth of the organisms was taken as minimum inhibitory concentration (MIC).

Minimum Bactericidal Concentration (MBC)

Spread plate technique was employed. A fresh solid medium was inoculated with the inoculums. The least concentration that showed no visible growth for 24 hours at 37ºC to the lowest concentration in which no growth occurred on the solid medium was recorded as minimum bactericidal concentration.

Statistical Analysis

Data collected were subjected to analysis of variance and means were separated using the least significant difference at 5% level of probability using SAS.

RESULTS

Phytochemical Screening of Plant Extracts

Table 1 shows the result of phytochemical screening of aqueous extracts of the three medicinal plants. Saponin and tannin were present in aqueous extracts of the three plants. Glycoside and alkaloids were only present in *D. microcarpum* and *C. edulis.*

Table 1: Result of phytochemical screening of aqueous extracts of *A. leiocarpus, D. microcarpum* and *C. edulis*

	Aqueous Extract		
Active compound	*A.leiocarpus*	*D. microcarpumC.edulis*	*C. edulis*
Alkaloid	-	+	+
Flavonoid	-	-	-
Saponin	+	+	+
Tannin	+	+	+
Glycoside	-	+	+

+ Present, − Absent

Table 2: shows the result of phytochemical screening of chloroform extract of the medicinal plants. Glycoside, tannins and alkaloids were present in all the three plant extracts

Table 2: Result of phytochemical screening of chloroform extract of *A. leiocarpus, D. microcarpum* and *C. edulis*

	Chloroform Extract		
Active compound	*A. leiocarpus*	*D. microcarpum*	*C. edulis*
Alkaloid	+	+	+
Flavanoid	-	+	+
Saponin	+	-	-
Tannin	+	+	+
Glycoside	+	+	+

Table 3: Antibacterial activity of aqueous extracts of plant samples at different concentrations

Extract	Concentration (mg/ml)	Zone of inhibition (mm) (+0.05)	Zone of inhibition (mm) (+ 0.05)
		S.aureus	*S. pyogenes*
A. leiocarpus	80	6	11
	60	8	10
	40	0	8
	20	0	0
	Control	0	0
D. microcarpum	80	10	20
	60	10	10
	40	0	8
	20	0	0
	Control	27	40
C. edulis	80	10	11
	60	8	8
	40	8	6
	20	0	0
	Control	41	32

Table 3 shows the antibacterial activity of the aqueous extracts of the plant samples. Ciprofloxacin, used as positive control, had the highest effect on *S. pyogenes* with a zone of inhibition of 20.0 mm. This effect was significantly higher ($p \leq 0.05$) than the effect of Ciprofloxacin on *S. aureus* which causes opportunistic skin infection. However, the concentration of 80 mg/ml was found to be significantly higher than 60 mg/ml, 40 mg/ml and 20 mg/ml ($p \leq 0.05$). *Detarium microcarpum* had the highest effect on *S. pyogenes* with a zone of inhibition of 20 mm. This effect was significantly higher ($p \leq 0.05$) than the effect of *A. leiocarpus* and *C. edulis* against both *S. aureus* and *S. pyogenes*, which cause opportunistic skin infection.

Table 4: Antibacterial activity of chloroform extracts of plant samples at different concentrations

Extract	Concentration (mg/ml)	Zone of inhibition (mm) (+0.05)	Zone of inhibition (mm) (+0.05)
		S. aureus	*S. pyogenes*
A. leiocarpus	80	*5*	10
	60	4	9
	40	6	7
	20	0	0
	Control	7	9
C. microcarpum	80	10	15
	60	9	12
	40	8	9
	20	8	8
	Control	50	32
C.edulis	80	10	10
	60	10	8
	40	8	6
	20	0	6
	Control	31	42

Table 4 shows that chloroform extract of plant samples was significantly higher ($p \leq 0.05$), with a zone of inhibition 10 mm, than the aqueous extract against *S. aureus*, which causes opportunistic skin infection. However, chloroform extracts of *D. microcarpum* was observed to be more effective in killing the test organisms which cause opportunistic skin infections. Ciprofloxacin which was used as positive control had the highest effect on *S. aureus* with a zone of inhibition of 50.0 mm. However, the concentration of 80 mg/ml was observed to be significantly higher than 60 mg/ml, 40 mg/ml and 20 mg/ml ($p \leq 0.05$).

Table 5: Minimum Inhibitory Concentration of aqueous extracts of *A. leiocarpus, D. microcarpum* and *C. edulis*

Organism	Plant sample	Concentration (mg/ml)			
		40	20	10	5
Streptococcus pyogenes	*A. leiocarpus*	+	_	_	_
Staphylococcus aureus	*A. leiocarpus*	+	+	_	_
Streptococcus pyogenes	*D. microcarpum*	+	+	_	_
Staphylococcus aureus	*D. microcarpum*	_	_	_	_
Streptococcus pyogenes	*C. edulis*	+	+	_	_
Staphylococcus aureus	*C. edulis*	+	_	_	_

Key: + = Growth, – = No growth

Result of MIC for aqueous extracts of the plant samples showed that the growth of test organisms only occurred at the highest concentrations of 40 mg/ml and 20 mg/ml.

Table 6: Minimum Inhibitory Concentration of chloroform extracts of *A. leiocarpus, D. microcarpum and C. edulis*

Organism	Plant sample	Concentration (mg/ml)			
		40	20	10	5
Streptococcus pyogenes	*A. Leiocarpus*	+	_	_	_
Staphylococcus aureus	*A. leiocarpus*	+	_	_	_
Streptococcus pyogenes	*D. microcarpum*	_	_	_	_
Staphylococcus aureus	*D microcarpum*	+	+	_	_
Streptococcus pyogenes	*C. edulis*	+	_	_	_
Staphylococcus aureus	*C. edulis*	+	_	_	_

Key: + = Growth, – = No growth

Result of MIC of chloroform extract of the plant samples showed that the growth of the test organisms was observed at the highest concentrations. The minimum bactericidal concentration of aqueous and chloroform extracts of *A. leiocarpus*, *D. microcarpum* and *C. edulis* were all positive, indicating that all the organisms were bacteriostatic. The growth of the test organisms was observed at all concentrations.

DISCUSSION

Antimicrobial activity of the extracts was enhanced by an increase in the concentration of the extracts, which corroborated the report of Mann *et al.* (2008), that the higher the concentration of the plant extract, the greater the zone of inhibition. Chloroform extracts showed greater activity than the aqueous, which might be due to higher solubility of the active component in chloroform than in water.

The zones of inhibition of the bacterial isolates varied. According to Presscot (2002), the effect of bioactive agent also varies with target organisms. Mann *et al*. (2008) reported that the position of the zone edge (diameter of zone of inhibition) is determined by the initial population density of the organisms. Their growth rate and diffusion of the antimicrobial agents explain clearly the difference in the zones of inhibition observed.

The activity of the extracts of *Anogeissus lieocarpus, Carissa edulis and Detarium microcarpum* against the test organisms justified their traditional usage as ethnomedicinal plants amongst the people of the study area and this activity could be as a result of the presence of bioactive compounds like saponins, tannins, alkaloids,

flavonoids and glycosides. Ciprofloxacin was used as the positive control against the test organisms, and was found
to have the widest zone of inhibition against *S. pyogenes*. It was observed that *Detarium microcarpum* had the widest zone of inhibition capable of killing the bacteria which cause opportunistic skin infection.

The MIC values obtained in this study varied and were lower than that of MBC. This shows that the concentration used in the study was able to inhibit the growth of the organisms rather than killing them (i.e. bacteriostatic at lower concentration and bactericidal at higher concentration). This agrees with the report of Abalaka *et al*. (2010). There is an urgent need to conserve these medicinal plants which have become endangered species. These plants are experiencing genetic erosion due to anthropogenic activities and environmental degradation (Obute and Osuji, 2002). Unfortunately, little or no conservation strategies are in place to safeguard them. As noted by Hussein and Deeni (1991), knowledge of what to conserve is necessary in deciding on a programme of action. The lower MIC value obtained in the research is an indication of good antibacterial activity of the stem exudates.

CONCLUSION

Anogeissus leiocarpus, *Detarium microcarpum* and *Carissa edulis* were reported to be used in the treatment of skin infections like eczema, ringworm, scabies, skin allergies, heat rash and other related skin infections. Crude chloroform extract used in this study confirmed its solubility in water. It can penetrate the outer covering of gram-positive bacteria, causing disturbed metabolism, cellular function, loss of cellular constituent and sometimes death. All the three plants were bactericidal; therefore, both aqueous and chloroform extracts were observed to be effective in killing *S. aureus* and *S. pyogenes*, which cause opportunistic skin infections.

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