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Effects of geographical location on the yield and bioactivity of *Anoigeissus leiocarpus*

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Abstract
Acetone extracts of the leaves of *Anoigeissus leiocarpus* collected from two different geographical locations from Nigeria were analysed for their phytochemicals, antioxidants, antibacterial, antimycoplasmal and cytotoxic activities. It was shown that plant from the colder region has higher yield and better activities than that from warmer region; having bacterial minimum inhibitory concentration (MIC) value of 0.08 mg/ml and mycoplasmal MIC value of 0.16mg/ml. The result showed that there were variations between the two plant extracts in their activities such as antimicrobial, phytochemicals, antioxidants and cytotoxicity.

Keywords: *Anoigeissus leiocarpus*; Antioxidant; Antibacterial; Antimycoplasmal; Cytotoxic

Introduction

The effectiveness of medicinal plants is sometimes affected by biochemical factors within the individual species, plant parts and even external factors such as climate, geographical location, season, nature of the soil, and growth conditions (Prance, 1994). Studies have shown that plants display variation in the concentration of their bioactive phytochemicals. The phytochemicals in different plants with anticancer activity have been reported to change quantitatively with season (Mukherjee *et al*., 2002; Elgorashi *et al*., 2002). More striking is the difference in variation in the phytochemicals and activity of the same plant parts from the same plant collected at different geographical location. Bioassays of extracts made from different plant parts, or of plant parts collected at different times or from different locations may yield new active compounds. Variation of the activity can also occur due to treatment after collection, i.e. storage and preparation (Houghton and Raman, 1998; Stafford *et al*., 2005). Therefore, the same medicinal plants from different location may not consistently produce the same chemicals in the same quantities.

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In this study we compared and report the variations in the percentage yield, the phytochemicals, the antioxidants, antimicrobial and cytotoxic activities of the acetone extracts of the leaves of *Anoigeissus leiocarpus* plant collected from two different locations.

Experimental

Plant preparation and extraction. The leaves of *Anoigeissus leiocarpus* used in this experiment were collected around Zaria in Kaduna State (between latitude 11°11' N and longitude 7°38' E) and around Jos in Plateau State (between latitude 80°24$^'$ N and longitude 80°32' N & 100°38' E), both in Nigeria, between February and March, 2007. Voucher specimens were deposited at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. Leaves were dried, ground in a mill and extracted by vigorously shaking with acetone at a ratio of 1:10 w/v. Fifty grams of each plant material was dissolved in five hundred millilitres of acetone and the filtrate collected using Whatman No 1 filter paper. This process was repeated 2 times by adding 250ml of acetone to the sediments and shaken for an hour so as to exhaustively extract the plant materials. The filtrates were collected and combined with the previous ones making a final ratio of 1:15 $^w/_v$. The clear homogenous filtrate was dried and concentrated under a fast stream of air at room temperature and the percentage yields of individual plant extract was determined. The extracts were then prepared in acetone at a concentration of 10mg/ml for each extract ready for use.

Phytochemical analysis. The chemical constituents of the plant extracts, from both locations, were qualitatively analyzed using aluminium-backed thin layer chromatography (TLC) plate. 10µL of 10mg/ml of each extract was spotted onto a TLC plate and developed in three different solvent systems of varying polarities. These were, a polar or neutral solvent [containing ethyl acetate/methanol/water (EMW) in the ratio 10:1.35:1], an intermediate polar or acidic solvent [containing chloroform/ethyl acetate/formic acid (CEF) in the ratio 5:4:1] and a non-polar or basic solvent [containing benzene/ethanol/ammonium hydroxide (BEA) in the ratio 9:1:0.1] for separation of the constituents (Kotze and Eloff, 2002). Developed plate was sprayed with vanillin-sulphuric acid (containing 0.1 g vanillin, 28 ml methanol and 1 ml sulphuric acid) and heated at 110 °C to optimal colour detection of chemical components of the extract.

Antioxidant analysis. The qualitative DPPH and quantitative MTT methods were used for screening of antioxidant compound in the plant extracts. Here, 100µg (i.e. 10µl of 10mg/ml) of each extract was spotted on a TLC plate and developed in the three solvent systems as described. The dried plate was sprayed with 0.2% DPPH (2,2, diphenyl-1-picrylhydrazyl) in methanol and the presence of antioxidant compounds was immediately observed as a yellow band against a purple background. For MTT assay, a two-fold dilution of 10 mg/ml of each extract was carried out with 100 µl of distilled water in a 96-well microdilution plate. Thereafter, 50 µl of 0.2 mg/ml of MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) was added to every well and the plate was incubated for 2-3 hours at 37°C after which the result was read.

Antibacterial and antimycoplasmal activities. The two plant extracts were evaluated for their antimicrobial activities. The method of Eloff (1998) was used to test for the MIC of the extracts on a Gram positive (*Staphylococcus aureus*, ATCC 29213) and a Gram negative (*Escherichia coli*, ATCC 27853) bacteria while the method of pH-dependant technique or glucose metabolic inhibition was used for mycoplasmal organism (*Mycoplasma mycoides* subsp.

mycoides, T1/44 strain). The bacteria were grown on Mueller Hinton broth while mycoplasma was grown on PPLO medium. The organisms were used at approximately 2.6 X 10^{12}, 3.0 X 10^{11} and 6.0 X 10^{12} cfu/ml for *S. aureus, E. coli* and *M. mycoides* subsp. *mycoides* respectively. Gentamicin and tylosin were used as reference drug positive control for antibacterial and antimycoplasmal effects respectively. Acetone was used as negative control. The minimum inhibitory concentration (MIC) result for antibacterial effect was taken after 1 hour of INT salt addition while antimycoplasmal effect was taken after 24-36 hours of incubation.

Bacteria bioautography. The bioautography procedure described by Begue and Kline (1972) was used to identify the relative front (R_f) of the active components of the plant extracts. Chromatogram of the two extracts were sprayed with a dense suspension of actively growing overnight bacteria (*Staphylococcus aureus* and *Escherichia coli*) cultures. This was incubated for 24 hours at 38° C under 100% relative humidity and then sprayed with 2mg/ml of p-iodonitrotetrazolium violet (INT). A clear zone against a red or purple background on TLC plates after about one hour incubation indicates bacteria growth inhibition by the particular compound(s) represented by the band (McGaw & Van Staden, 2000).

Cytotoxicity study. The cytotoxicity of the plant extracts was measured by MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide) assay (Mosmann, 1983), using the African monkey kidney (Vero) cell lines seeded into 96 wells of the microplates at a density of 0.5-10 x 10^3 cells/ml. Different concentrations of each extract (i.e. 0.001, 0.01, 0.1 and 1.0 mg/ml) were used. The growth medium plus DMSO (dimethyl sulphoxide) was used as negative control while berberine chloride (Sigma) was used as cytotoxic drug positive control. The absorbance was measured with ELISA microplate reader (Versamax, Molecular Devices) at 570 nm wavelength and the median lethal concentration (LC_{50}) was determined by plotting the graph of the mean absorbance against the mean log of concentrations.

Results and discussion

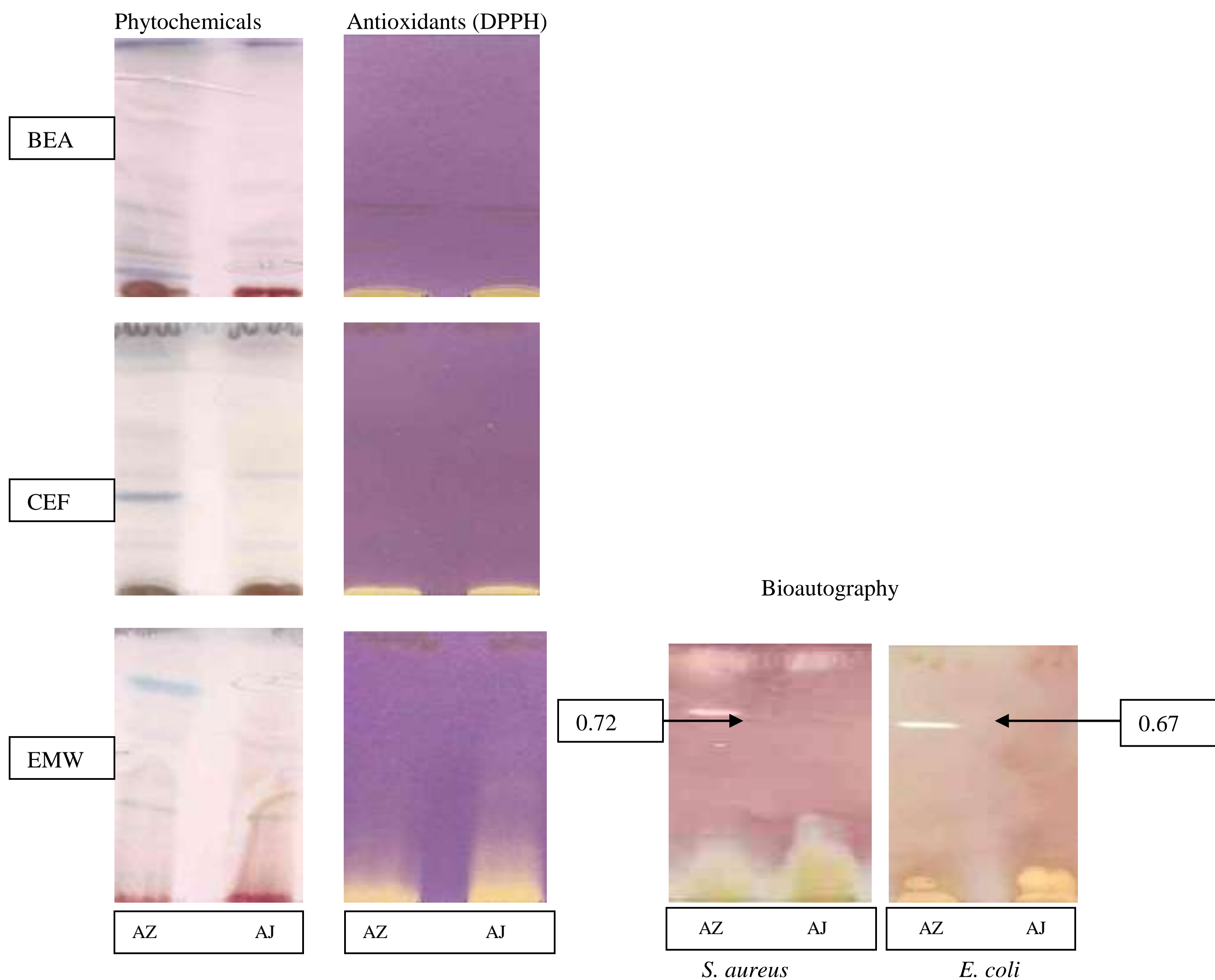
The amount and percentage yield of the same quantity of both plant extracts varies, with the Jos plant having a higher percentage yield of 15.15% as compared to the Zaria plant with 11.6% (Table 1). The difference in the yield may be attributed to the difference in the activities of the plants with the Jos plant having the lowest MIC's values of 0.08mg/ml and 0.16mg/ml for antibacterial and antimycoplasmal effects respectively as against that of Zaria with value of 0.16mg/ml and 0.31mg/ml respectively for both organisms. It was noted that there was not much difference in the chemical composition of both extracts eluted by a polar and an intermediate polar solvents except for Zaria plant which has more of non-polar compounds eluted by a non-polar solvent (BEA). This may be responsible for the lowest activity of the plant as these extra 'inactive' compounds may antagonize the activity of the active compound(s) in the crude extract. This observation was corroborated by the effect of each compound as seen in bioautography. There were some compounds active against *S. aureus* (with R_f value of 0.72) and *E. coli* (with R_f value of 0.67), eluted by polar solvent. This showed that there was at least one active compound which has antibacterial effect only when eluted, but with weak effect when in crude form. It was surprising that the Jos plant, which had better activity in crude form has no active compound (on both organisms) when separated. This may point out that the activity of the plant is enhanced in crude form when compounds are having a synergistic or additive effect with one another. The MTT

method of antioxidant determination showed the same values for both plant extracts but with little or no variation in the composition in both extracts as shown by DPPH method (Table 1 and Figure 1). The margin of safety for the use of the Jos plant on *S. aureus* was three times wider than that of Zaria plant signifying lower cytotoxic effect in Jos plant.

Table 1: Comparison of activities of the two plants against the microbes

Plant extract	Mass yield (g)	% yield	MIC (mg/mL)			LC_{50} (mg/mL)	Antioxidant (MTT)
			S. aureus	*E. coli*	*M. mycoides*		
A. leiocarpus (Jos)	7.58	15.15	0.08 ± 0	0.08 ± 0	0.16 ± 0	0.09589	<0.04
A. leiocarpus (Zaria)	5.98	11.6	0.16 ± 0	0.08 ± 0	0.31 ± 0	0.0756	<0.04

Plate 1: TLC Plates showing phytochemicals, antioxidants and bacteria bioautography



Key: AZ = *A. leiocarpus* from Zaria, AJ = *A. leiocarpus* from Jos
BEA = benzene/ethanol/ammonium hydroxide (9:1:0.1)
CEF = chloroform/ethyl acetate/formic acid (5:4:1) EMW = ethyl acetate/methanol/water (10:1.35:1)

These variations in the same plant may be explained by both intrinsic factors like age of the plant or part of the plant used and extrinsic factors like geographical climate, nature of the soil, season and processing methods. From our study, the variation in both plant extracts may be connected to the climatic condition of the two regions in which Jos is cooler (lower ambient temperature) than Zaria.

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