ASPIDOSPERMINE ALKALOID ISOLATED FROM THE LEAF EXTRACT OF *Anogeisus leiocarpus*

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Abstract
The powdered leaves (1 Kg) of Anogeisus leiocarpus was percolated with ethanol. The crude ethanol extract was subsequently fractionated using n-hexane, chloroform and n-butanol which lead to the formation n-hexane (FH01), chloroform (FC01) and n-butanol (FB01) soluble fractions respectively. Bioactive guided chromatographic isolation and purification of the chloroform soluble fraction afford a pure compound which was structurally deduced as an alkaloid belonging to aspidospermine group but having an isoquinoline moiety rather than the usual indole, base on its spectral characteristics.

Keywords: Aspidospermine, Alkaloid, Chromatography, *Anogeisus leiocarpus*, Isoquinoline.

Introduction

The plant *Anogeisus leiocarpus* belongs to the family combraetacaea consisting of 17 genera with 500 species with a great number belonging to combraetum and terminalia [Rendle, 1952]. The plant belongs to the order *Anogeisus* of dicotyledonous plants, a tree of tropical West Africa and largely distributed in the regions of Senegal, Sudan, Nigeria, Cote d'ivore, among others [Sofowora, 1993]. In Nigeria the predominant specie is *Anogeisus leiocarpus*, mostly found in the northern part of the Savannah vegetation zone. The leaves of *Anogeisus leiocarpus* are used locally among Hausa community in northern Nigeria as a feed for cattle, sheep and goat suffering from influenza disease or mucus infection [Ajaafar, 1982]. Decoction of the leaves with red potash is widely used for oral application as cough mixture. Similarly, decoction of the leaves with spider nut is taken orally against pulmonary tuberculosis. The flower of the plant is also known to be fried, grinded and later infused in warm water as a treatment against tape worm [Ajaafar, 1982]. The root and the stem have been used as chewing stick for dental hygiene. The powdered bark is applied to wound and the aqueous extract is used for diarrhea treatment in Senegal [Burkill, 1985]. A mixture of the powdered bark with *Terminalia* sp is applied to gum for tooth ache in Burkina-faso and Cote d'ivore [Burkill, 1985]. The bark is also found used in upper Guinea as a febrifuge in hot lotions and as an infusion for the treatment of leprosy in Burkina-faso. In Ghana decoction of the bark with red pepper is taken for body and chest pains. The powdered bark mixed with ordinary vaseline as an ointment is applied on the skin for skin complains in Cote d'ivore. In Sudan, decoction of the leaves is used for bathing as treatment for feverish cold. The bark and more often the fruits, leaves and seeds are used as taenicide for horses and donkeys [Burkill, 1985]. The plant was found to be named differently among the Nigerian natives, for example Hausa people call it Marke, Fulani call it Kojoli, Yaruba call it Ayin, Igbo call it Atare, among others [Keay, 1964].

The study was set out to investigate the effect of the leaves extract of *Anogeisus leiocarpus* on some micro-organisms with a view to isolating and characterization of the most active component(s).

Materials and methods

Plant collection: The leaves of *Anogeisus leiocarpus* (Combretacaea) were collected

from Dorayi in Gwale local government area of Kano state, Nigeria. The plant was identified in accordance with Sofowora, 1993. The sample was air-dried for two weeks, crushed, grinded, weighed and kept on shelf before percolation.

Fractionation: About One kilogram (1 Kg) of the air-dried powdered leaves of *Anogeisus leiocarpus* was percolated with two liters of ethanol with vigorous shaken at regular intervals for one week. It was decanted and filtered and the extracted was concentrated on rota vapor (R110) at 40^{o}C. The crude ethanol extract was label as FE01 and weighed 60.56 g. About 50.00 g of the FE01 was successively macerated with 500 ml of n-hexane in parts using 50 ml each. The combined n-hexane extract was evaporated to dryness on rota vapor at 40^{o}C [Abdu, 2006]. The residue of the n-hexane soluble fraction weighed 10.22 g and labeled as FH01. The n-hexane insoluble fraction (FH02, 38.97 g) was macerated with 500 ml of chloroform in parts using 50 ml each to obtain the chloroform soluble fraction. This weighed 25.17 g and labeled as FC01. The chloroform insoluble fraction (FC02, 14.10 g) was also macerated with 500 ml of n-butanol in parts to obtain the n-butanol soluble fraction (FB01) which weighed 5.56 g. The n-butanol in soluble fraction FB02 weighed 8.90 g. All the fractions (FE01, FH01, FC01 and FB01) were subjected to antimicrobial screening [Kirby, 1964] among which the chloroform soluble fraction proved to be the most active fraction.

Chromatographic separation: Both thin layer (TLC) and column chromatography were employed in trying to isolate and purify the most active component. A big column was packed wet using silica gel (60-120 mesh) after which a slurry of FC01 (10 g) was prepared and load on top of the adsorbent. The column was eluted using a gradient solvent system of petroleum ether (60-80^{o}) and ethylacetate. Later the column was washed with 100% methanol. Base on TLC analysis similar fractions were combined and evaporated to dryness on rota vapor. A small column was also run and base on TLC analysis semi pure compounds were isolated which were further purified using preparative TLC to obtain the most pure component.

Results and Discussion:

Isolation of the pure compound was done using both column and thin layer chromatography. Table 1 shows the results of the column chromatography of the leaf extract of the chloroform soluble fraction.

Table 1: Column chromatography of chloroform soluble fraction.

Fraction	Eluents (PE:EA)	Weight (mg)	Fraction	Eluents (PE:EA)	Weight (mg)
ALC 01	100 % PE	0	ALC 15	70 % EA/PE	128
ALC 02	5 % EA/PE	0	ALC 16	75 % EA/PE	136
ALC 03	10 % EA/PE	0	ALC 17	80 % EA/PE	62
ALC 04	15 % EA/PE	0	ALC 18	85 % EA/PE	72
ALC 05	20 % EA/PE	11	ALC 19	90 % EA/PE	73
ALC 06	25 % EA/PE	28	ALC 20	95 % EA/PE	77
ALC 07	30 % EA/PE	56	ALC 21	100 % EA	92
ALC 08	35 % EA/PE	59	ALC 22	10% MeOH/EA	85
ALC 09	40 % EA/PE	61	ALC 23	20% MeOH/EA	40
ALC 10	45 % EA/PE	78	ALC 24	30% MeOH/EA	65
ALC 11	50 % EA/PE	95	ALC 25	40% MeOH/EA	56
ALC 12	55 % EA/PE	103	ALC 26	50% MeOH/EA	44
ALC 13	60 % EA/PE	138	ALC 27	100% MeOH	59
ALC 14	65 % EA/PE	90			

Keys: ALC: *Anogeisus leiocarpus* chloroform fraction; PE: petroleum ether; EA: ethylacetate; MeOH: methanol

The column was run based the gradient solvent system (100 % petroleum ether to 100 % ethylacetate) and it was washed with methanol to remove the more polar compounds. Based on the thin layer chromatography (TLC) all the fractions were analyzed of which the following (ALC 12, ALC 13, ALC 14, ALC 15, ALC 20, ALC 21 and ALC 23) shows the presence of at least three similar spots. Based on that they were combined together and assigned a new code as ALC 12A which weighed 668 mg. The fraction, ALC 12A was further purified using a smaller column which afford to give three different fractions as ALC 12A-X (40 mg), ALC 12A-Y (72 mg) and ALC 12A-Z (56 mg). Fraction ALC 12A-X shows only one spot on TLC and this was further confirmed using a two dimensional TLC which confirmed that it is only one spot with the R_f value as 0.45.

Fraction ALC 12A-X which was found to be oily in nature was found to be a pure compound. The ^{1}H nmr ($CDCl_3$) in combination with IR absorption spectra were used to propose the structure of the compound. The summary of the ^{1}H nmr chemical shift values and the IR absorption frequency bands of the compound ALC 12A-X are given in table 2.

Table 2: ^{1}H nmr ($CDCl_3$) and IR absorption frequency bands of ALC 12A-X ALC 12A-X

δ/ MHz (CDCl$_3$):

0.85 (3H, s, -C*H*$_3$), 0.87 (3H, s, -C*H*$_3$), 1.25 (2H, t, *J* 6.5, -C*H*$_2$), 2.10 (2H, q, *J* 6.5, -C*H*$_2$), 2.24 (2H, t, *J* 6.5, -C*H*$_2$-*N*), 2.30 (2H, d, *J* 5.4, N-C*H*$_2$-*C*=), 2.66 (1H, s, -C*H*), 3.49 (1H, t, -C*H*), 3.66 (1H, t, -C*H*), 3.70 (2H, s, -C*H*), 3.76 (2H, s, -C*H*), 4.21 (1H, d, *J* 5.4, =C*H*), 4.29 (1H, q, *J* 5.4, =C*H*), 7.04 (1H, d, *J* 6.5, Ar-*H*), 7.46 (2H, m, Ar-*H*), 7.53 (2H, m, Ar-*H*) and 9.10 (1H, s, -C*H*O).

V$_{max}$/ cm^{-1}:

3169 (Ar-H stret.), 1710 (-CHO), 1620 (-C=N-) and 1150 (-C-N-C-).

From the data above (^{1}H nmr and IR), the proposed structure for the isolated compound is given in figure 1 below. The compound falls within the aspidospermine group of alkaloids but possessing the quinoline backbone moiety rather than the usual indole.

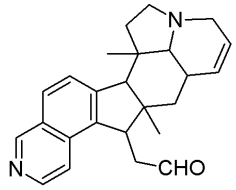

Figure 1: Proposed structure of ALC 12A_X

Conclusion:

The structure given in figure 1 is proposed based on ^{1}H nmr and IR spectra only. The exact structure of this isolate has not been established due the unavailability of facilities (such as ^{13}C

nmr, ^{15}N nmr and Mass spectroscopy). It is therefore recommended that further analysis should be done for the full characterization of the compound.

References

Abdu, K. and Johnbull, E.O. (2006): Phytochemical and antimicrobial screening of the ethanolic extract of the leaves of *Anogeisus leiocarpus*, *Bio. Environ. Sci. J. Trop.* **3** (6), 162-164.

Aja'afar, B.I. (1982): Northern Nigeria medicinal plants as sources of drugs, *B. Sc Thesis*, Department of Chemistry, Bayero University, Kano Nigeria.

Burkill H.M. (1985): *The useful plants of tropical Africa*, 2nd edition, vol. 1 Royal botanical garden, kew, pp. 389-391.

Keay, R.W.J., Onochie, C.F.A. and Stanfield, D.P. (1964): Nigerian trees, Dept. of forest research Ibadan, **1**, Pp 159-161.

Kirby, M.C. and Bauer, A.W. (1996): Antibiotic susceptibility testing by standardized single disc method, *Clinical Path*. **45**, 493-496.

Rendle, A.B. (1925): Classification of flowering plants. *Cambridge University press*, **II**, 380.

Sofowora, S.A. (1993): Medicinal plants and traditional medicine in Africa. *Spectrum books Ltd*, 2nd edition, 150-172.