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Chemical Constituents of *Pavetta Corymbosa* Leaves

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ABSTRACT

Two steroids, three triterpenoids and three Flavonoids: namely, lupenol, β-sitosterol Stigmasterol(1:1), α-amyrin, Ursolic acid, were isolated from the dichloromethane extract of the leaves of *Pavetta corymbosa*, while the ethylacetate soluble part of the Ethanolic extract afforded the Flavonoids: Quercetin,Kaempferol and Quercetin-7-O-rhamnoside. The structures were confirmed using spectroscopic techniques and are reported here for the first time.

Keywords: Steroids/Triterpenoids,Flavonoids,*Pavetta corymbosa.*

INTRODUCTION

Pavetta corymbosa is a shrub or a small tree 9m tall with trunk 30cm girth of dry forest, jungle grass savannah and coastal scrub,common across the region from Senegal to Western Cameroon and Nigeria [1]. The genus Pavetta have been used in ethnomedicine in western Africa as hypotensive, antimalarial, and as remedy for respiratory infections and wound healing [2-4]. *Pavetta corymbosa* is used in Nigerian ethnomedicine for treating malaria [5].The methanol extract of the aerial parts of the plant has been reported to be active against clinical isolates of Plasmodium falciparum [6]. Despite the reported biological studies on this plant, there is no documented report on the phytochemical constituents on this plant, as part of our efforts in screening Nigerian medicinal plants for bioactive plant metabolites, the dichloromethane and ethylacetate soluble extracts of the leaves of *Pavetta corymbosa*(DC) FC Williams were investigated phytochemically.

EXPERIMENTAL.

General experimental procedures

^{1}H-NMR and ^{13}C-NMR was recorded on Bruker DRX 600MHz and 125Mhz spectrophotometer respectively with TMS as internal standard. Column chromatography was carried out on a silica gel G 230-400μm(Flash) Fluka, while gel filtration was carried out using sephadex LH-20(Sigma). Thin layer chromatography (TLC) was carried out on precoated silica gel 60 F_{254} and visualization was carried out by spraying with 10% H2SO4,followed by heating at 105^{0}C for 5min. Preparative TLC were carried out on MERCK silica gel 60 F_{254} glass plates (0.5mm) respectively.

Plant material

Pavetta corymbosa leaves were collected in Samaru-Zaria-Nigeria in the month of July,2010 it was authenticated at the herbarium section of Biological Science department,Ahmadu Bello University,Zaria were a vouchered specimen(No.2763) has been deposited.

Extraction and isolation.

The air-dried powdered leaves (350g) were extracted with Dichloromethane (2x2.5L) to exhaustion at room temperature for seven days, the combined dichloromethane extract was concentrated to give a greenish mass (12.5g, 3.5%w/w). The marc was then extracted with 70% ethanol for 5 days to give a greenish mass (8.4g, 2.4%). 7.5 g of the ethanolic extract was suspended in water and partitioned with

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dichloromethane(1L),ethylacetate(1L) and N-butanol(1L) to give 3.5g of dichloromethane soluble part (DCM),ethylacetate soluble part(EA) 1.5g and n-butanol (NBT)3.7g respectively.

The dichloromethane extract (10.6g) was fractionated over flash column chromatography on silica 50g, (50cmx2.5cm), eluting with n-hexane, n-hexane:ethyl acetate mixtures(0-100:0) and then 10%methanol in ethyl acetate. 40 fractions were collected with an aliquot of 100ml each based on their TLC profile.Fractions 1-6 consists mostly fatty material and was not investigated. Fractions eluted with 15% ethylacetate in n-hexane (14-16,1.392g) were pooled together to give a white solid which was re-chromatographed on silica gel and eluted with 10% ethylacetate in n-hexane to give 20 fractions,fractions 8-11 (175mg was subjected to Preparative TLC using n-hexane:ethylacetate(5:1) as the solvent system to afforded compound 1, a white solid (54mg) lupenol, while fraction 12 gave compound II a white crystalline solid (3mg) which was found to be a mixture of β-sitosterol and stigmasterol(1:1).

Fraction 22-24 (180mg) eluted with 40% ethylacetate in n-hexane was purified over sephadex LH-20 eluted with dichloromethane to give compound III, a white powder(10.2mg) which was found to be α-amyrin.The dichloromethane soluble part of the ethanolic extract (2.2g) was subjected to flash column chromatography using silica gel (35g), and eluted with n-hexane, and n-hexane:ethylacetate mixtures, fractions eluted with 50% ethyl acetate in n-hexane afforded a white solid mass(100mg), which was purify over sephadex LH-20 eluted with dichloromethane to give 35 fractions, fraction 22-31 gave compound IV a white crystalline solid 7.5mg which was found to be ursolic acid.

The ethyl acetate soluble part(1g) of the ethanolic extract was fractionated over sephadex LH-20 eluting with pure methanol to give 30 fractions, fraction 22-24 gave compound V a yellow solid (3mg) which was found to be Quercetin. Fraction 15-17 gave 2 spots on TLC, this was re- purify over sephadex eluting with methanol to give compound VI Kaempferol (4mg) and compound VII Quercetin 7-O-rhamnoside(3.4mg).

RESULTS.

Compound I,a white amorphous solid.^{l}H-NMR ($CDCl_3$):δ=4.68(1H,d,j=19,H-29a),4.57(1H,d,j=1.9,H-29b),3.19(1H,m,H-3),1.70(3H(s),H-30);1.05(3H(s),H-27),0.99 3H(s),H-26);0.97 3H(s),H-23);0.85 3H(s),H-28);0.81 3H(s),H-25);0.78 3H(s),H-24.^{13}C-NMR (125MHz,$CDCl_3$):38.7(C-1);27.4(C-2);79.0(C-3);38.8(C-4);53.3(C-5);18.3(C-6);29.3(C-7);40.8(C-8);50.4(C-9);37.2(C-10);20.9(C-11);25.2(C-12);38.1(C-13);42.3(C-14);27.5(C-15);35.6(C-16);43.0(C-17);48.3(C-18);48.0(C-19);150.9(C-20);29.9(C-21);40.0(C-22);28.0(C-23);15.4(C-24);16.1(C-25);15.9(C-26);14.6(C-27);18.0(C-28);109.3(C-29);19.3(C-30).

Compound II, a white crystalline solid.^{1}H-NMR($CDCl_3$): δ=5.36,2H(m,H-6);5.15 ,1H(d,d,j=15.1,8.7Hz,H-22);5.02,1H(d,d,j=15.1,8.7Hz,H-23);3.54 2H(m,H-3);0.68 3H(s,H-18);1.01,3H(s,H-19);1.02 3H(d,j=6.63,H-21);0.85,3H(d,j=6.41,H-26);0.81,3H(d),j=7.55Hz,H-29);0.80,3H(d,j=6.41Hz,H-27). **^{13}C**-NMR(125MHz,$CDCl_3$):β-Sitosterol: 37.1(C-1);31.6(C-2);71.8(C-3);41.5(C-4);140.2(C-5);121.7(C-6);31.7(C-7);31.9(C-8);50.1(C-9);36.7(C-10);20.8(C-11);39.6(C-12);42.5(C-13);56.7(C-14);24.0(C-15);28.6(C-16);55.9(C-17);11.6(C-18);19.2(C-19);40.3(C-20);20.5(C-21);138.0(C-22);129.2(C-23);51.1(C-24(;32.0(C-25):19.0(C-26);21.2(C-27);25.4(C-28);12.0(C-29). Stigmasterol: 37.4(C-1);31.9(C-2);71.8(C-3);42.3(C-4);140.8(C-5);121.7(C-6);31.7(C-7);31.9(C-8);50.1(C-9):36.5(C-10);21.1(C-11);39.8(C-12);42.3(C-13);56.7(C-14);24.3(C-15);28.3(C-16);56.1(C-17);11.9(C-18);19.4(C-19);36.2(C-20);18.8(C-21);33.9(C-22);26.1(C-23);45.8(C-24);29.2(C-25);19.8(C-26);19.0(C-27);23.1(C-28);11.9(C-29).

Compound III a white solid.^{1}H-NMR($CDCl_3$);δ=5.21,lH,br(H-12);3.20 ,1H(m),H-3;1.13 3H(s),H-27;1.03 3H(s),H-23;1.00,3H(s),H-26;0.97 3H(s),H-25;0.94 3H(s),H-29;0.85 3H(s),H-30;0.80 3H(s),H-28;0.76 3H(s),H-24. ^{13}C-NMR(125MHz,$CDCl_3$): 38.7(C-1);27.3(C-2);79.0(C-3);38.8(C-4);55.3(C-5);18.4(C-6);32.7(C-7);38.9(C-8);47.7(C-9);37.2(C-10);23.5(C-11);121.7(C-12);145.2(C-13);41.7(C-14);26.2(C-15);27.0(C-16);32.5(C-17);47.3(C-18);46.9(C-19);31.1(C-20);34.8(C-21);37.2(C-22);28.1(C-23);15.5(C-24);15.6(C-25);16.8(C-26);26.0(C-27);28.4(C-29);33.3(C-29);21.5(C-30). Compound III was found to be α-amyrin by comparison of the spectra with literature.

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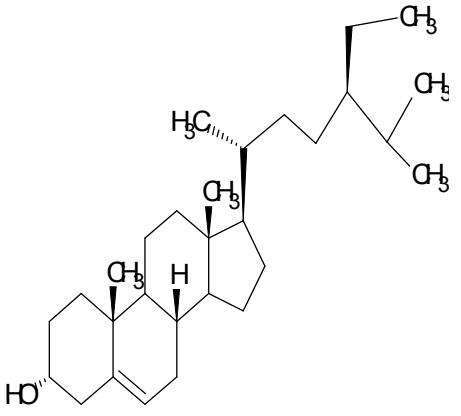
Compound IV, a white amorphous solid (10.2mg),^{1}H-NMR($CDCl_3$):δ=5.25,1H,H-12;3.20(m),H-3;2.02, 2.2 1H,d,j=3Hz,H-18;1.25 3H(s),H-23;1.14 3H(s);H-27;1.08 3H(s),H-26;0.98 3H(s),H-24;0.93 3H(s),H-20;0.91 3H(s),H-22;0.77 3H(s),H-25. ^{13}C-NMR (125MHz,$CDCl_3$):39.8(C-1);27.8(C-2);79.5(C-3);39.9(C-4);56.7(C-5);19.4(C-6);34.3(C-7);40.7(C-8);47.6(C-9);38.1(C-10);24.3(C-11);126.8(C-12);139.6(C-13);42.8(C-14);29.2(C-15);25.3(C-16);47.6(C-17);54.3(C-18);40.4(C-19);40.4(C-20);31.7(C-21);38.1(C-22);28.7(C-23);16.6(C-24);16.3(C-25);17.6(C-26);24.0(C-27);181.6(C-28);17.8(C-29);21.5(C-30). Compound IV was found to be Ursolic acid by comparison of the NMR spectra with literature.

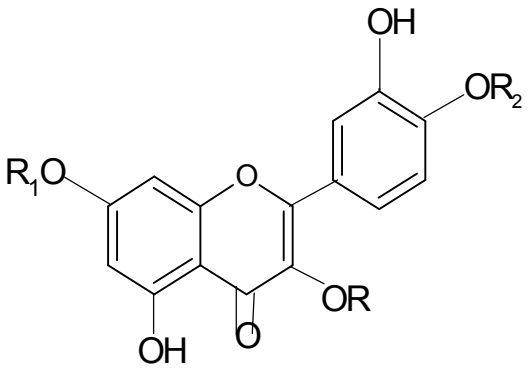
Compound V, a yellow solid (3mg).^{1}H-NMR(CD_3OD),δ=6.20,1H,d,j=2Hz,H-6;6.40 1H,d,j=2Hz,H-8,6.80 1H,d,j=8.2Hz,H-5^{1};7.51 1H,d,dj=2Hz,8Hz,H-6^{1} and 7.75 1H,d,j=2Hz,H-2^{1}) this was found to be Quercetin by comparison of the ^{1}H-NMR spectra with literature.

Compound VI, a yellow amorphous solid(4mg).^{1}H-NMR(CD_3OD),δ=6.20 (d,j=2Hz,H-6);6.40 (d,j=2Hz,H-8);6.90 2H(d,j=8Hz,H-3^{1}.5^{1}); and 8.10 2H(d,j=8Hz,H-2^{1},6^{1}). Compound VI was found to be Kaempferol by comparison of the NMR spectra with literature.

Compound VII, a yellow solid (3.4mg),^{1}H-NMR(CD_3OD),δ=6.40 1H,(d,j=2Hz,H-6);6.75 1H,(d,j=2Hz,H-8);6.90 1H(d,j=2Hz,H-5^{1});7.65 1H(d,d,j=2,8Hz,H-6^{1}) ; 7.75 1H(d,j=2Hz,H-2^{1});1.30 3H (d,j=6Hz,H-6 rhamnose);4.5 1H(s,H-1 rhamnose), was found to be Quercetin-7-O-rhamnoside by comparison of the NMR spectra with literature.

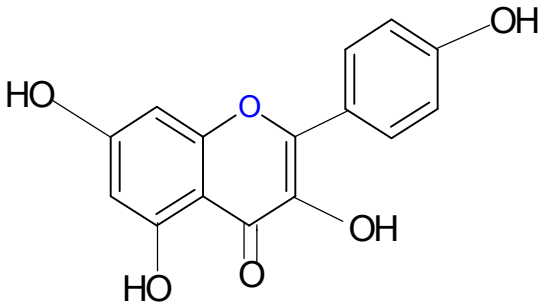


(II).β-Sitosterol; 22,23-dehydro(Stigmasterol)



Compound V: $R=R_1=R_2=H$

Compound VII: $R=R_2=H,R_1$=-rhamnoside



Compound VI

Fig.2

DISCUSSION.

Compound I appeared to be lupane type triterpenoid. The ^{1}H-NMR displayed a characteristic signal of isopropenyl group, a downfield singlet of vinylic methyl (Me-30) at δ=1.61 and a pair of broad singlets due to exomethylene protons of H-29 at δ=4.57 and 4.65 respectively.The double doublets signal at 3.17 is typical for a triterpenoid with a 3-hydroxy substitution, the spectral data ^{1}H-NMR and ^{13}C-NMR of compound I were in full agreement with Lupenol [7].

Compound II gave three vinylic proton signals at δ=5.36 (2H (m),H-6);5.15 (1H,d,d,H-22) and 5.02(d,d (H-23) and a proton signal at δ=3.54 (2H,H-3),the proton integration revealed a 2:1:1:2 ratio thus indicating compound II to be a mixture of two phytosterols. Compound II also gave six methyl proton signals with two singlets at δ=0.68(H-18) and 1.01(H-19) and doublet methyl signals at δ=0.8(H-27),0.81(H-29),0.85(H-26)and 1.02(H-21).The ^{13}C-NMR (Table2) revealed four olefenic carbon signals at δ=140.7,138.3,129.3 and 121.3 and one oxygen

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attached carbon signal at δ=71.8ppm.The remaining carbon showed signals having chemical shifts between 11-57ppm. The above spectral data seemed to be a mixture of β-sitosterol and Stigmasterol (Fig2). Direct comparison of the spectra data with those reported in literature [8,9],showed they are identical. Thus compound II was identified as a mixture of β-sitosterol and Sitgmasterol.

Compound III,The ^{1}H-NMR spectra display singlets of eight tertiary methyl indicative of oleanane skeleton with signal at δ=0.77(me-28),0.81(Me-29),0.85(Me-29,30),0.92(Me-24),0.95(Me-26),0.98(Me-23) and 1.12(Me-27).A downfield shift at δ=5.35 is assigned to the olefenic proton at (H-12),a doublet,doublet signal at δ=3.21 was assignable to the carbinylic proton(H-3).The ^{13}C-NMR spectra showed a pair of downfield signals at δ=123.5 and 145.2 represented the C-12 unsaturation.On the basis of these and comparison of the spectral data with iterature [10,11].Compound III was identified as α-amyrin(Fig1).

The ^{1}H-NMR of compound IV revealed a total of seven methyl protons five being tertiary at δ=0.67(H-23);0.75(H-26);0.86(H-25);0.89(H-24) and 1.03(H-27) and secondary methyl protons at δ=0.81(H-29) and 0.91(H-27). The olefenic proton (H-12) resonates at δ=5.25 and the carbinylic proton (H-3) was observed at δ=3.20(d,d) with the carbon signals at δ=77.8ppm. A doublet signal at δ=2.20ppm indicated that the C-18 and C-19 protons are trans to one another and such doublet is as a result of coupling between C-19 hydrogen and C-18 methyl protons [12].Spectral data of compound IV are consistent with Ursolic acid [13,14].

Compound V a yellow amorphous solid, the ^{1}H-NMR spectra data showed a 5,7-dihydroxy substituted ring A of the Flavonoid nucleus with metacoupled protons at δ=6.15 1H and 6.40 1H assigned to H-6 and H-8 of ring A of the Flavonoid nucleus[15],while signals at δ=6.90,7.65 and 7.72 all integrating for 1 proton are assigned to H-5^{1},H-6^{1}and H-2^{1} protons of ring B of the flavonoid nucleus, thus compound V was identified as Quercetin by comparison of the ^{1}H-NMR spectra with literature [15].

Compound VI a yellow solid, the ^{1}H-NMR spectra showed an A_2 B_2 aromatic protons signals at δ=6.90, d,J=8Hz and 8.1 d,J=8Hz assigned to H-3^{1},5^{1} and H-2^{1},6^{1} of ring B of the Flavonoid nucleus,metacoupled protons at δ=6.2 1H and 6.4 1H assigned to H-6 and H-8 of ring A of the flavonoid nucleus. Compound VI was identified as Kaempferol by comparison of the ^{1}H-NMR spectra with literature [15].

Compound VII, a yellow amorphous solid showed a similar protons with compound V except that the meta coupled protons are deshielded with signals at δ=6.40(H-6) and 6.75(H-8) indicating the presence of a sugar moiety at position 7 of the ring A of the flavonoid nucleus. The sugar proton was found to be rhamnose with the characteristic methyl signals at 1.31 (d,J=6Hz) and the anomeric proton at δ=4.1ppm, thus compound VII was identified as Quercetin-7-O-rhamnoside by comparison of the ^{1}H-NMR spectra with literature [16].

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