CHAPTER 5
CYTOTOXICITY ACTIVITY OF SELECTED SOUTH AFRICAN MEDICINAL PLANTS AGAINST VERO CELLS

5.1. Introduction

Epidemiological statistics show that cancer and infectious diseases like tuberculosis (TB) are important causes of mortality throughout the world (WHO, 1999). Secondary metabolites of plants possess many biological activities since they serve either as protective agents against various pathogens (e.g. insects, fungi and bacteria) or as growth regulatory molecules (e.g. hormone-like substances that stimulate or inhibit cell division and morphogens). These physiological effects make some of them potentially anti-cancerous, due to either their direct cytotoxicity on cancer cells or modulation of tumor development (Don *et al*., 2006). Cytotoxicity tests are part of developing a potential pharmaceutical product into a clinically acceptable drug. This provides a screening system to ascertain that the compounds being tested are not more harmful to the normal biological processes than the effects they are being tested for (Gebhardt, 2000). Various natural products have particular reactions against biological systems and the cytotoxic evaluation of plant extracts is essential before they could be considered for new drug development (Avila *et al*., 1997). Many plant extracts and isolated compounds have been tested *in vitro* for cytotoxicity by using different human cell lines (prostate, stomach, cancer, liver colon, etc) as well as animal cells such as monkey kidney cells (Lamidi *et al*., 2005; Al-Fatimi *et al*., 2005).

In this chapter, the cytotoxicity activity of the crude extracts from the selected medicinal plants against Vero cell lines is reported.

5.2. Materials and methods

5.2.1. Plant materials

Plant species and their parts were selected based on their traditional use for TB. The selection of plant species for this study was based on the information culled from published sources and traditional healers. (Chapter 3, Table 3.1; Chapter 4, section 4.2.1). Voucher specimens were deposited and identified at the H.G.W.J. Schweickerdt Herbarium (PRU), University of Pretoria, South Africa (Chapter 3, Table 3.1).

5.2.2. Preparation of plant extracts

Preparations of crude extracts were prepared according to Chapter 4, section 4.2.2.

5.3. Cells culture

5.3.1. Vero cell line

The cytotoxicity of all the crude ethanol extracts against Vero cells were tested following the method of Zheng *et al*., 2001. Cells were cultured in Eagle's minimal essential (MEM**),** supplemented with 1.5 g/L sodium bicarbonate, 2mM L-glutamine, 0.1 mM non-essential amino acids, 1.0 mM sodium pyruvate, 10 µg/mL penicillium, 10.0 µg/mL streptomycin, 0.25 µg/mL fungizone and 10% fetal bovine serum, at 37^{o}C in a humidified atmosphere with 5% CO_2. Cells were subcultured in a ratio of 1:6 every second to third day after the trypsinization of confluent cultures (Zheng *et al*., 2001).

5.4. Cytotoxicity assay

Cytotoxicity was measured by the 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-

[(phenylamino)carbonyl]-2-*H*-tetrazolium hydroxide (XTT) method using the Cell Proliferation Kit II (Roche Diagnostics GmbH). 100.0 µL of Vero cell lines ($1x10^5$ Cells/mL) were seeded into inner wells of the microtiter plates, while in the outer wells 200.0 µL of incomplete medium was added. The plates were incubated for 24 hours to allow the cells to attach to the bottom of the plate.

Dilution series were made of the extracts and the various concentrations (400.0 to 3.12 µg/mL) were added to the inner wells of the microtiter plate and incubated for 48 hours (Figure 5.1). Fifty microlitters of XTT reagent (1.0 mg/mL XTT with 0.383 mg/mL PBS) was added to the wells and the plates were then incubated for 1 - 2 hours. The positive control, (Zearalenone) was included at final concentration of 1.25 µg/mL. After incubation the absorbance of the colour complex was spectrophotometrically quantified using an ELISA plate reader, which measures the optical density at 450 nm with a reference wavelength of 690 nm. Referring to the control (medium with DMSO control), cell survival was assessed. The 'GraphPad Prism 4', statistical program was used to analyse the fifty percent inhibitory concentration (IC_{50}) values.

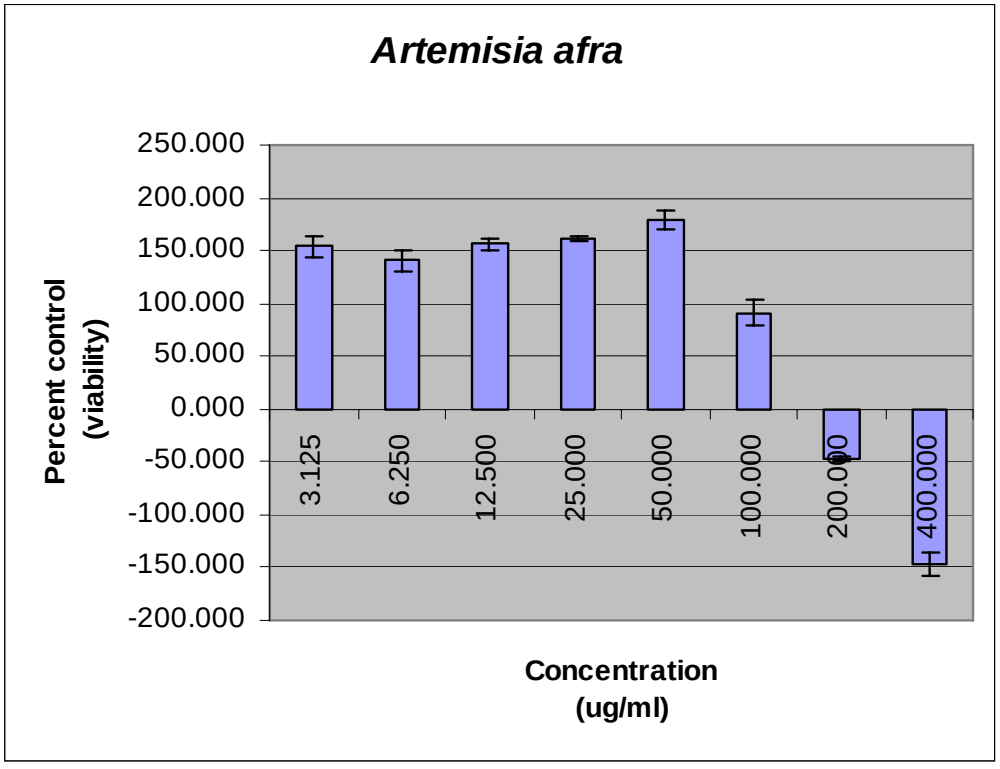
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		**A**								Media	DMSO	
C		400	200	100	50	25	12.5	6.25	3.125	Media	DMSO	
D		µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	Media	DMSO	
E		**B**								Media	DMSO	
F		400	200	100	50	25	12.5	6.25	3.125	Media	DMSO	
G		µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	µg/mL	Media	DMSO	
H												

Figure 5.1. Microtitre plate for cytotoxicity testing

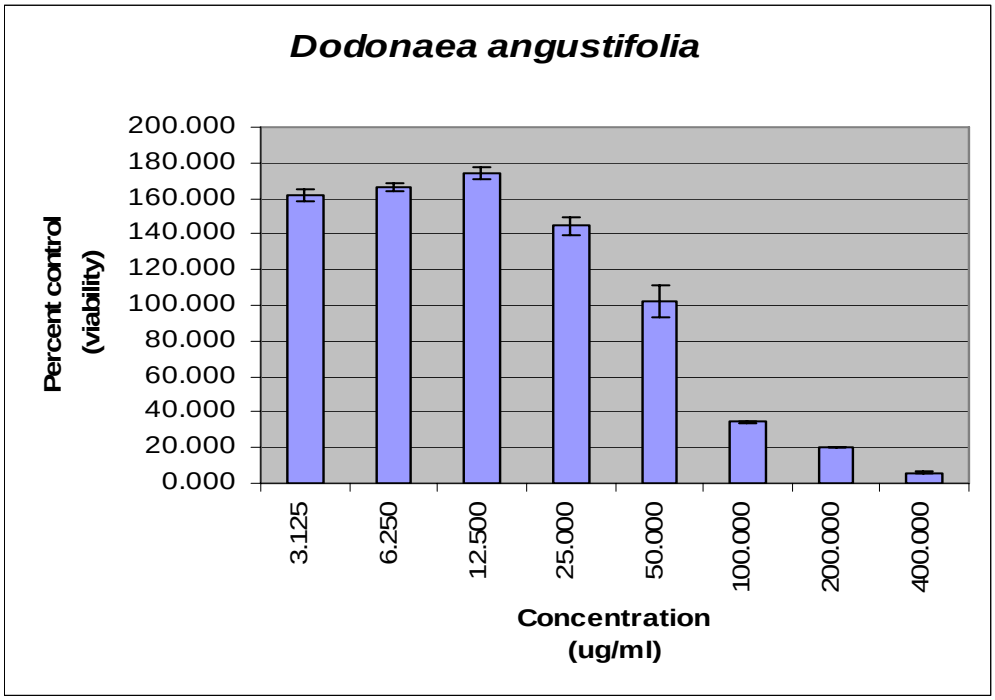
5.5. Results and Discussion

A cytotoxicity assay is a rapid and cost-effective tool to sort out the likely failures before a plant extract or a compound enters into the costly development process and also help to choose the optimal candidate. The results (Figure 5.2) obtained indicated that ethanol crude extracts of the selected plants have moderate toxicity. The cytotoxicity effects of the six ethanol plant extracts on Vero cells demonstrated marginal toxicity except for *S. cordatum*, which showed fifty percent inhibitory concentration (IC_{50}) at 2.67 µg/mL against the Vero cells. Ethanol extract of *P. africana* had the highest IC_{50} value (212.0 µg/mL) compared to other plant extracts. *G. africana* showed moderate toxicity exhibiting IC_{50} at 101.3 µg/mL (Table 5.1).

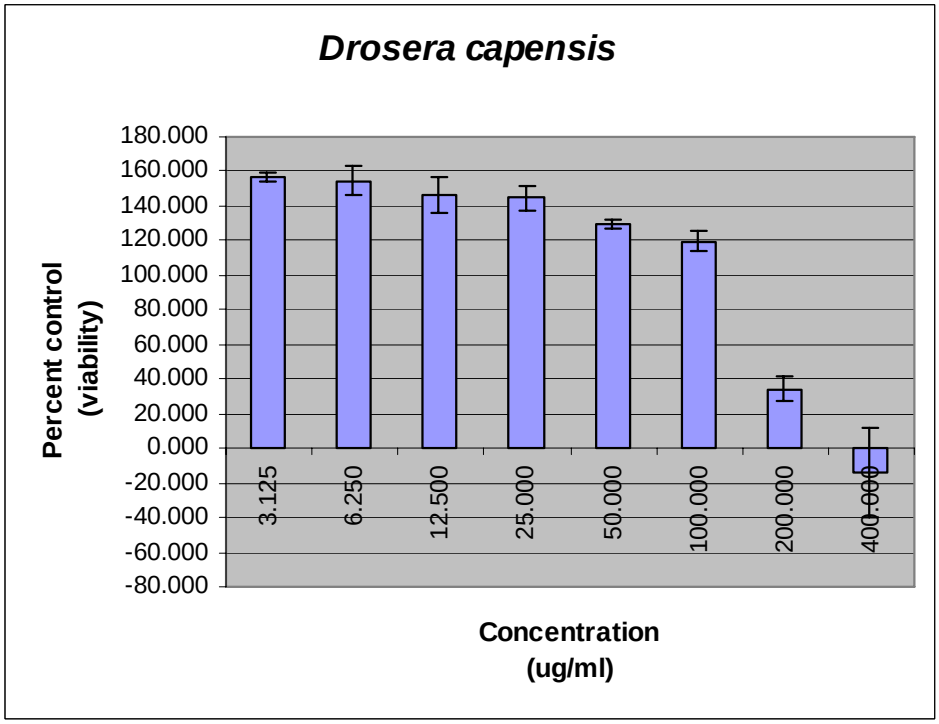
The dose-response curves of each plant extract against the proliferation of Vero cells are as follows:



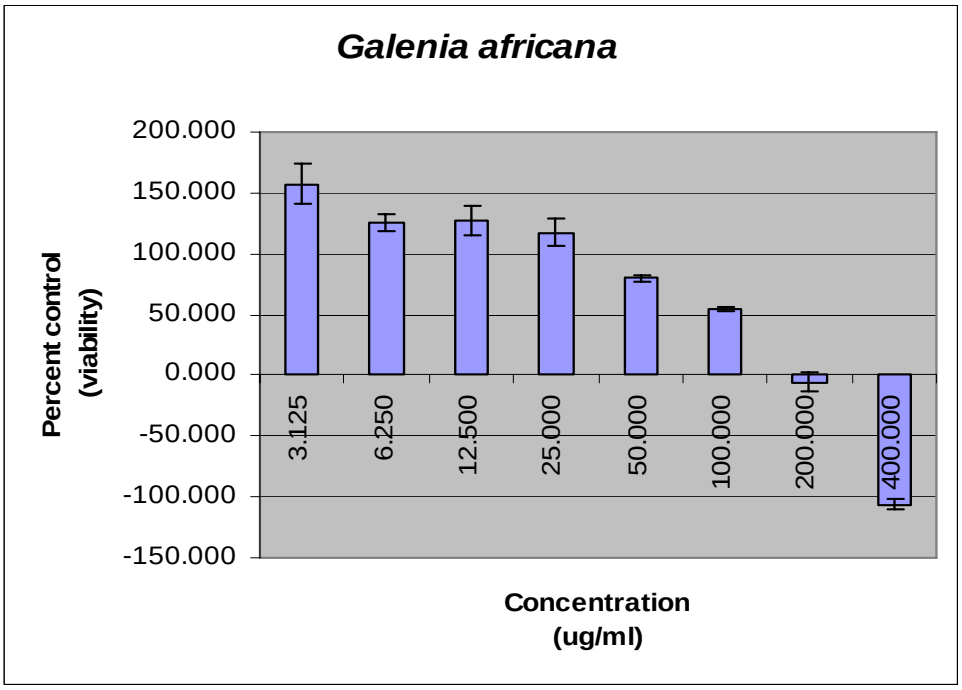
(a)


Dodonaea angustifolia
Percent control (viability)
200.000
180.000
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
3.125
6.250
12.500
25.000
50.000
100.000
200.000
400.000
Concentration (ug/ml)

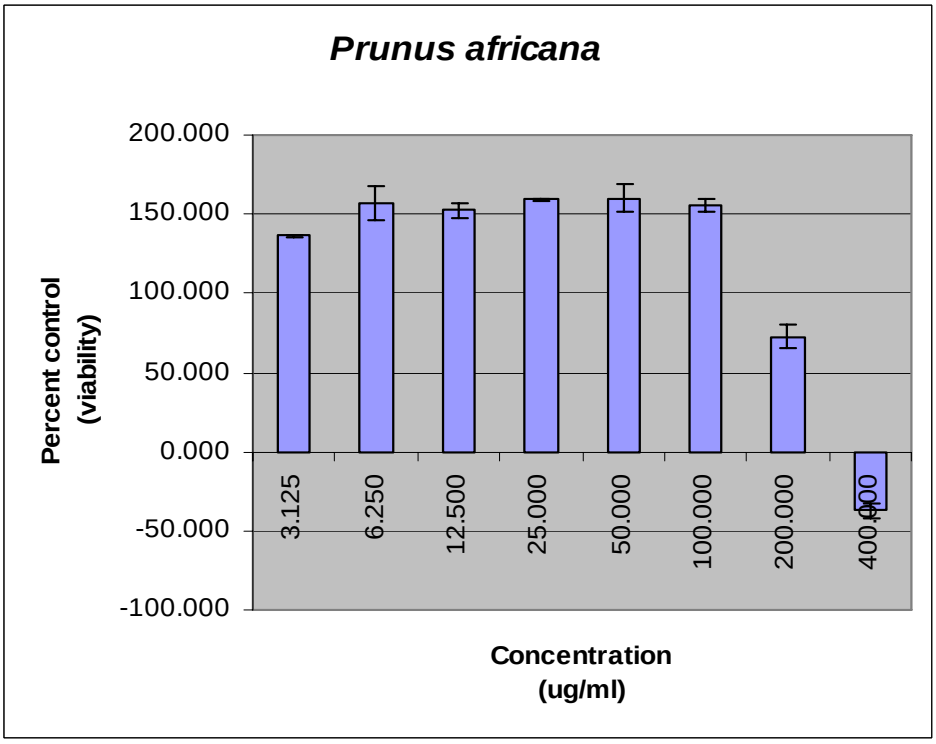
(b)


Drosera capensis
Percent control (viability)
180.000
160.000
140.000
120.000
100.000
80.000
60.000
40.000
20.000
0.000
-20.000
-40.000
-60.000
-80.000
3.125
6.250
12.500
25.000
50.000
100.000
200.000
400.000
Concentration (ug/ml)

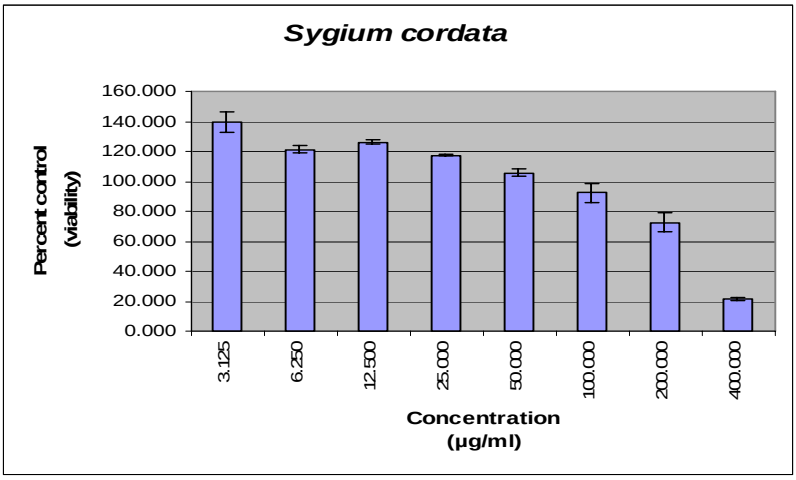
(c)


Galenia africana
200.000
150.000
100.000
50.000
0.000
-50.000
-100.000
-150.000
Percent control (viability)
3.125
6.250
12.500
25.000
50.000
100.000
200.000
400.000
Concentration (ug/ml)

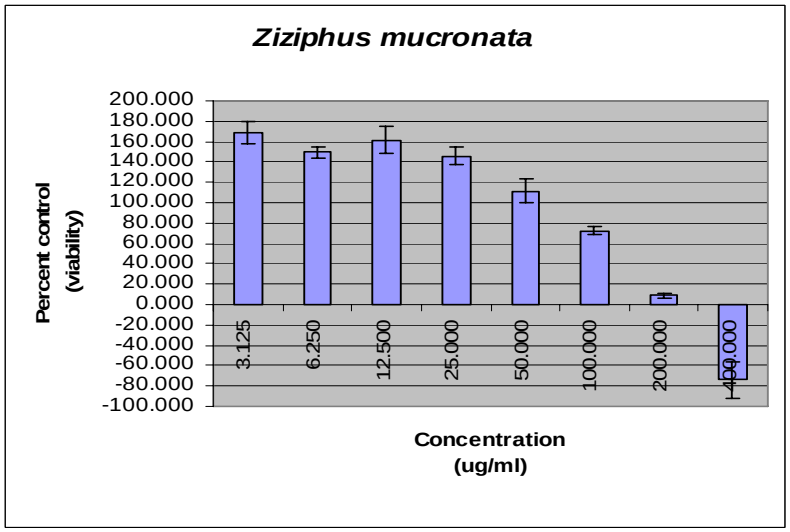
(d)


Prunus africana
200.000
150.000
100.000
50.000
0.000
-50.000
-100.000
Percent control (viability)
3.125
6.250
12.500
25.000
50.000
100.000
200.000
400.000
Concentration (ug/ml)

(e)



(f)



(g)

Figure 5.2. Effect of the ethanol crude extracts of selected South African medicinal plants on the proliferation of Vero cells

(a) ***A. afra***

(b) ***D. angustifolia***

(c) ***D. capensis***

(d) ***G. africana***

(e) ***P. africana***

(f) ***S. cordata***

(g) ***Z. mucronata***

Table 5.1. Cytotoxicity of the ethanol extracts of selected South African medicinal plants against Vero cells

Plant species	IC_{50}[a] (µg/mL ± SD)	MIC[b] (µg/mL)	SI[c]
A. afra	113.0 ± 2.05	na[d]	nd[e]
Dodonaea angustifolia	091.0 ± 1.10	5×10^3	0.018
Drosera capensis	141.4 ± 2.14	na	nd
G. africana	101.3 ± 2.19	1.2×10^3	0.084
P. africana	212.0 ± 2.33	na	nd
S. cordatum	002.67 ± 2.54	na	nd
Z. mucronata	118.2 ± 2.36	na	nd
Zearalenone (positive control)	002.31 ± 0.30	nd	nd

[a]IC_{50}, 50% inhibitory concentration of samples on Vero cell line.

[b]Minimum inhibitory concentration against *M. tuberculosis*.

[c]SI, selectivity index (*in vitro*): IC_{50} in Vero cells/MIC against *M. tuberculosis*.

[d]Not active at the highest concentration tested (5.0 mg/mL) against *M. tuberculosis*.

[e]Not determined.

5.6. Conclusion

Cytotoxicity screening of plant-derived extracts is a necessary aspect of the preliminary safety evaluation for further testing or compound isolation. This helps to ensure that the biological activity of the plant extract is not due to a general toxic effect. The *in vitro* cytotoxicity in early research efforts provides an important advantage in identifying potentially cytotoxic compounds. However, *in vitro* cytotoxic assays that can reliably predict *in vivo* toxicity of a drug is rare because it is the most difficult property to adequately investigate. Nonetheless, these assays do provide a useful tool to compare and rank new non-poisonous compounds to some extent (Hamid *et al*., 2004).

Dodonaea angustifolia and *G. africana* displayed activity against *M. tuberculosis* but they also displayed lower IC_{50} values in Vero cells. *A. afra* was evaluated against for anticancer activity against human cell lines (breast MCF7, renal TK10 and melanoma UACC62) and was found to have total inhibition growth of 9.73 µg/mL for UACC62 (Fouche *et al*., 2008). This is the first report on cytotoxicity of plant samples selected in this study on Vero cell lines.

CHAPTER 6

BIOASSAY GUIDED FRACTIONATION OF *G. AFRICANA* L. VAR. *AFRICANA*

6.1. Introduction

There is no information on isolated constituents from plants belonging to the genus Galenia. Preliminary chemical tests of *G. africana* showed presence of alkaloids but not of saponnins, tannins and glycosides. Compounds such as dihydroechinoidinin, 2', 4'dihydroxydihydrochalcone, 5,7-dihydroxy-flavanone (pinocembrin), 2',4'-dihydroxychalcone and 5-hydroxy-7-methoxy-flavanone (pinostrobin) have been isolated from *G. africana* (Figure 6.1). These compounds were investigated for antifungal properties (Vries *et al*.,2005). However, there is no current information regarding other secondary metabolites from *G. africana*.

G. africana has been associated with liver damage and severe ascites, a condition commonly referred to as "waterpens" in sheep and goats. Indigenous tribes chew the plant to relieve toothache and it has been reported that it can produce blisters on the mucous membrane of the mouth if used excessively. The plant is also used in the treatment of venereal diseases and a decoction as a lotion for skin diseases, including ringworm, and for the relief of inflammation of the eyes (Adamson, 1956). The objective in this chapter is to isolate, purify and characterize the secondary metabolites with antimycobacterial activity from the leaves of *G. africana*.

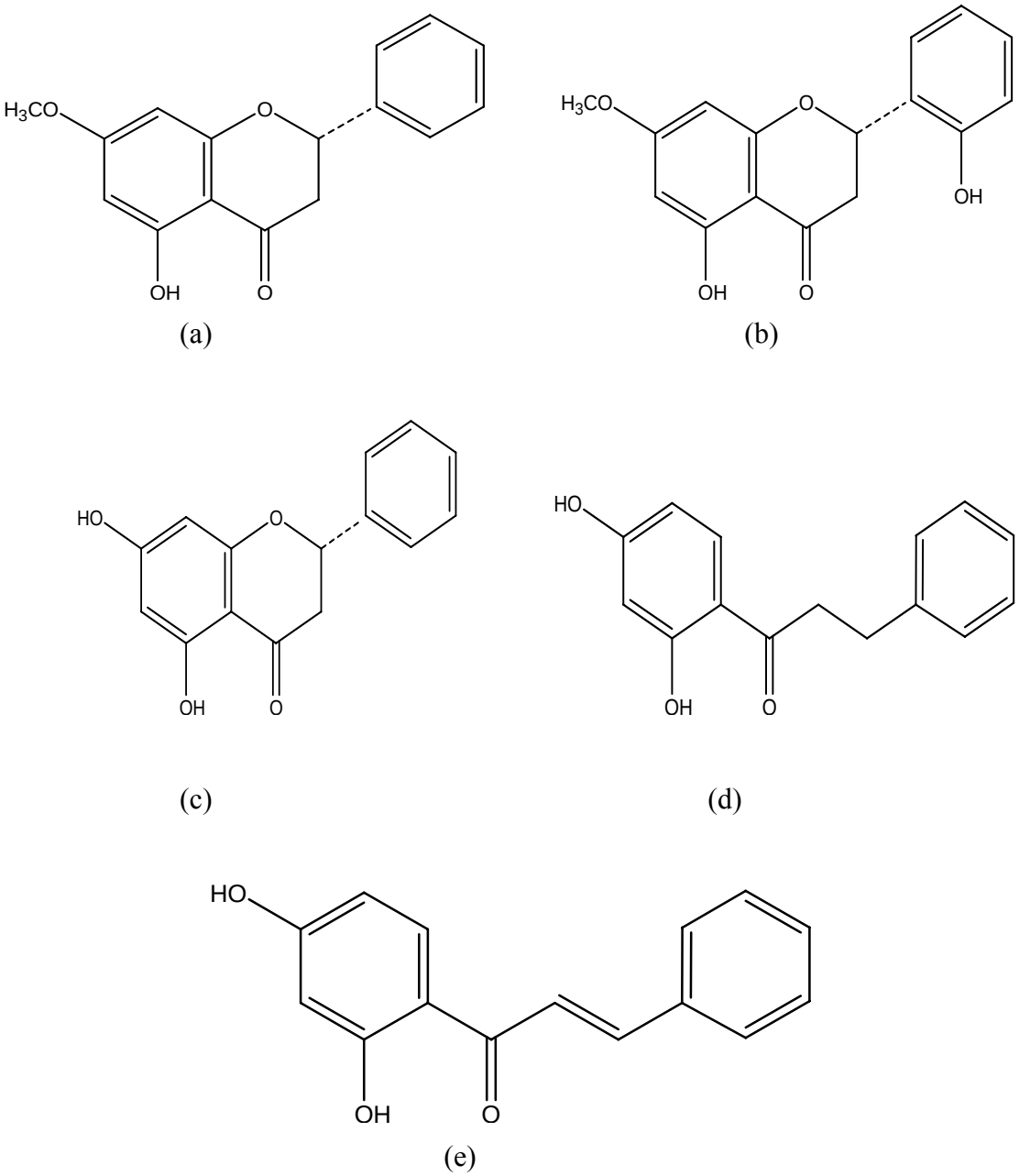


Figure 6.1. Chemical structure of compounds isolated from *G. africana*

(a) 5-hydroxy-7-methoxy-flavanone (pinostrobin)

(b) Dihydroechinoidinin

(c) 5,7-dihydroxy-flavanone(pinocembrin)

(d) 2',4'dihydroxydihydrochalcone

(e) 2',4'-dihydroxychalcone

6.2. Bioassay guided fractionation

6.2.1. Silica gel column and GPC-HPLC chromatography

Considering significant inhibitory activity shown by the ethanol extract of *G. africana* against *M. smegmatis* and *M. tuberculosis*, (Chapter 4), this plant species was selected for the identification of bioactive principles. The ethanol crude extract was filtered and evaporated under reduced pressure. The total concentrated extract (20.0 g) which appeared as a yellow-green solid, was soluble in ethanol (EtOH), methanol (MeOH), and dichloromethane (CH_2Cl_2), but insoluble in hexane ($CH_3(CH_2)_4CH_3$) and partially soluble in water (H_2O). A portion of the ethanol extract (6.0 g) was chromatographed on silica gel column chromatography PF_{254} (CC, size 70 x 120 cm, 2.5 kg, Figure 6.2) using $CH_3(CH_2)_4CH_3$/ethyl acetate (EtOAc) mixtures of increasing polarity (0 to 100%) followed by 100% EtOH. In total, 21 crude fractions (300.0 mL) were collected (Figure 6.3) and analysed on thin layer chromatographic (TLC) using different mixtures of $CH_3(CH_2)_4CH_3$: EtOAc. Similar fractions, according to the (TLC) profile, were combined which resulted into 6 main fractions (Figure 6.4). The fractions were assayed on a thin layer chromatogram (TLC silica gel 60 F_{254}) using $CH_3(CH_2)_4CH_3$ and EtOAc (8:2) as eluent and analysed under a UV light. The rest of the compounds remaining on the column were washed out with 2.0 L of 100% EtOAc. All 6 main fractions were tested against *M. smegmatis* and *M. tuberculosis* (Chapter 7).

The GPC-HPLC (gel permeation chromatography-high pressure liquid chromatography, 20.0 mm x 500.0 mm, flow rate of 1.0 mL/min, temperature of 40^{o}C and wavelength of 206nm) isolation was carried out by LC-908W (Japan Analytical Industry). GPC-HPLC is a normal HPLC method by the use of GPC column instead of octadecyl silane (ODS) or silica gel column. NMR spectra were recorded on JNM ECA-600 (JEOL). Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to tetramethylsilane (δ_H) and residual solvent signals (δ_C 49.0 and 29.8 for methanol-d_4 and acetone-d_6, respectively) as internal standards. Mass spectra were

measured on JMS AX-500 and AX-700 JEOL (Figure 6.6).

Schematic representation of the purification steps for the isolation of the compounds is illustrated in Figure 6.5.

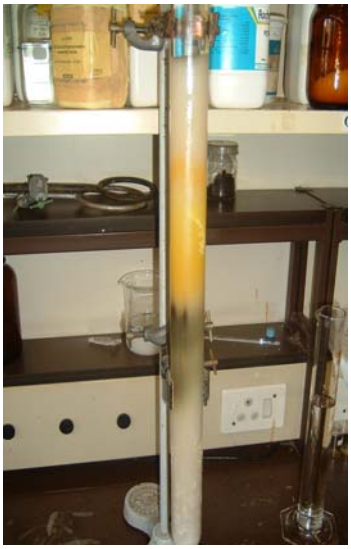
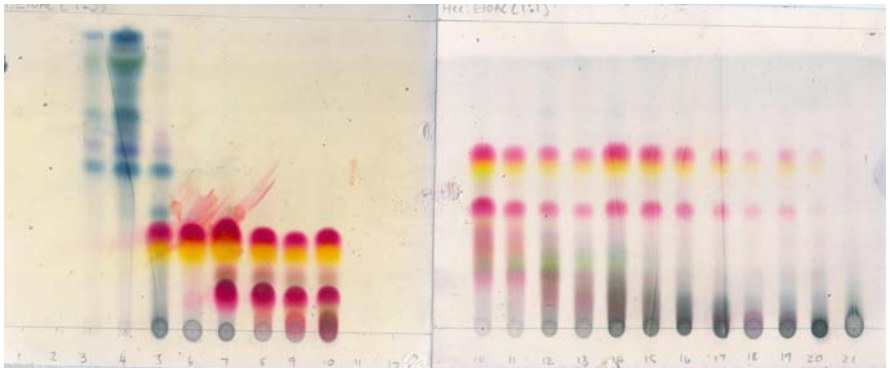


Figure 6.2. Chromatographic purification of the ethanol extract of *G. africana* using silica column chromatography PF_{254}



(a) **(b)**

Figure 6.3. TLC developed-plates of 21 main fractions obtained from silica column. Solvent systems: (a) $CH_3(CH_2)_4CH_3$: EtOAc (3:1)
(b) $CH_3(CH_2)_4CH_3$: EtOAc (3:2)
Detection:Vanillin in H_2SO_4

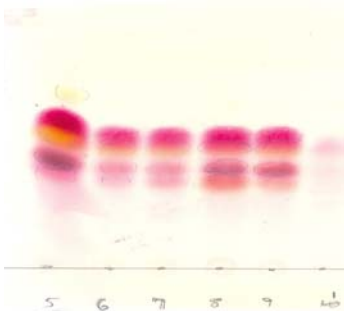


Figure 6.4. **TLC developed-plates of similar and combined fractions which resulted in 6 main fractions. Solvent systems: $CH_3(CH_2)_4CH_3$: EtOAc (8:2). Detection : Vanillin in H_2SO_4**

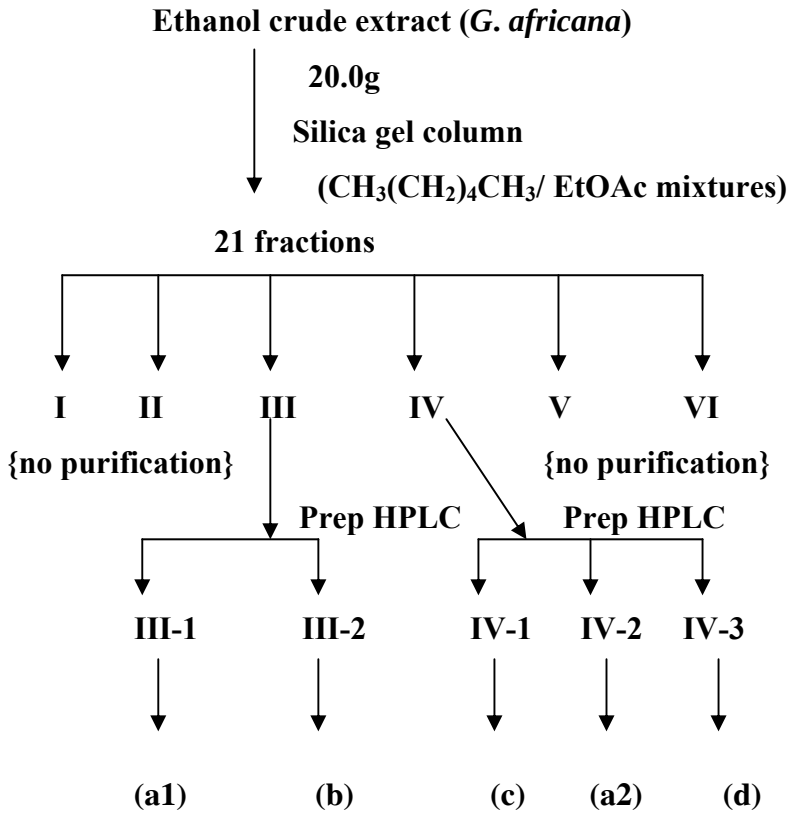


Figure 6.5. **Schematic representation of the purification steps for the isolation of the compounds from the ethanol extract of *G. africana***

(a1): (2*S*)-5,7,2'-trihydroxyflavanone

(b): (*E*)-3,2',4'-trihydroxychalcone

(c): (*E*)-2',4'-dihydroxychalcone

(a2): (2*S*)-5,7,2'-trihydroxyflavanone

(d): (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

6.3. Results and Discussion

Fractions I (81.0 mg) and II (56.0 mg) did not show presence of compounds whereas fractions III (112.9 mg), IV (201.2 mg) and V (0.28 mg) which had similar TLC profiles showed presence of different compounds. Fractions III and IV were subjected to further purification and were individually chromatographed on GPC-HPLC, eluting with $CHCl_3$-MeOH (4:1). The major fractions of fractions III and IV were shown by TLC and NMR to be mixtures containing varying proportions of chalcone, dihydrochalcone and flavanone. Fraction V was not sufficient and therefore it was not selected for purification. Three known and one novel compounds belonging to flavonoid and chalcone groups were isolated from fractions III and IV of the ethanol extract of *G. africana*. The structural analysis of the isolated compounds is as follows:

Fraction III (112.9 mg) was subjected to GPC-HPLC (column, JAIGEL-GS310 (f 20.0 mm x 500 mm); solvent, $CHCl_3$-MeOH (4:1); flow rate, 5.0 mL/min.) to give 2 fractions: Fraction III-1 (108.0 mg, t_R 13.6~23.4 min.), was a pure compound, **(2*S*)-5,7,2'-trihydroxyflavanone** (Figure 6.7a). Fraction III-2 (33.7 mg, t_R 25.1~29.0 min.), was subjected to GPC-HPLC (column, JAIGEL-W252 (f 20.0 mm x 500 mm); solvent, $CHCl_3$-MeOH (4:1); flow rate, 5 mL/min.) to give **(*E*)-3,2',4'-trihydroxychalcone** (Figure 6.7c).

Fraction IV (201.2 mg) was subjected to GPC-HPLC (column, JAIGEL-GS310 (f 20.0 mm x 500 mm); solvent, $CHCl_3$-MeOH (4:1); flow rate, 5.0 mL/min.) to give 3 fractions: Fraction IV-1 (11.0 mg, t_R 20.3~21.7 min.), was subjected to GPC- HPLC (column, JAIGEL-W252 (f 20.0 mm x 500 mm); solvent, $CHCl_3$-MeOH (4:1); flow rate, 5.0 mL/min.) to give **(*E*)-2',4'-dihydroxychalcone** (4.0 mg, t_R 12.2~16.5 min.) (Figure 6.7b). Fraction IV-2 (80.5 mg, t_R 24.0~26.9 min.), was a pure compound, **(2*S*)-5,7,2'-trihydroxyflavanone** (30.0 mg). Fraction IV-3 (28.3 mg, t_R 26.9~29.4 min.), was subjected to GPC-HPLC (column, JAIGEL-W252 (f 20.0 mm x 500 mm); solvent, $CHCl_3$-MeOH (4:1); flow rate, 5.0 mL/min.), and the peaks between tR 11.7~15.1 min. were separated by the recycle mode. After five times of cycles,

(*E*)-3,2',4'-trihydroxy-3'-methoxychalcone (18.0 mg) was isolated (Figure 6.7d). The aromatic signals and structure elucidation of each purified compound are as follows:

(2*S*)-5,7,2'-trihydroxyflavanone: colourless amorphous solid; $[\alpha]_D$ -114 (*c* 1.00, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 7.44 (1H, dd, *J* = 7.7, 1.4 Hz), 7.14 (1H, td, *J* = 7.7, 1.4 Hz), 6.86 (1H, td, *J* = 7.7, 0.8 Hz), 6.81 (1H, dd, *J* = 7.7, 0.8 Hz), 5.94 (1H, d, *J* = 2.2 Hz), 5.89 (1H, d, *J* = 2.2 Hz), 5.68 (1H, dd, *J* = 12.8, 3.1 Hz), 2.94 (1H, dd, *J* = 17.2, 12.8 Hz), 2.80 (1H, dd, *J* = 17.2, 3.1 Hz) (Figure 6.5b). ^{13}C NMR (150 MHz, CD_3OD) δ 198.0, 168.2, 165.4, 165.1, 155.2, 130.2, 127.6, 126.7, 120.7, 116.2, 103.3, 97.1, 96.2, 75.9, 42.9; EIMS *m/z* 272 $[M]^+$, 254 (100%), 253, 179, 153; HREIMS *m/z* 272.0667 (272.0685 calcd for $C_{15}H_{12}O_5$); CD (MeOH) λ, nm ([θ]): 286 (-43800), 315 (+5210), 325 (+6840) (Figure 6.5a).

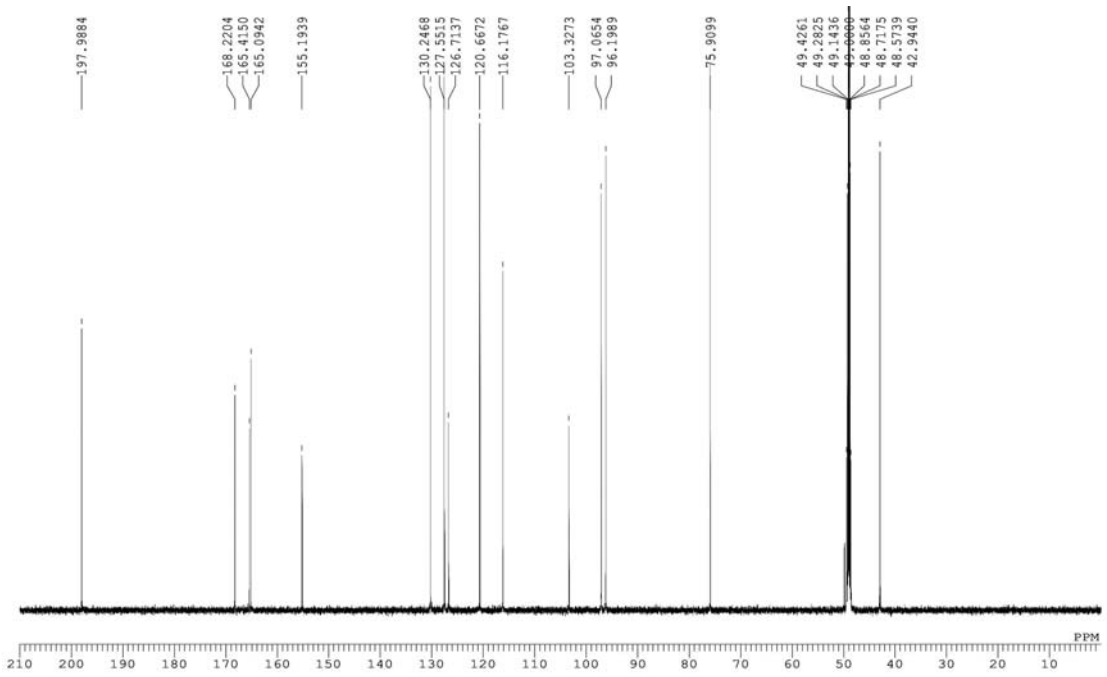
(*E*)-2',4'-dihydroxychalcone: yellow amorphous solid; the spectrum was of a chalcone structure, with ^{1}H NMR (600 MHz, acetone-d_6) δ 13.45 (1H, s), 8.15 (1H, d, *J* = 8.9 Hz), 7.95 (1H, d, *J* = 15.4 Hz), 7.87 (1H, d, *J* = 15.4 Hz), 7.84-7.87 (2H, m), 7.44-7.48 (3H, m), 6.48 (1H, dd, *J* = 8.9, 2.4 Hz), 6.37 (1H, d, *J* = 2.4 Hz) (Figure 6.5d). ^{13}C NMR (150 MHz, acetone-d_6) δ 192.9, 167.8, 166.1, 144.9, 136.1, 133.7, 131.5, 129.9 (2C), 129.8 (2C), 121.9, 114.6, 109.1, 103.9; EIMS *m/z* 240 $[M]^+$ (100%), 223, 163, 137; HREIMS *m/z* 240.0783 (240.0786 calcd for $C_{15}H_{12}O_3$) (Figure 6.5c).

(*E*)-3,2',4'-trihydroxychalcone (not reported from natural sources): yellow amorphous solid; ^{1}H NMR (600 MHz, acetone-d_6) δ 13.45 (1H, s), 8.14 (1H, d, *J* = 8.9 Hz), 7.87 (1H, d, *J* = 15.5 Hz), 7.79 (1H, d, *J* = 15.5 Hz), 7.32 (1H, dt, *J* = 7.7, 1.1 Hz), 7.26-7.30 (2H, m), 6.94 (1H, ddd, *J* = 8.0, 2.4, 1.1 Hz), 6.48 (1H, dd, *J* = 8.9, 2.5 Hz), 6.37 (1H, d, *J* = 2.5 Hz) (Figure 6.5f). ^{13}C NMR (150 MHz, acetone-d_6) δ 192.8, 167.7, 165.9, 158.8, 144.9, 137.7, 133.6, 130.8, 121.7, 121.1, 118.6, 116.1, 114.4, 108.9, 103.8; EIMS *m/z* 256 $[M]^+$ (100%), 239, 163, 137; HREIMS *m/z* 256.0722 (272.0736 calcd for $C_{15}H_{12}O_4$) (Figure 6.5e).

(*E*)-3,2',4'-Trihydroxy-3'-methoxychalcone (Novel compound): yellow amorphous solid; ^{1}H NMR (600 MHz, acetone-d_6) δ 13.67 (1H, s, 2-OH), 7.94 (1H, d, J = 9.1 Hz, H-6'), 7.87 (1H, d, J = 15.4 Hz, H-α), 7.80 (1H, d, J = 15.4 Hz, H-β), 7.33 (1H, dt, J = 7.7, 1.0 Hz, H-4), 7.26-7.30 (2H, m, H-2,5), 6.94 (1H, ddd, J = 8.0, 2.5, 1.0 Hz, H-6), 6.52 (1H, d, J = 9.1 Hz, H-5'), 3.85 (3H, s, 3'-OMe) (Figure 6.3h). ^{13}C NMR (150 MHz, acetone-d_6) δ 193.5 (C=O), 159.8 (C-2'), 158.8 (C-3), 157.9 (C-4'), 145.1 (C-β), 137.2 (C-1), 135.8 (C-3'), 130.8 (C-5), 127.7 (C-6'), 121.6 (C-α), 121.2 (C-4), 118.7 (C-6), 116.1 (C-2), 115.2 (C-1'), 108.4 (C-5'), 60.5 (3'-OMe); EIMS *m/z* 286 $[M]^+$ (100%), 225, 193, 166, 138; HREIMS *m/z* 286.0829 (272.0841 calcd for $C_{16}H_{14}O_5$) (Figure 6.5g).

HREIMS (*m/z* 286.0829 $[M^+]$) indicated the molecular formula of (*E*)-3,2',4'-Trihydroxy-3'-methoxychalcone as $C_{16}H_{14}O_5$. ^{1}H, ^{13}C NMR and HMQC spectra indicated that (*E*)-3,2',4'-Trihydroxy-3'-methoxychalcone was the kind of chalcones including a 1,3-disubstituted benzene ring (δ_H 7.33 (1H, dt, J = 7.7, 1.0 Hz), δ_H 7.26-7.30 (2H, m) and δ_H 6.94 (1H, ddd, J = 8.0, 2.5, 1.0 Hz)), a 1,2,3,4-tetrasubstituted benzene ring (δ_H 7.94 (1H, d, J = 9.1 Hz) and δ_H 6.52 (1H, d, J = 9.1 Hz), a methoxyl group (δ_C 60.5 and δ_H 3.85) and an α,β-unsaturated ketone (δ_C 193.5; δ_C 121.6 and δ_H 7.87 (1H, d, J = 15.4 Hz); δ_C 145.1 and δ_H 7.80 (1H, d, J = 15.4 Hz)). HMBC spectrum revealed the connection of these constituents to elucidate the structure of (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone (Figure 6.8)

Compounds, **(2*S*)-5,7,2'-trihydroxyflavanone** and **(*E*)-2',4'-dihydroxychalcone** were reported previously (Miyachi *et al*., 1987, Su *et al*., 2003, Miyaichi and Morimoto, 2006, Miyachi *et al*., 2006, Vries *et al*., 2005, Zampini *et al*., 2005 and Svetaz *et al*., 2007). **(*E*)-3,2',4'-trihydroxychalcone** was synthesized previously (Severi *et al*., 1998), but has not been reported from natural sources.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96.1989
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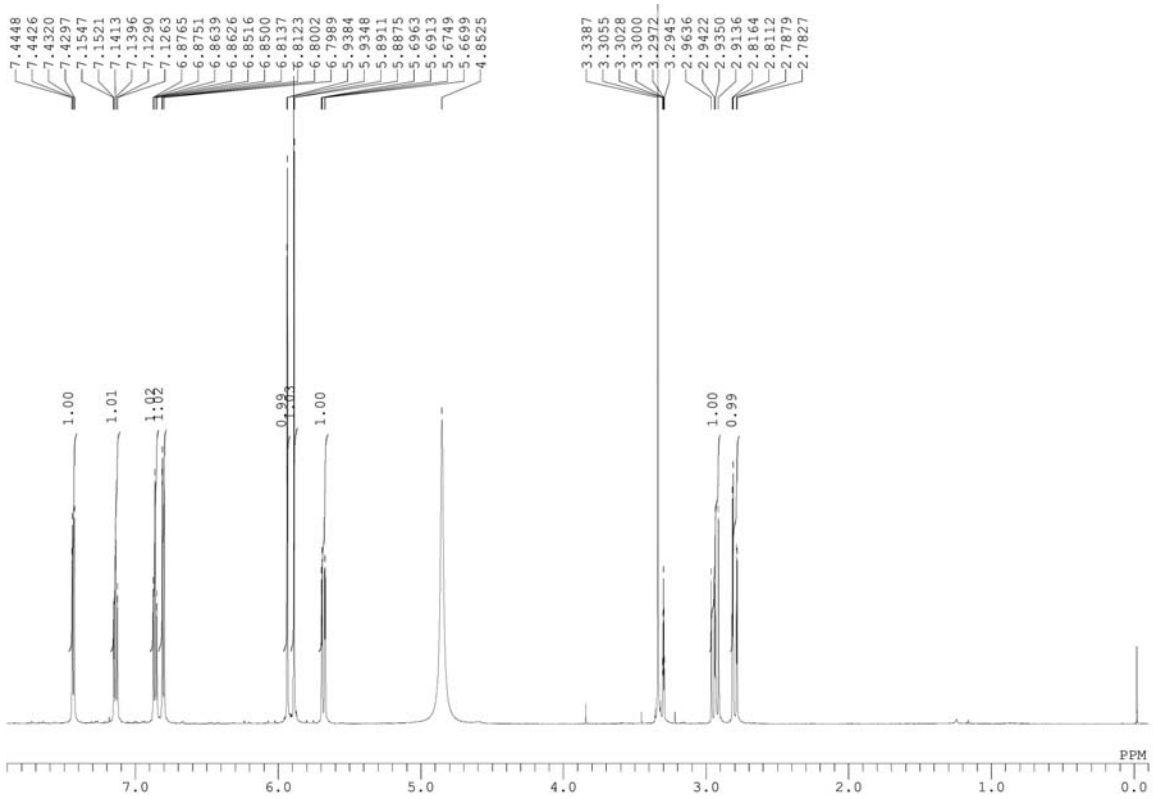
(a)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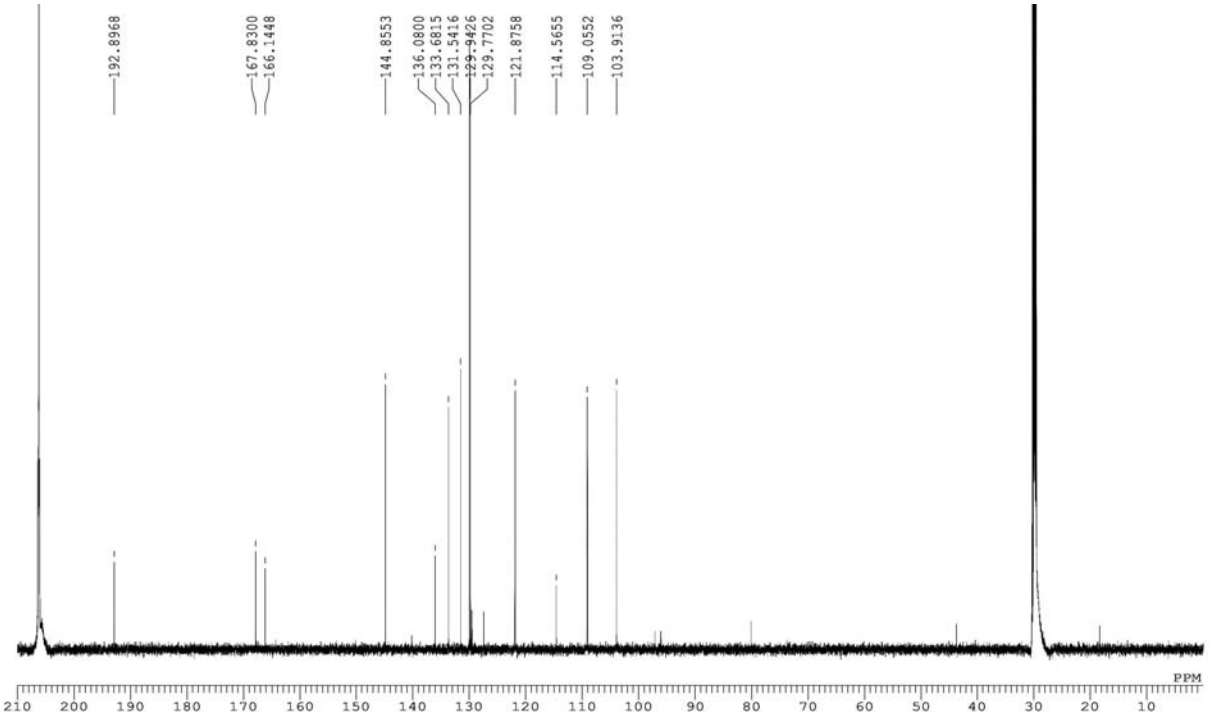
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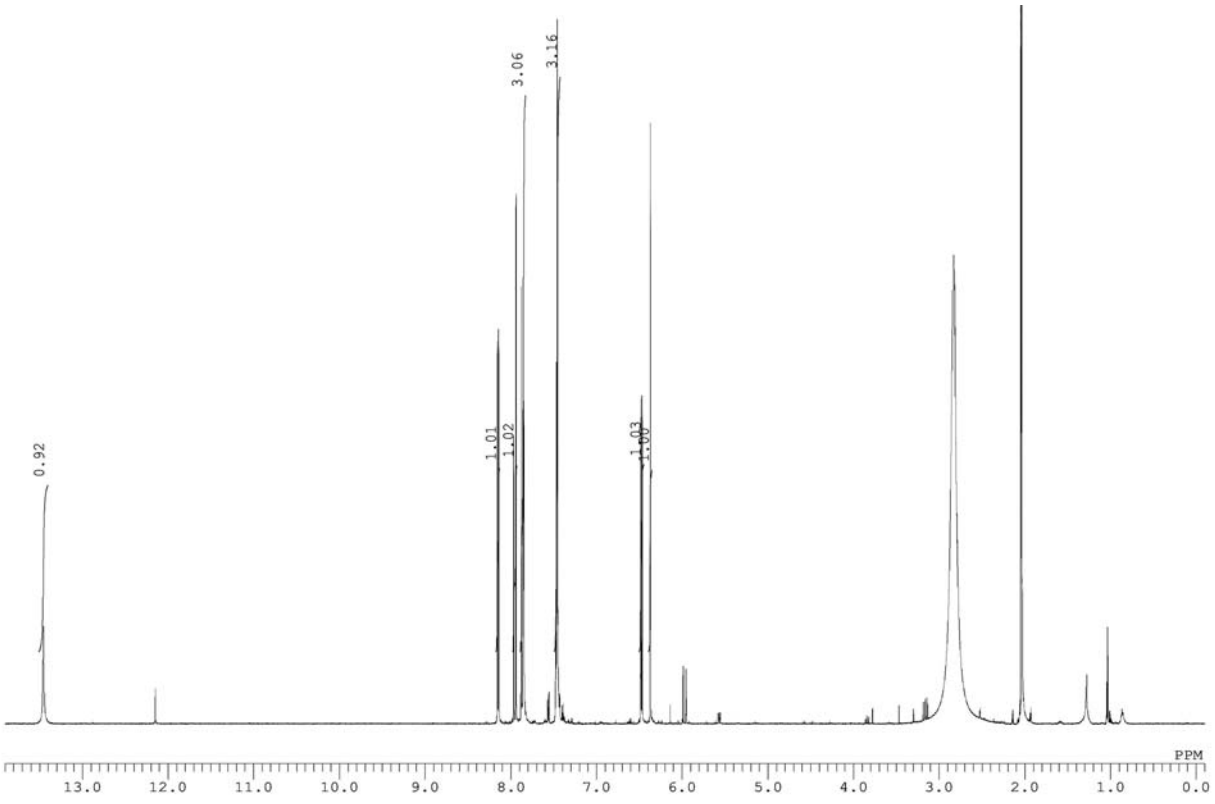
(c)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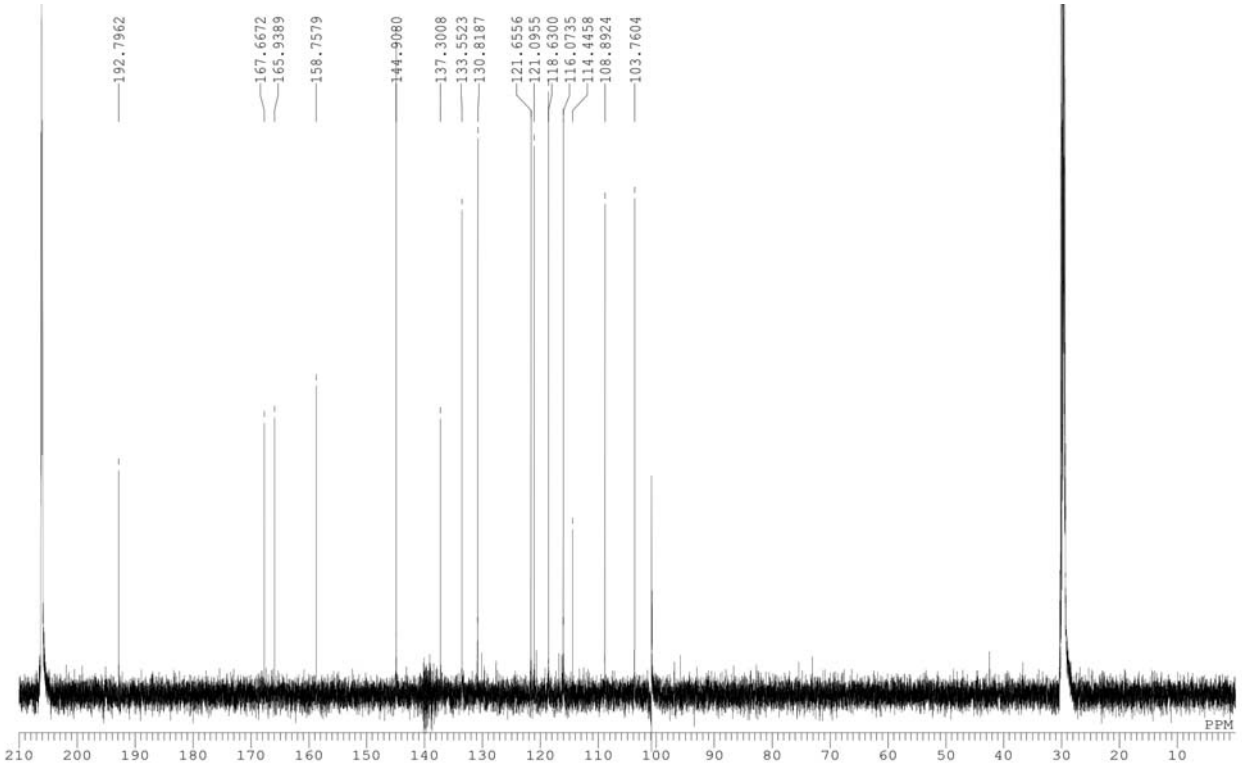
(d)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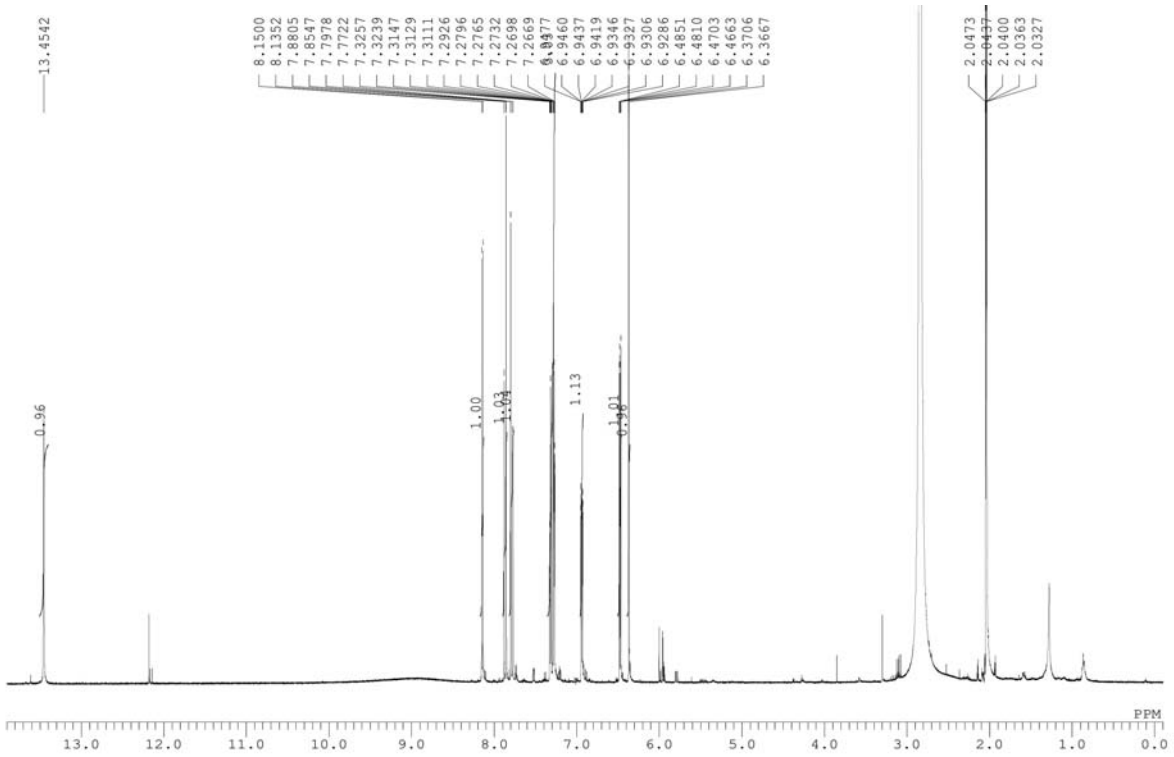
(e)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1.13
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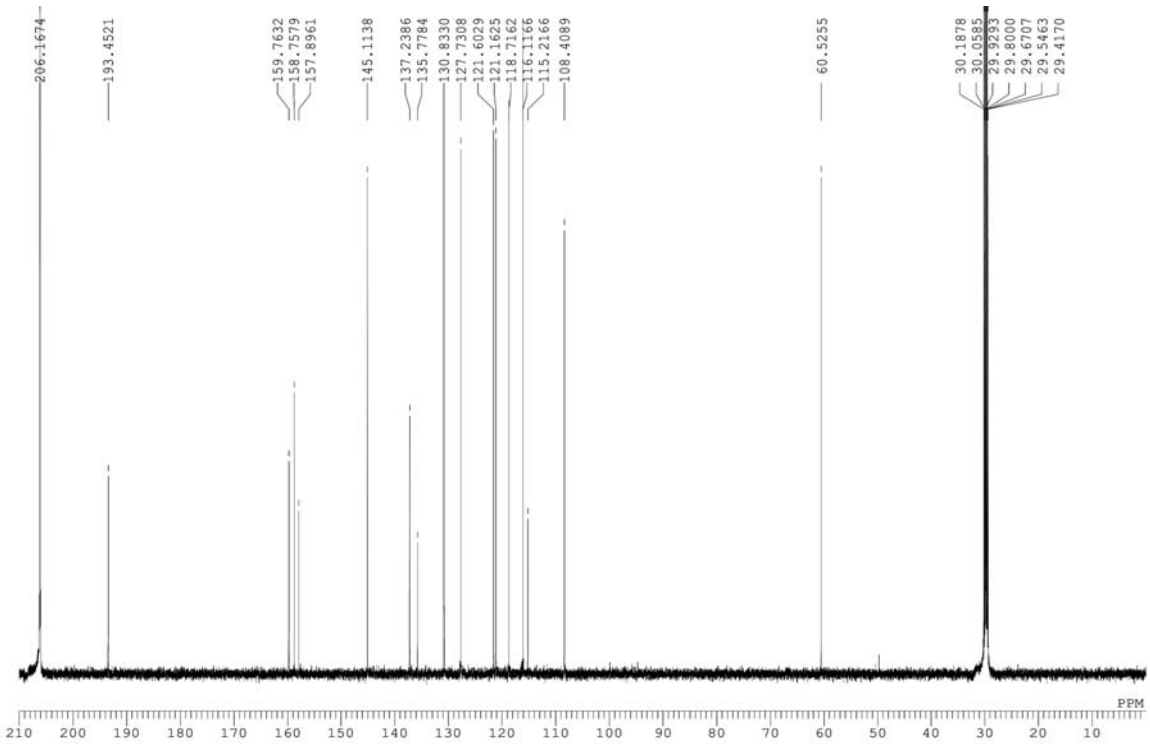
(f)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30.1878
30.0585
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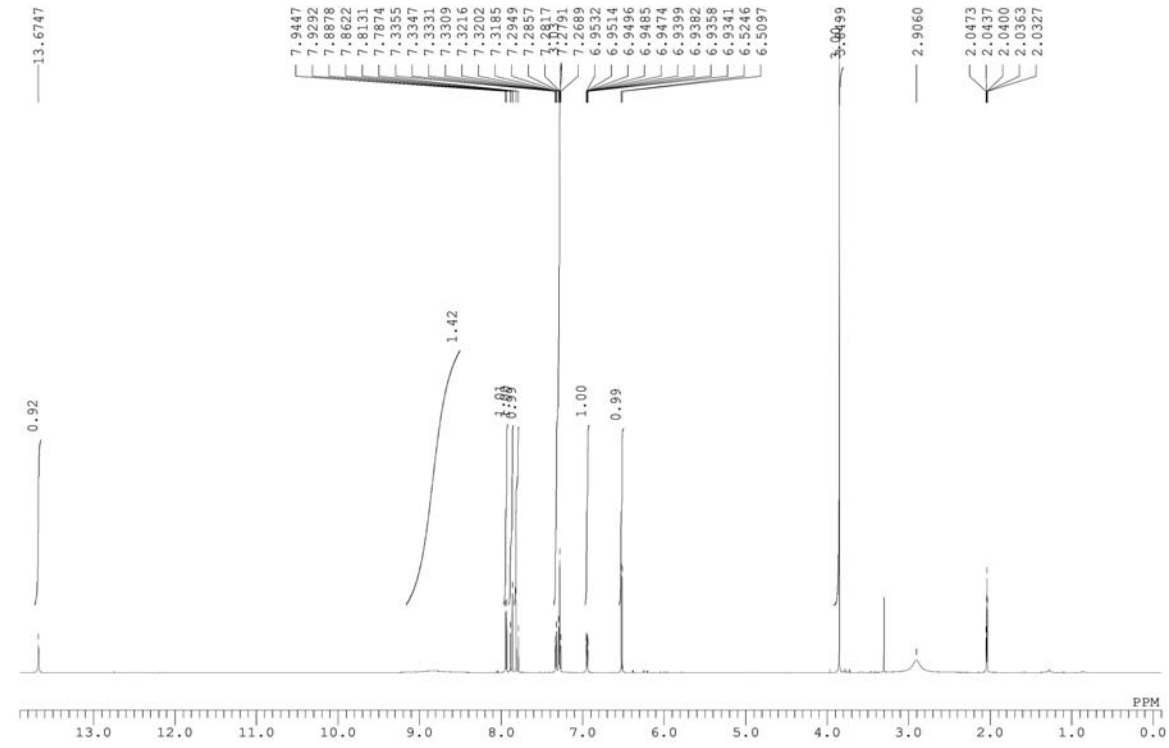
(g)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0.99
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PPM
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(h)

Figure 6.6 NMR spectrums of flavonoids isolated from the ethanol extract of *G. africana*

(a) ^{13}C NMR of (2*S*)-5,7,2'-trihydroxyflavanone

(b) ^{1}H NMR of (2*S*)-5,7,2'-trihydroxyflavanone

(c) ^{13}C NMR of (*E*)-2',4'-dihydroxychalcone

(d) ^{1}H NMR of (*E*)-2',4'-dihydroxychalcone

(e) ^{13}C NMR of (*E*)-3,2',4'-trihydroxychalcone

(f) ^{1}H NMR of (*E*)-3,2',4'-trihydroxychalcone

(g) ^{13}C NMR of (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

(h) ^{1}H NMR of (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

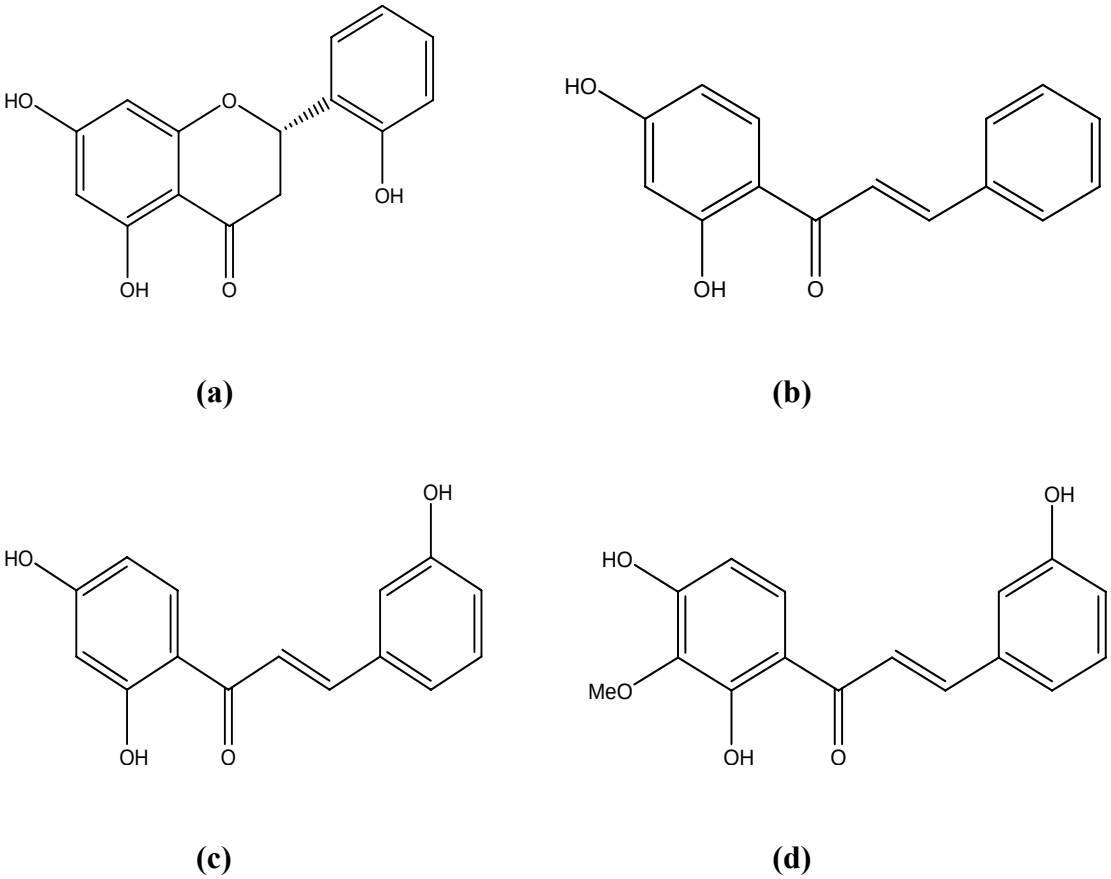

Figure 6.7. **Chemical structures of isolated compounds from the ethanol extract of *G. africana***

(a) (2*S*)-5,7,2'-trihydroxyflavanone

(b) (*E*)-2',4'-dihydroxychalcone

(c) (*E*)-3,2',4'-trihydroxychalcone

(d) (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

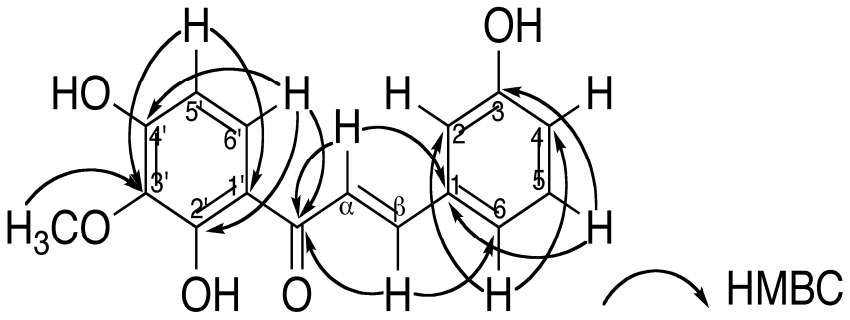

Figure 6.8. The HMBC spectrum of (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone

6.4. Conclusion

Nature has a proven ability to produce diverse and novel chemical prototypes with potential for possible drugs. Natural products like medicinal plants, play an important role in the discovery of potential innovative leads in drug development and in the development of high quality herbal drugs with proven efficacy. Rapid detection of biologically active natural products play a key role in the phytochemical investigation of crude plant extracts.

In order to perform an efficient isolation of compounds from any crude plant extracts, different techniques are used, such as low pressure column chromatography, ion-exchange methods, mass spectrometry, HPLC, planar chromatography and high-speed countercurrent chromatography to provide numerous structural data for isolation (Bohlin, 1998).

Due to the good inhibitory activity of the ethanol extract of *G. africana* amongst the other selected plants, the ethanol extract was selected for the isolation of compounds. Three known and one novel compounds belonging to flavonoid and chalcone groups were isolated. These compounds are: (2*S*)-5,7,2'-trihydroxyflavanone, (*E*)-2',4'-dihydroxychalcone, (*E*)-3,2',4'-trihydroxychalcone and (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone. Two compounds, (2*S*)-5,7,2'-trihydroxyflavanone and (*E*)-2',4'-dihydroxychalcone were previously isolated

by other researchers. Compound, (*E*)-3,2',4'-trihydroxychalcone has not been reported from natural sources but previously isolated by Severi *et al*., 1998 and a novel compound, (*E*)-3,2',4'-trihydroxy-3'-methoxychalcone is isolated from *G. africana* for the first time. Biological activity of some of the compounds isolated in the present study has been reported by other researchers previously.

Chalcones are a subset of compounds known as flavonoids. In a chalcone, two aromatic rings are joined by a 3-carbon unsaturated carbonyl system.