ANTI-TRYPANOSOMAL EVALUATION OF *Ximenia americana* ROOT BARK AND CHROMATOGRAPHIC-MASS SPECTROSCOPY PROFILE

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SUNDAY 5TH – THURSDAY 9TH AUGUST 2018

Introduction

- What is Trypanosomiasis?
- Mode of transmission - tsetse fly (*Glossina sp.)*
- Types of Africa trypanosomiasis
- Sleeping Sickness – Melarsoprol, Pentamidine, Suramin, Eflornithine
- Current treatment options for Sleeping Sickness are very limited with high toxicity
- Mode of administration is difficult, a situation that presents significant challenges in endemic areas.
- Natural products continue to be an important alternative source of chemotherapeutic agents and several have found applicable in the treatment of parasitic diseases.
- Biological and chemical understanding of any natural product is important in medicinal chemistry.
- *Ximenia americana* is one of the eight (8) species of the genus Ximenia which belongs to the family Olacaceae (Brasileiro et al., 2003).
- It is commonly known as “wild olive” in English and Yellow Plum or Sea Lemon in Australia and Asia; In Nigeria; Tsada (Hausa), Chabbuli (Fulani), Anomadze (Tiv), Igo (Yoruba) - (Keay, 1989; Folarin et al., 2013).

Objectives

- ❖ This study was aimed at carrying out biological evaluation of *Ximenia americana* root bark against *Trypanosoma brucei* and establishing the chromatographic-mass spectrometric profile
- Other specific objectives include;
- ❖ Phytochemical screening of crude extracts
- ❖ Bioactivity-guided fractionation of plant extracts
- ❖ Fractionation using chromatographic techniques
- ❖ Development of TLC-MS and LC-MS methods for identification

Methodology

- Plant collection and authentication
- Plant Extraction – Soxhlet extraction method
- Phytochemical Analysis
- Biological Assay

Chromatography and Mass spectrometric analysis

- Solid-phase extraction cartridge (SPE); C-18 silica with pore size 125Å
- TLC: Silica gel 60Å F_{254} TLC 20 x 20 cm analytical aluminium plates (Merck KGaA, 64271 Darmstadt, Germany)
- TLC-MS equipment (Advion Expression CMS-L) at capillary temperature 150^0C using acetonitrile: water, 95:5 v/v with 0.1% formic acid (flow rate 0.2mL/min). ESI mode (Full ESI-MS scan, positive ionization, drying gas temperature 350^0C, capillary voltage 150V).
- LC-MS: Thermo Scientific Accela LC system with PDA detector controlled by Xcalibur software and coupled to Orbitrap Q-exactive Focus mass spectrometer

Experimental Setting

Chromatographic conditions

xa' 1 10 -esi_02
Accela AS
Creator: Thermo
Last modified: 10/24/2016 by Thermo
Summary: (none)

Accela AS Method:

Reservoir 1:

Reservoir 2:

Reservoir 3:

Reservoir 4:

Wash Bottle:

Injection volume (ul) 5.000

Flush volume(ul): 250

Flush/Wash source is bottle.

Needle height from bottom(mm): 2.000

Wash volume (ul): 250

Flush speed (ul/s): 100.000

Post-Injection Valve switch time (min): 0.000

Syringe speed (ul/s): 8.000

Injection mode is partial loop

Tray temp control is off

xa' 1 10 -esi_02
Accela Pump

Method creator:	Thermo
Last modified:	10/24/2016 9:46:43 AM by Thermo
Instrument:	Accela Pump

Common settings:

Pressure units:	bar
Pressure stability:	10.00

Pump 1 settings:

Name:	Pump 1
Comment:	
Solvent A:	Ethyl Acetate
Solvent B:	0.1 % HCOOH
Solvent C:	MeOH
Solvent D:	ACN
Start settings:	Accela AS injection logic
Method finalizing:	First line conditions
Operating mode:	Low pressure (0..~7000 PSI)
Min pressure:	0.00
Max pressure:	1000.00

Pump 1 gradient table:

No.	Time	A%	B%	C%	D%	µl/min
0	0.00	0.0	80.0	20.0	0.0	400.0
1	5.00	0.0	50.0	50.0	0.0	400.0
2	10.00	0.0	50.0	50.0	0.0	400.0
3	15.00	0.0	5.0	95.0	0.0	400.0
4	18.00	0.0	5.0	95.0	0.0	400.0
5	18.50	0.0	80.0	20.0	0.0	400.0
6	23.00	0.0	80.0	20.0	0.0	400.0

Results and Discussion

Table 1: Biological activity of *X. americana* root bark against *T. brucei* after 3h incubation at room temperature

Extract		Concentration (µg/mL)		
		1000	500	250
Acetone	Parasitemia	36.67±1.53	37.00±1.00	40.00±0.00
	% Inhibition	8.33	7.50	-
	Significant difference	***	***	***
70% EtOH	Parasitemia	34.67±0.58	37.67±0.58	40.00±0.00
	% Inhibition	13.33	5.83	-
	Significant difference	***	***	***
EtOAc	Parasitemia	34.33±0.58	37.00±1.00	40.00±0.00
	% Inhibition	14.18	7.50	-
	Significant difference	***	***	***
MeOH	Parasitemia	0.33±0.58	1.00±0.00	5.00±1.00
	% Inhibition	99.18	97.50	87.50
	Significant difference	Ns	Ns	Ns
Positive Control	Parasitemia	38.67±0.58	40.00±0.00	39.67±0.00
	% Inhibition	-	-	-
	Significant difference	***	***	***
Isometamidium Chloride	Parasitemia	-	-	4.67±1.16
	% Inhibition	100	100	88.33

Parasitemia values are mean ± standard deviation; n = 3; significant different at *P<0.05* compared with isometamidium chloride (* = extremely significant; Ns =Not significant; - = Nil)**

Results and Discussion

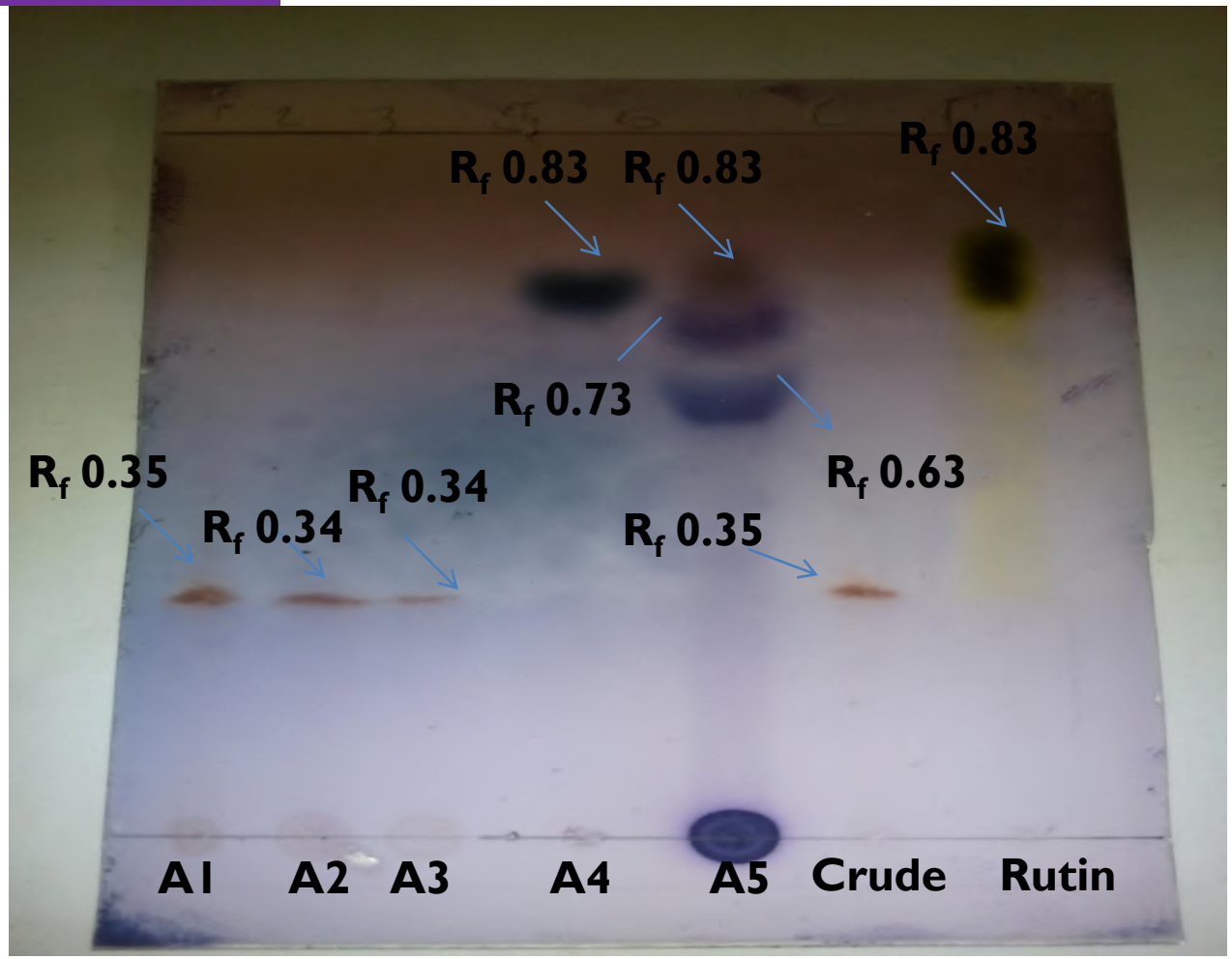
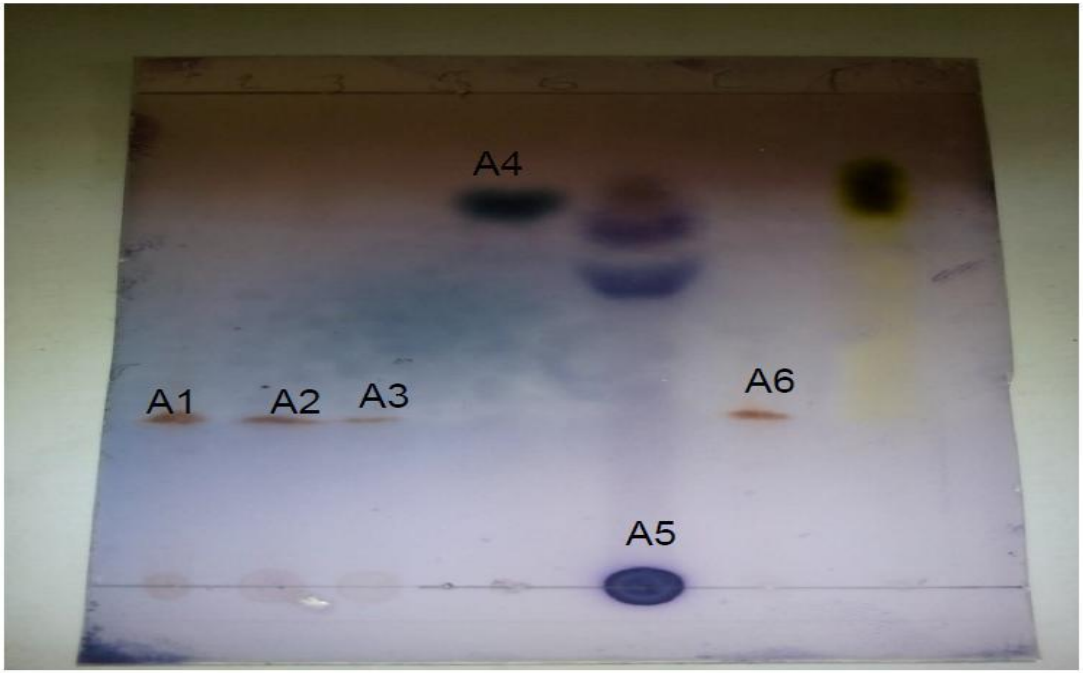


Fig. 1: TLC plate viewed with UV lamp at 366nm after spraying with sulphuric acid/anisaldehyde reagent

Results and Discussion

Spots were labelled as A1-A6. (A1, A2, A3 and A6 similar Rf) Each spot was analysed by TLC-MS to obtain the spectrum shown on the following slides

Solvent system for TLC: methanol: water: ethylacetate (25ml:5ml:2ml)
TLC plate : normal phase (Silica gel)
Derivatised with anisaldehyde-sulphuric acid reagent)



Results and Discussion

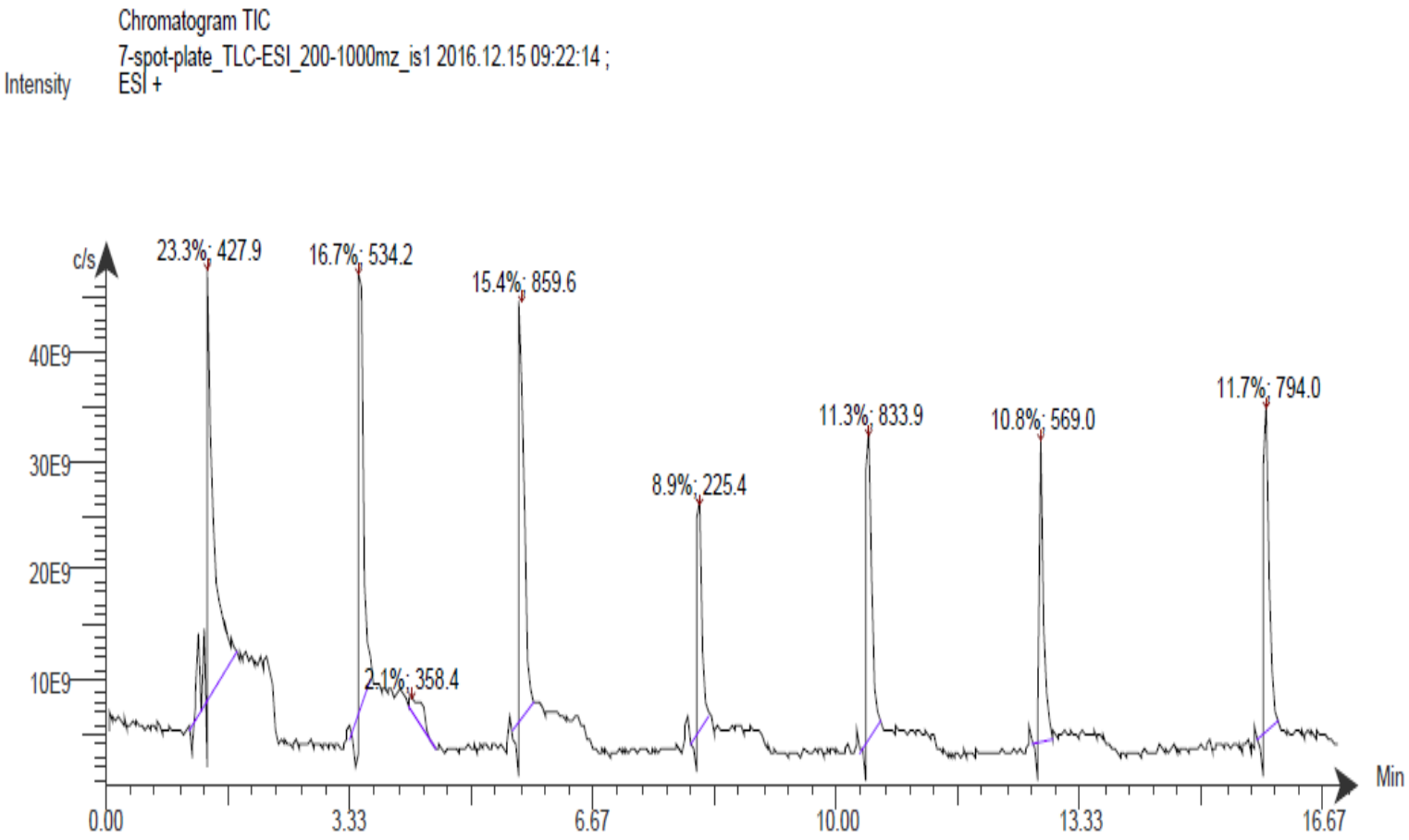


Fig. 2: TLC-MS Chromatogram in ESI+ mode for 7 Spots

Results and Discussion

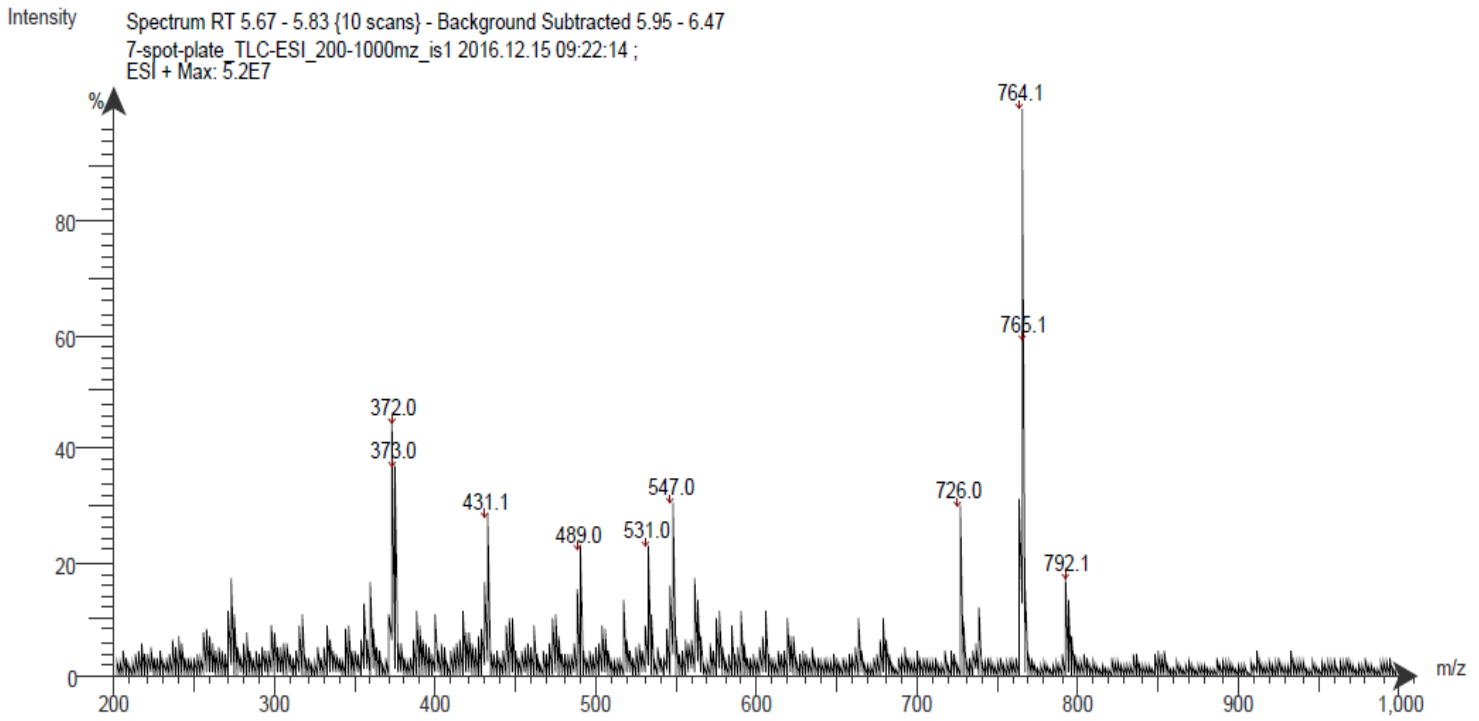


Fig. 3: TLC-MS spectrum (RT 5.67-5.83) using Advion spectrometer in +ESI

- Peak 792.1 m/z (Fig. 3) suggests large molecule trimers of a sesquiterpene (MW 264; $C_{15}H_{20}O_4$). Voss et al. (2006) isolated this compound from ethanol extract of *X. americana* stem using EI-MS however it was reported not to be active against human leukemia and human breast cancer.
- Peaks 344, 358 and 372 m/z on the spectra are residual peaks from a test solution

Results and Discussion

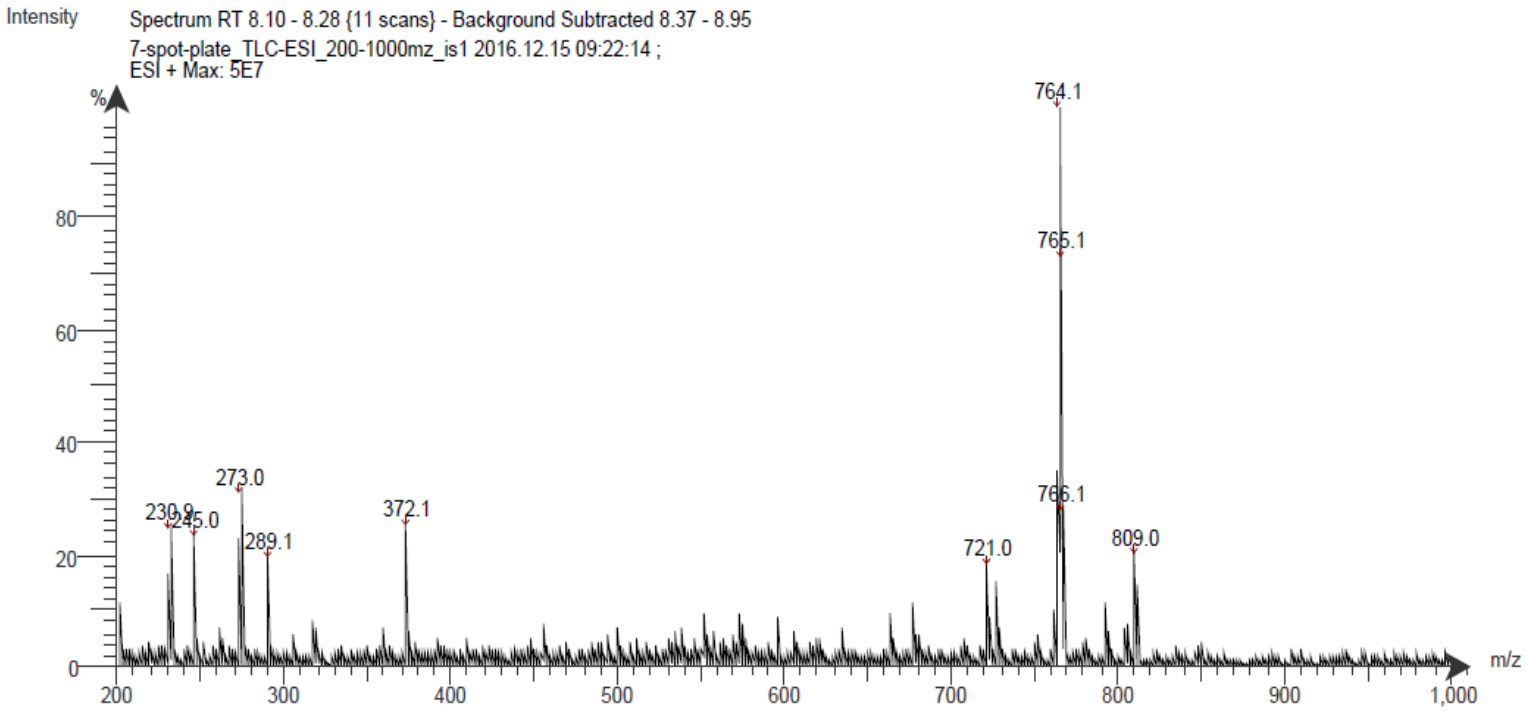


Fig. 4: TLC-MS spectrum (RT 8.10-8.28) using Advion spectrometer in +ESI

Lower peak observed at 289.1 (Fig. 4) suggests (M+H) for quercetin as earlier isolated from *X. americana* leaves by Le et al. (2012). The flavonol has been established to have high radical scavenging capacity, thus anti-trypanosomal activity of *X. americana* root bark may likely be attributed to antioxidant activity.

Results and Discussion

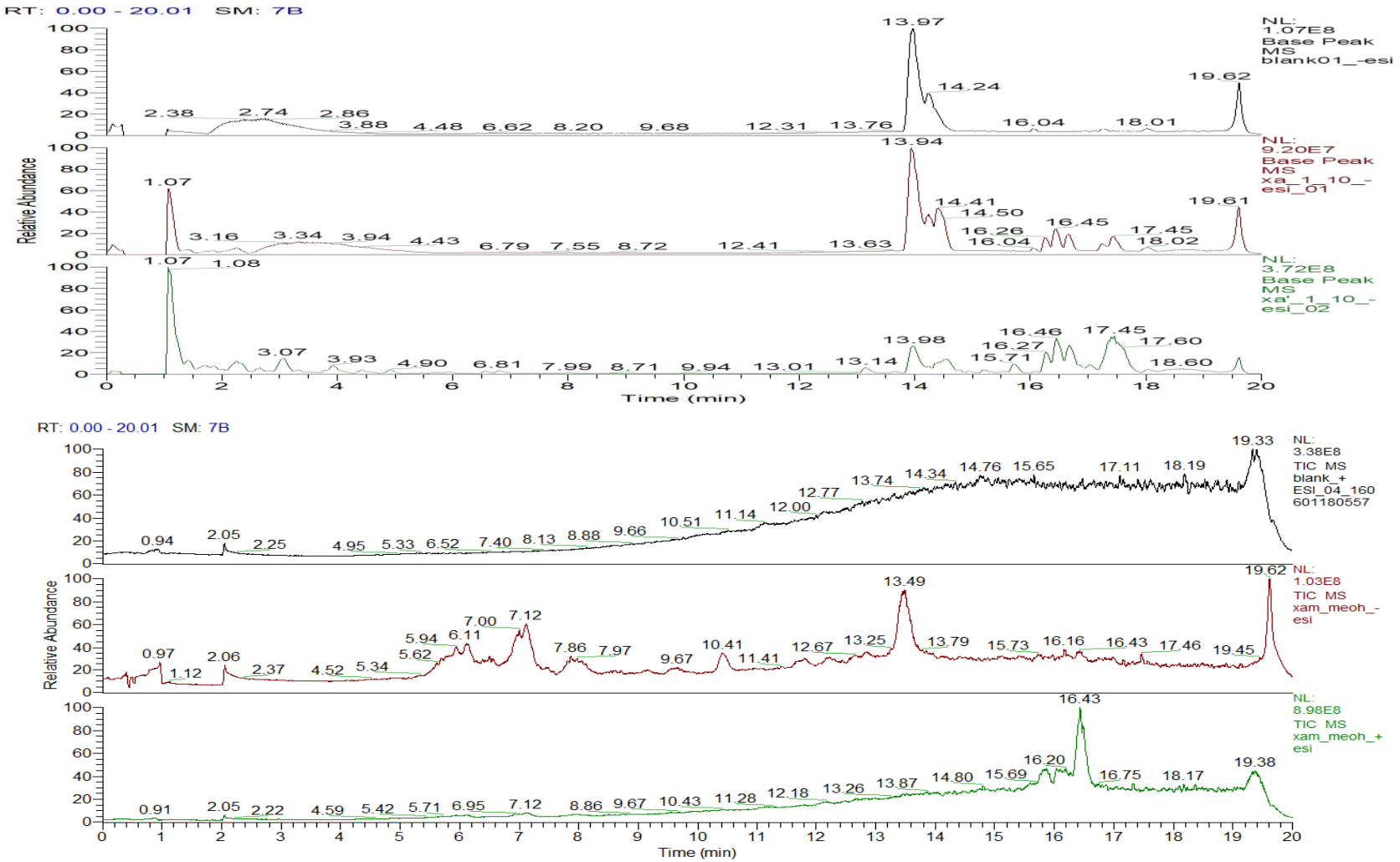


Fig. 5: LC-MS UV scan of *X. americana* methanol extract showing blank run and two concentrations of the extract at ESI negative and positive ionization modes

Results and Discussion

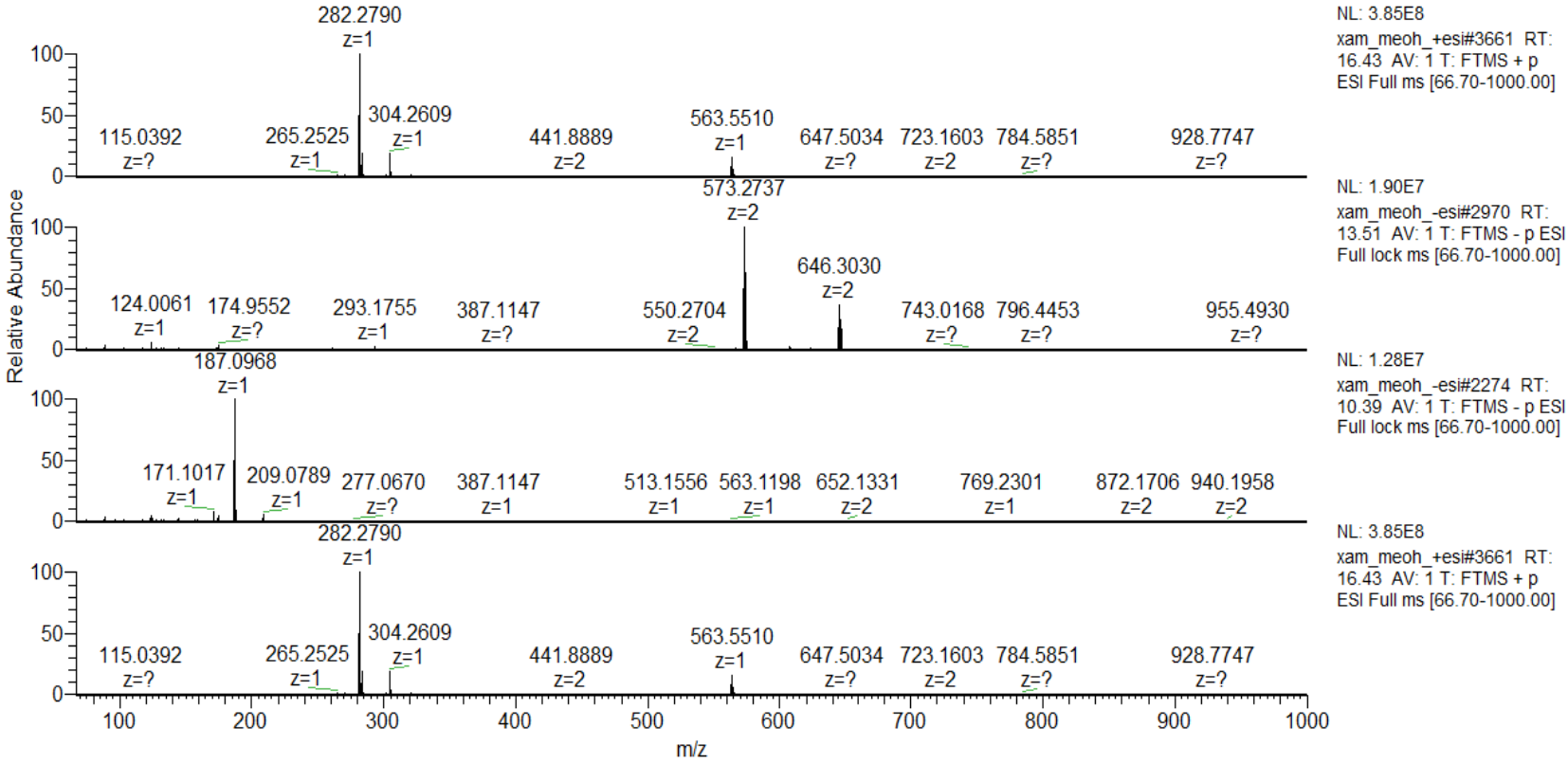


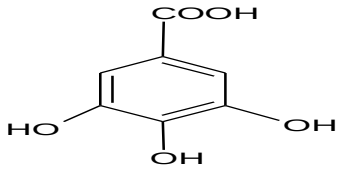
Fig. 6: High Resolution Mass spectrum of *X. americana* methanol extract

Mass spectrum in Fig. 6 using Fourier Transform Mass Spectrum (ESI-MS +) gives peak at retention time 16.43 to give molecular ion at peak 282.2790. This suggests 2',5-dimethoxyflavone (MW 282.1; $C_{17}H_{14}O_4$) with 304.2609 (Na adduct, M+ Na (22.0019) or a hydroxylated form of quercetin as reported in literature data bank (Massbank, Japan; High quality Mass Spectral Database).

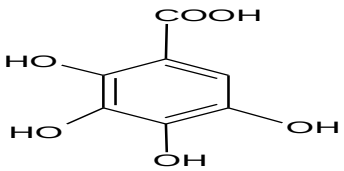
Results and Discussion

- Peak 304.2609 could represent hydroxylation of quercetin to form 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-4-one known as dihydroquercetin ($C_{15}H_{12}O_7$) in comparison with peak database by Massbank, Japan; High quality Mass Spectral Database.
- A Sesquiterpene (MW 264.25; $C_{15}H_{20}O_4$) (MW 265.25; M+H) was earlier reported by Voss et al. (2006). In Figure 6, a very intense signal at 187.0968 negative ion mode ESI-MS spectrum retention time 10.39 can be seen, which possibly originates from the (gallic acid – H) 171.1195 m/z on the spectrum by hydroxylation to form 2,3,4,5-tetraydroxybenzoic acid (MW 186.1189; $C_7H_6O_6$).

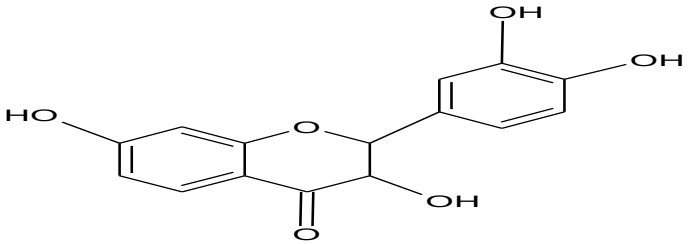
Results and Discussion



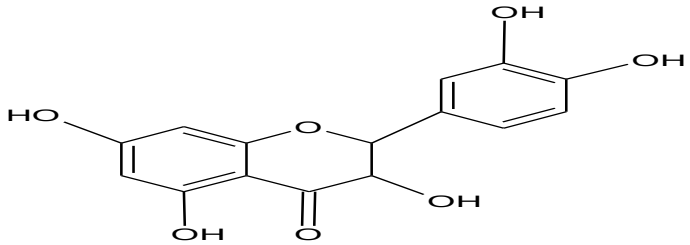
Gallic acid



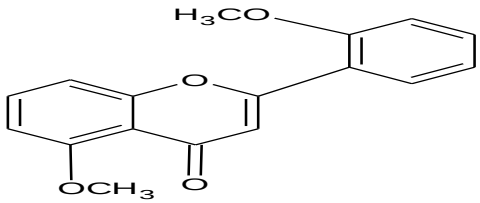
2,3,4,5–tetrahydroxybenzoic acid



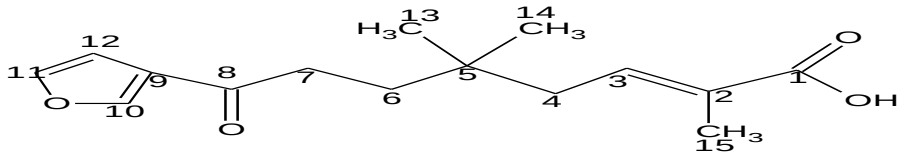
Quercetin



Dihydroquercetin



2',5-dimethoxyflavone



Sesquiterpene

Structures of suggested compounds

Conclusions & Recommendations

- Methanol extract derived from sequential extraction of *X. americana* root bark exhibited anti-trypanosomal activity against *Trypanosoma brucei brucei* with 99.18%, 97.5% and 87.5 inhibition at 3hrs incubation (room temperature) using 1000 µg, 500 µg and 250 µg, respectively.
- The results obtained showed that at 1000 µg, the inhibitory activity of methanol extract and isometamidium chloride are comparable with 95%CI [-1.10, 1.77]
- Chromatographic and spectroscopic techniques used in this study provided data for compounds similar to some library data for gallic acid, quercetin, dihydroquercetin, 2,3,4,5-tetrahydobenzoic acid and 2',5-dimethoxyflavone.
- The TLC and LC chemical profile could aid identification and contribute to dereplication process in compound identification
- Overall, the results obtained from this study contributes to current knowledge about the plant and provides information on the *in vitro* inhibitory effect on *T. brucei*.
- The information could also be of great benefit in standardization and quality control of herbal medicine
- However, *in vivo* study is required to ascertain the toxicity of the active extract and anti-trypanosomal effect in addition to further structural analysis to confirm the suggested structures.

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Acknowledgements

- African Centre of Excellence in Phytomedicine Research and Development (ACEPRED), Unijos.
- My supervisor Dr. (Mrs.) Odumosu
- All Lecturer - Pharmaceutical Chemistry, Unijos
- All staff - Pharmaceutical Chemistry, Unijos
- My lovely wife