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In Vitro ANTITRYPANOSOMAL ACTIVITY OF CAMPESTEROL AND ETHYL OLEATE FROM THE METHANOL EXTRACT OF *Abrus precatorius* Linn

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ABSTRACT

Abrus precatorious (fabaceae) is an important medicinal plant used to treat tetanus, rabies, fever, and jaundice. The plant was investigated in present research for the presence of secondary metabolites, and the antitrypanosomal activity against *Trypanosomes brucei brucei* for the development of new and cost effective alternative strategies for the treatment of trypanosomiasis. The plant was air dried and extracted with organic solvent in a polarity gradient of petroleum ether, ethyl acetate and methanol. The column chromatographic CC of the methanol extracts leads to the isolation of campesterol and ethyl oleate which were characterized using Fourier transform Infra – red FT-IR, Gas Chromatography mass spectrometry GC-MS and Nuclear Magnetic Resonance NMR analysis and comparison with literature data. An appreciable *in vitro* antitrypanosomal activity was attained by the campesterol and ethyl oleate by drastically reducing the motility rate of the *Trypanosomes brucei brucei* at a concentration of 50 mg/ml within 80 and 110 minutes respectively. These results suggest that the plant contain potent antitrypanosomal activity agents that could be developed as drugs for African animal trypanosomiasis and also shows the ethno – pharmacological usefulness of the plant

Keywords: *Abrus precatorious*, Trypanosomiasis, Campesterol, Ethyl oleate

INTRODUCTION

African Trypanosomiasis (also known as Sleeping sickness) is an insect borne parasitic disease of human and livestock caused by the protozoa Trypanosome (WHO, 2014) It is threatening about 60 million people in west and central Africa as well as some part of east Africa (Duman *et al.,* 1999). The control and treatment of these disease by the used of chemotherapy has been faced with problems ranging from the toxicity of the drugs to emergence of drug resistant trypanosome strains as well as the limited availability and affordability of pharmaceutical medicine to the resource poor population and farmer in Africa (Afewark *et al.,* 2000). Therefore there is the needs for a new, safe, effective and cheap anti - trypanosomal medicine most probable from plant source as most plants has provides basis for traditional treatment of different type of diseases.

Abrus precatorious (Fabaceae) is a slender perennial climber that twines around trees, shrub and hedges, it flowers are purple pink in color and are borne in cluster, the fruits are pod bearing characteristic red seeds with black spot around the hilum (Tabsum *et al*., 2016) it is a wild plant that grows in a fairly dry regions in tropical and sub- tropical areas of the world. *Abrus precatorious* commonly called crab's eye or crab's eye creeper, idon zakara (Hausa) has been used traditionally used for treatment of diseases such as fever, cough and cold (Nasir *et al*., 2013), used as nerve tonic (Elisabetsky *et al*., 1992), the leaves are used as laxative and aphrodisiac

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while the seeds are used to treat diarrhea, dysentery and diabetic (Manago and Alumanah, 2005)

The bioactivity properties of this plant includes anti – inflammatory (Anam, 2001), anti tumor (Bhutia *et al*., 2008) anti – diarrheal (Nwodo and Ahumanid, 1991) anti – helmintic (Molgaard *et al*., 2001) and anti – malarial activities (Limmatvapiral *et al.,* 2004).

A number of phytochemicals have been reported from different parts of *Abrus prectoroius* ranges from alkaloids (Batto and Kumar, 2009), fatty acid (Krishnaveni *et al.,*2004) Flavonoids (Jaya and Arnet 2016) Triterpenes (Sumeet *et al.* 2013) to glycosides (Daniels 2006). However these paper reports the isolation and characterization of campesterol (a sterol) and ethyl oleate (fatty esters) from methanol extract of *Abrus precatorious* and it anti trypanosomal activity of the compounds against *Trypanosamal brucei brucei*

MATERIALS AND METHODS

Collection of Plant Materials

Fresh aerial part of *Abrus precatorious* was collected 9th March, 2019 at Kufena village, Zaria Local Government Area of Kaduna State, Nigeria. It was identified and deposited at the herbarium Department of Plant Biology, Bayero University, Kano, Nigeria with an accession number BUKHAN 0064. The plant material was washed with clean water, air dried and pounded into powder.

Extraction

The coarse powder of the aerial part of the *Abrus precatorious* (231.24 g) was sequentially extracted with solvent in an increasing polarity of petroleum ether, ethyl acetate and methanol. The extracts were concentrated at 40^{o}C using rotary evaporator to give petroleum ether extract (APPE), ethyl acetate extract (APEA) and methanol extract (APME).

Methanol extracts (APME) (5.4g) was subjected to column chromatography (46.5g silica gel, column size 25x1.5cm) using petroleum ether, ethyl acetate and methanol step gradient to afford one hundred and seventy four fractions (APME 1 – 174). Fractions were pooled together based on their thin layer chromatography (TLC) profile. APME 97 and APME 132 were monitored by TLC on petroleum ether – ethyl acetate (1;1) and (3;7) solvent system respectively and visualize using iodine vapor

Characterization of Isolated Compound

^{1}HNMR (400MHz) ^{13}CNMR (100MHz) and 2DNMR experiment such as Distortionless enhancement polarition by transfer DEPT, correlation spectroscopy COSY were carried out using Agilent NMR spectrometer in $CDCl_3$. The Gas Chromatography – mass spectrometry GCMS were performed on Agilent GCMS system (GC: 5890 series II: MSD 5972). The fused- silica HP-5 capillary column (30 m × 0.25 mm, ID, film thickness of 0.25µm) was directly coupled to the mass spectroscopy MS. The carrier gas was helium with a flow rate of 1.2 ml min^{-1}. The IR spectrum was measured on shimadzu FT – IR instrument model 8400s

In vitro Antitrypanosomal Assay

The stabilite of *Trypanosomes brucei brucei* were obtained from Nigerian Institute for trypanosomiasis Research, Vom, Plateau state, Nigeria and passes into mice by injecting. The *in vitro* antitrypanosomal activity was performed by blood incubation infectivity test (BIIT) using 96 well micro plates as described by mergia *et al*, 2014 with a slight modification. About 40mg of the isolated compounds (campesterol and ethyl oleate) were dissolved in 2% phosphate buffered

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saline glucose (PBSG) solutions to make an effective concentrations of 100, 50, 25, 12.5, and 16.5mg/ml. 200µl of infected blood were added to each of the above, Similarly A positive control was prepared by dissolving diminazene aceturate in 2% PBSG while the negative control contain only PBSG solution. The blood was checked at 10 minutes interval for 2 hours for the inactivity of the parasite in the blood when mixed with the test samples using USCAMEL monocular microscope at ×400 magnification. The shorter the time of cessation of motility of the parasite, the more active the extract was considered to be, under this in vitro system, the parasite survived for 4 hours when no extracts or isolates were present. The validation of these *in vitro* antitrypanosomal activity was done by injecting intraperitoneally the parasite suspension of the in vitro test from micro plate into five healthy mice, and the level of parasitaemia was assessed every day by collecting blood from the tail of each mouse and check for the presence of trypanosomes using the wet blood film by microhaematocrit buffy coat technique. The loss of infectivity of the trypanosomes to mice was concluded if no trypanosomes were detectable within 21 days (Maikai, 2011). The effect of the extracts and isolated compound in prolongation of establishment of infection was monitored by comparing with the negative control.

Statistical Analysis

MINITAB version 14 made by Triola Statistics company USA was used as statistical tool, the p-value of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The extract obtained was gummy and brownish in color, the methanol extract (5.36g, 2.31%) was subjected to gravity column chromatographic separation using petroleum ether – ethyl acetate – methanol gradient over silica gel. The elution afforded 174 fractions

Fraction 97 (APME 97) revealed a single spot with Rf value of 0.25 (Petroleum Ether; Ethyl acetate 1;1) as a colorless solid (40 mg), The FT IR spectral data analysis of the isolated compound showed the presence of hydroxyl group at 3434 cm^{-1} , alkene C=C at 1622 cm^{-1}, indicating unsaturation in the molecule, two peaks at 2857 cm^{-1} and 2928 cm^{-1} indicate SP^3 C – H stretching, the - CH_3 and – CH bending appears at 1378 cm^{-1} and 1402 cm^{-1} respectively. The C – O single bond stretching appears at 1048 cm^{-1}.

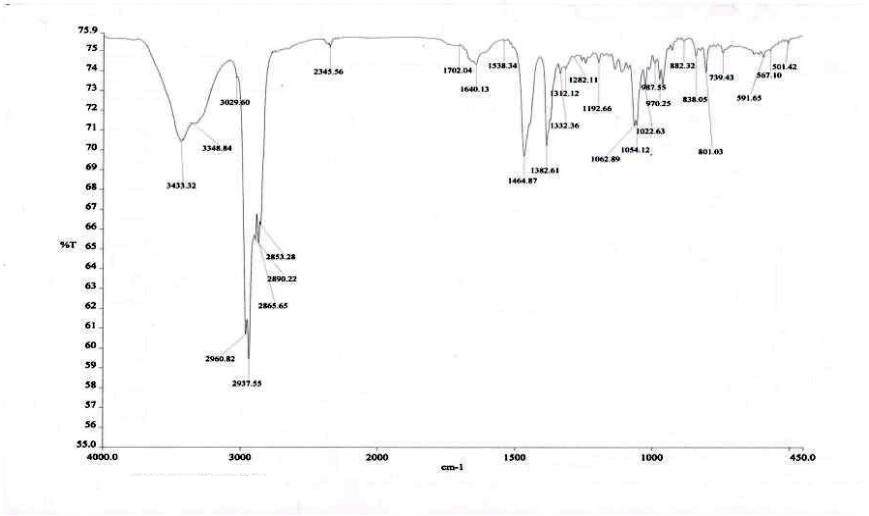

Figure 1: FT –IR spectral of APME 97

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The [1]HNMR revealed methyl, methylene and methine protons between 0.72 – 2.40 ppm, a triplet signals appeared at 5.24 ppm which indicates the presence of an olefinic proton at C – 6 owing to the double bond between C – 5 and C – 6. The angular methyl protons at C – 18, C - 19, resonates at δ = 0.72 ppm (s) and 1.20 ppm (s) respectively, while the doublet at 1.05 ppm, 0.86 ppm and 0.78 ppm are for C – 21,C – 25 and C – 27 respectively.

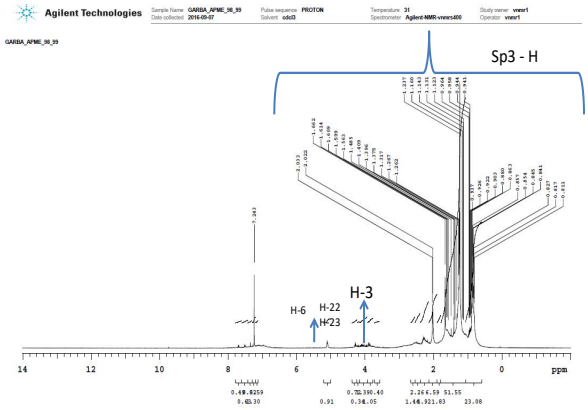


Figure 2: [1]H NMR spectral of APME 97

The [13]CNMR spectrum revealed a total of twenty-eight carbons among which twenty-five corresponds to methyl, methylene and methine between 13.76 to 62.00 ppm. An Olefinic carbon and a deshielded quaternary carbon were observed at 125.00 ppm and 135.20 ppm corresponding to C – 6 and C – 5 respectively. The carbon at C – 3 resonates at 76.71 ppm as a result of deshielding by an electronegative oxygen.

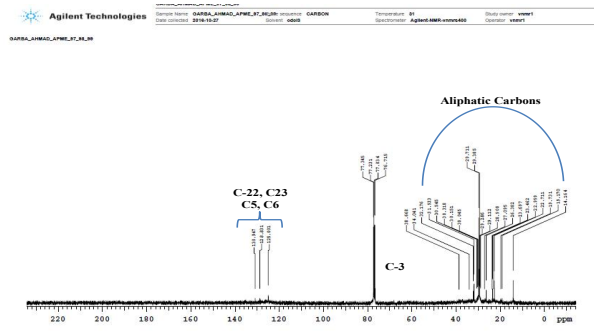


Figure 3: [13]C NMR spectral of APME 97

2D – NMR experiments exhibited proton correlations in the H^1 – H^1 COSY spectrum between H-6 and H - 7, therefore the compound APME 97 was identified as campesterol [17 – (5,6 – dimethylheptan-2-yl) – 10,13-dimethyl – 2,3,4,7,8,9,11,12,14,15,16,17,-dodecahydro – 1H – cyclopenta[α] phenanthen – 3 – ol] as the data compared with literature (Gangwal *et al*.,2010, Jung *et al*., 2007)

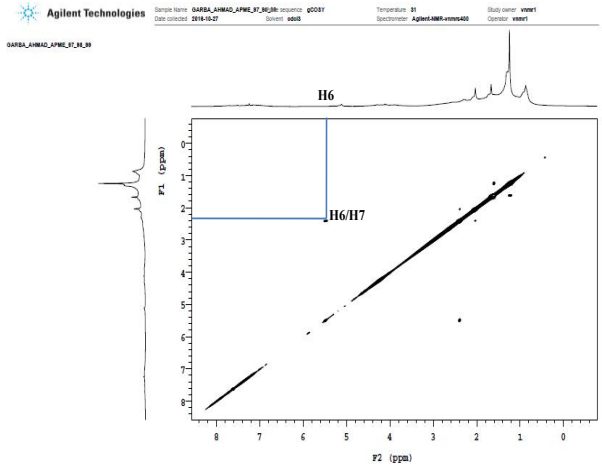
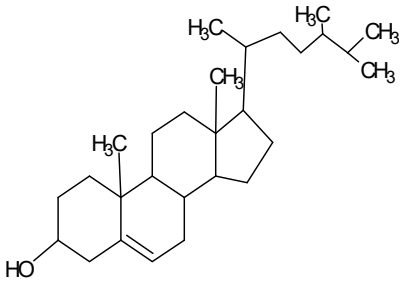


Figure 4: COSY for APME 97

Campesterol is one of the most common plants sterols found abundantly in seeds nuts cereals beans legumes and vegetable oils (Philips *et al*, 2005), campesterol is similar in structure to cholesterol, therefore they have cholesterol – lowering effects (Plat and Mensink, 2001). Campesterol has been reported to have anti - carcinogenic (li *et al.,* 2001) anti - bacterial and anti fungal activities (Padmaja *et al*., 1993).



Campesterol

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Analysis of APME 132

A yellowish oil (7mg, Rf = 0.58, petroleum ether; ethyl acetate 3:7) which displayed a prominent ester carbonyl absorption at 1734 cm^{-1} in the IR spectrum, the aliphatic carbon – hydrogen absorption bands was observed at 2714 and 2850 cm^{-1} while the olefenic C – H bending at 1463 cm^{-} [1].

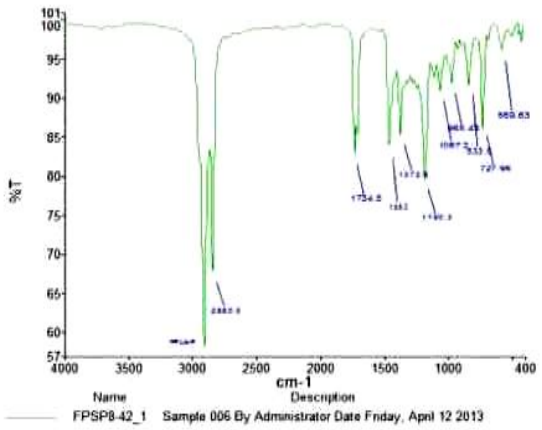


Figure 5: FT –IR spectral of APME 132

The ^{1}HNMR displayed aliphatic protons in the ranges of 0.88 to 2.03 ppm, the olefinic protons appeared at 5.11 ppm while the proton adjacent to the carbonyl functional group will resonates at 2.03 ppm and a proton assignable to – O – CH_2 appeared at 4.09 ppm.

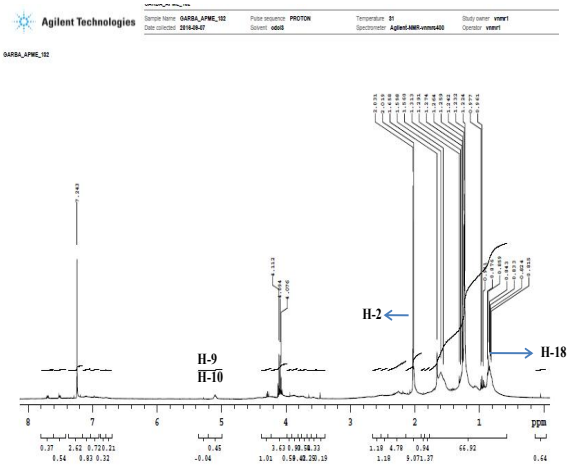


Figure 6: ^{1}H NMR spectral of APME 132

The ^{13}CNMR revealed a total of twenty carbons of which nineteen carbons correspond to methyl, methylene and methine carbon atoms between 14.16 to 32.92 ppm, and the deshielded carbon observed at 150.8 ppm correspond to C – 9 and C – 10, the ester carbonyl carbon signal was observed at 176.5 ppm while oxy – methylene signal was displayed at 67.8 ppm.

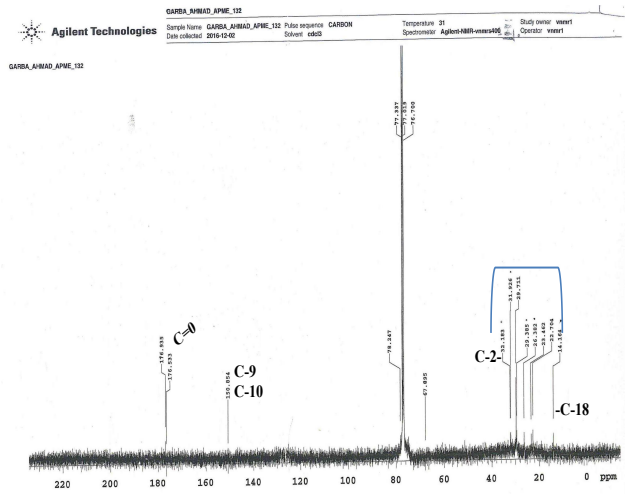
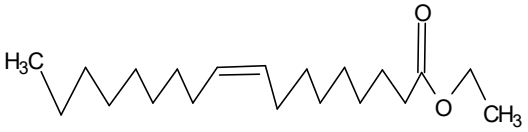


Figure 7: ^{13}C NMR spectral of APME 132

The mass spectrum shows a molecular ion peak with loss of ethyl group hydride ion $[M - 29]^{+n}$ at m/z at 281. It is apparent that the m/z values of 281 correspond to molecular weight of oleate moiety. Therefore, the compound correspond to ethyl oleate as it is in the literature (Akin – osanaiye *et al*., 2011).



Ethyl oleate

The fragmentation pattern is

m/e =281 m/e =29

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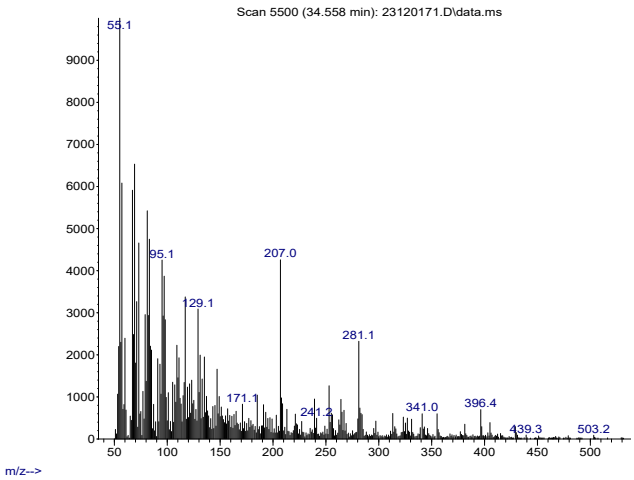

Figure 8: Mass Spectrum of APME 132

Ethyl oleate has been isolated from plants such as *Samnae saman* (Isiaka *et al*, 2006), and has been found to be produced by the human body during ethanol intoxication (Dan and Laposata, 1997). It has also been identified as primer pheromone in honey bees (Leoncini *et al.,* 2004).

In vitro antitrypanosomal activity

The parasite motility constitutes a reliable indicator of the trypanosomes well being. A complete elimination or reduction in motility of trypanosomes when compare to the control could be taken as an index of trypanocidal activity (Mergia *et al*, 2014).

TABLE 1: Effect of *A. precatorius* extracts and isolated compounds on *Trypanosome brucei brucei*

Treatment	**Time of cessation or drastic reduction in motility (mins)**					
	Sample	100mg/*ml*	50mg/*ml*	25mg/*ml*	12.5mg/*ml*	6.25mg/*ml*
Abrus precatorius isolated compounds	Campesterol	70 **	80 **	NA	NA	NA
	Ethyl oleate	70 **	110 **	NA	NA	NA
	Diminazene aceturate	40 **	NA	NA	NA	NA
Positive control	2% tween 80 PBSG					
Negative control		NA	NA	NA	NA	NA

*Ceased motility, **: Drastically reduced motility, NA: No action,

The isolated compounds, Campesterol and ethyl oleate does not eliminate the motility at the concentration of 12.5mg/ml, however increasing the concentration to 50mg/ml drastically reduced the motility within 80 and 110 minutes respectively, however, it may appears that the isolated compounds belong to the group of compounds that acts by static action affecting growth and multiplication of the trypanosome rather than eliminating them completely (Atawodi and Shehu, 2011). The complete immobility of the parasites *in vitro* may not necessarily indicate that the parasites may have lost their infectivity due to unfavorable conditions causes by the drugs. The parasites might have recovered and become infective with suitable physiological condition (Yusuf *et al,* 2012)

The trypanosomal activity of the methanol extract of *Abrus precatorious* is lower when compare with that of *Ximenia Americana* (Maikai, 2010) and *Clutia absyunia* (Ermias, 2015) which at effective concentration of 9mg/ml and 4mg/ml inhibits motility of *Trypanosome congolenses* within 45 minutes and 40 minutes, respectively. This might be due to the fact that *Trypanosome congolenses* was used instead *Trypanosome brucei brucei*

CONCLUSION

Campesterol and Ethyl oleate were isolated from the plant *Abrus precatorious*.which

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shows a potent antitrypanosomal activity against *Trypanosome brucei brucei*, therefore it could be used as potential source of drug in chemotherapy of African animal trypanosomiasis.

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