Search for Schweinfurthins and other Secondary Metabolites from *Macaranga Occidentalis* (Euphorbiaceae) and Evaluation of Possible Anticancer Activity

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CAiSMD

OUTLINE

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INTRODUCTION

- Cancer is the abnormal division of cells which may eventually spread into other tissues (Metastasis).

- It is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020

(Ferlay et al,, 2020) .

- Cancer arises from the transformation of normal cells into tumour cells in a multi-stage process that generally progresses from a pre-cancerous lesion to a malignant tumour. These changes are the result of the interaction between a person's genetic factors and three categories of external agents, including:

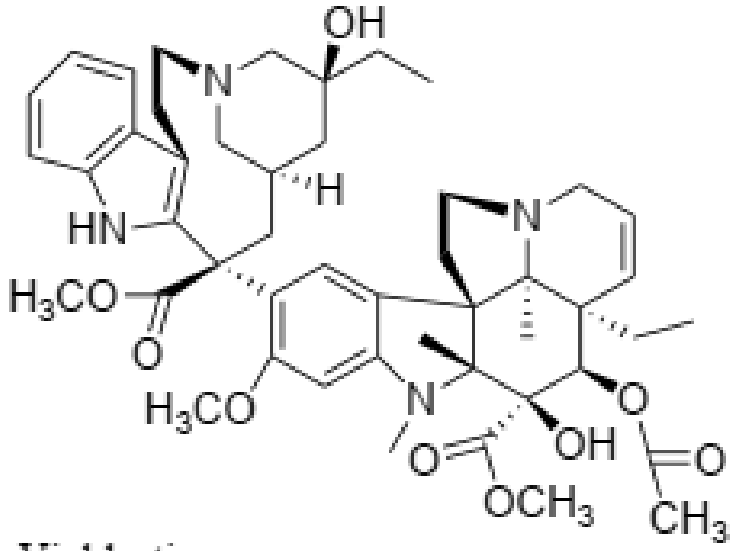
- Physical Carcinogen (UV radiation)
- Biological Carcinogen (Infections from Bacteria, viruses and parasites.
- Chemical Carcinogen (Alcohol, Tobacco)

- It has been estimated that approximately over half of the pharmaceuticals in clinical use today are derived from natural products.

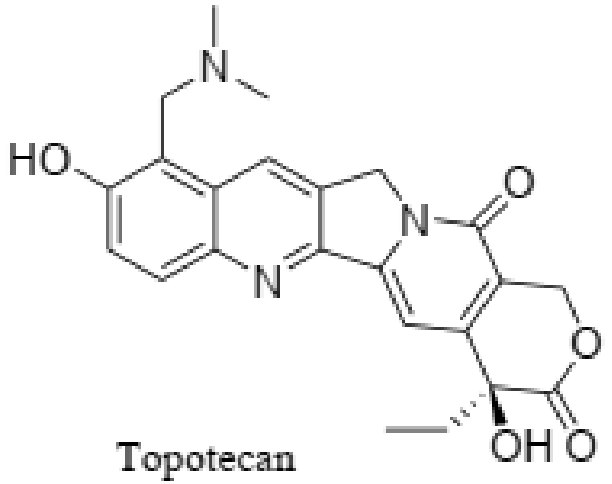
- Some natural product derived drugs that are a hallmark of modern pharmaceutical care include Quinine from *Cinchona* spp, Theophylline, Penicillin from microorganisms, Morphine from *Papaver somniferum*.

- It is estimated that over 60% of anti- cancer agents presently in clinical use are derived from natural sources, including plants, marine and micro-organisms. (Cragg and Newman, 2005).

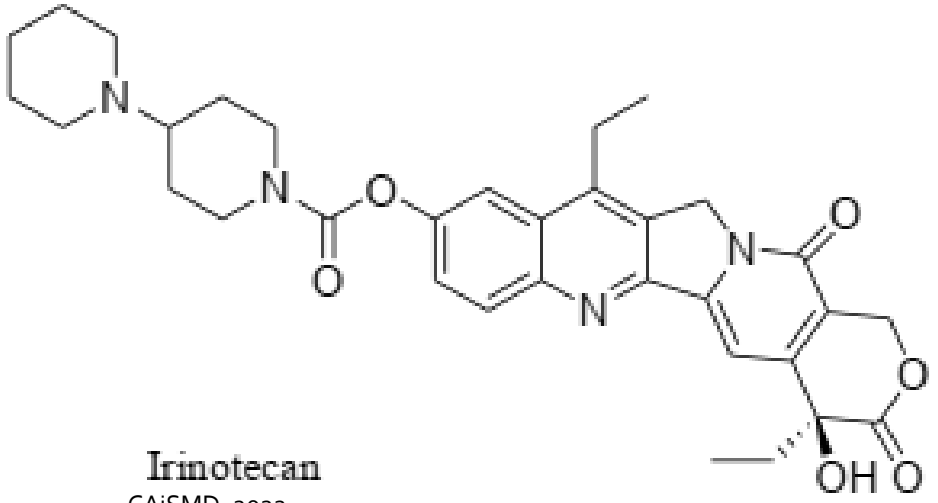
- Some examples of anticancer drugs originating in plants include irinotecan, topotecan, camptothecin (from *Camptotheca acuminata*), vinblastine and vincristine (from *Catharanthus roseus*), and etoposide (*Podophyllum peltatum*) (Pan *et al.*, 2010).



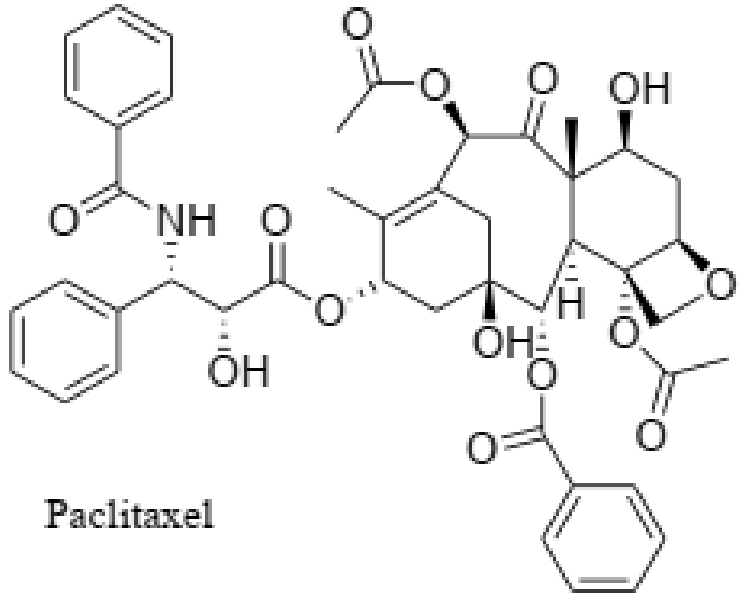
Vinblastine



Topotecan



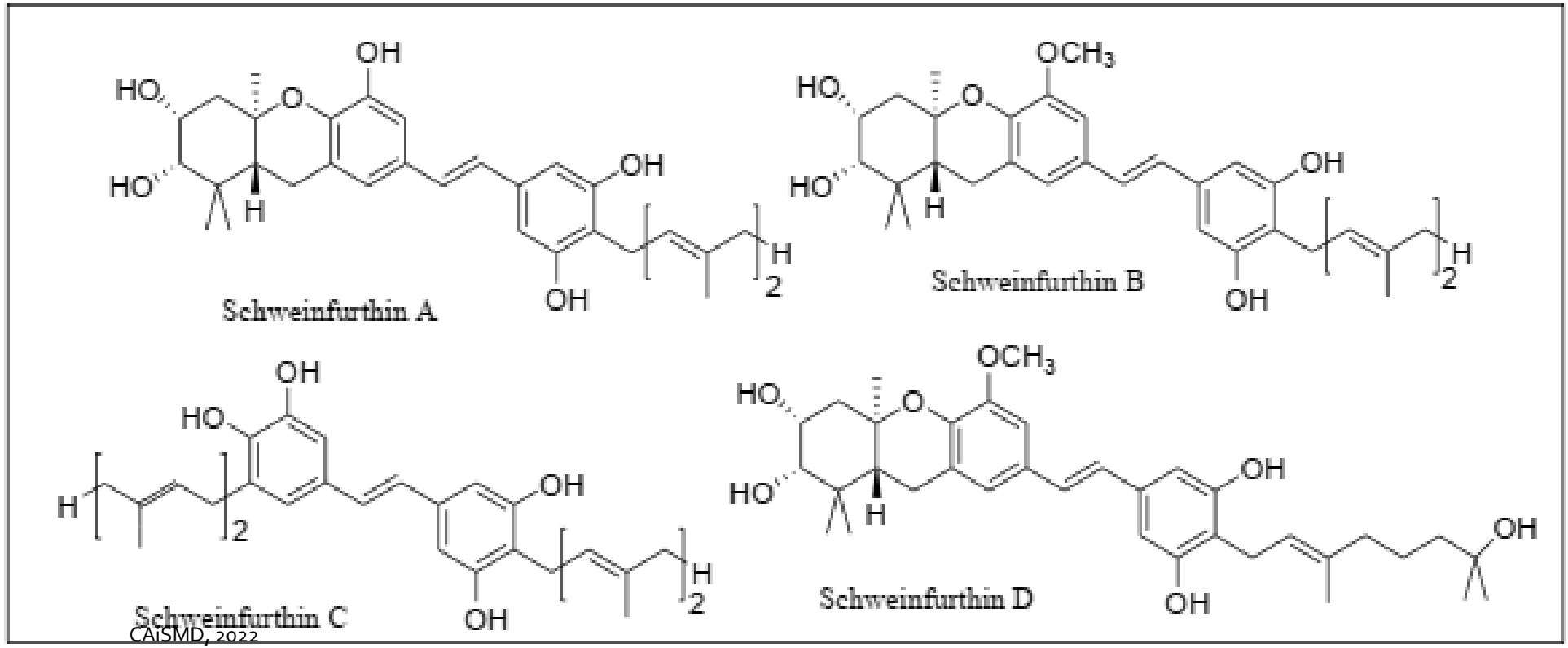
Irinotecan

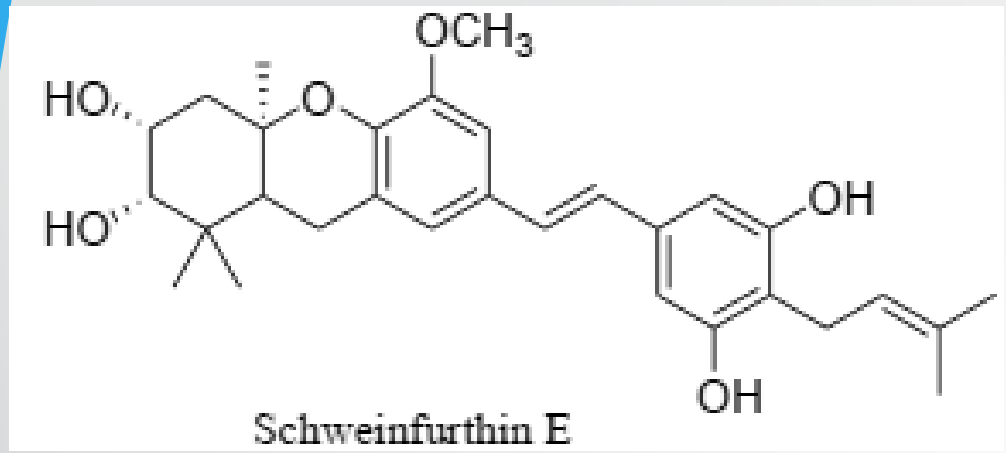


Paclitaxel

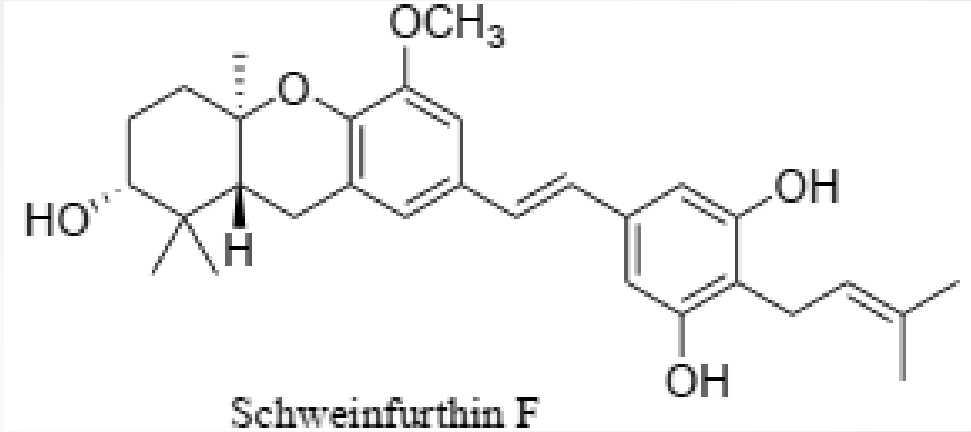
RATIONALE

- The schweinfurthins are a promising group of anticancer prenylated stilbenes which were obtained from *M. schweinfurthii* and other *Macaranga* species (*M. tanarius, M. alnifolia*).
- Various schweinfurthins display selectivity for CNS tumour and leukemia cell lines in the NCI 60-cell assay and they appear to act by a novel mechanism of action (Beutler *et al.*, 2006).

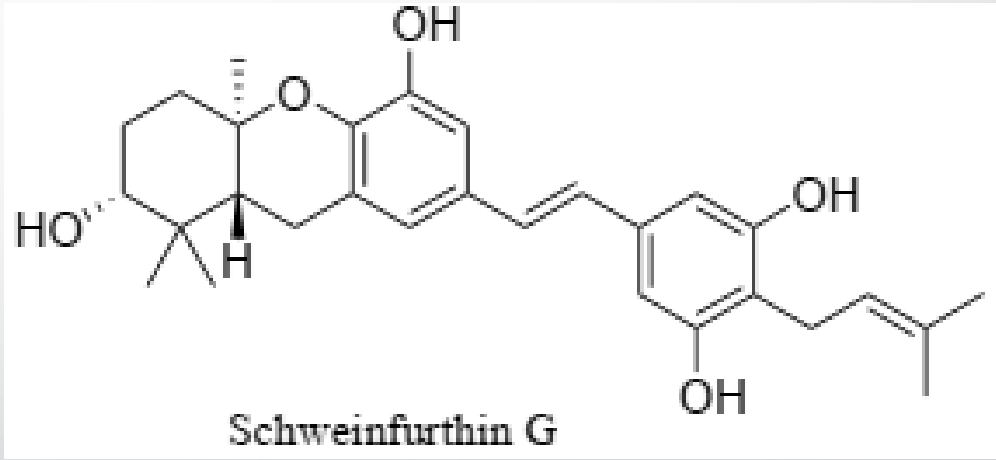




Schweinfurthin E



Schweinfurthin F



Schweinfurthin G

- Schweinfurthins from *M. alnifolia*
  Yoder *et al.*, 2007

This therefore prompted the phytochemical investigation of other Macaranga species in a bid to isolate the Schweinfurthins and other possible anticancer secondary metabolites.

OBJECTIVES

- The overall goal of the study is to identify other Macaranga species and to possibly isolate the Schweinfurthins and other secondary metabolites from them exhibiting anti-cancer activity.

Specifically:

- subsequently macerate parts of *Macaranga occidentalis* using methanol and exhaustively extract them using methylene chloride and hexane as solvents.

• carry out phytochemical screening of the extracts.

• isolate and purify the compounds using chromatographic methods notably column chromatography.

• compare the isolated compounds with Schweinfurthins.

• Elucidate the structures of the purified compounds pure compounds by using routine spectroscopic methods (GC-MS, 1H-NMR spectroscopy) and solubility test.

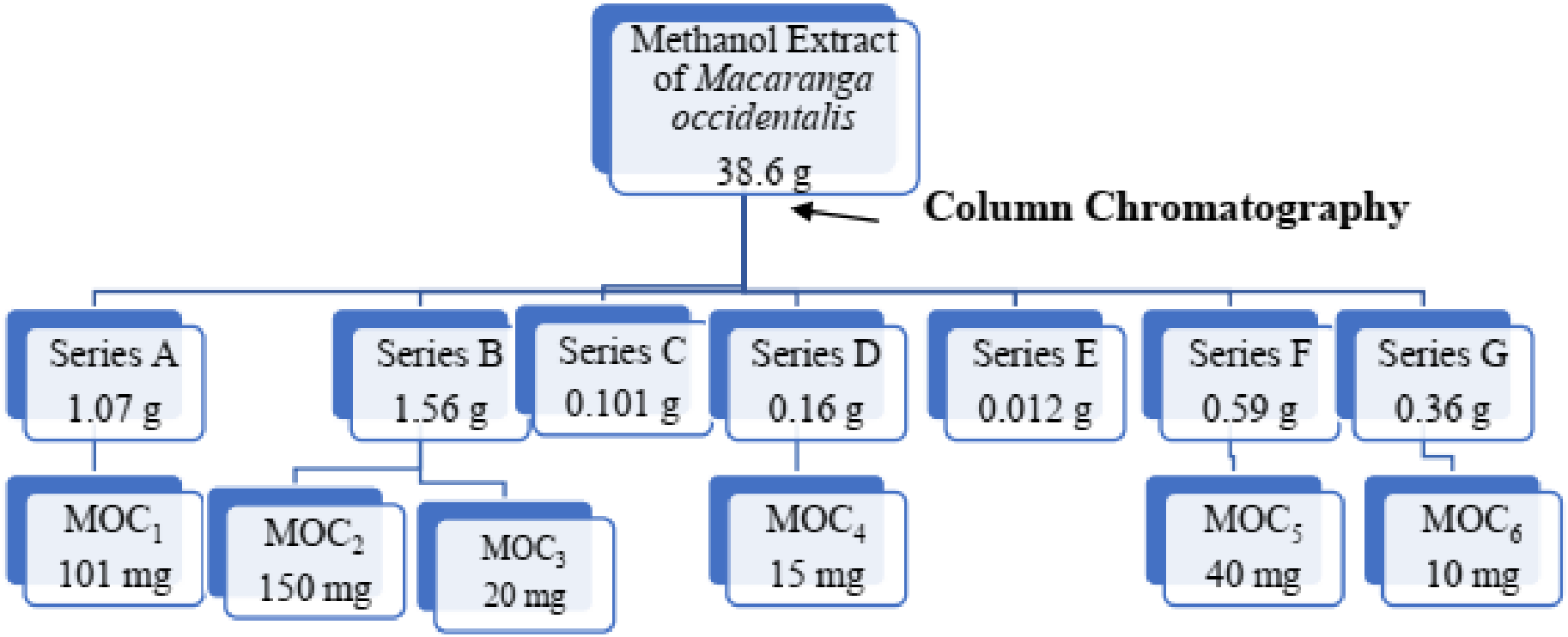
• screen the isolated compounds for anti-cancer activities.

MATERIALS & METHODS

- *Macaranga occidentalis* is found mostly in Equatorial Guinea, Nigeria and Cameroon. In Cameroon, it is found precisely at Oku, Kupe and Mount Cameroon (Patricia *et al.*, 2013).

- In traditional medicine, the decoction of the leaves and stem of *M. occidentalis* is consumed by pregnant women for stomach wash (Jiofack *et al.*, 2010).

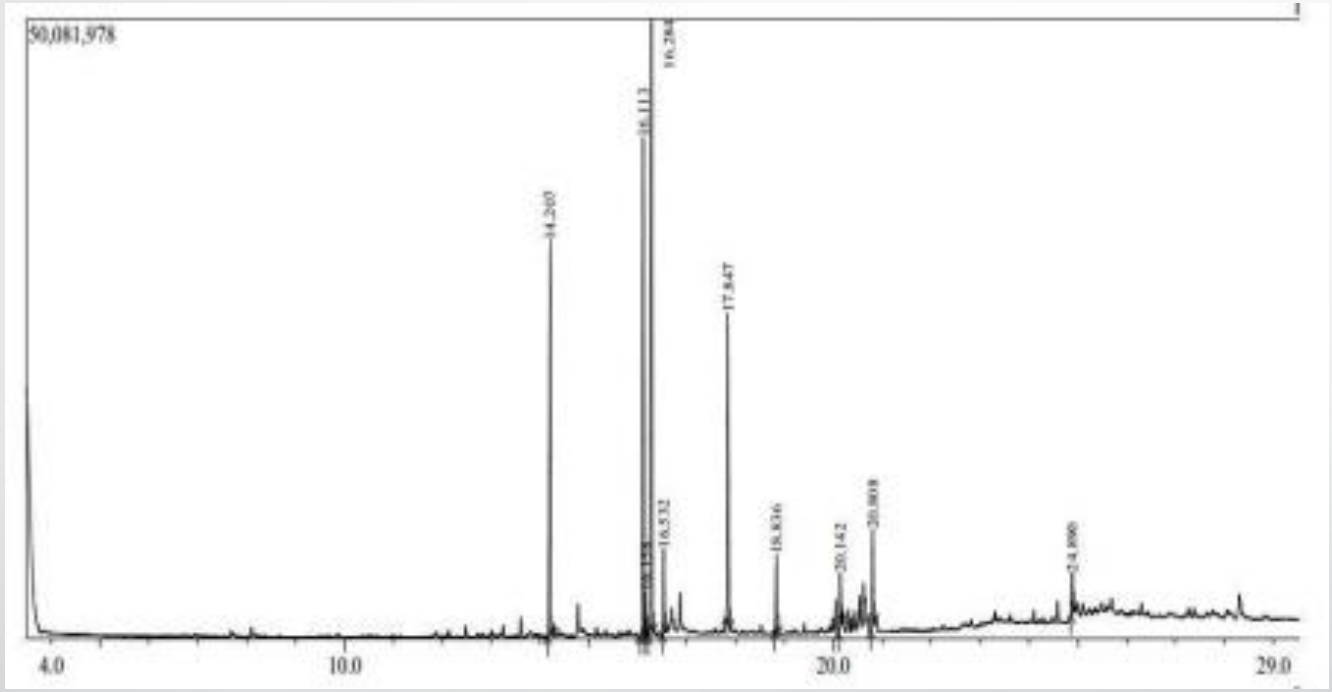
- The stem bark of *Macaranga occidentalis* was harvested from the Likombe forest, at the foot of Mount Cameroon in the South West Region of Cameroon, September 2017. The material was chopped, air- dried and ground to give 2.5 kg of fine powder.



Scheme 1: Fractionation Sequence of the Stem Bark Methanol Extract of *Macaranga occidentalis*.

RESULTS & DISCUSSION

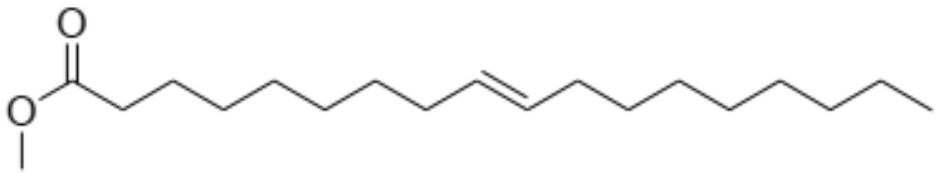
- MOC1 was obtained as an oily fraction from Series A (Fraction 13-14). The oil had a Rf value of 0.45 on the TLC plate. The oil was collected in a vial and stored at 5 ˚C. MOC1 was soluble in hexane and dichloromethane.



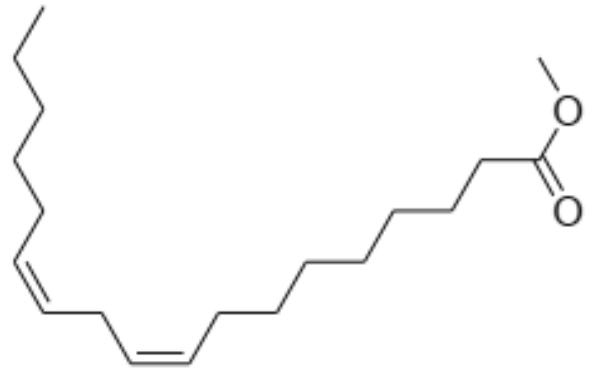
GC-MS Chromatogram of MOC_1

Table 1: GC-MS data of MOC1 with retention time and m/z ratios.

Peak No.	Retention time (R. Time)	% Area	m/z Ratio	Name
1	14.207	17.25	74.10	Hexanedecanoic acid-E-methyl ester
2	16.113	25.17	55.10	**9-Octadecenoic acid-E-methyl ester**
3	16.158	1.63	55.10	11-Octadecenoic acid methyl ester
4	16.284	28.95	67.10	**9,12-Octadecadienoic acid (Z, Z) methyl ester**
5	16.532	3.17	79.10	**9,12,15-Octadecatrienoic acid (Z, Z, Z) methyl ester**
6	17.847	12.63	319.10	RT :17.847
7	18.836	3.12	129.10	Hexanedioic acid, bis (2-ethylhexyl) ester
8	20.142	2.39	317.30	RT:20.142



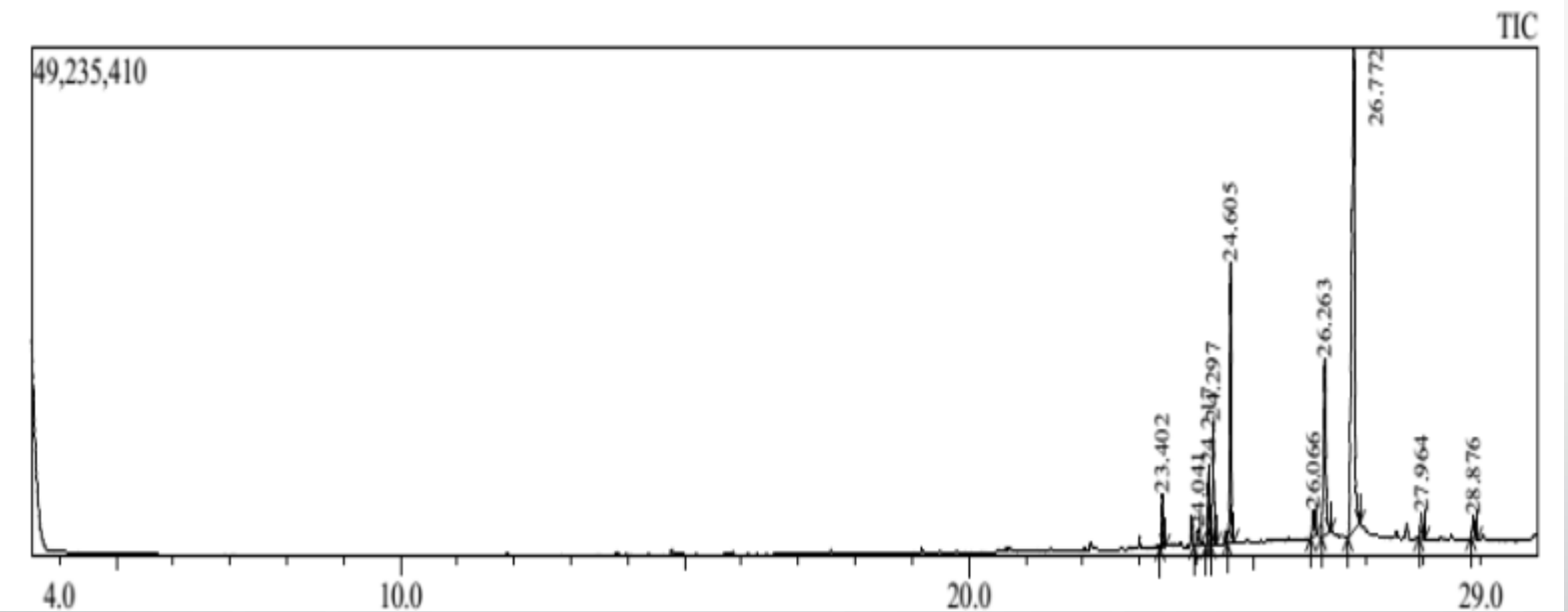
9-Octadecenoic (E)-methyl ester



9,12-Octadecadienoic acid (Z,Z)-methyl ester

Isolation and identification of MOC2

- Compound MOC2 precipitated as white solids in Hexane/Ethyl acetate at polarity 90:10. It was purified by washing several times using hexane (100 %) to afford 150 mg white powder.
- The GC-MS analysis of the compound showed it to be a mixture of phytosterols, notably **ß-Sitosterol acetate, Campesterol, Stigmasterol, γ-Sitosterol.**



GC-MS Chromatogram of MOC_2

Table 2: Phytosterols in MOC_2 with their retention times

Peak No.t	Retention time (R. Time)	%Area	Name
1	23.402	2.69	ß-Sitosterol acetate
2	24.297	6.08	ß-Sitosterol acetate
3	24.605	15.18	ß-Sitosterol acetate
4	26.066	2.35	Campesterol
5	26.263	13.97	Stigmasterol
6	26.772	50.81	γ-Sitosterol
7	27.964	1.78	**RT :27.967**

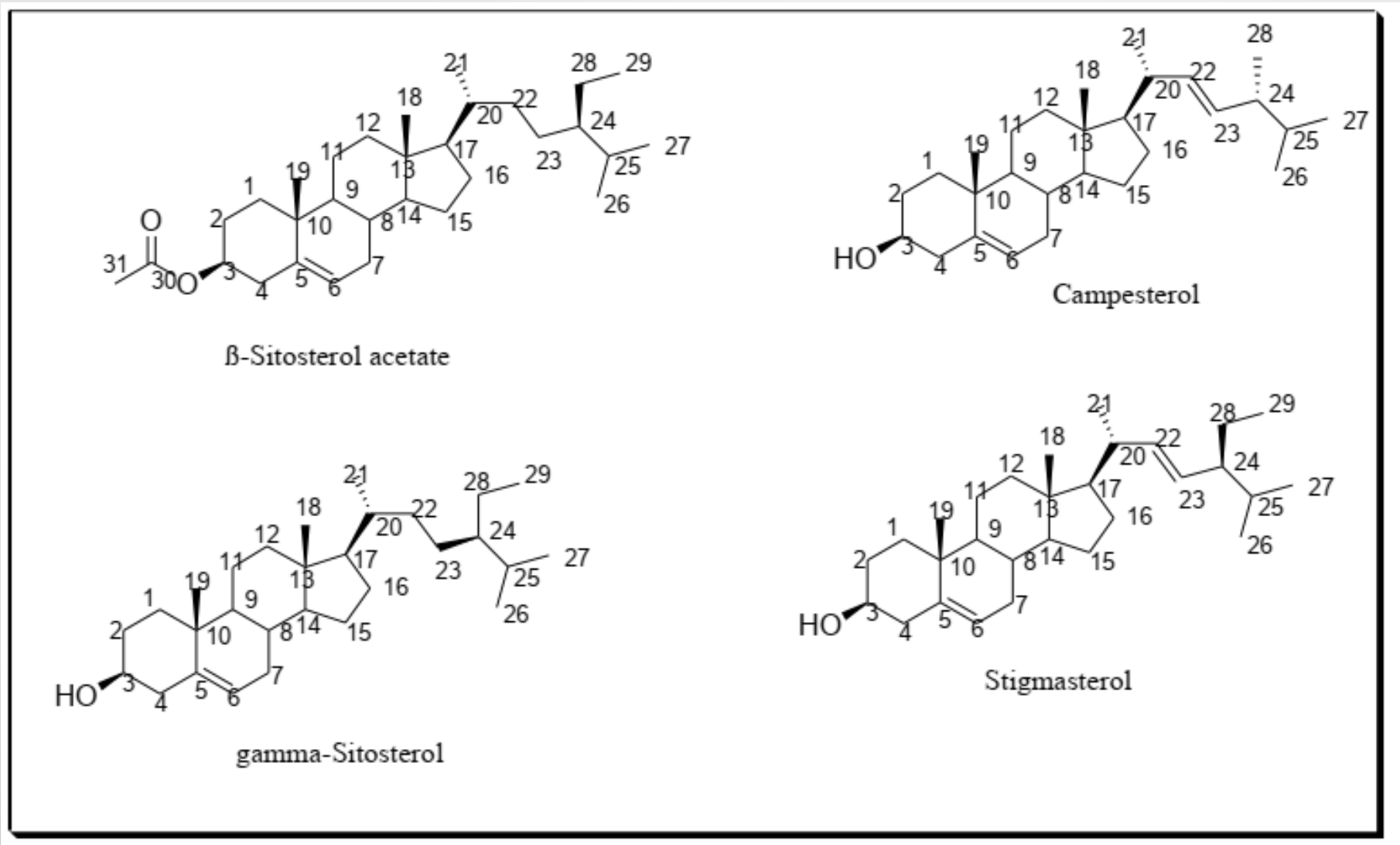


Figure 2: Phytosterols from MOC2

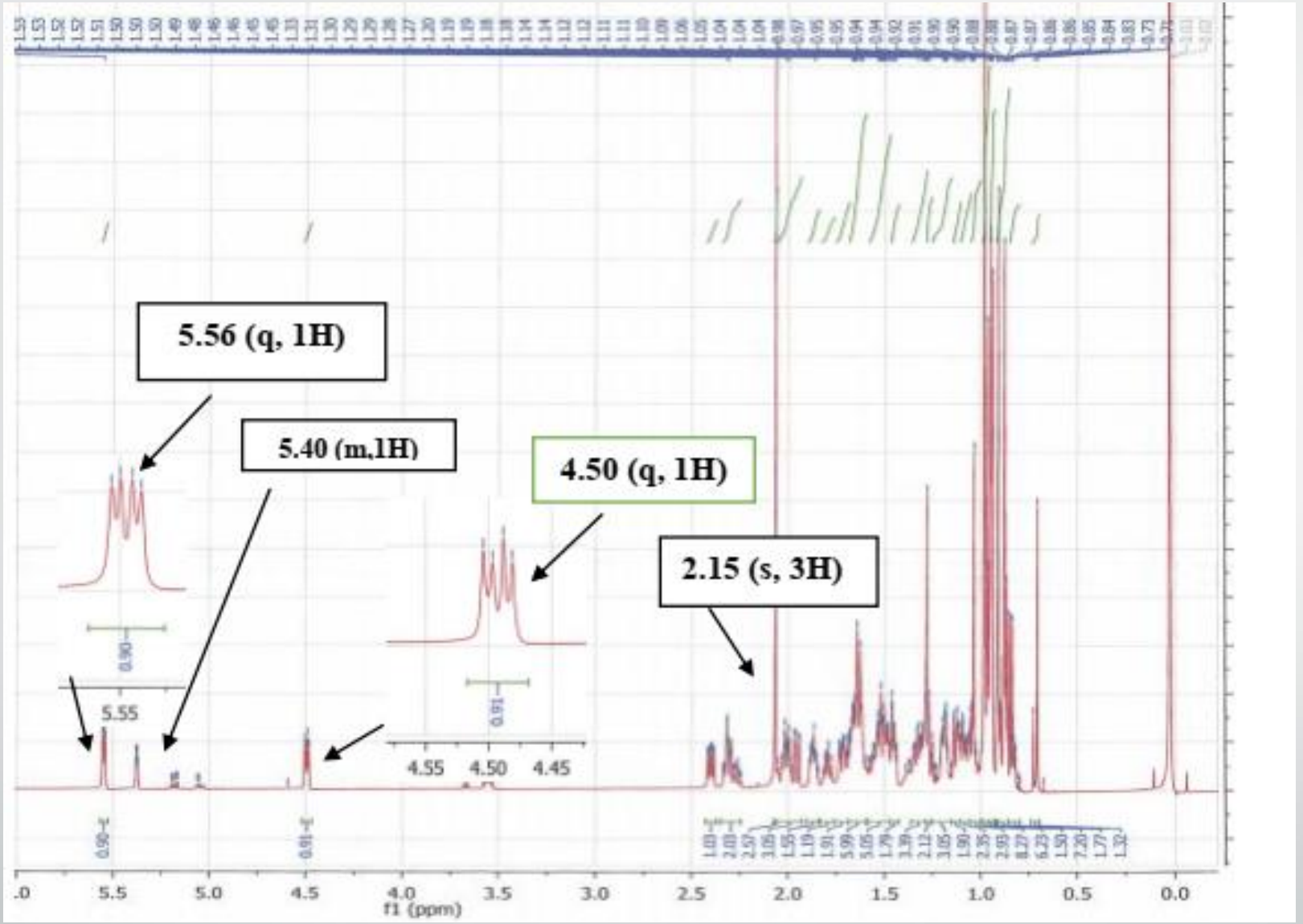
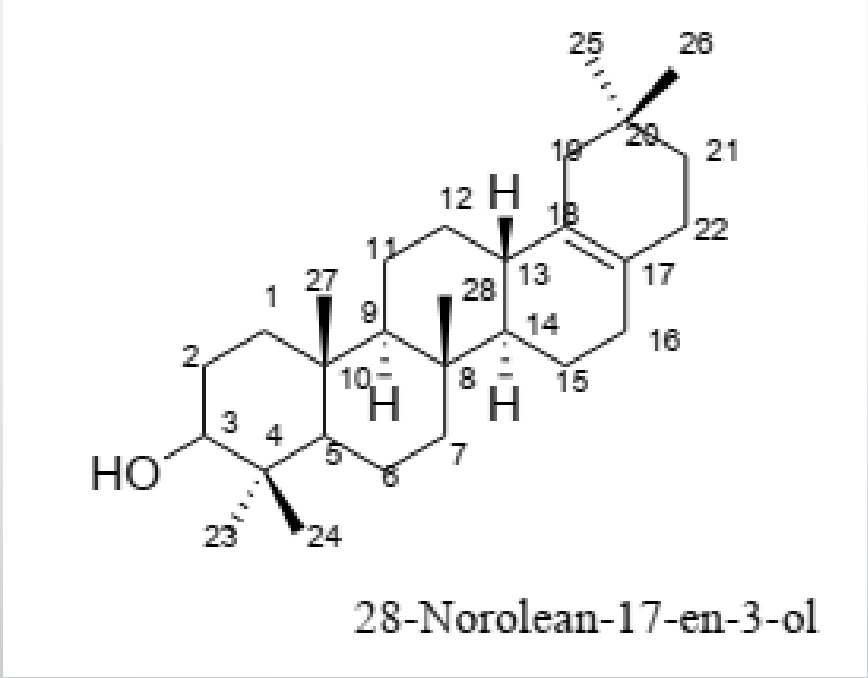


Figure 3: $_1$H-NMR ($CDCl_3$) of MOC_2.

Compound MOC_2 therefore precipitated as a mixture of ß-Sitosterol acetate, Campesterol, Stigmasterol, γ-Sitosterol. CAiSMD, 2022

Identification and isolation MOC3

- Compound MOC3 precipitated in Hexane/Ethyl acetate at a polarity of 88:12. It was purified by washing several times using hexane (100 %) to afford 20 mg white powder. It showed a positive Liebermann-Buchard test for Triterpenoids. The GC-MS analysis of the compound showed it to be a pentacyclic triterpenoid called **28- Norolean-17-en-3-ol** with retention time 28.876 and melting point 362 ˚C.



28-Norolean-17-en-3-ol

CONCLUSION

- The Stem bark of *Macaranga occidentalis* was evaluated for the presence of Schweinfurthins, which are promising lead compounds for the treatment of cancer.

- This rather led to the isolation of six compounds among which three were purified and have been identified by GC-MS analysis and NMR spectroscopy to be a mixture of **Linoleic acid (Z,Z) methyl ester** (9,12-Octadecadienoic acid-(Z, Z)-methyl ester) and **Methyl-E-Oleate** (9-Octadecenoic acidE-methyl ester), a mixture of phytosterols **(Stigmasterol, ß-Sitosterol acetate, γ-Sitosterol and Campesterol)** and **28-Norolean-17-en-3-ol.**

- The Schweinfurthins could not be identified from these isolated compounds.