

Supplementary Data: Ethical Approval and Gas Chromatography-Mass Spectrometry for the project entitled “*The Isolation of Hypoglycaemic Compounds from Desmodium canum and their Synergistic Effect on Blood Glucose Levels in Euglycaemic Sprague-Dawley Rats*”

Kemmoy Lattibeaudiere¹, Roy Porter², Ruby Lisa Alexander-Lindo¹

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Supplementary File 1: Ethical Approval Obtained in 2016 for the Project as Described in Manuscript.



THE UNIVERSITY OF THE WEST INDIES
MONA CAMPUS
Faculty of Medical Sciences
Office of the Dean

Dean: Horace Fletcher, MB, BS, DM (O&G), FRCOG, FACOG
Professor of Obstetrics and Gynaecology

May 3, 2016

Dr. Lisa Lindo
Section of Biochemistry
Department of Basic Medical Sciences
The University of the West Indies
Mona, Kingston 7

Dear Dr. Lisa Lindo,

Re: Kemmoy Lattibeaudiere research proposal entitled- Investigating the effects of various plant extracts on testosterone levels, blood glucose concentration, blood pressure and penile erection in Sprague-Dawley rats. AN 07, 15/16

Thank you for submitting the above mentioned proposal for review by the UWI Ethics Committee.

The proposal was reviewed and approved, having met the required ethical standards. The extended approval period commences on May 4, 2016 and will end on May 3, 2017. However, as per the intention of the principal investigator to conduct the same study using 35 additional plants listed in the proposal, Dr. Lindo should be requested to submit to the Ethics committee the following:

1. A summary page outlining the specific plant selected at each phase of the study
2. Name(s) of student(s) assigned to carry out the additional investigations on the other plant species

Mona, Kingston 7, Jamaica W.I.

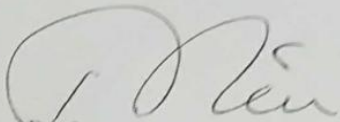
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Fax: (876) 977-2289, (876) 977-3470

3. A completed "Animal Checklist" for the use of additional Sprague-Dawley rats to conduct further investigations using any of the other plants listed in this AN7 15/16

Yours sincerely,



Professor Horace Fletcher
Chairman UWI Ethics Committee

**Supplementary File 2: Ethical Renewal Granted in 2017 for the Continuation of the Project
as Described in the Manuscript**



THE UNIVERSITY OF THE WEST INDIES
MONA CAMPUS
Faculty of Medical Sciences
Office of the Dean

Dean: Horace Fletcher, MB, BS, DM (O&G), FRCOG, FACOG
Professor of Obstetrics and Gynaecology

May 11, 2017

Dr. Lisa Lindo
Section of Biochemistry
Department of Basic Medical Sciences
The University of the West Indies
Mona, Kingston /

Dear Dr. Lisa Lindo,

Re: Kemmoy Lattibeaudiere research proposal entitled- Investigating the effects of various plant extracts on testosterone levels, blood glucose concentration, blood pressure and penile erection in Sprague-Dawley rats. AN 07, 15/16

I thank you for submitting the above mentioned proposal for review by the UWI Ethics Committee.

The proposal was reviewed and approved, having met the required ethical standards. The extended approval period commences on May 11, 2017 and will end on May 12, 2018.

Yours sincerely,

Professor Horace Fletcher
Chairman UWI Ethics Committee

Mona, Kingston 7, Jamaica W.I.

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The Gas Chromatography-Mass spectrometric analysis of the semipurified fraction, KLF6.2E-M was done as described in text, section 2.2.3. The fraction was compared to butanedioic acid (succinic acid; >99% purchased from Sigma) and oleic acid (>99% purchased from Sigma). Figure S1 shows the analysis of the succinic acid standard derivitized with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). A retention time (RT) of 8.90 min was observed from the GC analysis. Mass spectral analysis of the compound shows varying fragments with the most abundant peak being 147 m/z followed by 73 m/z. The varying fragments presented a molecular 'fingerprint' for said compound.

Acquired : 25 Jun 19 2:52 pm using AcqMethod JRC
Instrument : GC/MS Ins
Sample Name: Succinic Acid + BSTFA
Misc Info : Deriv. with BSTFA, DB5-MS, 1uL
Vial Number: 7

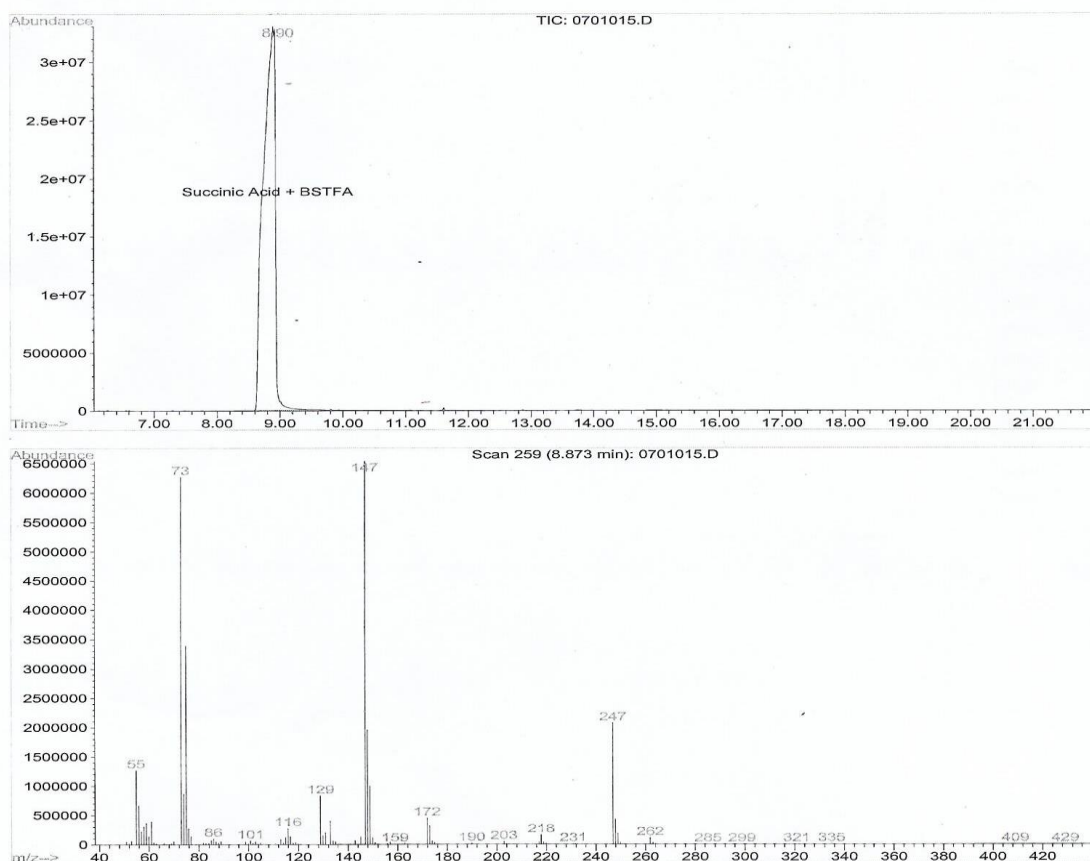


Figure S1: Gas Chromatography-Mass spectrum analysis of Butanedioic acid, bis(trimethylsilyl) ester

A similar analysis of oleic acid-BSTFA was done and displayed a RT of 14.08 min. Mass spectrum displayed the fragments with the most abundant peak being 73 m/z (Figure S2). The mass spectrum once again provides a 'fingerprint' for oleic acid which is then compared to the sample, KLF6.2E-M to confirm the presence of oleic acid. The data can be found in Figure S3

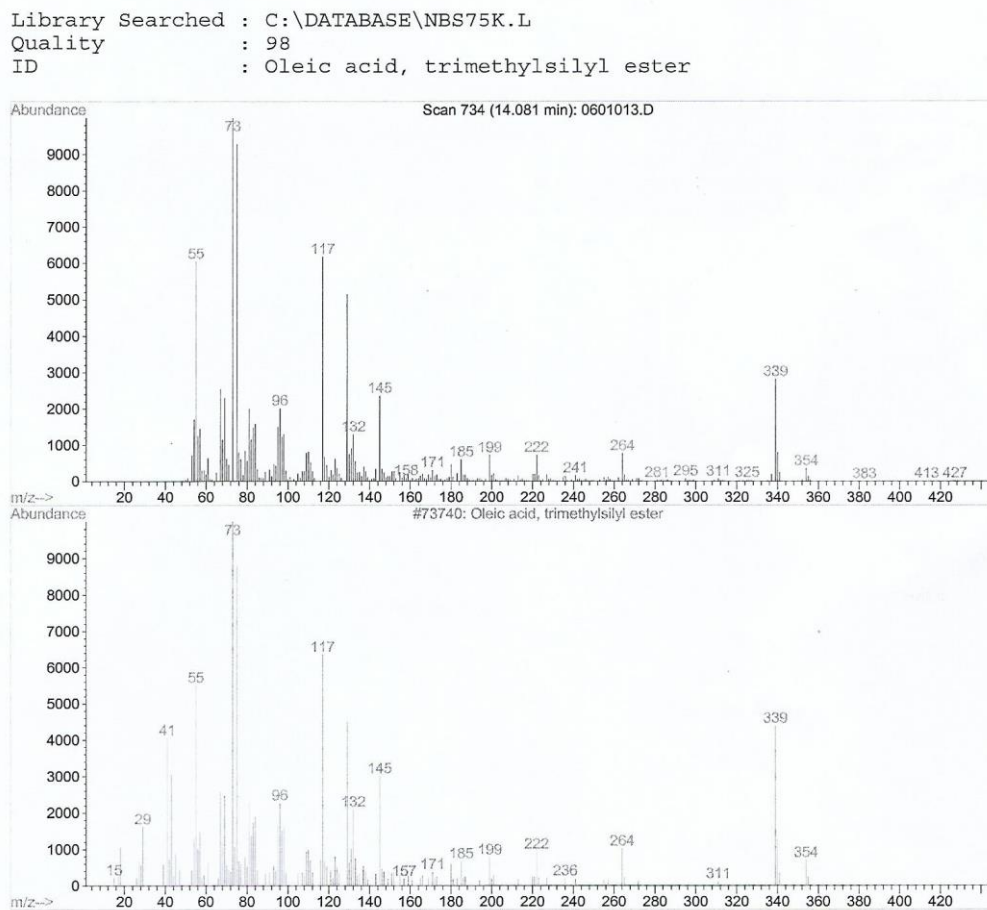


Figure S2: Mass spectrum analysis of Oleic acid, bis (trimethyl silyl) ester

A comparison to KLF6.2E-M derivitized with BSTFA was made with these standards, where it was observed that these compounds. It was observed that both succinic acid (RT of 8.93 min) and oleic acid (RT of 14.12 min) were the two most abundant compounds present within the fraction. Other compounds were also observed in lower quantity, however, succinic acid and oleic acid are believed to be synergistically responsible for the hypoglycaemic activity documented.

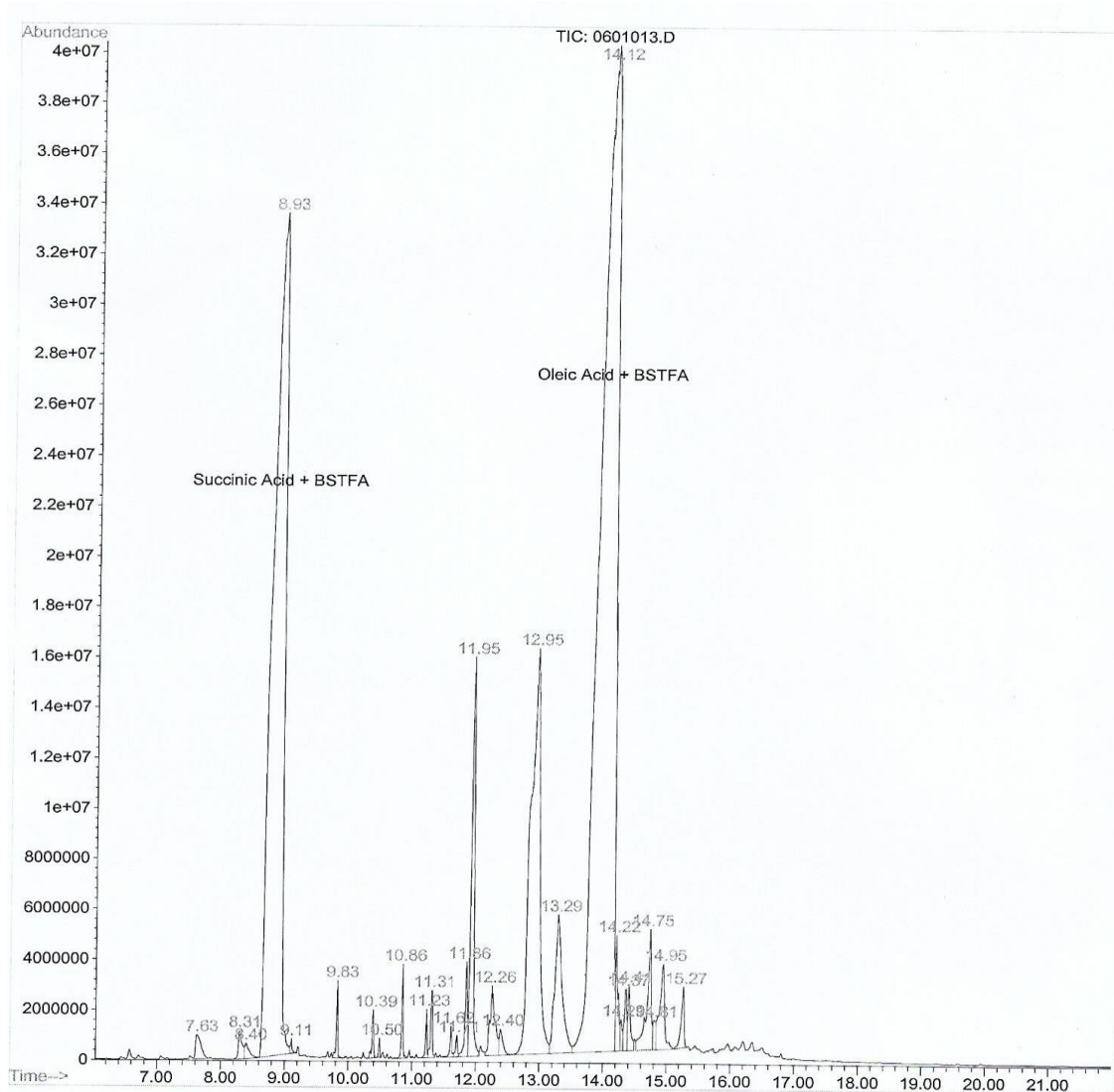


Figure S3: Gas chromatography-Mass Spectrum analysis of KLF6.2E-M indicating the presence of succinic acid and oleic acid