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# Chemical composition of *Sterculia oblonga* leaf petroleum spirit extract and its testosterone level enhancing property in male Albino rats

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**Abstract**

*Sterculia oblonga* is an African timber tree and medicinal plant which is locally used to enhance male fertility. The present study is aimed at identification of the chemical constituents of its leaf petroleum spirit extract. It is also to determine the testosterone level enhancing property of the extract in white albino rats. Leaves of *Sterculia oblonga* were dried in open air under laboratory conditions. It was ground and Soxhlet-extracted for 3h. The extracted solution was distilled down to get the petroleum spirit extract. The individual constituents were separated by gas chromatography and identified by mass spectrometric analysis. The rats were fed with different doses of the extract for one week. The level of testosterone in their blood serum after one week was determined using UV-spectrophotometer at $\lambda_{(max)}$ of 450 nm. The petroleum spirit extract contains twenty five compounds and it increased the testosterone level in the blood serum of the white albino rats. The petroleum spirit extract contained twenty five compounds, twenty four of which are being reported for the first time in *Sterculia oblonga*. The extract exhibited testosterone level enhancing property which is dose dependent.

**Keywords:** *Sterculia oblonga*, petroleum spirit extract, chemical composition, testosterone level

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## 1. Introduction

*Sterculia oblonga* is a timber tree growing in tropical rain forests of Central and West Africa. It is used for flooring beam, plank and furniture. The seeds are eaten in Cameroon and the ark decoction is used against stomach ache. It has yellow colored heartwood [1]. The wood contains pith and blast fiber. The properties of the wood make it good for production of good quality printing paper [2]. Yellow *Sterculia* is used in Cameroon for making of musical drum [1]. Dried *Sterculia oblonga* wood contains cellulose, 36.2-43%; furfural, 11.5-12.4%; pentosans, 19.8-2.1%, lignin, 18.2-22.7% and ash 1.3-3.2% [1]. It also contains tannins, 1240 $mgg^{-1}$; cyanide, 1.66 $mg100g^{-1}$ and lipid 4.8% [3, 4]. *Sterculia oblonga* leaf essential oil has been shown to contain thirty three natural products which include dodecanoic acid, 2-sec-butyleyelohexane, 2-Z-decenol and disoctylphalate. The essential oil exhibited testosterone level enhancing property [5]. The present study is focused on identification of the chemical constituents of its leaf petroleum spirit extract. It is also aimed at determining the testosterone level enhancing property of the petroleum spirit extract of the leaf and hence its suitability as a drug for control of male faction infertility.

## 2. Materials and methods

### *2.1. Collection of samples*

*Sterculia oblonga* leaves were harvested from Ikot Omini, Calabar, Cross River State, Nigeria in the month of March and was authenticated by staff of the Herbarium unit, Botany Department of the University of Calabar. The white albino rats were procured from the animal house in the Physiology Department, University of Calabar.

### *2.2. Extraction of the leaf*

The leaves were rinsed with distilled water and dried under room conditions for two weeks. About 300g of the powdered leaf was weighed out, carefully transferred into a Soxhlet extractor and extracted with petroleum spirit (60-80$^{o}$C) for 3h. The extracted solution was distilled down over a hot water bath to give a dark syrupy petroleum spirit extract.

### *2.3. GC-MS analysis*

A very dilute solution of the extract in hexane was used for the GC-MS analysis. The constituents of the extract were separated by gas chromatography while the individual constituents were identified by mass spectrometric analysis. The compounds identification was done by comparison of

the obtained mass spectra with those in the standard mass spectra of organic compounds from National Institute of Standard and Technology (NIST) library [6].

### *2.4. Biological activity*

Nine white albino rats each weighing 150g were selected from the animal house. Another set weighing 160g each were also selected for the work. The petroleum spirit extracted was administered orally at a dose of 1.6g $kg^{-1}$ of body weight for 150g rats and a dose of 3.0g $kg^{-1}$ body weight for the 160g rats. It was administered twice daily to the rats over a period of one week. They were sacrificed after a week to collect their blood. The blood was left to clot for the serum to separate out. The specimen was centrifuged to separate the serum from the blood cells.

Six micro-plates wells for the petroleum spirit extract and 7$^{th}$ one for control were used. Appropriate serum reference (10µL) were pipetted and assigned into the cells. 50µL of the working testosterone enzyme reagent was added. The micro-plates were swirled gently for 30 seconds to mix. The micro-plates were covered and incubated for 60min at room temperature. The contents of the micro-plates were discarded by decantation and blotted dry with absorbent paper. 350 µL of washing buffer was added and decanted. This was repeated twice to make a total of three washes. Manual plate washer was employed. 100µL of working substrate solution was added. All the reagents were added in the same order to all the wells to minimize difference of reaction time between different wells. The mixture was incubated for 15min at room temperature. Finally 50µL of stop solution was added to each well and gently mixed for 20 seconds. The absorbance was read at 450nm within 30min of addition of the stop solution. A calibration graph of absorbance versus concentration was used for the estimation of testosterone level.

## 3. Results

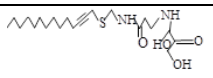
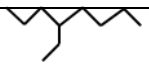
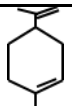
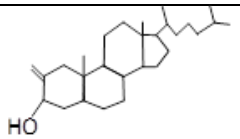
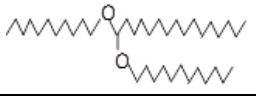
### *3.1. Chemical composition*

Table 1 shows that the petroleum spirit extract of *Sterculia oblonga* contains twenty five natural products which include Z-phythol, 17-pentatricatene, 3-ethyl-5-(2-ethylbutyl)-octadecene, heptacosane, 2-hexadecanol, E-3-7, 11, 15-tetramethyl-2-hexadecene-1-ol etc. Seventeen of these compounds are oxygenated.

### *3.2. Biological activity*

Table 2 shows that the petroleum spirit extract caused rise in testosterone level in the male white albino rat blood serum. The table further showed that the blood serum of the rats fed with the extract contained higher levels of testosterone than the control rats which were not fed with the crude drug (i.e. extract). There was increase in testosterone level with increase in the dose of drug administered. None of the rats died indicating that the crude drug is not lethal to them.

**Table 1:** Gas chromatography-mass spectroscopy analysis of petroleum spirit extract of *Sterculia oblonga* leaf

| S/N | Compound Name | Retention Time (Minutes) | Molecular Formula | Relative Molecular Mass | Base Peak | Percentage composition | Chemical Structure |
|---|---|---|---|---|---|---|---|
| 1 | Z,Z,-2,5-Pentadecadien-1-ol | 8.014 | $C_{15}H_{27}OH$ | 219 | 57 | 0.41 |  |
| 2 | 2-myristgnoyl pantetheine | 8.602 | $C_{25}H_{44}N_2O_5S$ | 484 | 57 | 0.39 |  |
| 3 | Octane, 4-ethyl | 10.973 | $C_{10}H_{22}$ | 142 | 57 | 3.22 |  |
| 4 | D-Limonene | 13.944 | $C_{10}H_{16}$ | 136 | 68 | 1.91 |  |
| 5 | Octadecane, 6-methyl- | 17.316 | $C_{18}H_4O$ | 256 | 57 | 1.17 |  |
| 6 | Cholestan-3-ol, 2-methylene-, (3β, 5α) | 19.499 | $C_{27}H_{45}O_2$ | 401 | 57 | 0.18 |  |
| 7 | Hexadecane, 1,1,-bis(dodecyloxy) | 24.116 | $C_{40}H_{82}O_2$ | 594 | 82 | 0.62 |  |

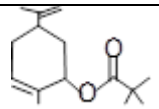
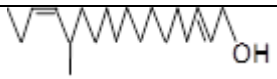
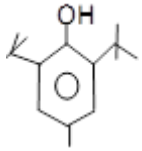
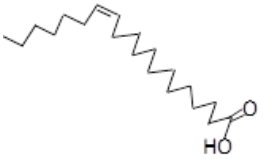
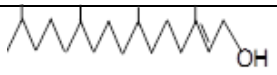
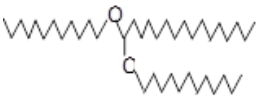
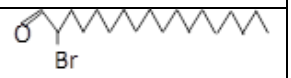
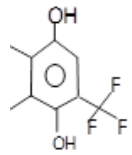
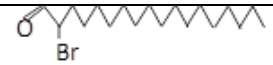
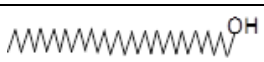
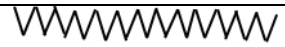
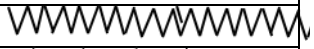
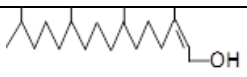
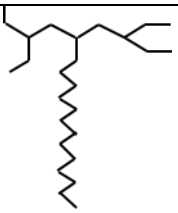
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 8 | Limonene-6-ol, pivalate | 25.573 | $C_{13}H_{23}CO_2H$ | 224 | 119 | 1.36 |  |
| 9 | 12-Methyl-E,E-2,13-octadecadien-1-ol | 29.189 | $C_{18}H_{32}OHCH_3$ | 280 | 69 | 0.90 |  |
| 10 | 4-Methyl-2,6-di isobuthylphenol | 31.003 | $C_{15}H_{24}OH$ | 221 | 205 | 3.58 |  |
| 11 | Cis-13-Eicosenoic acid | 36.163 | $C_{19}H_{37}CO_2H$ | 310 | 97 | 7.45 |  |
| 12 | 2-Hexadecanol | 37.333 | $C_{16}H_{33}OH$ | 242 | 57 | 1.24 |  |
| 13 | Tetradecane, 2,6,10-trimethyl- | 37.402 | $C_{17}H_{36}$ | 240 | 57 | 3.94 |  |
| 14 | E-3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 37.777 | $C_{20}H_{39}OH$ | 296 | 68 | 11.11 |  |
| 15 | Hexadecane, 1,1-bis(dedecyloxyl)- | 37.840 | $C_{40}H_{82}O_2$ | 594 | 57 | 2.50 |  |
| 16 | R-Octadecanal, 2-bromo | 37.990 | $C_{18}H_{34}OB_r$ | 345 | 57 | 1.76 |  |
| 17 | Phen-1,4-diol,2,3-dimethyl-5-trifluoromethyl | 38.052 | $C_9H_9F_3O_2$ | 163 | 149 | 1.33 |  |
| 18 | S-Octadecanal, 2-bromo | 38.140 | $C_{18}H_{34}OB_r$ | 345 | 57 | 3.22 |  |
| 19 | 1-Heptatriacotanol | 38.440 | $C_{37}H_{73}OH$ | 534 | 69 | 2.80 |  |
| 20 | 2-Hexadecanol | 38.928 | $C_{15}H_{30}OH$ | 227 | 57 | 4.54 |  |
| 21 | Heptacosane | 38.959 | $C_{27}H_{56}$ | 280 | 57 | 5.41 |  |
| 22 | 17-Pentatraicontene | 39.547 | $C_{35}H_{70}$ | 490 | 57 | 2.62 |  |
| 23 | Z-Phytol | 39.654 | $C_{20}H_{39}OH$ | 296 | 71 | 9.13 |  |
| 24 | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | 40.129 | $C_{26}H_{54}$ | 366 | 57 | 5.24 |  |
| 25 | 17-Pentatriacontene | 40.098 | $C_{35}H_{70}$ | 490 | 57 | 10.89 |  |

**Table 2:** Effect of dose of *Sterculia oblonga* leaf petroleum spirit extract on testosterone level in male albino rats

| Dose gkg$^{-1}$ body weight | % mortality | Testosterone level ngcm$^{-3}$ |
|---|---|---|
| 1.6g kg$^{-1}$ | 0% | 0.65 ± 0.05 |
| 3.0g kg$^{-1}$ | 0% | 0.73 ± 0.03 |
| 0.0g kg$^{-1}$ | 0% | 0.57 ± 0.01 |

## 4. Discussion

The petroleum spirit extract of *Sterculia oblonga* leaf contains twenty five compounds which include Z-phytol (9.31%), 17-pentatriacotene (10.89%), 3-ethyl-5-(2-ethylbutyl)-octadecane (5.24%), heptacosane (5.41%), 2-hexadecanol (4.54%), 5-2-bromo ocatadecanal (3.22%), E-3,7,11,15-tetramethyl-2-hexadecen-1-ol (11.11%) 2,6,10-trimethyltetradecane (3.94%), cis-13-eicosenoic acid (7.45%), 4-methyl-2,6-di isobutyl phenol (3.58%), and cholestan-3-ol-2-methylene (3β, 5α) (0.18%) etc. With exception of cis-13-eicosenoic acid which was recently reported in *Sterculia oblonga* leaf essential oil [5], the remaining twenty four compounds are being identified for the first time in *Sterculia oblonga*.

None of the rats died after seven days of administration of the leaf petroleum spirit extract. These points to the fact that the extract is apparently innocuous to them. At the daily dose of 1.6 g kg$^{-1}$ of body weight, the level of testosterone increased from 0.57 to 0.65 ng cm$^{-3}$ in control and sample respectively. At a daily dose of 3 g kg$^{-1}$ of body weight, the testosterone level increased from 0.57 to 0.73 ng cm$^{-3}$ in the blood serum of the rats after seven days of the drug administration. This further shows that the effect of the drug is dose dependent as increase in the administered dose resulted in increased testosterone level. Since the level of serum testosterone is known to be directly related to male fertility [7, 8], the extract increases fertility in male rats. The increase in serum testosterone level on drug administration is by enhancing the production of luteinizing hormone, enhancing the viability of Leydig cells, reducing testicular oxidative injury, enhancing StAR gene [9]. The present study therefore serves as a scientific backing to the local use of *Sterculia oblonga* for control of infertility in man. The use of herbs in health care delivery generally has the advantage of having less hazardous side effect and it is environmentally friendly [10]. One is often tempted to believe that the major constituents of a plant are responsible for its observed biological activity but such activities are known to be modulated by the minor constituents [11, 12].

## Conclusion

The petroleum spirit extract of *Sterculia oblonga* leaf contained twenty five compounds. Twenty four of these are being identified for the first time in this plant species. The extract increased the level of serum testosterone and hence increased male fertility. This may lead to development of new drug for control of male infertility.

## Conflict of interest

The authors declare no conflict of interest.

## Author's contribution

Frank N. I. Morah–contribution to the conception and design, acquisition of data, analysis and interpretation of data, drafting of article, revising it critically for important intellectual content, final approval of the version to be published.

Emmanuel A. Bisong-acquisition of data, analysis and interpretation of data, revising the article critically for important intellectual content, final approval of the version to be published

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