Turk J Pharm Sci 2022;19(2):180-186
DOI: 10.4274/tjps.galenos.2021.48264

ORIGINAL ARTICLE

13, 14-Epoxyoleanan-3-ol-acetate: A Male Fertility-Enhancing Constituent from Hexane Fraction of *Momordica charantia* L. (Cucurbitaceae)

Oluwasegun ADEDOKUN[1]*, Adebayo GBOLADE[1], Bunyaminu AYINDE[2]

[1]Igbinedion University, College of Pharmacy, Department of Pharmacognosy, Okada, Nigeria
[2]University of Benin, Faculty of Pharmacy, Department of Pharmacognosy, Benin City, Nigeria

ABSTRACT

Objectives: Male infertility has been associated with oxidative stress-induced and/or microbial induced in some men. The use of medicinal plants to overcome oxidative stress-induced infertility cannot be over emphasized. Hence, the aim of this research was to isolate the antilipid peroxidation (an index of usage for treating oxidative stress-induced male infertility) bioactive principle from *Momordica charantia* using bioactivity-guided isolation.

Materials and Methods: *n*-Hexane fraction from the crude ethanol extract obtained by Soxhlet extraction of aerial parts (without fruit) of bitter melon, *M. charantia*, was assessed for *in vitro* lipid peroxidation, followed by bioactivity-guided isolation of bioactive principles using *in vitro* lipid peroxidation as an index of aphrodisiac and male fertility enhancer.

Results: Fractionation of the active *n*-hexane fraction using vacuum liquid chromatography (VLC) gave five pooled fractions on the basis of their thin layer chromatography (TLC) characteristics (*n*-hexane: EtOAc, 2:3, sulphuric acid spray). *In vitro* activity of the most active VLC fraction C was less than that of the positive control, vitamin E. Further fractionation of VLC-C by open column chromatography on silica gel led to the isolation of a compound which was purified by preparative-TLC. The purified compound, 10 mg/mL (R_f 0.54, TLC silica gel, *n*-hexane: ethyl acetate; 2:3) was equipotent with vitamin E (25 mg/mL) in reducing peroxidation of polyunsaturated fatty acids *in vitro*. Structural elucidation by NMR (^1H, ^{13}C) and mean mass spectroscopy confirmed the identity of the new bioactive compound as 13, 14-epoxyoleanan-3-ol-acetate.

Conclusion: This study scientifically validates the traditional claim of *M. charantia* as an aphrodisiac or male fertility enhancer and suggests that 13, 14-epoxyoleanan-3-ol-acetate might be responsible for the observed activity.

Key words: *Momordica charantia*, *n*-hexane fraction, *in vitro* lipid peroxidation assay, 13, 14-epoxyoleanan-3-ol-acetate, vitamin E, VLC

INTRODUCTION

Sexual dysfunction is a serious medical and social problem that occurs in 10-25% of men and 25-63% of women.[1,2] Among men aged 40-70 years, estimated 34.8% have moderate to complete erectile dysfunction.[2] This condition can be managed *via* psychotherapeutic and pharmacotherapeutic approaches.[1,3]

Free radicals are present in seminal plasma, some of the most prevalent reactive oxygen species (ROS) are hydroxyl, superoxide, and hydrogen peroxide radicals. During oxidative stress, excessive production of the ROS or free radicals in seminal plasma tends to have a destructive effect of the sperm cells and in turn induce oxidative stress induced male infertility. Moreover, antioxidants in seminal plasma aid in scavenging the harmful effect of the ROS *via* the donation of electrons to the electron-impaired radicals, thereby reduce their influence.[4] Spermatozoa in mammals are rich in polyunsaturated fatty acids (PUFA) as a result of that they are very prone to membrane lipid

*Correspondence: adedokun.oluwasegun@iuokada.edu.ng, Phone: +2348035485083, ORCID-ID: orcid.org/0000-0001-7264-3695
Received: 09.07.2020, Accepted: 10.08.2021

peroxide ion and ROS attack. Moreover, a balance is maintained between the amount of ROS produced and that scavenged arises, when this disturbed equilibrium toward pro-oxidants in semen and vaginal secretions can induce an oxidative stress on spermatozoa, which in turn can cause its damage and cause infertility.[4]

Theoretically, oxidative stress-induced sperm cells results in decreased sperm motility, presumably by a rapid loss of intracellular adenosine triphosphate leading to axonemal damage, decreased sperm viability and increased midpiece morphology defects, with deleterious effects on sperm capacitation and acrosome reaction.[5] However, the key mechanism of ROS-induced sperm damage leading to infertility is principally induced by the effect peroxidation of sperm membrane lipid.[6] Among the medicinal plants used in treating numerous diseases including male sexual dysfunction and infertility is *Momordica charantia* L. (Cucurbitaceae).[6-10] It is a climbing vine commonly found in the tropics and subtropics. It is also a tropical vegetable employed in ethnomedicine for the treatment of various diseases including diabetes, malaria and dysentery, and as a stomachic, stimulant, emetic, antibilous, and laxative.[11]

Previously, *M. charantia* has been investigated for analgesic and antipyretic,[7,8] antimicrobial,[9] and anti-HIV activities. Bioactive compounds responsible for the widely investigated antidiabetic and hypoglycemic activities have been linked to cucurbitanetriterpenoids.[11-13] The cucurbitane-type triterpenoids have been extensively isolated from various parts of *M. charantia*.[11-15]

Until date, literature information is unavailable on male fertility enhancing the potential of *M. charantia*, and only a mention of traditional use of the Nigerian plant as an aphrodisiac is known.[7,9,16] We therefore investigated *in vitro* fertility activity (using lipid peroxidation as index) of the most active hexane fraction of the aerial parts (it contain no fruit) by lipid peroxidation assay, and isolated the bioactive constituent to rationalize the traditional claim of the plant as an aphrodisiac plant by the people of Esan community in Edo State, Nigeria.

MATERIALS AND METHODS

Plant collection and authentication

M. charantia used in the research was collected from wild in the Ewu community of Esan-Central Local Government Area of Edo state (Nigeria) in January 2012 and authenticated at the herbaria in Paxherbal Laboratories, Ewu (Nigeria) by Professor J. C. Okafor and the Federal Forestry Research Institute of Nigeria, Ibadan, Oyo State (FHI 109577). Voucher specimens were also deposited in these herbaria and at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria.

Extraction and solvent partitioning

1.2 kg of the dried aerial part of the plant was extracted to exhaustion with absolute ethanol in a Soxhlet apparatus. The extract was reduced *in vacuo* to yield a residue that was refrigerated at 4°C until needed. The crude ethanol extract was partitioned into *n*-hexane, chloroform, and water using a separatory funnel and total yield of the fractions was determined.

Chromatographic studies

Vacuum liquid chromatography (VLC): n-Hexane fraction (21.00 g) was packed onto a sintered glass Buchner filter funnel, loaded with silica gel for analytical thin layer chromatography (TLC) without binder and eluted with *n*-hexane, chloroform, and methanol mixtures to yield 17 fractions (x 200 mL each). The fractions were bulked according to their TLC profile (*n*-hexane: EtOAc, 2:3; H_2SO_4 spray reagent) into 5 main fractions (A-E), weighed and bio-assayed *in vitro.*

Bottom of form

Column chromatography (CC)/preparative-TLC: Conventional open CC (30 cm long and 5 cm diameter) of the most active VLC fraction C was done. The silica gel used was 70-230 mesh size (0.063-0.200 mm) particle size. The silica gel was loaded on top of the column and allowed to settle. VLC fraction C (3 g) was diluted, adsorbed onto silica gel and poured on top of the column which was eluted with *n*-hexane, chloroform, ethyl acetate, and methanol mixtures. Eluates (151 fractions x 10 mL) were collected into test tubes and bulked according to their TLC characteristics (silica gel, *n*-hexane: ethylacetate, 2:3; H_2SO_4 spray reagent) into 5 main fractions CC (A-E), dried, and weighed. The most active fraction, *i.e.* CC-D (0.63 g), was subjected to prep-TLC (commercial type) with *n*-hexane: ethyl acetate (4:6). The bands were scrapped, eluted with methanol, filtered, and evaporated in a fume closet to yield needle-shaped crystals.

Spectroscopic studies

1D NMR (^{1}H and ^{13}C NMR) as well as 2D NMR (DEPT, HMBC) experiments were performed on the isolated compound. Mean mass spectroscopy (MS) was recorded on an Agilent Technologies S973 network mass selective detector and the spectrum was compared with database NIST02 reference spectra library.

In vitro lipid peroxidation assay

Fresh but frozen Titus fish (*Scomber japonicum*) were purchased from the Ekpoma market in Benin City, washed, and fleshy muscular parts will be used. The tissue homogenate was prepared as described earlier as the modified method of Luotola and Luotola.[17] Twenty grams of raw fish muscle tissue was turned into a paste in a mortar. 200 mL distilled water was added and the mixture was cooked at a temperature of 100°C for 15 min followed by thoroughly blending in an electric blender (10% w/v). This, in turn, was filtered and unbroken cells along with cell debris were removed by centrifugation at 1500 rpm for 15 min. The supernatant thus obtained was termed as a homogenate and used for the *in vitro* lipid peroxidation study. A lipid peroxidation study was conducted almost immediately after the homogenate preparation. 1 mL of each VLC fraction (100 mg) was added to 1 mL of the fish homogenate and mixed together. Thiobarbituric (TBA) reactivity in the homogenate

was determined by following a modified method of Luotola and Luotola.[17] 3 mL of 20% trichloroacetic acid was added, mixed, and centrifuged for 15 min. Then, TBA (0.67% w/v, 1 mL) was added to 2 mL supernatant, mixed, and kept in a boiling water bath for 10 min, which, after, cooled down to room temperature. TBA chromogen (intensity of the pink coloured complex) was measured at 532 nm against blanks using a ultraviolet spectrophotometer (Thermo spectronic, Genesys 20 model). Vitamin E was used as the reference. A graph of absorbance against concentration will be plotted using the data obtained for pure vitamin E. Thiobarbituric acid reactive substances (TBARS) of the extract were evaluated from the standard curve and expressed as nmol TBARS *per* mg of tissue. This procedure was repeated for the positive control (vitamin E, 25 mg/mL), negative control (5% Tween 80, 1 mL), column fractions, and the isolated compound (10 mg/mL). All results were replicated three times and the mean was determined.

Statistical analysis

All data collected from the entire study was analyzed using Microsoft excel and Statistical Package for Social Sciences (SPSS) version 17 (when needed). All values in the test were presented as mean ± standard error of mean (SEM). Statistical differences between the means of the various groups were evaluated by One-Way ANOVA and tested at 0.05 level of significance. The results were considered statistically significant if the *p* values were 0.05 or less.

RESULTS

Lipid peroxidation of bulked VLC fractions of the hexane fraction

From an earlier study described by Adedokun et al.[18], the *n*-hexane fraction was the most active aphrodisiac agent, *in vivo* and *in vitro* of the three fractions from the crude ethanol extract of *M. charantia.* The result of lipid peroxidation of the entire bulked VLC fractions (VLC-A to VLC-E) is shown in Table 1.

The values above represent the mean ± SEM of 5 replicates. Values with superscripts indicate significant different relative to the negative control (5% Tween 80) at $p \leq 0.05$ across the column for each time using One-Way ANOVA (non-parametric).

From Table 1, VLC-C showed the highest degree of inhibition of polyunsaturated fatty acids in the fish tissue over time with lowest and highest amounts of malondialdehyde observed at 0 min and 240 min as 98.00 ± 0.01 and 171.00 ± 0.08 respectively, significantly different from negative control at $p \leq 0.05$ at a similar time interval as shown in Table 1.

Lipid peroxidation of isolated compound and vitamin E

The result of comparative lipid peroxidation study of isolated compound X (10 mg/mL) with both vitamin E (26 mg/mL) as well as the negative control (5% Tween 80) is shown in Figure 1. No significant difference was observed in the activity of compound X (10 mg/mL) and positive control (vitamin E) 26 mg/mL using an *in vitro* model.

Spectroscopic analysis of compound X

Compound X was subjected to spectroscopic analysis to identify the nomenclature of the unknown bioactive compound, NMR studies (^{1}H, ^{13}C, DEPT, and HMBC) and MS, as shown in Figures 2-6.

DISCUSSION

Bioactivity-guided studies by VLC with the *n*-hexane, chloroform, and methanol mixtures gave five bulked VLC fractions: -VLC-A (2.8 g, 13.4%), VLC-B (2.6 g, 12.5%), VLC-C (3. 1 g, 14.7%), VLC-D (11.6 g, 55.2%), and VLC-E (0.8 g, 4.0%). In *in vitro* aphrodisiac screening of the VLC fractions (100 mg/mL each), ability of the fractions to reduce lipid peroxidation significantly increased with time (Table 1).

The potency of the fractions can be ranked as the following: VLC-C › VLC-D › VLC-A › VLC-B › VLC-E. Of all five VLC fractions, VLC-C had the highest potential in reducing the destruction of sperm, giving 171 nmoles malondialdehyde *per* gram of fish tissue in 240 min, which is less than 28 nmoles malondialdehyde *per* gram of fish tissue produced by the positive control, vitamin E, implying 16% potency.

VLC-C, upon open CC and elution with *n*-hexane, ethyl acetate, methanol mixtures gave six bulked fractions CC (A-E) of which

Table 1. ***In vitro*** **lipid peroxidation of bulked VLC fractions of hexane fraction**

Sample	n moles of malondialdehyde/gm tissue/time				
	0 min	60 min	120 min	180 min	240 min
VLC-A (100 mg/mL)	300.00 ± 0.01	324.00 ± 0.03	334.00 ± 0.01	335.00 ± 0.11	330.00 ± 0.13
VLC-B (100 mg/mL)	327.00 ± 0.21	448.00 ± 0.14	500.00 ± 0.16	548.00 ± 0.22	523.00 ± 0.20
VLC-C (100 mg/mL)	98.00 ± 0.01*	124.00 ± 0.07*	137.00 ± 0.09*	161.00 ± 0.09*	171.00 ± 0.08*
VLC-D (100 mg/mL)	206.00 ± 0.08	334.00 ± 0.04	224.00 ± 0.09	241.00 ± 0.05	290.00 ± 0.05
VLC-E (100 mg/mL)	647.00 ± 0.01	635.00 ± 0.03	633.00 ± 0.02	610.00 ± 0.04	614.00 ± 0.05
Vitamin E (positive control, 25 mg/mL)	25.17 ± 0.02*	40.67 ± 0.23*	27.92 ± 0.05*	28.33 ± 0.07*	28.08 ± 0.09*
5% Tween 80 (negative control, 1 mL)	275 ± 0.05	282 ± 0.03	295 ± 0.01	299 ± 0.06	302 ± 0.02

The values above are mean of three replicates. n= 3. n ± SEM. Samples with superscript * indicate significant difference at $p<0.05$ relative to negative control using One-Way ANOVA (Kruskal–Wallis test) while samples with no superscript * indicate no significant difference at $p<0.05$ relative to negative control using One-Way ANOVA (Kruskal–Wallis test). VLC: Vacuum liquid chromatography

CC-D (1 g) was the most active *in vitro.* A bioactive compound (50 mg) was finally isolated from CC-D by prep-TLC (hexane: ethyl acetate, 6:4). It gave R_f 0.54 (TLC silica gel, *n*-hexane: ethyl acetate; 2:3, brown on spraying with concentrated H_2SO_4). *In vitro* antilipid peroxidation bioassay of this compound showed a high degree of reduction of the peroxidation of PUFA that was stable over the experimentation period and parallel that of 25 mg of vitamin E (α-tocopherol) producing 30 nmoles malondialdehyde *per* gram fish tissue in 240 min (Figure 1).

Spectroscopic information from NMR studies (^1H, ^{13}C, DEPT, and HMBC) and MS suggested the bioactive compound to be a pentacyclic triterpene, 13, 14-epoxyoleanan-3-ol-acetate (M^+ 470, $C_{31}H_{50}O_3$) as shown in Figures 2-7 below.

This is the first time this compound has been reported in *M. charantia* and according to the literature, no direct pharmacological property has been associated with this compound.

To date, only the structurally-related oleanan compounds, soyasaponins I-III have been isolated from the Japanese *M. charantia.*[19] However, Venktesh et al.[20] recently reported the isolation of 3-hydroxy-21-normethyl-19-vinylidenylursane, an *in vivo* aphrodisiac compound, from a Bombacaceae plant, *Durio zibenthinus* fruit. A wide variety of cucurbita triterpenes and triterpene saponins have been isolated from *M. charantia.* Notable among these triterpenes are di- and tri-hydroxycucurbitadienes with antidiabetic activity, momordicolide (10E)-3-hydroxyl-dodeca-10-en-9-olide and momordicophenoide A, cucurbitane-type triterpenoid saponins such as momordicosides M, N, O, L, F1, F2, G, L, A, K, U, V, and W.[21-23] However, none of these triterpenes is associated with aphrodisiac activity.

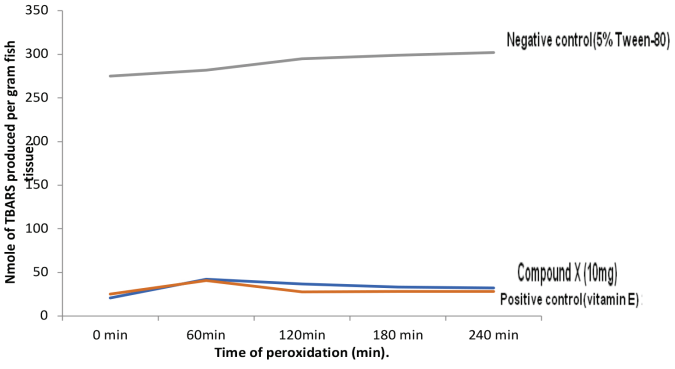

Figure 1. *In vitro* lipid peroxidation of bioactive compound and vitamin E (25 mg/mL)

According to Kumar et al.[10] *M. charantia* has been reported to negatively affect fertility in both male and female animals, without mention of any active constituent. The present study has established improvement in sexual activity of normal male rats by *M. charantia*. Apart from *M. charantia*, other Nigerian medicinal plants with reported *in vitro* lipid peroxidation activity are *Syzygium aromaticum* flower bud and *Fadogia agrestis* stem and *Terminalia catappa* seeds from elsewhere.[17,24-27] Reviews on aphrodisiac and male fertility enhancing plants have been published.[2,3,7,8] antioxidant activity of *M. charantia* might be connected with its ability to reduce lipid peroxidation, and hence protect sperm.[26,27] It therefore has potential for treating sperm-related male infertility. *M. charantia* is one of a traditional aphrodisiac plant that has not yet been fully explored. This study is a continuation of our investigation into the aphrodisiac activity of *M. charantia*.

CONCLUSION

This investigation establishes *in vitro* male fertility enhancing activity for the aerial part of *M. charantia* and suggested bioactivity to be due to a pentacyclic triterpene 13, 14-epoxyoleanan-3-ol-acetate isolate from the most active *n*-hexane fraction for the first time. It has not been previously isolated from Cucurbitaceae or elsewhere. The *in vitro* aphrodisiac activity of the compound compared with that of vitamin E. Its structure was confirmed by NMR, MS, and by comparison with a computer database. The study further lends credence to the ethnopharmacological claim

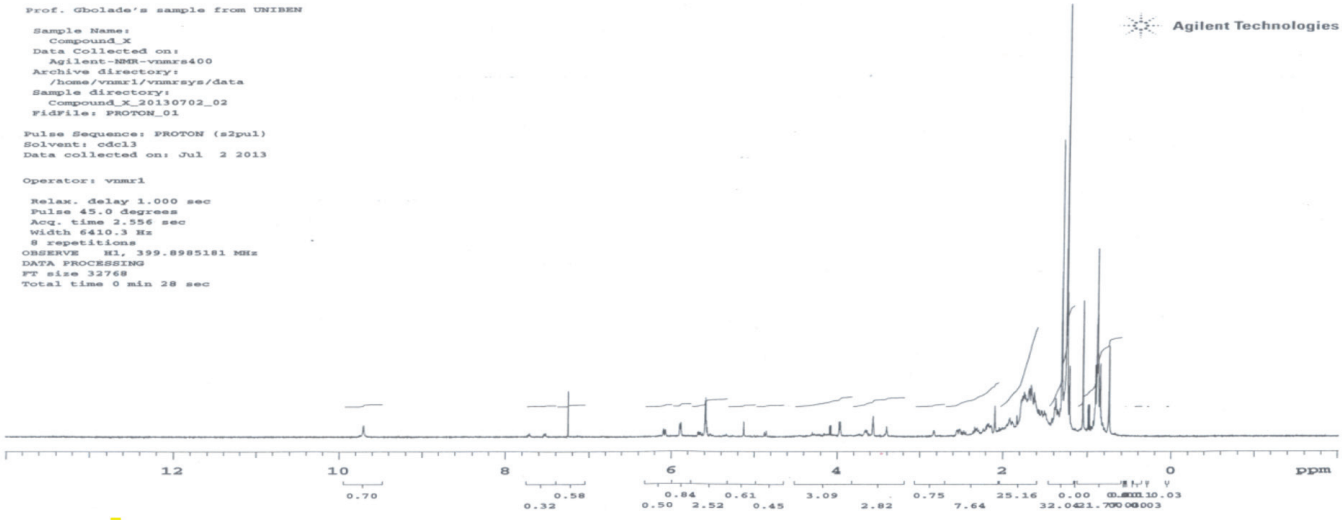

Figure 2. ^1H NMR of isolated compound X

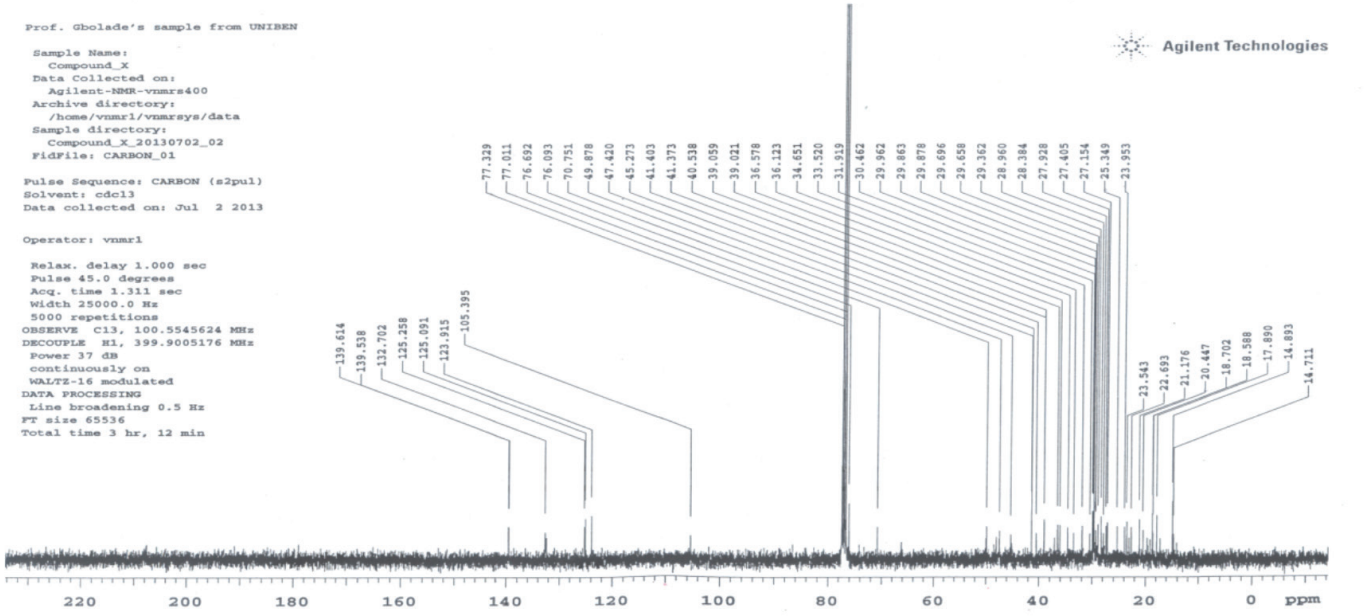

Figure 3. ^{13}C NMR of isolated compound X

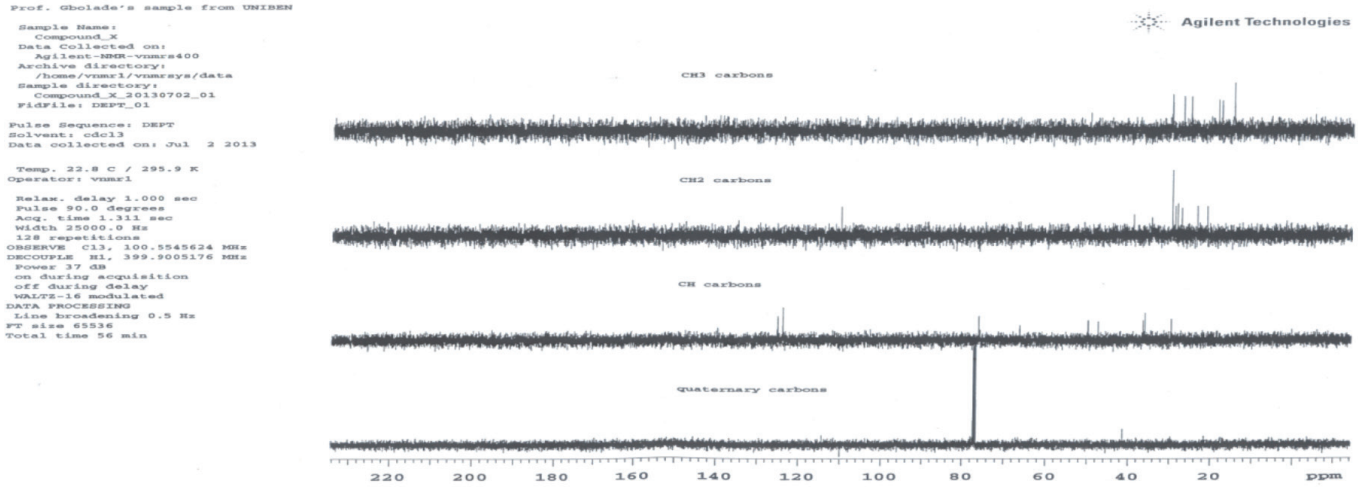

Figure 4. DEPT of isolated compound X

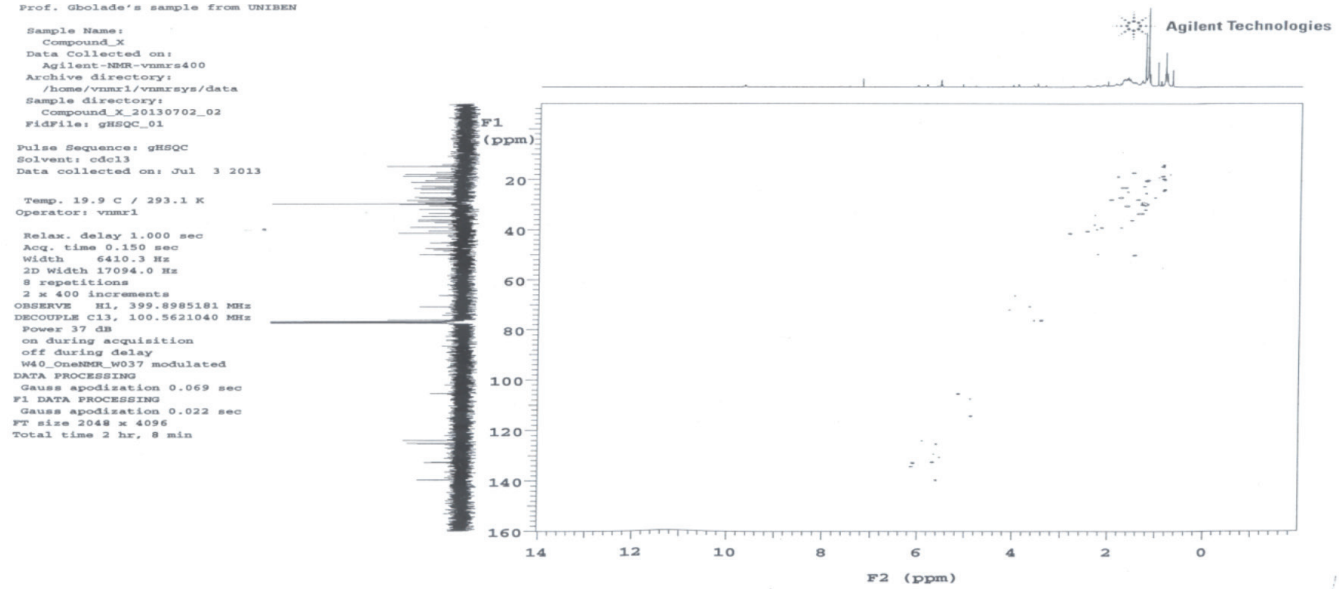

Figure 5. HMBC of isolated compound X

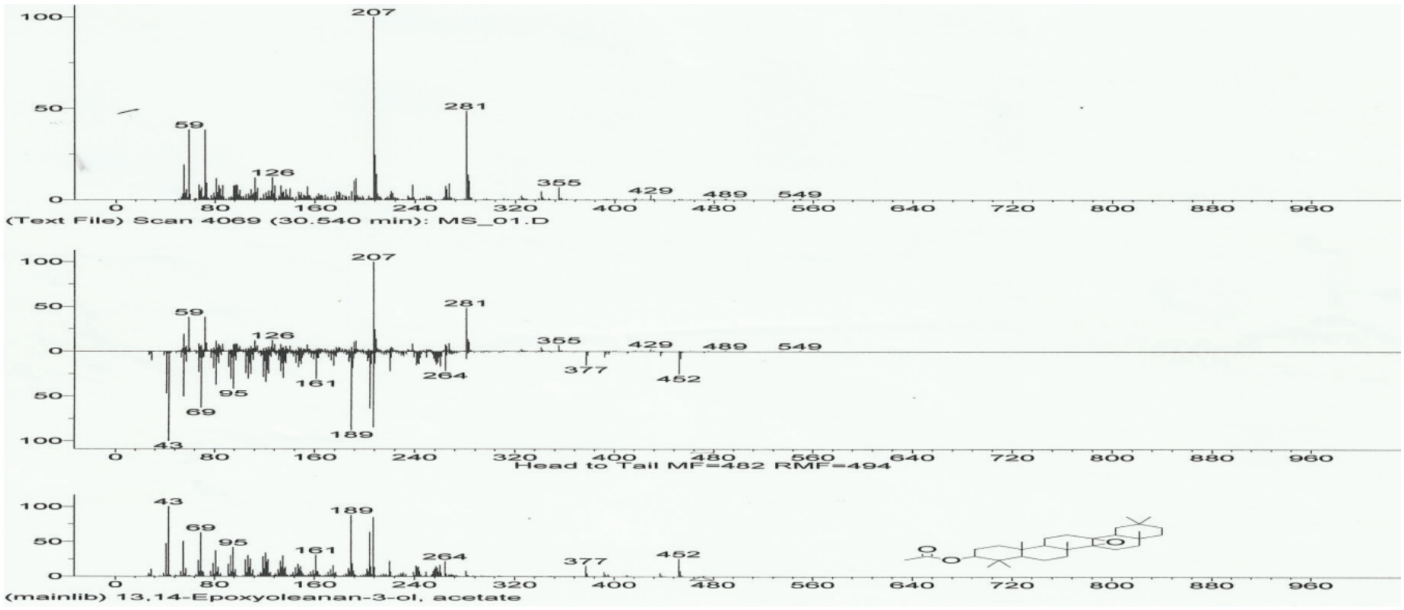

Figure 6. GC-MS of isolated compound X
GC-MS: Gas chromatography-mass spectrometry

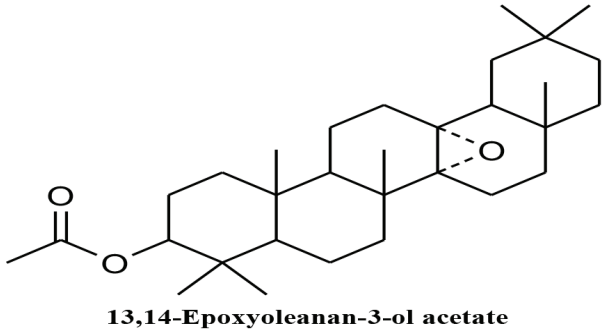

Figure 7. Suggested structure of compound X

of the plant as an aphrodisiac by the people of Esan Central Local Government Area of Edo State in Nigeria.

ACKNOWLEDGEMENTS

The authors are grateful to the Rev. Fr. Anslem Adodo and Paxherbal Laboratories, Ewu, Edo State, for access to laboratory facilities. We also thank the organizers of the WANNPRES 2014 congress for the opportunity given to present some findings of this research.

REFERENCES

1. Guay AT, Spark RF, Bansal S, Cunningham GR, Goodman NF, Nankin HR, Petak SM, Perez JB; American Association of Clinical Endocrinologists Male sexual dysfunction task force. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the evaluation and treatment of male sexual dysfunction: a couple's problem-2003 update. Endocr Pract. 2003;9:77-95. Erratum in: Endocr Pract. 2008;14:802-803.
2. Patel DK, Kumar R, Prasad SK, Hemalatha S. Pharmacologically screened aphrodisiac plant- a review of current scientific literature. Asian Pac J Trop Biomed. 2011;(Suppl)S131-S138.
3. Malviya N, Jain S, Gupta VB, Vyas S. Recent studies on aphrodisiac herbs for the management of male sexual dysfunction-a review. Acta Pol Pharm. 2011;68:3-8.
4. Dandekar SP, Nadkarni GD, Kulkarni VS, Punekar S. Lipid peroxidation and antioxidant enzymes in male infertility. J Postgrad Med. 2002;48:186-89; discussion 189-190.
5. de Lamirande E, Gagnon C. Reactive oxygen species and human spermatozoa. I. effects on the motility of intact spermatozoa and on sperm axonemes. J Androl. 1992;13:368-378.
6. Alvarez JG, Storey BT. Spontaneous lipid peroxidation in rabbit and mouse epididymal spermatozoa: dependence of rate on temperature and oxygen concentration. Biol Reprod. 1985;32:342-351.
7. Singh R, Singh S, Jeyabalan G, Ali A, Semwal A. Medicinal plants used to treat sexual dysfunction: a review. Int J Rec Adv Pharm Res. 2013a;3:29-35.
8. Singh R, Singh S, Jeyabalan G, Ali A. An overview of traditional medicinal plants as aphrodisiac agent. J Pharmacogn Phytochem. 2012;1:43-56.
9. Gupta M, Sharma S, Guatam AK, Bhadauria R. *Momordica charantia* Linn. (Karela): nature's silent healer: a review. Int J Pharm Sci Rev Res. 2011;11:32-37.
10. Kumar DS, Sharathnath KV, Yogeswaran P, Harani A, Sudhakar K, Sudha P and Banji D. A medicinal potency of *Momordica charantia*. Int J Pharm Sci Rev Res. 2010;1:95-100.
11. Li QY, Chen HB, Liu ZM, Wang B, Zhao YY. Cucurbitane triterpenoids from *Momordica charantia*. Magn Reson Chem. 2007;45:451-456.
12. Chen J, Tian R, Qiu M, Lu L, Zheng Y, Zhang Z. Trinorcucurbitane and cucurbitanetriterpenoids from the roots of *Momordica charantia*. Phytochemistry. 2008;69:1043-1048.
13. Leung L, Birtwhistle R, Kotecha J, Hannah S, Cuthbertson S. Antidiabetic and hypoglycaemic effects of *Momordica charantia* (bitter melon): a mini review. Br J Nutr. 2009;102:1703-1708.

14. Chang CI, Chen CR, Liao YW, Cheng HL, Chen YC, Chou CH. Cucurbitane-type triterpenoids from *Momordica charantia*. J Nat Prod. 2006;69:1168-1171.

15. Chang CI, Chen CR, Liao YW, Cheng HL, Chen YC, Chou, CH. Cucurbitane-type triterpenoids from the stems of *Momordica charantia*. J Nat Prod. 2008;71:1327-1330.

16. Yakubu MT, Akanji MA, Oladiji AT. Aphrodisiac potentials of aqueous extract of *Fadogia agrestis* (Schweinf. Ex Hiern.) stem in male Albino rats. Asian J Androl. 2005;7:399-404.

17. Luotola MT, Luotola JEI. Effect of α-tocopherol on the peroxidation of cod liver oil. Life Chem Reports. 1985;3:159-163.

18. Adedokun, OA, Gbolade, AA, Ayinde, BA. Effect of ethanol crude and solvent fractions of *Momordica charantia* Linn (Curcubitaceae) on male sexual performance. Niger J Pharm Sci. 2021.

19. Murakami T, Emoto A, Matsuda H, Yoshikawa M. Medicinal foodstuffs. XXI. Structures of new cucurbitane-type triterpene glycosides, goyaglycosides-a, -b, -c, -d, -e, -f, -g, and -h, and new oleanane-type triterpene saponins, goyasaponins I, II, and III, from the fresh fruit of Japanese *Momordica charantia* L. Chem Pharm Bull. 2001;49:54-63.

20. Venktesh P, Hariprasath K, Soumya V, Prince-Francis M, Sanker S. Isolation and aphrodisiac screening of the fruits of *Durio zibenthinus* Linn. Asian J Biol Sci. 2010;3:1-17.

21. Harinantenaina L, Tanaka M, Takoaoka S, Oda M, Mogami O, Uchida M, Asakawa Y. *Momordica charantia* constituents and antidiabetic screening of the isolated major compounds. Chem Pharm Bull. 2006; 54:1017-1021.

22. Liu Y, Ali Z, Khan IA. Cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia*. Planta Med. 2008;74:1291-1294.

23. Nguyen XN, Phan VK, Chau VM, Ninh KB, Nguyen XC, Le MH, Bui HT, Tran HO, Nguyen HT, Kim YH. Cucurbitane-type triterpene glycosides from the fruits of *Momordica charantia*. Magn Reson Chem. 2010;48:392-386.

24. Tajuddin, Ahmad S, Latif A, Qasmi IA. Effect of 50% ethanolic extract of *Syzygium aromaticum* (L.) Merr. & Perry. (clove) on sexual behaviour of normal male rats. BMC Complement Altern Med. 2004;4:17.

25. Ratnasooriya WD, Dharmasiri MG. Effects of *Terminalia catappa* seeds on sexual behaviour and fertility of male rats. Asian J Androl. 2000;2:213-219.

26. Lin KW, Yang SC, Lin CW. Antioxidant constituents from the stem and fruits of *Momordica charantia*. Food Chem. 2011;127:609-614.

27. Talukder EU, Aklima J, Bin Emran T, Islam S, Rahman A, Bhuiyan RH. *In vitro* antioxidant potential of *Momordica charantia* fruit extracts. Br J Pharm Res. 2013;3:963-971.