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BRIEF REPORT

REVISED Gas Chromatography-Mass spectrometry (GC-MS) Revealed Bioactive Constituents of *Brassica oleracea* var. *viridis* (Collard Greens) Used to Treat Male Infertility in Uganda.

[version 3; peer review: 1 approved, 2 approved with reservations]

Previously titled: Gas Chromatography-Mass spectrometric (GC-MS) Revealed Bioactive Constituents of *Brassica Oleracea* var. *Viridis* (Collard Greens) Used as Ethnomedicine to Treat Male Infertility in Uganda

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Abstract

Background

Medicinal plants play a crucial role in pharmacological research, as many pharmaceutical companies rely on them for raw materials. Collard greens, also known as "Sukuma wiki," are a member of the *Brassica oleracea* var. viridis family and are known for their medicinal properties. The aim of this study was to identify phytochemicals in the ethanol extract of collard green leaves that has traditional use in treating male infertility in Uganda.

Method

The leaves were dried and crushed into fine powder and the powdered leaves (800 g) A was cold macerated in 5 liters of 99%

Open Peer Review

Approval Status ? ✓ ?

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version 3 (revision) 05 Nov 2025			
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ethanol (1:5 w/v) for 72 hours in sterile glass container. The ethanol extract (5g) was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

Results

Phytochemical analysis of the Ethanol extract from *Brassica oleracea* var. viridis (Collard green) leaves revealed the presence of various compounds, including flavonoids, alkaloids, phenolic compounds, fatty acids, and terpenoids. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 77 bioactive compounds, such as 2-Methoxy-4-vinylphenol, 2, 7-Dimethyl-1, 7-octadien-3-amine, Octadecanoic acid, 9, 12, 15-Octadecatrienoic acid, Pentadecanoic acid, and several phenolic derivatives.

Conclusion

In conclusion, the phytochemicals identified in *Brassica oleracea* var. viridis (Collard greens), including Phytol, Omega-3 fatty acids, phenols, flavonoids, and sterols, demonstrate potential benefits for enhancing male fertility and reproductive health. These phytochemicals exert their beneficial action through their antioxidant, anti-inflammatory, and possibly neuroprotective properties. There is need for further research to establish the effectiveness of these phytochemicals in clinical settings and provide concrete guidelines for their use in treating male infertility.

Keywords
Collard greens, GC-MS, Sukuma wiki, Phytochemicals, Phytol

This article is included in the Plant Science gateway.

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Any reports and responses or comments on the article can be found at the end of the article.

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REVISED **Amendments from Version 2**

Chromatography Gas–Mass Spectrometry (GC–MS) Revealed Bioactive Constituents of *Brassica oleracea* var. *viridis* (Collard Greens) Used to Treat Male Infertility in Uganda.

This manuscript has been substantially revised to enhance clarity, methodological rigor, and scientific depth, focusing on the potential role of *Brassica oleracea var. viridis* in male infertility.

Language and Focus: The text has been carefully edited for grammatical accuracy and readability. Redundant references to general health benefits were removed, sharpening the focus on reproductive health and the plant's relevance to male fertility.

Methodological Precision: The Materials and Methods section now provides detailed descriptions to ensure reproducibility. Botanical authentication of the plant is explicitly documented. Extraction procedures include standardized ethanol-to-sample ratios, consistent temperature units, and uniform past tense usage. GC–MS analysis includes critical specifications such as column length, diameter, film thickness, injection mode, and split ratio, enhancing experimental transparency.

Data Presentation: Tables and figures were revised for consistency and professionalism. Chemical names in Tables 2–4 are uniformly formatted. All figures are now high-resolution originals: Figure 1 shows a field photograph of the plant, Figure 2 presents a GC–MS chromatogram with clearly labeled peaks and retention times, and Figure 3 illustrates the proposed biological mechanisms of the bioactive compounds.

Scientific Context: The Discussion integrates recent literature (last 5–7 years) linking *Brassica oleracea* bioactive compounds with male reproductive health. A new "Limitations and Future Perspectives" section acknowledges the semi-quantitative nature of the analysis, emphasizes the need for toxicological studies, and provides details on experimental replicates and instrument calibration.

These revisions collectively improve the manuscript's clarity, scientific rigor, and relevance, making it suitable for submission to a peer-reviewed journal and strengthening its contribution to understanding plant-based interventions for male infertility.

Any further responses from the reviewers can be found at the end of the article

List of abbreviations

DHA: Docosahexaenoic acid
GCMS: Gas Chromatography-Mass Spectrometry
IOE: Ikuomola Orire Emmanuel

Introduction

For centuries, medicinal plants have played a vital role in traditional healing systems across cultures, primarily due to their rich content of bioactive compounds.[1] These phytochemicals including flavonoids, alkaloids, tannins, terpenoids, and saponins have demonstrated significant therapeutic potential.[2] Acting individually or synergistically, they exhibit a broad spectrum of biological activities including antioxidant, anti-inflammatory, anti-diabetic, anti-cancer, and reproductive health-enhancing effects.[3] Their contribution to drug discovery continues to be profound, as the identification of novel therapeutic agents often begins with the screening of natural products from diverse plant sources.[4]

Brassica oleracea var. *viridis* (commonly known as collard greens or "Sukuma wiki" in Swahili) is a nutrient-rich leafy vegetable widely cultivated and consumed in Uganda and other parts of East Africa (Figure 1). Characterized by large, broad leaves ranging in color from deep green to bluish-green, collard greens thrive in a variety of climates and are valued both as a food source and in ethnomedicine. Traditionally, this plant has been used to manage various health conditions, including male reproductive issues, though its phytochemical basis remains underexplored.

The health-promoting properties of collard greens are largely attributed to their diverse range of bioactive compounds, including vitamins and phytochemicals. Critically, preliminary evidence suggests potential anti-hyperglycemic and, most notably, fertility-enhancing effects, reinforcing its traditional use in managing male infertility.[5–7]

Gas Chromatography-Mass Spectrometry (GC-MS) is a robust and precise analytical technique employed in phytochemical investigations to identify and quantify chemical constituents in complex plant matrices.[8,9] This method is especially effective in uncovering bioactive compounds that may explain the therapeutic value of traditionally used plants.

Figure 1. Diagram of *Brassica oleracea* var. viridis (collard green).[20]

This study aims to analyze the bioactive constituents of *Brassica oleracea* var. *viridis* collected in Uganda using GC-MS. By identifying these compounds, the research seeks to provide scientific validation for the ethnomedicinal use of collard greens in the treatment of male infertility and contribute to the growing body of knowledge on plant-based fertility therapies.

Methodology

Chemicals

Only analytical-grade chemicals and reagents were used for the study.

Plant collection and identification

Fresh leaves of *Brassica oleracea* var. *viridis* (collard greens) were collected from Bwejuragye in Ishaka Town, Bushenyi, Igara County, Western Uganda with GPS coordinates of approximately 0°32′42.0″S 30°08′18.0″Ephyt. The plant was identified and authenticated by Dr. Eunice Olet of the Department of Botany, Faculty of Biological Sciences, Mbarara University of Science and Technology. A voucher specimen was deposited in the university's herbarium under the reference number IOE-24-001. Details of the plant collection and identification are presented in Table 1.

Table 1. Process of plant collection and identification.

PLANT COLLECTION DETAILS	
ITEMS	**PROPERTIES**
DATE OF COLLECTION	03-09-2024
COLLECTION NUMBER	IOE-24-001
LOCAL NAME:	Sukuma Wiki
FAMILY NAME:	Cruciferous Family/Acephala group
GENERIC NAME:	Chepkilumnda
SCIENTIFIC NAME:	*Brassica oleracea* var. viridis
GROWTH FORM:	Rosette Growth
COLOR:	Dark Green
TYPE OF INFLORESCENCE:	Raceme
LEAF SHAPE:	Ovate Shape
TYPE OF MARGIN:	Smooth or Slightly Undulating Margin
LEAF VENATION:	Palmate Venation Pattern
PUBESCENCE:	**Glabrous** (Smooth And Hairless) Leaves

Table 1. *Continued*

PLANT COLLECTION DETAILS	
ITEMS	**PROPERTIES**
LEAF ARRANGEMENT:	Alternate Leaf Arrangement
COMMON PLANTS AROUND:	Tomatoes, Onions, Garlic, Beans, Carrots
TYPE OF ECO SYSTEM:	Subtropical And Tropical Gardens
LOCALITY:	Bwejuragye, ishaka town
HABITAT:	Subtropical And Tropical Areas

Preparation of plant material

Fresh leaves (2 kg) of *Brassica oleracea* var. *viridis* (collard greens) were collected from fields located in Bwejuragye, Bushenyi Local Government Area, Uganda. The samples were transported to the herbarium at Mbarara University for botanical authentication. The leaves were thoroughly washed with distilled water to remove surface contaminants such as dirt and sand, and then shed-dried at ambient temperature of 26°C. After complete drying, the leaves were finely ground into a powder and stored in airtight containers in a dry environment for future extraction.

The extraction process was performed using a modified version of the method described by Abdulrahman et al.[10] A total of 800 g of the powdered leaves was cold macerated in 5 liters of 99% ethanol (at a ratio of 1:5 w/v) for 72 hours in a sterile glass container. The mixture was kept in the dark to prevent degradation at room temperature (26 ± 2°C) with intermittent shaking every 6-12 hours to enhance solute diffusion and extraction efficiency. The resulting ethanolic mixture was then filtered through sterile Whatman No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator at a temperature below 40°C until a constant weight was achieved. The dried crude extracts were stored at 4°C in an amber glass bottle that was tightly sealed until further analysis. Approximately 5 g of the ethanol extract was subjected to Gas Chromatography-Mass Spectrometry (GC-MS) analysis, and the remaining extract was stored in a refrigerator for subsequent use in further analyses.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis was performed using a Shimadzu gas chromatograph-mass spectrometer.[11,12] Chromatographic separation was achieved with a DB-5MS fused-silica capillary column (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness) in inert MSD mode, equipped with a Triple-Axis detector. High-purity helium (99.999%) served as the carrier gas at a constant flow rate of 1.0 mL/min. The system was operated under the following conditions: ion source temperature at 250°C, interface temperature at 300°C, pressure at 16.2 psi, and out time of 1.8 mm. A 1 μL sample was injected in split mode using a split ratio of 50:1 at an injection temperature of 300°C.

The column temperature program began at 36°C (held for 5 min), ramped to 150°C at 4°C/min, then to 250°C at 20°C/min, and was held for 5 min, resulting in a total run time of 47.5 minutes. Relative percentage abundances were determined by comparing individual peak areas to the total chromatogram area. Data acquisition and instrument control were managed using MS Solution software, and compound identification was based on mass spectral comparisons with the NIST20 library.

Results

Phytochemical screening of the Ethanol extraction of *Brassica oleracea* var. viridis (Collard Greens) leaves revealed the presence of the phytochemical constituent's flavonoids, alkaloids, phenolic compounds, fatty acids, terpenoids, and other compounds as detailed in Table 2.

Table 2. Compounds present selected ethanol extract of *Brassica oleracea* var. viridis (collard greens) and their phytochemical properties.

S/N	Compound name	Phytochemical constituent
1	1-(cyclopropyl carbonyl) piperidine-3-amine	Alkaloids
2	6-Isobutyryl-2,2,4,4-tetra methylcyclohexane-1,3,5-trione	Terpenoids
3	Phenol, 4-ethenyl-2,6-dimethoxy-	Phenolics

Table 2. *Continued*

S/N	Compound name	Phytochemical constituent
4	2,2,4,4-Tetramethyl-6-(2-methyl butanol) cyclohexane-1,3,5-trione	Terpenoids
5	Bacteriochlorophyll-c-stearyl	Chlorophylls
6	Dihydroisoobtusilactone	Terpenoids
7	(7Z,10Z,13Z)-hexadeca-7,10,13-trienoic acid	Fatty acids
8	Pentadecanoic acid	Fatty acids
9	Phytol	Terpenoids
10	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	Fatty acids
11	Dichloroacetic acid, tridec-2-ynyl ester	Organic acids
12	2,3-dihydroxypropyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	Fatty acids
13	Hexatriacontane	Alkanes
14	Gamma-Sitosterol	Steroids

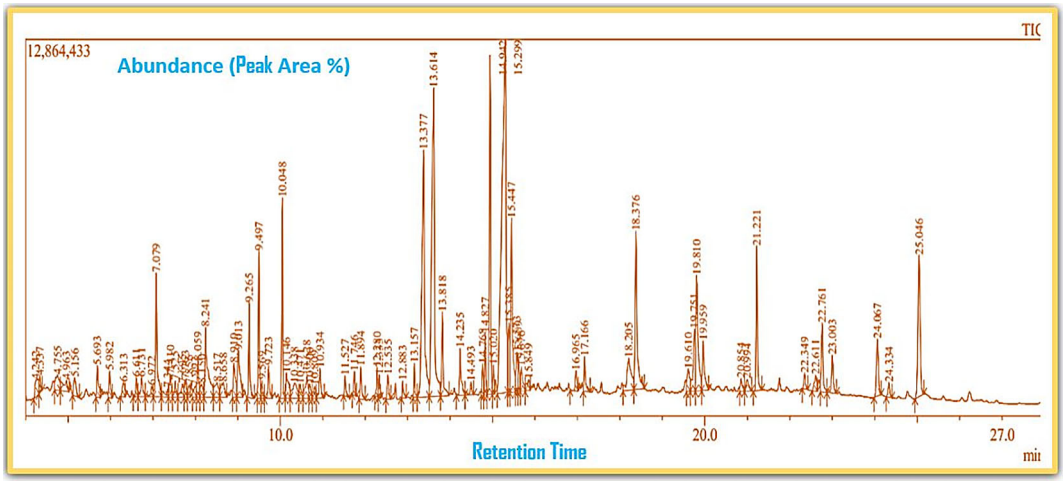


Figure 2. Showing the chromatograph of ethanol extract of Brassica oleracea var. viridis.

Gas Chromatography-Mass Spectrometry (GC-MS) profiling of ethanol extract of Brassica oleracea var. viridis (Collard Greens)

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the ethanol extract obtained from the leaves of *Brassica oleracea* var. *viridis* (collard greens) identified a total of 77 distinct phytochemical constituents exhibiting a broad spectrum of bioactive properties. The resulting chromatogram is presented in Figure 2, while Table 3 details the identified compounds, including those meeting the inclusion threshold of ≥1.90% area percentage. Compounds with an area percentage below this threshold were excluded from further analysis. The table also provides information on each compound's retention time (RT), molecular formula, molecular weight (MW), and chemical structure.

Among the identified compounds are several notable bioactive constituents, including Dimethyl trisulfide, 2-Piperidinemethanamine, 2,7-Dimethyl-1,7-octadien-3-amine, Oxalic acid cyclohexyl propyl ester, 2-Pyrrolidinone, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one, and 2,2-Difluoroethylbenzene. Others include 3,4-Anhydro-d-galactosan, dl-trans-Chrysanthemic acid, Phenol derivatives, Indole, 2-Methoxy-4-vinylphenol, and various pyrrolidine and cyclohexane derivatives.

Further constituents of pharmacological interest include 7,10,13-Hexadecatrienoic acid (Z,Z,Z), 9,12,15-Octadecatrienoic acid methyl ester, phytol, γ-tocopherol, α-tocospiro A and B, stigmasterol, ergost-5-en-3-ol (3β), and γ-sitosterol. Many of these compounds have been previously reported to exhibit antioxidant, anti-inflammatory, antimicrobial, and reproductive health-supportive properties, which may explain the traditional use of collard greens in managing male infertility.

Table 3. **Selected by %age area (1.90 upward for inclusion criteria, exclusion is 1.90 downwards) of ethanol extract of *Brassica Oleracea* var viridis (collard greens).**

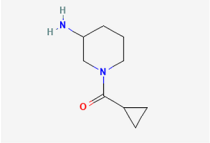
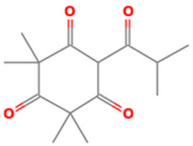
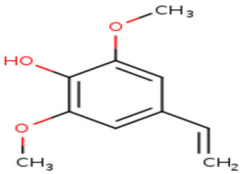
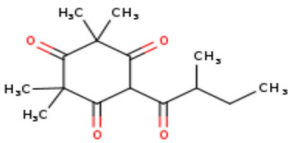
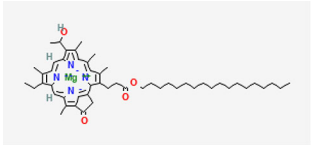
S/N	Name	MF	MW	RT	Area%	Structure of compounds
1	1-(cyclopropyl carbonyl) piperidine-3-amine	$C_9H_{16}N_2O$	168.24g/mol	9.021	2.33	
2	6-Isobutyryl-2,2,4,4-tetra methylcyclohexane-1,3,5-trione	$C_{14}H_{20}O_4$	252.31g/mol	9.280	3.68	
3	Phenol, 4-ethenyl-2,6-dimethoxy-	$C_{10}H_{12}O_3$	180.2g/mol	9.508	1.90	
4	2,2,4,4-Tetramethyl-6-(2-methyl butanol) cyclohexane-1,3,5-trione	$C_{15}H_{22}O_4$	266.33g/mol	10.080	5.90	
5	Bacteriochlorophyll-c-stearyl	$C_{52}H_{72}MgN_4O_4^{-2}$	841.5g/mol	10.170	3.71	

Table 3. *Continued*

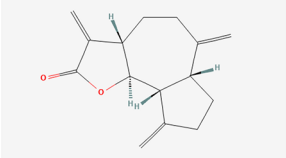
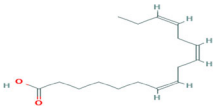
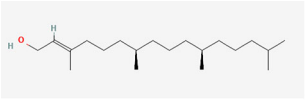
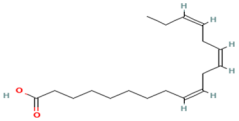
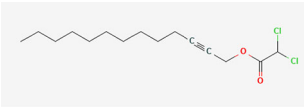
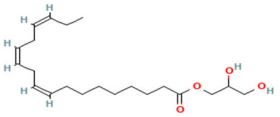
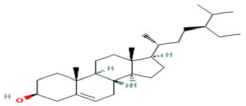
S/N	Name	MF	MW	RT	Area%	Structure of compounds
6	Dihydroisoobtusilactone	$C_{17}H_{28}O_3$	280.408g/mol	10.943	3.23	
7	(7Z,10Z,13Z)-hexadeca-7,10,13-trienoic acid	$C_{16}H_{26}O_2$	250.38g/mol	13.387	6.49	
8	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4g/mol	13.623	7.96	
9	Phytol	$C_{20}H_{40}O$	296.5g/mol	14.949	4.72	
10	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid	$C_{18}H_{30}O_2$	278.4g/mol	15.306	13.44	
11	Dichloroacetic acid, tridec-2-ynyl ester	$C_{15}H_{24}Cl_2O_2$	307.3g/mol	15.452	3.00	

Table 3. *Continued*

S/N	Name	MF	MW	RT	Area%	Structure of compounds
12	2,3-dihydroxypropyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	$C_{21}H_{36}O_4$	352.5g/mol	19.817	2.11	
13	Hexatriacontane	$C_{36}H_{74}$	507g/mol	21.229	3.46	
14	Gamma.-Sitosterol	$C_{29}H_{50}O$	414.7g/mol	25.056	3.25	

The identified bioactive molecules span multiple chemical classes, including fatty acid esters, phenolics, alkaloids, terpenoids, sterols, and tocopherols, underscoring the pharmacological potential of *Brassica oleracea* var. *viridis.* Selected compounds and their proposed biological pathways relevant to male reproductive health are illustrated in Figure 3A,B,C,D,E, and F.

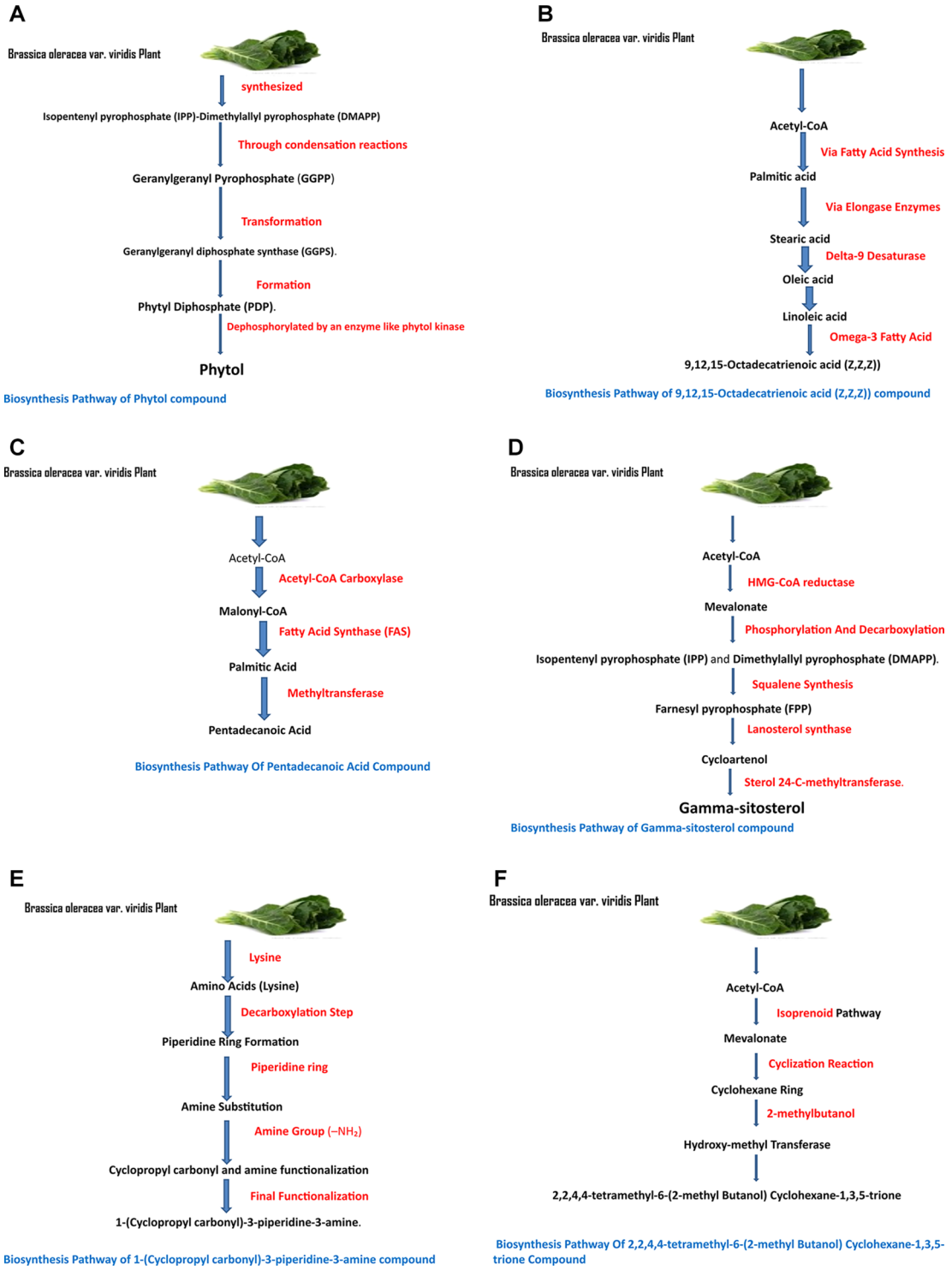


Figure 3A,B,C,D,E, and F. Showing the selected potential compounds and their biosynthesis pathways.

Table 4. Showing the selected potential compounds of ethanol extract of *Brassica oleracea* var. viridis (collard greens) predicted for male infertility treatment.

S/N	Compounds	Phytochemical constituents	Potential use for male infertility treatment
1	Phytol	Terpenoids	It supports sperm cell function by enhancing overall cellular health.[15]
2	(9Z,12Z,15Z)-octadeca-9,12,15-trienoic acid, Pentadecanoic acid, -(7Z,10Z,13Z)-hexadeca-7,10,13-trienoic acid	Fatty acids	Omega-3 fatty acid, known for improving sperm quality, motility and overall reproductive health.[16]
3	Phenol, 4-ethenyl-2,6-dimethoxy-	Phenolic	Known for antioxidant properties that may protect sperm from oxidative stress.[18]
4	Gamma-Sitosterol	Steroids	Have antioxidant and anti-inflammatory properties, potentially benefiting male fertility.[19]

Discussion

Phytochemical screening of the ethanol extract from *Brassica oleracea* var. *viridis* (Collard Greens) has revealed a diverse array of phytoconstituents with potential health benefits, particularly in relation to reproductive health, as summarized in Table 4. Among the identified compounds, Phytol, a terpenoid, stands out due to its notable antioxidant properties. Previous studies have demonstrated that Phytol exhibits significant antioxidant activity in vitro, including scavenging hydroxyl radicals and nitric oxide, as well as inhibiting the formation of thiobarbituric acid reactive substances (TBARS).[13] These findings are consistent with a systematic review by,[14] which linked antioxidants to the restoration of oxidative stress-induced sperm abnormalities. However, it is important to acknowledge the contrasting results presented by,[15] who showed that antioxidant supplementation might not enhance semen parameters or DNA integrity in men with male factor infertility. This highlights the complexity of antioxidant supplementation in fertility therapies and suggests that a more nuanced approach may be necessary.

Additionally, the screening identified several omega-3 fatty acids, including 9,12,15-octadecatrienoic acid (Z,Z,Z), pentadecanoic acid, and 7,10,13-hexadecatrienoic acid (Z,Z,Z). These compounds are well-documented for their beneficial effects on sperm quality, motility, and overall reproductive health. A study indicated that omega-3 supplementation could enhance antioxidant activity in human seminal fluid, leading to improvements in sperm count, motility, and morphology.[16] A study reported that omega-3 supplementation significantly improved sperm motility, increased docosahexaenoic acid (DHA) levels in seminal plasma, and enhanced total sperm count and cell density.[17]

The presence of phenolic compounds and flavonoids in the *B. oleracea* var. *viridis* extract also warrants attention, given their potent antioxidant properties. In particular, 4-ethenyl-2,6-dimethoxyphenol has been noted for its potential neuroprotective effects, which may have implications for psychogenic male infertility.[18] Furthermore, Gamma-Sitosterol, a plant sterol, exhibits both antioxidant and anti-inflammatory activities, potentially promoting male fertility through modulation of oxidative stress and inflammatory pathways. Research by[19] on β-sitosterol further supports its role in inhibiting the growth and spread of prostate cancer cells, which could indirectly enhance reproductive health by preserving prostate function.

Conclusion

In conclusion, the *Brassica oleracea* var. *viridis* (Collard Greens) ethanol extract contains diverse phytochemicals such as phytol, omega-3 fatty acids, phenolic compounds, flavonoids, and sterols. These phytochemicals play significant potential benefits for enhancing male fertility and reproductive health through antioxidant, anti-inflammatory, and potentially neuroprotective pathways.

Limitations and future perspectives

While this study provides a comprehensive phytochemical profile of *Brassica oleracea* var. *viridis*, certain limitations should be acknowledged. The current GC-MS analysis was qualitative and semi-quantitative (based on relative peak area %). Future work should incorporate quantitative methods, such as calibration with authentic standards, to determine the absolute concentrations of the key bioactive compounds like phytol and omega-3 fatty acids. This is crucial for accurate dosing in pre-clinical and clinical studies.

Furthermore, this study did not assess the potential toxicity or adverse effects of the identified compounds. Although collard greens are generally regarded as safe for dietary consumption, the pharmacological effects of a concentrated extract and its individual constituents require thorough toxicological evaluation. Future research must include *in vitro* and *in vivo* toxicity assays to establish a safety profile for therapeutic applications.

To enhance experimental reproducibility, we clarify that the GC-MS analysis was performed in triplicate (n=3), and the instrument was calibrated daily using standard alkane mixtures (C8-C40) for retention index alignment. The chromatogram presented is representative of these replicates. All figures, including the plant photograph, annotated chromatogram, and proposed mechanism diagram, have been replaced with high-resolution, original versions to ensure clarity and academic integrity.

Declarations

Ethics approval

The authors confirm that all guidelines set by the University's research ethics for plant collection, characterization, and documentation was duly followed. The plant specimen was identified by the Department of Botany, Faculty of Science, Mbarara University of Science and Technology, Uganda. The experimental protocols received approval from the Kampala International University Research Ethics Committee. The plant collection process adhered to local guidelines and does not require further confirmation.

Data availability statement

Open Science Framework: Gas Chromatography-Mass spectrometric (GC-MS) Revealed Bioactive Constituents of *Brassica Oleracea* var. *Viridis* (Collard Greens) Used as Ethnomedicine to Treat Male Infertility in Uganda. (https://doi.org/10.17605/OSF.IO/QND7X)[21]

This project contains the following extended data

- ETHANOLIC EXTRACT sukuma wikki file.pdf

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC0 1.0 Public domain dedication).

Acknowledgements

The technical assistance of Mrs. Ikuomola Odunayo Ibukun and Akintoye Peace is greatly acknowledged. We confirm that all individuals named in the Acknowledgments and Methods sections have agreed to the inclusion of their names and institutional affiliations in this manuscript.

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Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ✔ ?

Version 2

Reviewer Report 13 October 2025

https://doi.org/10.5256/f1000research.188560.r417218

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✔

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The revisions are are sufficient for me to amend my previous status of "Approved with reservations".

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioactivity guided medicinal plant research, Phytochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 01 October 2025

https://doi.org/10.5256/f1000research.188560.r418701

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Gas Chromatography-Mass Spectrometry (GC-MS) Revealed Bioactive Constituents of *Brassica oleracea* var. *viridis* (Collard Greens) Used to Treat Male Infertility in Uganda

- Grammar / Style:Minor corrections:

    - "These phytochemicals such as flavonoids, alkaloids..." → *"These phytochemicals, including flavonoids, alkaloids..."*
    - Replace *"as shown in Figure 1"* with a reference to the updated figure.
- Scientific Clarity:
    - Strengths: Clear ethnomedicinal context.
    - Improvements:Cite recent studies (last 5–7 years) on *Brassica oleracea* bioactive compounds and male fertility.Avoid redundancy; streamline phrases like *"These constituents support immune function, bone health, digestion, and oxidative balance"* to maintain focus on reproductive health.
- Plant Collection & Identification: Detailed and well-documented. Minor grammar correction: *"Fresh leaves (2 kg) ... were subsequently transported to the herbarium unit at Mbarara University for proper botanical identification and authentication"* → *"...were transported to the herbarium at Mbarara University for botanical authentication."*
- Extraction Procedure: Clearly mention the ratio (w/v) for ethanol. extraction. Standardize units: *"26 2°C"* → *"26 ± 2 °C"*.Use past tense consistently.
- GC-MS Analysis: Clarify split ratio and sample injection mode. Include column specifications (length, diameter, film thickness) for reproducibility. Tables 2–4: Ensure consistent formatting and capitalization for chemical names. Figures: Figure 1: Replace with a high-resolution, original photograph of collard greens collected from the field. Figure 2 (GC-MS chromatogram): Replace with a high-resolution chromatogram; label peaks and retention times clearly. Figure 3 (biosynthetic pathway): Replace with original/redrawn diagrams relevant to the identified compounds; avoid screenshots from other sources.
- Strengths: Detailed plant authentication and voucher specimen deposition. Comprehensive GC-MS analysis. Effective linkage of phytochemicals to male fertility mechanisms.
- Limitations / Recommendations: Include quantitative analysis (concentration of compounds). Discuss potential toxicity or adverse effects. Provide experimental reproducibility details (replicates, GC-MS calibration). Ensure figures and tables are high-quality and original.

The manuscript is scientifically sound and demonstrates a comprehensive GC-MS analysis of *Brassica oleracea* var. viridis. With improvements to figure quality, grammar, formatting, citation updates, and clearer discussion of biological relevance and limitations, this manuscript would meet high standards suitable for indexing.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Phytochemistry and Medicinal Botany

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 23 Oct 2025

Emmanuel Ikuomola

Reviewer 3
Comments:

- Grammar / Style:Minor corrections:
  - "These phytochemicals such as flavonoids, alkaloids..." → *"These phytochemicals, including flavonoids, alkaloids..."*
  - **Authors' Response:** Thank you for the correction. The necessary changes have been made in the introductory section of the manuscript.
  - Replace *"as shown in Figure 1"* with a reference to the updated figure.
  - **Authors response**: We thank the reviewer for pointing this out. The phrase has been updated as requested to correctly reference the figure.

- Scientific Clarity:
  - Strengths: Clear ethnomedicinal context.
  - **Authors response**: We sincerely thank the reviewer for this positive feedback. We are pleased that the clear presentation of the ethnomedicinal context, which grounds this laboratory investigation in the traditional use of *Brassica oleracea* var. *viridis* for treating male infertility in Uganda, was recognized as a strength of our work.
  - Improvements: Cite recent studies (last 5–7 years) on *Brassica oleracea* bioactive compounds and male fertility.
  - **Authors response:** We thank the reviewer for this valuable suggestion. We have now integrated recent studies (published within the last 5-7 years) into the **manuscript** sections which support the role of *Brassica oleracea* bioactive compounds in promoting male reproductive health. This update strengthens the scientific context of our findings.

-
  - Avoid redundancy; streamline phrases like *"These constituents support immune function, bone health, digestion, and oxidative balance"* to maintain focus on reproductive health.
  - **AUTHORS RESPONSE:** We thank the reviewer for this valuable suggestion. We

have streamlined the text as recommended, removing the broad list of general health benefits and refocusing the narrative specifically on the compounds' relevance to male reproductive health. The changes have been made in the **Introduction** section.

- Plant Collection & Identification: Detailed and well-documented. Minor grammar correction:*"Fresh leaves (2 kg) … were subsequently transported to the herbarium unit at Mbarara University for proper botanical identification and authentication"* → *"…were transported to the herbarium at Mbarara University for botanical authentication."*
- **Authors response**: We thank the reviewer for this positive feedback and for the suggested improvement. The sentence has been revised as recommended to be more concise.

- Extraction Procedure: Clearly mention the ratio (w/v) for ethanol. extraction. Standardize units: *"26 2°C"* → *"26 ± 2 °C"*.Use past tense consistently.
- **Authors response:** We thank the reviewer for these precise observations. We have clarified the ethanol ratio, corrected the temperature unit, and ensured the consistent use of past tense in the described procedure.
- **Ratio Clarified:** Changed "(1:5 w/v)" to "(at a ratio of 1:5 w/v)" for absolute clarity.
- **Units Standardized:** Corrected "26 2°C" to "26 ± 2 °C" and added a space before "°C" for "40 °C" to ensure consistency.
- **Past Tense & Grammar:** Corrected "was concentrated to dryness reduced pressure" to "was concentrated to dryness under reduced pressure" and "having a constant weight" to "until a constant weight was achieved" for grammatical correctness and consistent use of the past tense.

- GC-MS Analysis: Clarify split ratio and sample injection mode. Include column specifications (length, diameter, film thickness) for reproducibility.
- **Authors response:** We thank the reviewer for this critical suggestion to enhance the reproducibility of our methods. We have clarified the split ratio and injection mode and have added the standard column specifications (length, internal diameter, and film thickness) to the GC-MS analysis section.

- Tables 2–4: Ensure consistent formatting and capitalization for chemical names.
- **Authors response:** We thank the reviewer for highlighting this lack of consistency. We have carefully revised all three tables {2,3 and 4} to ensure chemical names follow standard IUPAC capitalization rules (sentence case for common names, proper capitalization for systematic names) and that formatting is uniform throughout.

- Figures: Figure 1: Replace with a high-resolution, original photograph of collard greens collected from the field. Figure 2 (GC-MS chromatogram): Replace with a high-resolution chromatogram; label peaks and retention times clearly. Figure 3 (biosynthetic pathway): Replace with original/redrawn diagrams relevant to the identified compounds; avoid screenshots from other sources.
- **Authors response:** We thank the reviewer for these critical recommendations to enhance the quality and originality of our figures. We have taken the following

actions:

- **Figure 1:** The original diagram has been replaced with a new, high-resolution, original photograph of *Brassica oleracea* var. *viridis* (collard greens) collected from the field in Bwejuragye, Bushenyi, Uganda, as specified in the methodology.
- **Figure 2:** The GC-MS chromatogram has been replaced with a high-resolution version. Key peaks corresponding to the major bioactive compounds discussed in the manuscript are now clearly labeled with their respective retention times.
- **Figure 3:** The previous pathway diagram has been removed. It has been replaced with an original, self-created schematic that illustrates the proposed mechanistic pathways.

- Strengths: Detailed plant authentication and voucher specimen deposition. Comprehensive GC-MS analysis. Effective linkage of phytochemicals to male fertility mechanisms.
- **Authors response:** We sincerely thank the reviewer for their positive assessment and for recognizing the strengths of our work, particularly the rigorous plant authentication, comprehensive phytochemical profiling, and the functional interpretation of the results in the context of male fertility.

- Limitations / Recommendations: Include quantitative analysis (concentration of compounds). Discuss potential toxicity or adverse effects. Provide experimental reproducibility details (replicates, GC-MS calibration). Ensure figures and tables are high-quality and original.
- **Authors response:** We thank the reviewer for these critical and constructive points. We have addressed them by adding a dedicated "Limitations and Future Perspectives" section to the manuscript. The key additions are summarized below.

Key additions

1. **Added a New Section:** A "Limitations and Future Perspectives" section has been inserted before the conclusion.
2. **Addressed Quantification:** Explicitly stated the qualitative/semi-quantitative nature of the study and recommended quantitative analysis for future work.
3. **Addressed Toxicity:** Acknowledged the lack of toxicity data and recommended future toxicological studies.
4. **Addressed Reproducibility:** Specified that the analysis was done in triplicate and mentioned instrument calibration.
5. **Confirmed Figure Quality:** Reiterated that all figures have been replaced with high-quality, original versions as per the reviewer's previous comment.

Competing Interests: The Authors declares no competing interests

Version 1

Reviewer Report 05 September 2025

https://doi.org/10.5256/f1000research.181158.r390229

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COMMENTS:

1. The topic needs to be revised if possible and, should read: " Gas Chromatography - Mass spectrometry.....(not "spectrometric").
2. The revision of the topic is suggested because ethnomidicine is the basis for suggesting that the *Brassica oleracea* var. Viridis is used to treat male infertility in Uganda and no previous scientific evidence is referenced or alluded to. Also, reports of the ethnobotanical survey should be included, if available.
3. Please note that:
   - Scientific names are always italicized
   - The genus is always capitalized
   - The species is never capitalized
4. The method in the abstract says the "point of this study was to find out...."
   - What bioactive compounds might be in the ethanol extract of collard green leaves
   - how well they work as antioxidants.
   - .....may be responsible for their traditional use in treating male fertility

Note that every objective (as shown above) must have clearly defined materials and methods but the only materials and methods seen is that of the GC-MS which addresses only the first objective which is to "find out what bioactive compounds might be in the ethanol extract of collard green leaves"
The second objective mentioned, which was to "find out how well they work as antioxidants" was not attended to by any materials and method carrying out the antioxidant capacity assays such as DPPH, ABTS, FRAP, NO or any other of the tests for radical scavenging activity, lipid peroxidation, reducing power; TEAC and TRAP are assays with mechanisms specifically for antioxidant activity
5. In the introduction, the statement below, must be punctuated with an appropriate reference compulsorily because the later part of the sentence is the crutch on which the justification for the research work is resting on:
"Additionally preliminary evidence suggests potential anti-hyperglycemic and fertility-enhancing effects, reinforcing its traditional use in managing male infertility" (reference not included)

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioactivity guided medicinal plant research, Phytochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 Sep 2025

Emmanuel Ikuomola

"We sincerely thank the reviewer for their constructive feedback and valuable recommendations. The authors' detailed responses to the comments are provided below."

Reviewer Comments:

1. The topic needs to be revised if possible and, should read: " Gas Chromatography - Mass spectrometry.....(not "spectrometric").

Author's **Response**: The topic has been revised to read "Gas chromatography-Mass spectrometry" as suggested

2. The revision of the topic is suggested because ethnomedicine is the basis for suggesting that the *Brassica oleracea* var. Viridis is used to treat male infertility in Uganda and no previous scientific evidence is referenced or alluded to. Also, reports of the ethnobotanical survey should be included, if available. Author's **Response**: The topic has been revised the word ethnomedicine has been deleted to read "Gas Chromatography-Mass spectrometry (GC-MS) Revealed Bioactive Constituents of *Brassica Oleracea* var. *Viridis* (Collard Greens) Used to Treat Male Infertility in Uganda. However, the report of the ethnobotanical survey is not available.

3. Please note that:
   - Scientific names are always italicized
   - The genus is always capitalized
   - The species is never capitalized

Author's **Response:** The botanical name has been corrected to show the genus and species in small letters.

4. The method in the abstract says the "point of this study was to find out...."
   - What bioactive compounds might be in the ethanol extract of collard green leaves
   - how well they work as antioxidants.
   - .....may be responsible for their traditional use in treating male fertility

Note that every objective (as shown above) must have clearly defined materials and methods but the only materials and methods seen is that of the GC-MS which addresses only the first objective which is to "find out what bioactive compounds might be in the ethanol extract of collard green leaves"
The second objective mentioned, which was to "find out how well they work as antioxidants" was not attended to by any materials and method carrying out the antioxidant capacity assays such as DPPH, ABTS, FRAP, NO or any other of the tests for radical scavenging activity, lipid peroxidation, reducing power; TEAC and TRAP are assays with mechanisms specifically for antioxidant activity.
Author's **Response**: The objective has been modified to focus on only the GC-MS. The sentence now reads "The aim of this study was to identify bioactive compounds in the ethanol extract of collard green leaves that has traditional use in treating male infertility in Uganda".

5. In the introduction, the statement below, must be punctuated with an appropriate reference compulsorily because the later part of the sentence is the crutch on which the justification for the research work is resting on:
"Additionally preliminary evidence suggests potential anti-hyperglycemic and fertility-enhancing effects, reinforcing its traditional use in managing male infertility" (reference not included).
Author's **Response**: The supporting references have been included in the introduction as suggested. The reference numbers are 6 and 7.

Competing Interests: "I confirm that there are no competing interests that could be perceived as influencing the validity or importance of the peer review reports."

Reviewer Report 25 June 2025

https://doi.org/10.5256/f1000research.181158.r388513

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General comments

- The abstract is well-structured but can benefit from clearer language, more detail about methods, and precise emphasis on the significance of findings.
- Clarify the methodology details, especially regarding extraction and GC-MS parameters.
- Highlight the potential health implications of identified compounds more explicitly.
- Consider adding quantitative data if available, such as relative abundances of key compounds.
- Ensure consistency in terminology, e.g., "bioactive compounds" vs. "phytochemicals."
- Improve readability with more formal academic language, especially in the conclusion.

1. Introduction to sample collection:

- Clarify why the specific location was chosen, if relevant.
- Correctly cite the method if it is referenced and complete the citation.

2. Botanical identification:

- Specify the authority or botanist who confirmed the identification for scientific rigor (e.g., "identified and authenticated by Dr. XYZ using standard taxonomic keys").

3. Sample preparation details:

- Mention how many leaves were collected or the approximate weight of the fresh leaves before drying.
- Specify the drying conditions (e.g., "dried at ambient temperature (~25°C) in the shade" for accuracy).

4. Extraction process:

- Complete the citation for the methodology or clarify if it's a modified version (e.g., "based on the method described by [Author], [Year]").
- Mention the solvent volume in a consistent manner ("5.5 liters of 99% ethanol," which is clear).

5. Maceration process:

- Indicate the container type used and mention if the mixture was shaken periodically during the maceration.
- Clarify whether the mixture was kept in the dark to prevent light degradation.

6. Filtration and concentration:

- Specify the conditions of the rotary evaporator, such as temperature (e.g., "at 40°C") and pressure.
- Mention how the dryness was confirmed (e.g., no further solvent smell or weight stabilization).

7. Storage:

- Clarify storage conditions (e.g., temperature, container type).

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: bioactivity analysis, phytochemistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 21 Sep 2025

Emmanuel Ikuomola

"We sincerely thank the reviewer for their constructive feedback and valuable recommendations. The authors' detailed responses to the comments are provided below."

General reviewer comments
The abstract is well-structured but can benefit from clearer language, more detail about methods, and precise emphasis on the significance of findings.
Clarify the methodology details, especially regarding extraction and GC-MS parameters.

Author's **Response**: The abstract has been modified to contain method of extraction and conclusion emphasized the significance of the phytochemicals identified in the extract.
Author's **Response:** The method of extraction has been clarified by including the temperature, volume of solvent used and the storage of the extract obtained in page 4.
Highlight the potential health implications of identified compounds more explicitly.
Consider adding quantitative data if available, such as relative abundances of key compounds.
Ensure consistency in terminology, e.g., "bioactive compounds" vs. "phytochemicals."
Author's **Response:** The quantitative data is not available however table three shows the retention time and area under curve that represents a little bit of abundance of the

compounds.
The terminology "bioactive compounds" has been replaced with “phytochemicals” as suggested for consistency.

Improve readability with more formal academic language, especially in the conclusion.
1. Introduction to sample collection:
Clarify why the specific location was chosen, if relevant.
Author’s **Response**: This specific location was chosen since it has more farmland where the plant is cultivated. The GPS location has been included in the text for clarity.
Correctly cite the method if it is referenced and complete the citation.
Author’s **Response:** There is no referenced method of picking the plant. It was just by plucking the leaves from the plant.

2. Botanical identification:
Specify the authority or botanist who confirmed the identification for scientific rigor (e.g., "identified and authenticated by Dr. XYZ using standard taxonomic keys").
Author’s **Response:** The plant was identified and authenticated by Dr. Eunice Olet of the Department of Botany, Faculty of Biological Sciences, Mbarara University of Science and Technology. A voucher specimen was deposited in the university's herbarium under the reference number IOE-24-001.

3. Sample preparation details:
Mention how many leaves were collected or the approximate weight of the fresh leaves before drying.
Author’s **Response:** The quantity of the fresh leaves has been mentioned to be 2 kg in page 4.
Specify the drying conditions (e.g., "dried at ambient temperature (~25°C) in the shade" for accuracy).
Author’s **Response:** The drying condition has been specified as shed dried at ambient temperature of 26℃.

4. Extraction process:
Complete the citation for the methodology or clarify if it’s a modified version (e.g., "based on the method described by [Author], [Year]").
Author’s **Response**: The sentence has been completed to read “The extraction process was performed using a modified version of the method described by Abdulrahman et al.[10]” in page 4.
Mention the solvent volume in a consistent manner ("5.5 liters of 99% ethanol," which is clear).
Author’s **Response**: The solvent volume has been modified to read “A total of 800 g of the powdered leaves was cold macerated in 5 liter of 99% ethanol (1:5 w/v).”

5. Maceration process:
Indicate the container type used and mention if the mixture was shaken periodically during the maceration.
Author's **Response:** The container has been specified as "sterile glass container". The mixture was shaken periodically during the maceration process. This has been stated in page 4.
Clarify whether the mixture was kept in the dark to prevent light degradation.
Author's **Response**: The mixture was kept in the dark as stated in page 4.

6. Filtration and concentration:
Specify the conditions of the rotary evaporator, such as temperature (e.g., "at 40°C") and pressure.
Author's **Response:** The temperature is stated as below 40°C and under reduced pressure. This is stated in the relevant section on page 4.
Mention how the dryness was confirmed (e.g., no further solvent smell or weight stabilization).
Author's **Response:** The filtrate was evaporated to dryness having obtained a constant weight as stated in page 4.

7. Storage:
Clarify storage conditions (e.g., temperature, container type).
Author's **Response:** The storage condition of the extract has been mentioned as follows in page 4. "The dried crude extracts were stored at 4°C in an amber glass bottle that was tightly sealed until further analysis".

Competing Interests: "I confirm that there are no competing interests that could be perceived as influencing the validity or importance of the peer review reports."

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