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Research Article

GC-MS analysis, antimicrobial and antiulcer evaluations of ginger-infused virgin coconut oil

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Abstract

Medicinal herb-infused oil is utilized in folkloric medicine due to its efficacy in ameliorating various diseases of humans; even with this evidence, most herbs are still underutilized and poorly investigated. This prompted the investigation of ginger-infused virgin coconut oil's chemical composition and antimicrobial and antiulcer activities (GIVCO). The virgin coconut oil was produced using the natural fermentation method. Dried ginger and VCO (1:10) were infused for three days and then filtered to obtain ginger-infused virgin coconut oil. GC-MS was used to detect GIVCO chemical constituents. The agar well diffusion method was used for antimicrobial evaluation test organisms. The ethanol and indomethacin-induced ulcer models were used for antiulcer evaluation. The GC-MS analysis identified the presence of lauric acid methyl ester, Myristic acid, Palmitic Acid, Oleic acid, Capric acid, Stearic acid, Caryophyllene, Docosahexaenoic Acid methyl ester. At 100 mg/ml, inhibition zone diameter (IZD) ranged from 10-16 mm for various strains of bacteria and 10-21 mm for various strains of fungi. The effect of GIVCO on tested organisms compared favorably to that of standard drugs. The acute toxicity study of GIVCO is atoxic. The antiulcer activity demonstrated a dose-dependent effect; at 100 mg/ml, the GIVCO protected the intestine with a %UI of 80 and 63 for ethanol and indomethacin model against the standard omeprazole with 50% UI. The study demonstrated the potential of GIVCO as an alternative medicine against antimicrobial infections and the prevention of stomach ulcers.

Keywords: infused oil; ginger; phytochemicals; ulcer, microorganisms; virgin coconut oil

Introduction

The efficacy of medicinal plants, especially herbal preparation in the treatment of diseases, has been regarded as the best alternative to synthetic medicine for health care management. The exploitation of medicinal plants started in ancient times and is invaluable as a rich source of therapeutic agents for the prevention and management of diseases all over the globe. Plants being used as nutritional supplements and medicine are more likely to yield pharmacologically active compounds, which are crucial for maintaining a healthy body [1,2]. The chemical compounds present in plants with the potential to maintain good health are known as phytochemicals. These phytochemicals have been in use from time immemorial for the treatment of different health disorders and are considered relatively safer compared to conventional drugs [3]. With the increasing popularity of plants as a safe and cheaper alternative to conventional therapeutic agents, the exploitation of plants as a whole or in the form of drugs has many

prospects for ameliorating health challenges. Herbal-infused oil is an easy herbal preparation that captures the benefits of herbs for many uses. The infusion of oil with herbs can transform them into medicinal preparations. Various dried herbs and organic carrier oils (virgin coconut oil, Jojoba oil, and olive oil) are the best choices as they have a long shelf life. Herbal-infused oil has demonstrated significant activities against disease conditions, as reported [4,5]. This formed the research background to investigate the potential of GIVCO to alleviate the burden of resistance pathogenic organisms arising from different strains.

However, this study aimed to evaluate the antimicrobial and antiulcer effects of GIVCO by utilizing the beneficial health effects of ginger rhizome and carrier oil properties of virgin coconut oil as an extracting solvent. The infused oil from this study is expected to be a putative therapeutic agent.

Materials and Methods

Materials

Collection and preparation of plant materials

Zinger officinale rhizomes and fresh Cocos nucifera nut were sourced from Ogbete main market Enugu, Nigeria. The samples were authenticated in the Department of Pharmacognosy Enugu State University of Science and Technology, Agbani, Enugu State.

Test organisms

The microorganisms were selected based on the ubiquitous infection they cause in the human population. They comprise two Gram-positive (*Staphylococcus aureus* and *Streptococcus pneumonia),* two Gram-negative *(Escherichia coli, Klebsiella pneumonia) bacteria,* and the fungi used were *Aspergillus niger, Aspergillus flavus, Epidermophyton floccosum* and *Candida albican* strains isolated from wound, ear infection, and high vaginal swap (HVS). These microorganisms were clinical isolates sourced from Adonai Research Laboratory and Biomedical Services, Nsukka, Enugu state. The tubes containing the bacterial isolates were incubated at 37 °C for 24 h, while the fungi isolates were incubated at 28 °C for 48 h. The inoculum was standardized by adjusting its turbidity to correspond with the 0.5 McFarland standard to have a comparable density equivalent to approximately 108 CFU/ml.

Experimental animal

Twenty (20) (male and female) Albino Wister rats with body weights ranging from 116 gm to 130 gm were used for the experiment. The rats were allowed to acclimate in the experimental lab for 7 days, giving them access to a standard pellet diet and water ad libitum. The food was withdrawn 12 h before the experiment; however, they were allowed free access to water.

Methods

Processing of Z. officinale powder

Fresh Z. officinale rhizomes were washed thoroughly to remove soil particles. They were sliced into tiny shapes to aid easy drying and allowed to dry under shade for 10 days. An electric blender pulverized the dried rhizomes to a fine, coarse powder. The powdered sample was stored in a cool, dry cupboard and is awaiting further processing.

Processing of Virgin coconut oil (VCO)

The extraction of coconut oil begins with carefully cracking a fresh C. nucifera nut to collect the mesocarp. This mesocarp is thoroughly washed and sliced into small, box-shaped pieces, facilitating a smoother grinding process. A mechanical blender is employed to pulverize these pieces into delicate flakes. Following this, the coconut milk is extracted from the chaff using Mushin cloth and placed in an airtight plastic bucket, left undisturbed for 24 h. This step is crucial as it separates three distinct layers: the cord, oil, and water. The cord forms the upper layer and is gradually and carefully scooped out. Next, the oil is extracted precisely to avoid mixing it with the water layer beneath. The oil sample is then stored in a dry container and is awaiting further processing. This extraction method, utilizing

fermentation, not only optimizes the quality of the oil but also ensures a sustainable approach to producing a valuable product.

Preparation of GIVCO

10 gm of finely powdered ginger was weighed out into a conical flask. 100 gm of virgin coconut oil was added to the conical flask and stirred thoroughly. The mixture was heated in a water bath at 60°C for 30 min and allowed to stand for 72 h at room temperature. Thereafter, it was filtered using a funnel and cotton wool-clogged funnel to extract the GIVCO. The GIVCO sample was stored in a dry container and is awaiting further processing.

GC-MS profiling of ginger infused VCO

The ginger-infused oil was analyzed using GCMS-QP2010 PLUS (SHIMADZU, JAPAN). The capillary column type was DB-IMS [30 m (length) X0.25µm (diameter) X0.25µm (film thickness)]. The carrier gas used was helium at a constant flow rate of 19.9 ml/min and an average velocity of 36.2 cm/s; the pressure was 56.2 KPa. The initial column temperature was set at 6°C 0 for 1 min and increased by 3 °C/min up to 180 °C and to the final temperature of 280 °C at the rate of 6 °C/min; volume injected was 1.0 µl at 250 °C in the splitless mode. Mass spectra were obtained by EI at 70 eV over the scan range 10-1000 m/z. The compounds were identified by comparison of their mass spectra with those of the NIST mass spectral library.

Acute toxicity study

The acute toxicity study was conducted following established protocols [6]. In the initial phase, GIVCO was administered at 10, 100, and 1000 mg/kg to three distinct groups of three mice each. Notably, after 24 h, no fatalities were recorded, indicating a positive initial safety profile. In the second phase, we advanced to higher doses of 1600, 2900, and 5000 mg/kg. During the 24 h observation period, we carefully tracked any signs of mortality and potential behavioral changes. Encouragingly, no deaths or signs of toxicity were observed, further supporting the safety and tolerability of GIVCO at these tested doses.

Ethanol-Induced Ulcer Model

The ethanol-induced ulcer model was adopted as previously reported [7]. The study involved dividing rats into four groups, with each group comprising five rats. Group A served as the negative control, receiving ethanol without any treatment. Group B acted as the positive control and was treated with omeprazole to evaluate its protective effects. Group C was administered a treatment of 50 mg/kg bodyweight of GIVCO, while Group D received 100 mg/kg bodyweight of GIVCO, allowing for a comparison of dosage effects. To ensure that the treatments could be evaluated effectively, Groups B, C, and D were treated one hour prior to the administration of ethanol. Following a one-hour period post-ethanol exposure, the rats were euthanized through cervical dislocation for humane considerations. Their stomachs were then carefully excised and opened along the greater curvature. This careful dissection allowed for thorough examination under a dissecting microscope to assess ulcer formation and quantify ulcer scores. Subsequent calculations involved determining the ulcer index (UI) and the percentage of ulcer inhibition, providing valuable insights into the protective effects of the treatments administered.

Indomethacin- induced ulcer model

The indomethacin-induced ulcer model, as reported previously, was adopted [8]. The study divided rats into four groups, each comprising five rats. Group A served as the negative control, receiving 30 mg/kg indomethacin without any treatment. Group B acted as the positive control and was treated with omeprazole to evaluate its protective effects. Group C was administered a treatment of 50 mg/kg body weight of GIVCO, while Group D received 100 mg/kg body weight of GIVCO, allowing for a comparison of dosage effects. Groups B, C, and D were treated one hour before ethanol administration to ensure that the treatments could be evaluated effectively. Following one-hour post-ethanol exposure,

the rats were euthanized through cervical dislocation for humane considerations. Their stomachs were then carefully excised and opened along the greater curvature. This careful dissection allowed for thorough examination under a dissecting microscope to assess ulcer formation and quantify ulcer scores. Subsequent calculations involved determining the ulcer index (UI) and the percentage of ulcer inhibition, providing valuable insights into the protective effects of the treatments administered.

Table 1. compounds detected by GC-MS analysis of GIVCO.

Name of compounds	Retention time (min)	Molecular formula	Molecular mass (g/mol)
Limonene	2.49	$C_{10}H_{16}$	136.2
Benzaldehyde	4.04	C_7H_6O	106.1
Eucalyptol	5.86	$C_{10}H_{18}O$	54.25
Cymene	8.51	$C_{10}H_{14}$	134.2
Caryophyllene	14.06	$C_{15}H_{24}$	204.4
Stearidonic acid methyl ester	14.63	$C_{19}H_{38}O_2$	298.5
Humulene	20.44	$C_{15}H_{24}$	204.4
Lauric acid	21.07	$C_{12}H_{24}O_2$	200.3
Docosahexaenoic acid methyl ester	22.46	$C_{22}H_{32}O_2$	328.5
2-Bromo-5-methoxytoluene	22.80	C_8H_9BrO	201.1
Geranyl geranyl alcohol	23.18	$C_{20}H_{34}O$	209.5
Docosapentenoic acid methyl ester	23.80	$C_{23}H_{36}O_2$	344.5
Myristic acid	24.02	$C_{14}H_{28}O_2$	228.4
3,5-diphenyl-4-methyl-3-penten-2-one	24.19	$C_{18}H_{18}O$	250.3
Methyl stearate	26.63	$C_{19}H_{38}O_2$	298.5
Caprylic acid	27.21	$C_8H_{16}O_2$	144.2
2,5-dimethyoxythiophenol	28.22	$C_8H_{10}O$	122.2
Palmitoleic acid	28.53	$C_{16}H_{30}O_2$	254.4
2,4,5-trimethoxybenzaldehyde	28.57	$C_{10}H_{12}O_4$	196.2
Elaidic acid	29.15	$C_{18}H_{34}O_2$	282.5
Capric acid	29.34	$C_6H_{12}O_2$	172.3
Eicosapentaenoic acid methyl ester	29.47	$C_{21}H_{32}O_2$	316.5
Eugenol	30.06	$C_{10}H_{12}O_2$	164.2
Lauric acid methyl ester	31.39	$C_{13}H_{26}O_2$	214.3
Palmitic acid	33.03	$C_{16}H_{32}O_2$	256.4
Isopropyl palmitate	34.65	$C_{19}H_{38}O_2$	298.5
Stearic acid	36.424	$C_{18}H_{36}O_2$	284.5
Oleic acid	36.645	$C_{18}H_{34}O_2$	282.5

Antimicrobial evaluation (agar well diffusion method)

The antimicrobial evaluation of the GIVCO was assayed using the agar well diffusion method. Sixteen Petri dishes were set up, and the plate lids labeled Sa1, Sa2, St1, St2, Ec1, Ec2, Kb1, Kb2, Ca1, Ca2, Ca3, Ca4, Ca5, Af, An and Ef which represents Staphylococcus aureus strain 1, Staphylococcus aureus strain 2, Streptococcus sp. strain, Streptococcus sp. strain 2, Escherichia coli strain 1, Escherichia coli strain 2, Klebsiella pneumoniae strain 1, Klebsiella pneumoniae strain 2 respectively. Ca1 and Ca2, Candida albican from the vaginal swab, Ca3 and Ca4, Candida albican from diabetic wound swab, Ca5 Candida albican from ear swab in otitis media, Aspergillus flavus Af, Aspergillus niger An, and Epidermophyton floccosum Ef respectively. Each plate was divided into sections with codes 1,2,3,4, and 5 representing the sample concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 10 mg/ml of standard drugs, respectively. The nutrient agar weighing 4.5 gm was dissolved in 160 ml of water in a conical flask. The SDA weighing 10 gm was dissolved in 160 ml of water in a conical flask, and 62.5 mg of chloramphenicol capsule was added to inhibit bacteria growth. The solutions were sterilized by heating in an autoclave for about 15 min at 120 °C and then cooled. 0.1 ml of the microorganisms was added carefully to the plates as labelled. 20 ml of molten agar was added to each plate containing the microorganisms with SDA for all fungi and nutrient agar for bacteria. It was appropriately swirled and allowed to solidify. The cork borer was used to make five holes of 8mm diameters on the sections labeled on the plate, and an additional hole was made in the middle for the control drug. The different concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml of GIVCO, virgin coconut oil, ginger powder solution, and 0.1 ml control drugs were added to the labeled bored holes using a micropipette.

The plates were allowed to stand undisturbed for a few minutes to allow diffusion. Then, they were stacked adequately in an incubator cabinet to prevent uneven heating and incubated for 24 h (37 °C) for bacteria and 48 h (28 °C) for fungi, respectively. At the end of the incubation period, the inhibition zonal diameter of microbial growth on the plates was observed, and the results were recorded in millimeters.

Statistical analysis

The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by multiple comparison tests. The values are expressed as means ± SEM and differences in the values were considered significant at 5 % level.

Results and Discussion

GC-MS Analysis

The GC-MS detected the presence of fatty acid esters as the major chemical constituents in GIVCO. The compounds identified, when compared with the NIST MS library, showed limonene, Benzaldehyde, lauric acid, Eucalyptol, Stearidonic acid methyl ester, caryophyllene, Cymene, Myristic acid, Docosahexaenoic acid methyl ester, Geranyl geranyl alcohol, Palmitoleic acid, Caprylic acid, Palmitic acid, Humulene, Lauric acid methyl ester, Methyl stearate, Eicosapentaenoic acid methyl ester, Omega-3-arachidonic acid methyl ester, and 2-phenyl-1-propanol as presented in Table 1. These novel findings are sure to pique the interest and stimulate further research in this area.

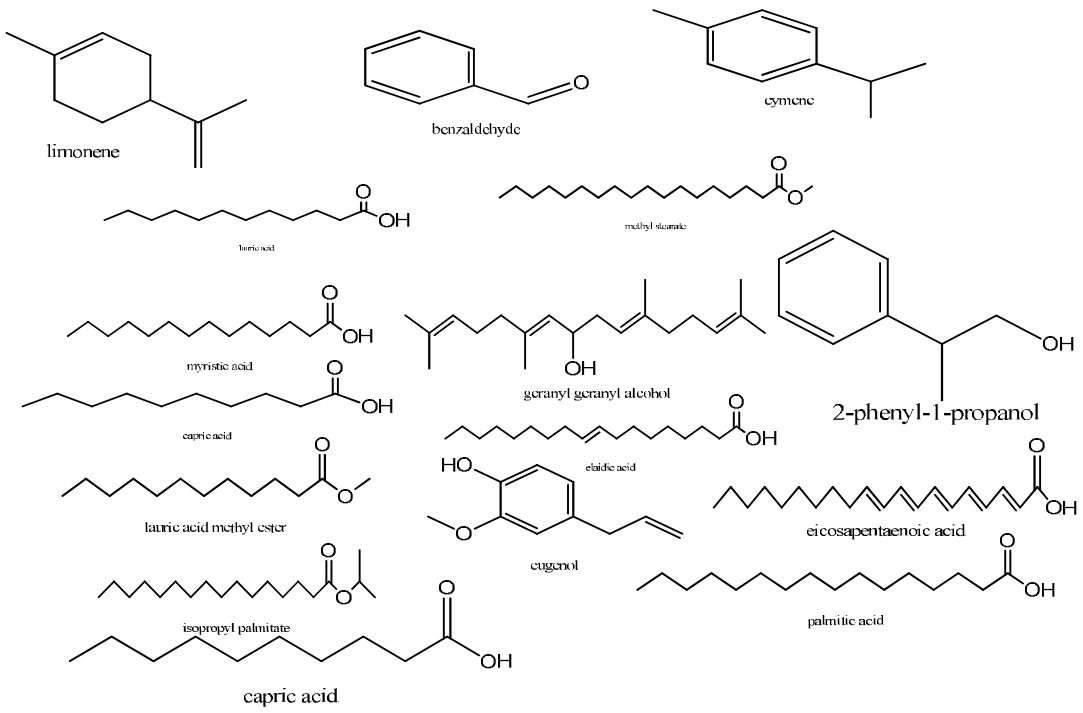

Figure 1. Structure of compounds identified in GIVCO.

Antimicrobial determination

The result showed that GIVCO has a significant effect against the two strains of S. aureus and S. typhi. One strain of E. coli whereas E. coli strain 2 and two strains of K. pneumoniae was not inhibited even at the high dose of 100 mg/ml, unlike the standard drug that showed potent inhibition even at a much lower dose, as presented in Table 2. In Table 3, the GIVCO exhibited a reasonable effect against the five C. albicans A. flavus strains. A potent inhibition was recorded against E. floccosum, whereas A. niger was not inhibited even at the high dose of 100 mg/ml. There is somewhat comparable effect between the standard drug and GIVCO.

Table 2. Effect of GIVCO on bacteria species.

Test samples	Conc. mg/ml	Sa1	Sa2	St1	St2	Ec1	Ec2	Kb1	Kb2
GIVCO	100	16	12	12	10	16	0	0	0
	50	14	10	10	10	14	0	0	0
	25	9	0	9	6	9	0	0	0
	12.5	0	0	0	0	0	0	0	0
Levofloxacin	0.01	36	36	36	36	36	36	36	36

Key: Sa1, Sa2, St1, St2, Ec1, Ec2, Kb1, Kb2, ca1, ca2, ca3, ca4, ca5, Af, An, Ef which represents Staphylococcus aureus strain 1, Staphylococcus aureus strain 2, Streptococcus sp. Strain 1, Streptococcus sp. strain 2, Escherichia coli strain 1, Escherichia coli strain 2, Klebsiella pneumoniae strain 1, Klebsiella pneumoniae strain 2.

Table 3. Effect of GIVCO on fungi species.

Test samples	Conc. mg/ml	Ca1	Ca2	Ca3	Ca4	Ca5	Af	An	Ef
GIVCO	100	21	20	14	10	16	13	0	28
	50	18	24	11	10	14	11	0	15
	25	12	15	9	6	9	9	0	10
	12.5	8	9	0	0	0	0	0	8
Fluconazole	0.01	45	45	45	45	45	45	45	45

Key: Ca1 and Ca2, Candida albican from vaginal swab, Ca3 and Ca4, Candida albican from diabetic wound swab, Ca5 Candida albican from ear swab in otitis media. Af, Aspergillus flavus, An, Aspergillus niger and Ef, Epidermophyton floccosum

Acute toxicity study

The acute toxicity study showed no mortality, even at 5000 mg/ kg within 24 h of administration of GIVCO. However, there was a visible sign of soft stool in the first phase, whereas in the second phase, there was a visiblesign of diarrhea.

Table 4. Initial acute oral toxicity test result.

Dose	10 mg/kg	100 mg/kg	1000 mg/kg
Surviving rat	3/3	3/3	3/3

Table 5. Final acute oral toxicity test result.

Dose	1600mg/kg	2900mg/kg	5000mg/kg
Surviving rat	1/1	1/1	1/1

Effect of GIVCO on ethanol induced ulcer in rat

The high dose of GIVCO exhibited the best antiulcer activity on the induced ulcer model with a percentage ulcer index of 80 %. There is a significant difference between the high dose of GIVCO and the standard drug, omeprazole. The low dose and standard drug showed no significant difference.

Table 6. Effect of GIVCO on ethanol induced ulcer.

Group	Treatment	Ulcer index	% ulcer index
Group A (Negative control)	Received 1mL water	10.7±0.2	-
Group B (Positive control)	30mg/kg Omeprazole + 1mL ethanol	5.36±0.2*a	50
Group C (Low dose)	50mg/kg GIVCO + 1mL ethanol	5.32±0.2a	50
Group D (High dose)	100mg/kg GIVCO +1mL ethanol	2.38±0.2*	80

Key: GIVCO= Ginger-infused virgin coconut oil, Values are expressed as Mean SD of each rat group (n=5). Mean values on the same column with (*) are significant at P < 0.01. While mean value with (a) are non-significant at P<0.01 and 0.05 respectively.

Effects of GIVCO on Indomethacin induced ulcer in rat

The effects of GIVCO on stomach ulcers caused by indomethacin demonstrated reasonable activity. The statistical analysis showed no significant difference between high and low doses of GIVCO, as indicated by a percentage ulcer index of 63 %. Comparing the standard ulcer drug omeprazole, there is a disparity in effect, indicating a significant difference.

Table 7. Effect of GIVCO on indomethacin induced ulcer.

Group	Treatment	Ulcer index	% ulcer index
Group A (Negative control)	Received 1mL water	10.7±0.2	-
Group B (Positive control)	30mg/kg Omeprazole + 30mg/kg indomethacin	5.36±0.2*	50
Group C (Low dose)	50mg/kg GIVCO +30mg/kg indomethacin	3.92±0.78*a	63
Group D (High dose)	100mg/kg GIVCO +30mg/kg indomethacin	3.94±0.78*a	63

Key: GIVCO= Ginger-infused virgin coconut oil, Values are expressed as Mean±SD of each rat group (n=5). Mean values on the same column with (*) are significant at P < 0.01. While mean value with (a) are non-significant at P<0.01 and 0.05 respectively.

Discussion

With the progress made in the use of synthetic drugs to combat the increasing rate of various ailments coupled with their adverse effects, much has not been done to alleviate the burden of health challenges, especially in developing countries. Therefore, the need for alternative therapeutic agents from indigenous herbs is crucial. GIVCO has demonstrated potential as an antimicrobial agent against different strains of bacteria and fungi. The antibacterial evaluation of GIVCO, at 100 mg/ml, showed a significant effect against Staphylococcus aureus 1 and 2 strain, Streptococcus sp. strains 1 and 2, and Escherichia coli strain 1 with IZD range of 10-16 mm Table 2. The result of the test sample was not comparable to the standard drug Levofloxacin. This could be because test samples could not diffuse well into the agar medium due to their compositions. For the fungi evaluation, the standard drug fluconazole showed the widest and clearest inhibition zone compared to other test samples. GIVCO had a significant antifungal effect on test organisms. At 100 mg/ml, GIVCO inhibited the growth of five different Candida albican strains, Aspergillus flavus, and Epidermophyton floccosum, with an IZD range of 10-28 mm, respectively Table 3. However, infused ginger oil showed more potent antifungal activity on Candida albican of different strains, Epidermophyton floccosum, and Aspergillus flavus. The potent inhibition recorded against Epidermophyton floccosum and candida albican strains indicated using GIVCO to treat skin and nail infections. The observed antimicrobial properties showed that virgin coconut oil could extract chemical constituents in ginger that are responsible for treating diseases associated with pathogenic organisms. Ginger has been reported to possess phytochemicals that inhibit microbial infections [9,10].

The acute toxicity study of GIVCO showed no mortality in the animals, though some physiological change was observed in Tables 4 and 5. At a concentration of 100 mg/kg body weight, the consistency of the droppings changes with 30 min of administration. The higher the dose, the rate at which the droppings get watering. This effect suggested the potential of GIVCO as a putative, purgative, and laxative agent.

A peptic ulcer is a localized lesion of the gastric or duodenal mucosa wall occurring at a site where the mucosal epithelium is exposed to aggressive factors, majorly chronic alcohol consumption, and misuse of non-steroidal anti-inflammatory drugs (NSAIDs). The ulcers induced by ethanol have been implicated in stimulating the formation of leukotriene C4 (LTC4), mast cell secretory products, and reactive oxygen species, resulting in the damage of gastric mucosa [11,12]. Moreover, NSAIDs, unlike ethanol, usually induce ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway [13,14]. The GIVCO demonstrated a reasonable effect on ethanol and NSAID-induced ulcers, as reported in Tables 6 and 7. The percentage ulcer index of GIVCO at 100 mg/kg outperforms the standard drug, suggesting a better antiulcer agent compared to omeprazole. The GIVCO ameliorates gastric ulcers caused by ethanol (80 % ulcer reduction) and indomethacin (63 % ulcer reduction) significantly at $p<0.01$, respectively.

The phytochemicals detected by GC-MS analysis of GIVCO, as presented in Table 1 and Figure 1, indicated the presence of phytochemicals of medicinal importance. The copious concentration of medium-chain fatty acid and its ester bioactive metabolites in GIVCO contributed to this study's observed antimicrobial and antiulcer properties. The fatty acids detected in the GIVCO, palmitic acid, myristic acid, oleic acid, and lauric acid, possess antimicrobial, antioxidant, and anti-inflammatory activities [15-18].

Conclusion

This study demonstrated that GIVCO contains phytochemical constituents, which contribute to the observed antiulcer and antimicrobial activity. Furthermore, this study has established the therapeutic potential of GIVCO in the treatment and management of diseases, thus recommending its utilization in the management of selected diseases.

Authors contribution

IJA designed the research work, JOO sourced and prepared the plant materials, and NHO drafted the first manuscript. CCA evaluated and interpreted the antimicrobial result. RCO produced the ginger-

infused virgin coconut oil, RMO assessed and analyzed the antiulcer result, and FBCO interpreted the GCMS chromatogram result.

Declaration of interest

The authors declare no conflict of interest.

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