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Pharmacological activities and phytochemical evaluation of coconut crude oil and upon exposure to ozone

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Abstract

Coconut oil is eatable oil with many nutritional and cosmetic applications. In this investigation coconut oil was subjected to 0 to 5 L/min of ozone for 3 h and the chemical composition of both crude and ozonized oil was valued via Gas Chromatography-Mass Spectrometry (GC–MS). Some biological tests were done including antibacterial action versus *Helicobacter pylori*, anti-biofilm activity versus *H. pylori*, anti-hemolytic activity in the existence of *H. pylori*, anti-Alzheimer action, and cytotoxic effect towards A-413 cancer cell line to determine the activity of coconut oil and upon exposure to ozone. Fifteen compounds were detected in the coconut oil crude and ozonized oils where the fatty acid esters were the most common molecules in crude coconut oil, whereas alkenes were the most predominant compounds in ozonized coconut oil. A slight elevation of antibacterial action towards *H. pylori* from 23.0 ± 0.1 to 28.2 ± 0.5 mm was displayed upon exposure of the coconut oil to ozone. Both crude and ozonized coconut oil showed a bactericidal effect with MICs = 62.5 ± 0.1, 125.0 ± 0.2 µg/mL and MBCs = 15.62 ± 0.2, 31.25 0.2 µg/mL for crude and ozonized oil, respectively. A significant elevation in anti-biofilm activity was found upon using 25% of MBCs of ozonized oil relative to crude oil. A dramatic rise was observed in anti-hemolytic activity upon using 25 and 75% of MICs of ozonized oil relative to crude one. A notable elevation of anti-Alzheimer impact was evident upon exposing coconut oil to ozone. Besides, the cytotoxic impact towards A-431 cells was slightly increased after exposing the oil to ozone. The current results suggest a new technique to expose coconut oil to ozone to improve some of its in vitro pharmaceutical applications.

Keywords Coconut, Ozone, *H. pylori*, Gas chromatography, Anti-hemolytic, Anti-Alzheimer

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Introduction

Natural products have traditionally used as significant pharmaceuticals in a multiplicity of therapeutic topics (Qanash et al. 2022; Bakri et al. 2024). Examining various compounds libraries is seen to be a viable method for finding new candidates for the development of innovative medications (Alawlaqi et al. 2023; Alsalamah et al. 2023). Recent advancements in numerous scientific domains have prompted an upsurge of interest in natural product evaluation (Almehayawi et al. 2024; Qanash et al. 2024; Rao et al. 2024). These incorporate not only the use of bioinformatics research, genetics, and genomic mining, but also the exploration of novel environmental niches and development of new methodologies for extracting, investigating and modifying the chemical composition of substances for beneficial uses (Privalsky et al. 2021; Barth et al. 2024).

Helicobacter pylori has a significant effect on communities as well as the global economy. It is imperative that we develop a successful strategy to combat *H. pylori* to prevent possible associated conditions (Yahya et al. 2022; Qanash et al. 2023a). Natural products are desirable as compatible and substitute therapies because of their lesser harmful effects and less costs; natural substances have long been considered essential prospective for anti-*H. pylori* therapies (Al-Rajhi et al. 2023a, b).

Alzheimer's is a degenerative condition that starts with minor memory impairment, impacting regions of the brain responsible for thinking, remembering, and language. This can significantly hinder an individual's capacity to perform everyday tasks (Tomášková et al. 2016). Although there is no known remedy for Alzheimer's, there are medications besides non-pharmacological therapies that may slow the disease's progress and alleviate its signs (Lima and Medeiros 2024). Several natural products were applied in management of Alzheimer's illness (Adedayo et al. 2020; Al-Rajhi et al. 2023a, b).

Oil of coconut, which is prepared from *Cocos nucifera* tree, is widely employed throughout many regions of the world for edible and commercial purposes. The traditional method of producing coconut oil in West Africa consists of grinding and compressing copra to release the oils (Boateng et al. 2016). This is carried out in sizable mills, and the oil is sold openly. The amazing nutritional and functional qualities of coconut oils can be used to the benefit of underdeveloped nations in Africa. For a very long time, West African communities have used coconut oil as a main source of dietary fat and as a medicine to treat various ailments (Yang et al. 2024). Many nations currently sell ozonized oil, although there is not much data accessible about its chemical composition, and biological properties. Characterization and classification of ozonized vegetable oils depend on an understanding of their physical and chemical characteristics (Ugazio et al. 2020; Radzimierska-Kaźmierczak et al. 2021). The ionization process is monitored and the efficacy of the ozonized vegetable oils is assessed using analytical techniques such as acidity and iodine levels (Díaz et al. 2006). GC–MS which has been applied to analyze both unsaturated and saturated fatty acids in oils from vegetables, is the most common method for analyzing fatty acids in organic substances (Al-Rajhi and Abdel Ghany 2023; Qanash et al. 2023b). In this study, the GC–MS testing was used to determine the constituents of the oils both before and after treatment by ozone. The current word was aimed to investigate the activities of the crude and ozonized coconut oils including anti-*H. pylori*, anti-biofilm, hemolytic activity, anti-Alzheimer, and cytotoxicity towards epidermoid carcinoma (A431) cell line.

Materials and methods

Ozonation of the coconut oil

Ozone gas was produced using an electrical barrier shock plasma reactor at faculty of the science Al-Azhar University. A 2.0 L drechsel container containing 1.0 L of coconut oil was submerged in a –4 °C chilling bath at the output of the plasma reactor. The ozone was bubbled in the coconut oil for 3 h at a rate of 0 to 5 L/minute, resulting in a semisolid form. The coconut oil was removed from the drechsel container after ozonation, transferred to an empty glass container, measured, and kept at 5 °C for storage (Elvis and Ekta 2011; Khalifa et al. 2022).

Gas Chromatography-mass spectrometry (GC–MS) for the crude and ozonized oil

GC–MS testing was done out through an auto-sampler (570-Thermo-fisher, USA), Rt-570 column (100.0 m×0.26 mm×0.21 µm; Thermo-Fisher, USA), and flame-ionization detector. Chromatography system (X-caliber data acquisition and software, Thermo-Fisher, USA) was implemented for details getting from the FID. Helium was applied as the transporter gas with rip administration (100:1). The Pre-run time out 10 min. The Equilibration time 0.5 min. 2 Ramps were done where, ramp 1 rate: 3°C/min ant its final temperature was 200 °C, while: ramp 2 rate:3°C/min. The Initial heat was 45°C ant its final temperature was 280 °C. The evaluations were performed out in systemized heat mode start at 100 to 280 °C and followed by isothermal for 14.0 min. The carrier flow 1.5 ml/min (Radzimierska-Kaźmierczak et al. 2021).

Antimicrobial action

To test the influence of both crude and ozonized coconut oil on *H. pylori*, a strain of *H. pylori* (ATCC 43504) was generously given by Al-Azhar University's Microbiology Department via prof. Tarek Abdelghany. Using the well agar diffusion procedure, the in vitro anti-*H. pylori* capabilities were done. In summary, 100 µL of *H. pylori*

solution (1.0×10^8 CFUs/ml) was applied to Mueller Hinton agar dishes that had 10.0% sheep blood in them. Next, a 6 mm size circular cut was aseptically punched employing a sterile cork borer. Next, a volume (100 µL) of the samples at the specified dose was added to the well. Antibiotics namely amoxicillin (0.05 mg/mL) and clarithromycin (0.05 mg/mL) were utilized as positive controls, while DMSO served as the non-effective negative control. The dimension of the inhibitory area was tested following a 72-h incubation period at 36°C in a microaerophilic container with humid conditions (Santiago et al. 2022).

Evaluation for minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The micro-dilution broth procedure was applied to test MIC of the specimens being studied by employing nutritional broth for bacterial. To find their final levels, which varied from 0.98 to 1000 µg/mL, the specimens under inquiry were diluted twice. 200 µl of the specimen dilutions under examination in broth medium were added to each well of the 96-well micro-titrate plate for preparing it. Following the generation of the inoculum using fresh bacterial cultures that met the turbidity standards of the 1.0 McFarland standard, 2.0 µL of sterile 0.9% NaCl was put to each well to achieve level of 3.0×10^6 CFU/ml. *H. pylori* was then incubated for 72 h at 36°C. The minimum inhibitory concentration (MICs) were detected by visually measuring the specimens' levels at which the standard strain's growth was completely inhibited. On each microplate, there was a positive control (an inoculum without the investigated specimens) and a negative control (tested specimens without an inoculum) (Huang et al. 2021).

MBC was estimated by sub-culturing 100 ml of the bacterial culture onto Mueller–Hinton agar developed with 10% blood plates/ well with total growth suppression, from the final positive, and from the development control. Following a 72-h incubation period at 35°C, the MBC was found to be the lowest concentration of specimens that did not promote microbial growth. To ascertain whether the tested had a bactericidal or bacteriostatic action that prevented microbial growth, the MBC/MIC ratios were computed (Huang et al. 2021).

Anti-biofilm action

The samples' impact on the development of biofilm was judged using 96-well polystyrene flat bottom dishes. By adding 280 µL of freshly seeded trypticase soy yeast broth (TSY) to every single well of a microplate, a ultimate level of 10^6 CFU/mL was ascertained. Then, the microplate was cultivated in MBC at the 75, 50, and 25% sub-lethal levels that were already established. As controls, wells with medium and those with just alcohol and no specimens were utilized. For 48 h, dishes were kept at 36°C. Following the discarding of the supernatant, each well's free-floating cells were thoroughly cleaned using sterile distilled water. The biofilm that had progressed was colored for fifteen minutes at room temperature operating a water-based solution of 0.10% crystal violet after the dishes were allowed to air dry for thirty minutes. Following the incubation procedure, the extra color was again eliminated by rinsing with sterile distilled water a further three times. Afterwards 15 min of incubation and the incorporation of 250 µL of 96% ethanol to the wells in order to ultimately destroy the dye attached to the cells, absorbance was measured at 560 nm using a microplate reader (Jeong et al. 2021).

Anti-hemolytic activity

To measure the hemolysis activity of samples treated with bacteria in sub-MIC (25% and 50% of MIC). After adjusting the cultures of tested bacteria to an OD_{600} of 0.40, they were centrifuged at 22,000×*g* for 15 min, using a 25%, 50%, and 75% MIC besides untreated cultures. The fresh erythrocyte suspension (2.0%) in 0.9 mL saline was mixed with 500 µL of supernatants, and the mixture was incubated for two hours at 35°C. The mixture was then rotated at 12,000×*g* for fifteen minutes at 5°C. A negative control of un-hemolyzed erythrocytes was formed by incubating erythrocytes in LB broth (Sigma, USA) under the same conditions as the positive control, which was produced via addition of 0.1% Sodium Dodecyl sulphate to an erythrocyte suspension. Measurement of absorbance at 550 nm was operated to gauge hemoglobin production (Mogrovejo et al. 2020).

Testing for anti-Alzheimer impact

Ten mg/10 ml of each component were mixed in a solution of phosphate buffer (pH 7.5), resulting in 100 µg/mL at the end. Earlier every test, the stock solutions were diluted by a 20 mM sodium phosphate buffer solution (pH 7.6) to a range of concentrations. The DTNB-phosphate-ethanol reagent: adding 50 mL of 0.1 mM phosphate buffer (pH 7.6) to 12.7 mg of DTNB dissolved in 120 mL of 96% ethanol. Ten microliters of the tested specimens in 0.3% DMSO, seventy-nine microliters of 20 mM sodium phosphate buffer (pH 7.6), and one microliter of the enzyme solution (final concentrations of 0.196 to 100 µg/ml for the compounds studied, and 0.3 units/mL for BChE) were combined and kept for fifteen minutes. After adding 10 µL of the substrate solution—final butyrylthiocholine iodide level: 4 mM—to the combination, it was incubated for 30 min. By putting 900 µL of DTNB-phosphate-ethanol, the process was stopped. The absorbance was determined using a reader for microplates at 410 nm (Cai et al. 2019).

Anti-proliferative activity

The cytotoxic impact of ozonized and crude oil on A-431 cells (cell type: epithelial; disease: epidermoid carcinoma) was detected by MTT procedure after the oil was dissolved in DMSO. Using conventional levels, the result is a blue color whose values directly connected with the quantity of living cells. At 560 nm, the absorbance was measured using a computerized microplate analyzer (Tecan Life Science Infinite F50, USA). The cells were put at 35°C for a further 24 h after the samples in levels from 1000 to 31.25 µg/mL was added after 24 h of attachment till merging. 100 µL of MTT solution (5.0 mg/mL) was incorporated after the fresh medium was supplied, and it was left at 35°C for four hours. A microscope (OMAX, USA) connected with a CCD camera to observe the cells (Examinati et al. 2008).

Statistical testing

Each experiment has been run three times, and the outcomes are shown as the average ±SD. The *t*-test was performed to evaluate the difference between means using Graph Pad Prism V5 (San Diego, CA, USA) software. Findings with $p < 0.05$ were referred to substantial change.

Results

Various molecules in crude and ozonized coconut oil using GC–MS

The investigation of crude coconut oil by GC–MS shown the occurrence of 15 compounds which consisted of seven fatty acids esters including: 2-(Decanoyloxy) propane-1,3-diyl dioctanoate, Glyceryl trilaurate, 1-(hydroxymethyl)-1,2-ethanediyl ester, Decanoic acid, 2-[(1-oxooctyl)oxy]-1,3-propanediyl ester, Decanoic acid, 1-[[(1-oxooctyl)oxy]methyl]-1,2-ethanediyl ester, 2-Lauroylgylcerol and Glyceryl 2-caprate dicarpylate; (Three alkenes) which were: Cyclohexane, nitro, Cyclohexane, bromo and 1-Butene, 2,3,3-trimethyl; (one alkene hydrocarbon) which was: Tetradecane; (One fatty acid) which was: Dodecanoic acid, (One pytosterol) which was ç-Sitosterol, (one alcohol) which was: 1,5-Heptadien-4-one, 3,3,6-trimethyl, (one glyceride) which was: 1-Dodecanoyl-3 myristoylglycerol as shown in (Fig. 1a, Table 1). While, testing ozonized coconut oil by GC–MS revealed the existence of fifteen compounds as well which were: (Four Alkene) which were: Pentane, 2,2-dimethyl, Cyclohexane, nitro, 1,8-Cineole 1,8-Epoxy-p-menthane cajeputol 1,8-epoxy-p-menthane, Hexadecane; (one hydroxyl-dicarboxylic acid) which were: D-(-)-Citramalic acid; (Three fatty acid ester) which were: Decanoic acid, 1-[[(1-oxooctyl)oxy]methyl]-1,2-ethanediyl ester, Glyceryl trilaurate and 9-Octadecenoic acid (Z)-, oxiranylmethyl ester; (two fatty acids) which were: Dodecanoic acid, Tetradecanoic acid; (two methane monoterpenoids) which were: Terpinen-4-ol, Alpha-terpinyl acetate; (one terpenoid) which was caryophyllene oxide; (one α, β-unsaturated aldehyde) which was: Cinnamaldehyde, (E)-; and (one diarylmethane) which was: p-Cresol, 2,2'-methylenebis[6-tert-butyl-as depicted in (Fig. 1b, Table 1).

Assessment of the impact of ozone on anti-*H. pylori* of coconut oil

The antibacterial activity of standard drugs, crude coconut oil, and ozonated coconut oil against *H. pylori* was evaluated. The inhibition zone of standard drugs versus *H. pylori* was 24.3±0.6 mm, whereas the crude coconut oil showed slightly lower anti-*H. pylori* with inhabitation diameter of 23.0±0.1 mm. Finally, exposure of coconut oil to ozone increased the anti- *H. pylori* inhibition zone to 28.2±0.5 mm as depicted in (Fig. 2). The MIC and MBC values of the standard drug detected as 31.25 µg/mL revealed its bactericidal impact versus *H. pylori*. Besides, the MIC value of crude coconut oil was 62.5 µg/mL and its MBC versus *H. pylori* was 125 µg/mL. On the other hand, the MIC value of ozonized coconut oil was 15.62 µg/mL, and its MBC was 31.25 µg/mL revealing its bactericidal action of both crude and ozonized coconut oil as shown in (Table 2, Fig. 3).

Evaluation of the role of ozone on anti-biofilm action of coconut oil

Anti-biofilm impact activity (%) of cured and ozonized coconut oil towards *H. pylori* were examined using (25, 50 and 75%) of MBC. There was a significant difference of anti-biofilm activity of cured and ozonized coconut oil ($P \leq 0.05$) upon using 25% of MBC towards *H. pylori*. While, upon using 50% of MBC of crude and ozonized coconut showed activities = 67% and 84% respectively (Fig. 4). Lastly, using 75% of MBC of crude and ozonized coconut had activities = 78 and 92% consecutively as shown also in (Fig. 4).

Detection of the effect of ozone on anti-hemolytic activity

The released hemoglobin was evaluated upon using 25, 50 and 75% of MIC of crude and ozonized coconut oil was relative to the positive control. There was substantial difference ($P \leq 0.05$) of activity upon using 25 and 75% crude and ozonized coconut oil. While, a slight difference of activity could be seen upon using 50% of crude and ozonized coconut oil which were 5.4±0.2 and 4.9±0.5% consecutively as shown in (Fig. 5).

Assessment of the impact of ozone on anti-Alzheimer action

The butylcholinesterase (BuChE) inhibition activity of both crude coconut oil and upon exposure to ozone was determined. There was a substantial rise ($P \leq 0.05$) in the

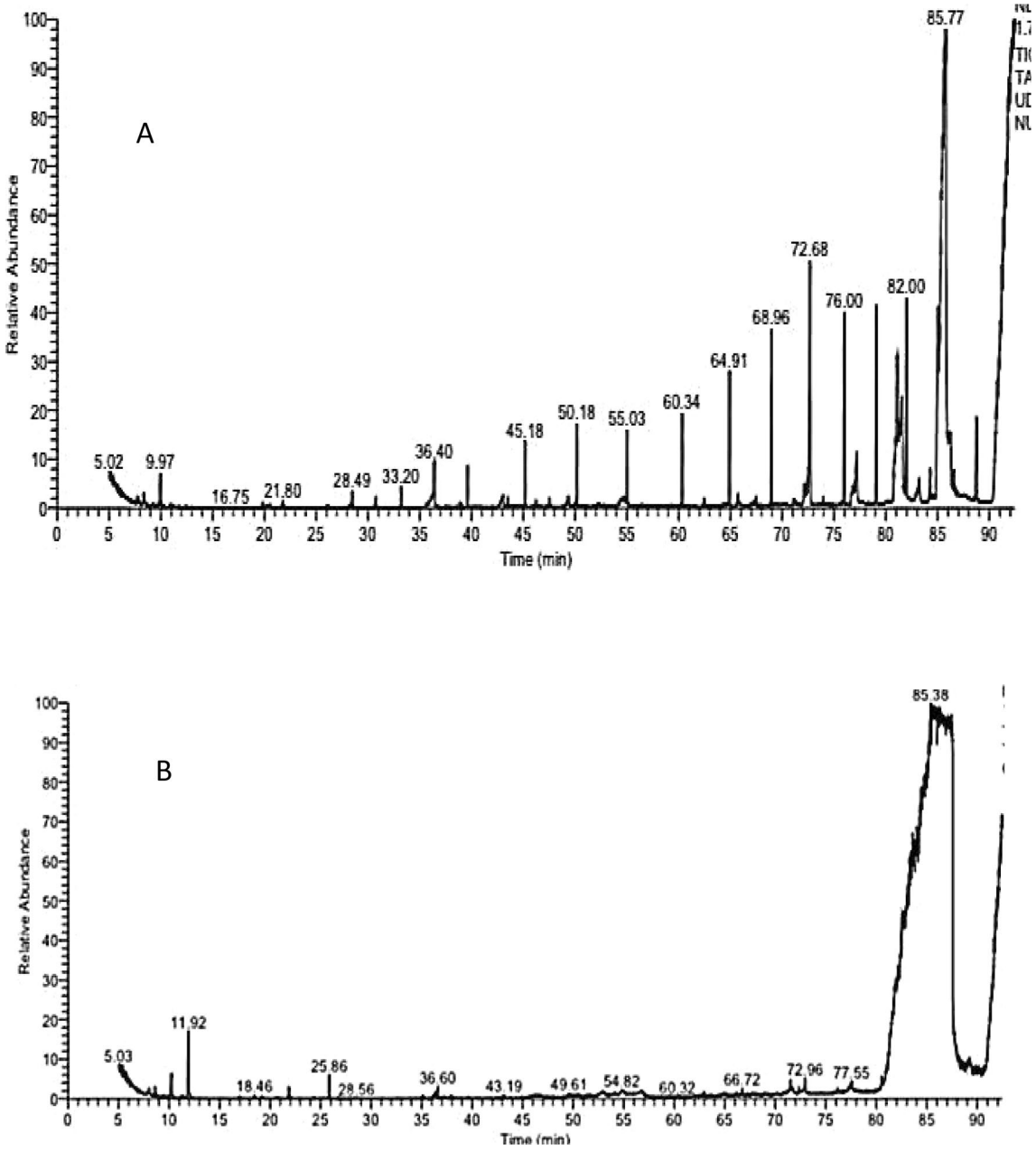

Fig. 1 GC- MS showing separation of various compounds in (**A**) Crude coconut oil, (**B**) Ozonized coconut oil

action after exposing the coconut oil to ozone, Where IC_{50} for crude, ozonized and standard were 9.33±0.3, 2.59±0.2, and 0.51±0.1 µg/mL respectively (Figure 6).

3.6 Determination of the role of ozone on anti-proliferative activity

The cytotoxic impacts of crude and ozonized coconut oil were tested using MTT assay towards A431 cells and it could be seen that upon using different concentrations from 1000 to 31.25 of the oil to ozone slightly elevate its cytotoxic impact where IC_{50}= 484.96 ± 5.4 and 467.11 ± 4.15 µg/mL for crude and ozonized oil respectively as shown in Table 3 and Fig. 7.

Discussion

Ozonized oils are gaining more attention from scientists and being used in medical centers as a result of the ongoing search for potent biomedical therapies that can treat illnesses with manageable negative consequences (Pietrocola et al. 2018). Vegetable oils contain unsaturated triacylglycerides that may combine with ozone which consider a potent oxidizing agent (Boland-Nazar et al. 2016). At ambient temperature, all of the ozonides produced

Table 1 Different separated compounds in crude and ozonized coconut oils

RT	Peak name	Molecular weight	Molecular formula	Peak area %	*RT (O3)	Peak name	Molecular weight	Molecular Formula	Peak area %
5.02	1-Butene, 2,3,3-trimethyl-	98	C_7H_{14}	2.055	5.03	Pentane, 2,2-dimethyl	100	C_7H_{16}	0.944
9.97	Cyclohexane, nitro-	129	$C_6H_{11}NO_2$	2.096	8.60	D-(-)-Citramalic acid	148	$C_5H_8O_5$	0.826
16.75	1,5-Heptadien-4-one, 3,3,6-trimethyl	152	$C_{10}H_{16}O$	0.610	10.24	Cyclohexane, nitro-	129	$C_6H_{11}NO_2$	1.7936
21.80	Cyclohexane, bromo	162	$C_6H_{11}Br$	0.814	11.92	1,8-Cineole 1,8-Epoxy-p-menthane cajeputol 1,8-epoxy-p-menthane	154	$C_{10}H_{18}O$	4.956
28.49	Tetradecane	198	$C_{14}H_{30}$	0.814	18.46	Terpinen-4-ol	154	$C_{10}H_{18}O$	0.472
36.40	Dodecanoic acid	200	$C_{12}H_{24}O_2$	2.442	21.87	Cinnamaldehyde, (E)-	132	C_9H_8O	0.826
80.80	Glyceryl 2-caprate dicarpylate	498	$C_{29}H_{54}O_6$	0.936	25.86	Alpha-terpinyl acetate	196	$C_{12}H_{20}O_2$	1.475
81.10	Glyceryl trilaurate	638	$C_{39}H_{74}O^6$	10.177	35.10	Caryophyllene oxide	220	$C_{15}H_{24}O$	0.236
81.58	2-Lauroylgylcerol	484	$C_{29}H_{56}O_5$	12.823	36.4	Dodecanoic acid	200	$C_{12}H_{24}O_2$	0.472
84.29	ç-Sitosterol	414	$C_{29}H_{50}O$	1.933	36.6	Hexadecane	226	$C_{16}H_{34}$	0.5428
85.34	Decanoic acid, 2-[(1-oxooctyl) oxy]-1,3-propanediyl ester	526	$C_{31}H_{58}O_6$	3.460	43.19	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	0.177
85.78	Decanoic acid, 1-[[(1-oxooctyl) oxy]methyl]-1,2-ethanediyl ester	526	$C_{31}H_{58}O_6$	45.552	49.61	Decanoic acid, 1-[[(1-oxooctyl)oxy] methyl]-1,2-ethanediyl ester	526	$C_{31}H_{58}O_6$	0.3422
86.26	1-Dodecanoyl-3-myristoylglycerol	484	$C_{29}H_{56}O_5$	4.070	54.58	Glyceryl trilaurate	638	$C_{39}H_{74}O_6$	1.4986
90.72	1-(hydroxymethyl)-1,2-ethanediyl ester	581	$C_{37}H_{72}O_4$	0.610	60.32	p-Cresol, 2,2'-methylenebis[6-tert-butyl-	340	$C_{23}H_{32}O_2$	0.3422
91.23	2-(Decanoyloxy)propane-1,3-diyl dioctanoate	493	$C_{29}H_{54}O_6$	11.601	64.83	9-Octadecenoic acid (Z)-, oxiranyl-methyl ester	338	$C_{21}H_{38}O_3$	85.196

*RT, Retention time

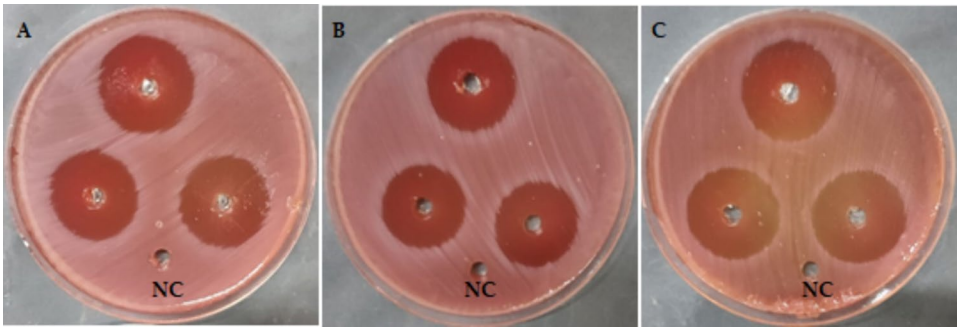

Fig. 2 Antimicrobial action of (**A**) Standard drug; (**B**) Crude coconut oil; and (**c**) Ozonized coconut oil towards *H. pylori* (Data are illustrated as means ± SD)

Table 2 Anti-*H. pylori* with MIC and MBC results of crude and ozonized coconut oil. (The outcomes were tabulated as means ± SD); The MBC/MIC of the specimens ≤ 4 proposed their bactericidal impact towards *H. pylori*

Treatments	Inhibition area (mm)	MIC (µg/mL)	MBC (µg/mL)	MIC/MBC Index
Standard drug*	24.3 ± 0.6	31.25 ± 0.1	31.25 ± 0.1	1
Crude oil	23.0 ± 0.1	62.5 ± 0.1	125.0 ± 0.2	2
Ozonized oil	28.2 ± 0.5	15.62 ± 0.2	31.25 ± 0.1	2

*Standard drug (0.05 mg/ml clarithromycin), Index > 4 showed their bacteriostatic action

through vegetable oil ozonation are liquid or semisolid, and it is necessary to determine whether or not the quality of them will be sufficient for use under typical conditions for preservation (Cirlini et al. 2012; Moureu et al. 2016).

In the our study, ozonation has been done using a designated system to produce a flow rate of ozone of 0 to 5 L/minute of ozone and the crude coconut oil has been treated through this protocol. According to many reports, a number of factors affect the general efficacy of ozonated derivatives, including: (i) the kind and caliber of ozone producers; (ii) the ozonation circumstances, including reactor type and duration, substance kind and quantity, existence of water and/or catalysts; and (iii) the ozonizer's effectiveness, including output O3 level,

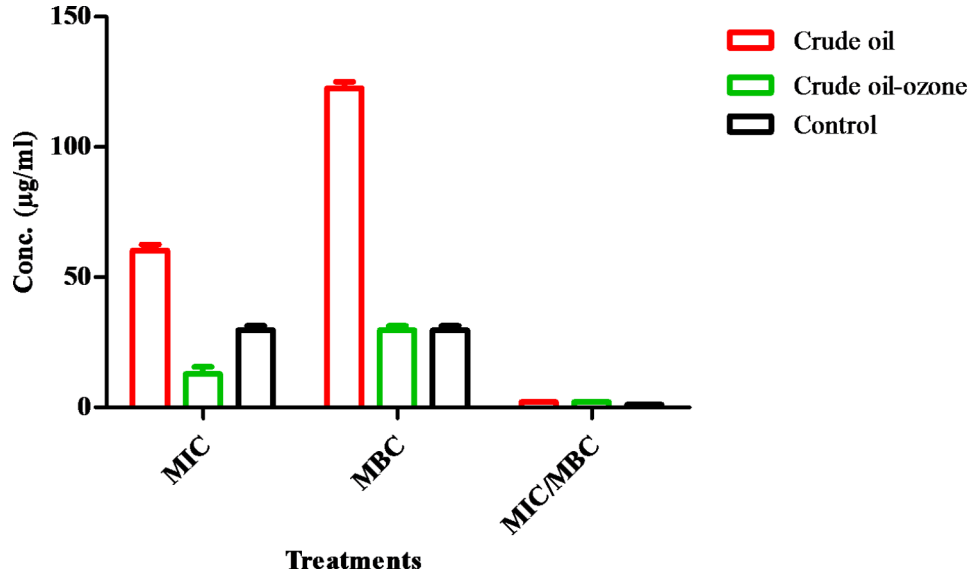

Fig. 3 Comparison of MIC, MBC and MIC/MBC upon using crude and ozonized oil of coconut oil relative to control (clarithromycin) (outcomes are drawn as means ± SD)

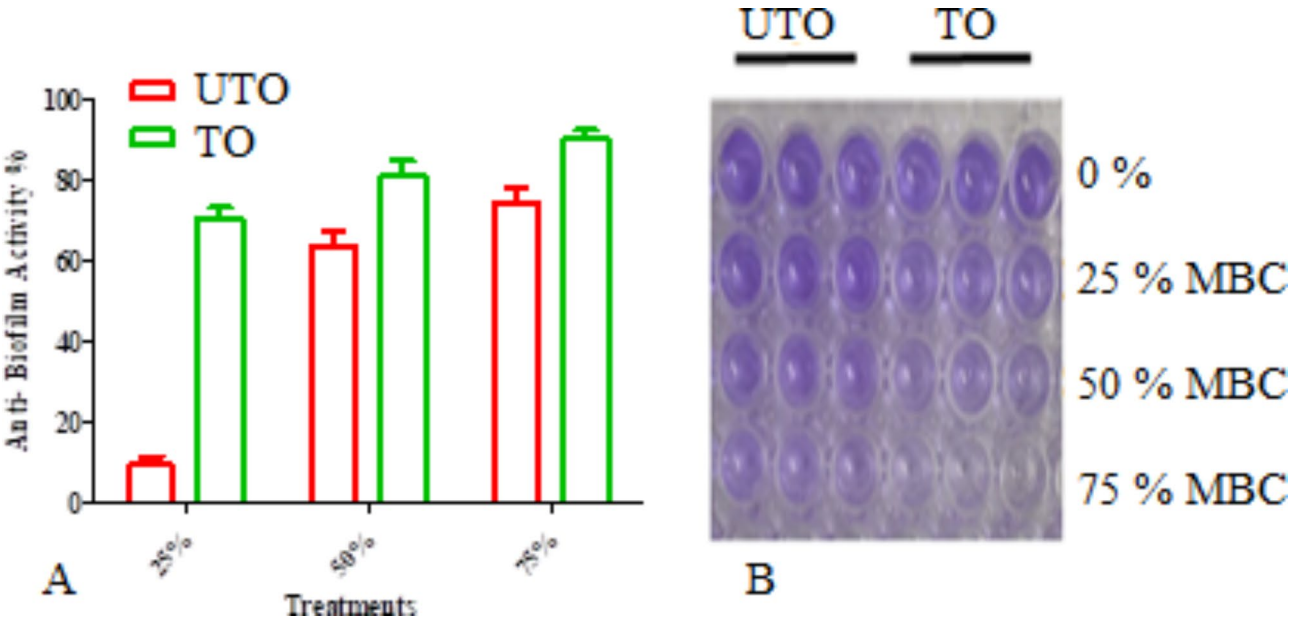

Fig. 4 Anti-biofilm action (%) towards *H. pylori* upon applying 25, 50 and 75% of MBC of crude (UTO) and ozonized (TO) coconut oil (results are illustrated as means ± SD) (**A**) with the stained plate

oxygen supply, and oxygen transporter (Napolitano et al. 2004; Kim et al. 2009; Jacinto et al. 2023). From the published investigation, the oxygen for medical purposes is quite effective in ozonation of sunflower oil (Díaz et al. 2012). Longer ozonation reaction durations are associated with larger degrees of unsaturation in the interaction between ozone and carbon–carbon double bonds (Moureu et al. 2015). This aspect undoubtedly affects the ozonation kinetics as a sequence of the source of the green oils and the chemical structure of their fatty acids (De Almeida et al. 2016).

In the present study exposure to crude coconut oil to ozone led to alteration of the chemical composition of the oil which contained seven fatty acids esters, three alkenes, one alkene hydrocarbon, one fatty acid, one glyceride in crude cocnut oil to ozonized oil which contained four Alkene, one hydroxyl-dicarboxylic acid, three fatty acid ester, two fatty acid, two methane monoterpenoids, one terpenoid and one α, β-unsaturated aldehyde and one diarylmethane. Using GC-MS investigations of the volatile portion of oil exposed to ozone, researchers examined the breakdown of fatty acid ozonides; they

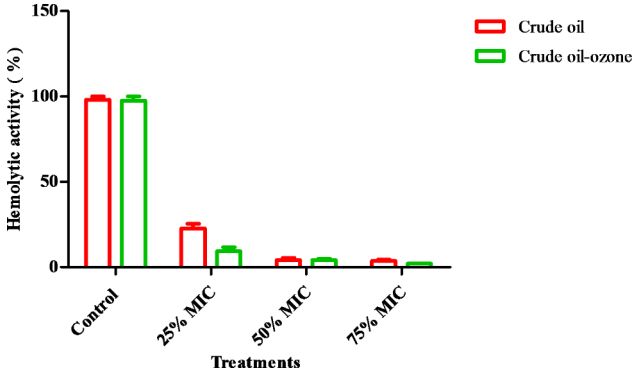

Fig. 5 Anti-hemolytic action upon applying 25, 50 and 75% of MIC of crude and ozonized coconut oil (results are illustrated as means ± SD)

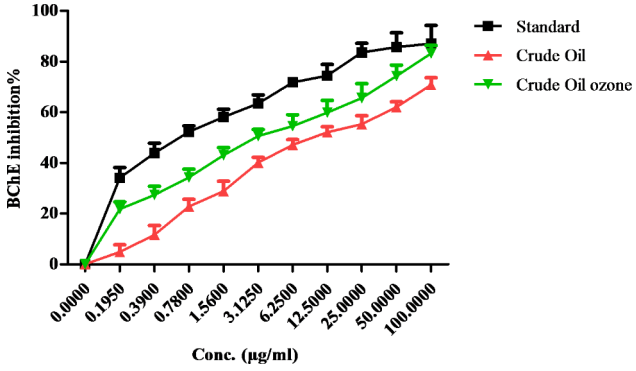

Fig. 6 Anti-Alzheimer of crude and ozonized coconut oil compared to rivastigmine as a standard drug (results are illustrated as means ± SD)

Table 3 Determination of cytotoxic effect of different coconut oil and after exposing to O_3 on A431 cells (Data are tabulated as means ± SD)

Treatment	Dose (µg/mL)	Mean O.D	± SE	Viability %	Toxicity %
Ozonized	1000	0.088667	0.002333	11.97	88.03
	500	0.326667	0.00441	44.08	55.92
	250	0.735667	0.001453	99.28	0.72
	125	0.739	0.000577	99.73	0.27
	62.5	0.740333	0.000333	99.91	0.09
	31.25	0.740667	0.001202	99.96	0.04
	0.0	0.741	0.000577	100	0
	IC_{50}	467.11 ± 4.15 µg/mL			
Control	1000	0.088667	0.002333	11.97	88.03
	500	0.326667	0.00441	44.08	55.92
	250	0.735667	0.001453	99.28	0.72
	125	0.739	0.000577	99.73	0.27
	62.5	0.740333	0.000333	99.91	0.09
	31.25	0.740667	0.001202	99.96	0.04
	0.0	0.741	0.000577	100	0.0
	IC_{50}	484.96 ± 5.4 µg/mL			

discovered degradation products include furyl derivatives, saturated and unsaturated aldehydes, and carboxylic acids. However, it is important to keep in mind that glyceryl oil-based ozonides can be impacted by light and/or humidity, which can facilitate a number of disintegration processes, the main one leading to the creation of aldehydes (Sega et al. 2010; Guerra et al. 2015; Ozturk et al. 2017).

In this investigation exposing coconut oil to ozone led to a slight increase in anti-*H. pylori* impact. Many studies illustrated the possibility of using crude coconut oil as adjuvant therapy in animals infected with *H. pylori* and developed gastric ulcer [Selvarajah et al. 2015; Meng et al. 2019). It has been shown that adding ozone can improve the qualities of oils and its antimicrobial actions (Martínez et al. 2005). Ozone's stronger oxidizing qualities, which cause bacterial outer structures to break down, and become more permeable and allow ozone to enter (Zeng and Lu et al. 2018; Borges et al. 2017).

The exposing of coconut oil to ozone led to intensify the anti-biofilm role of the oil towards *H. pylori*. Unique and potent antimicrobial medications for the management of biofilms have been appeared in response to the growing crisis of antibiotic resistance and formation of biofilms of bacteria. Through the application of oxidizing chemicals that are effective towards microorganisms without causing antibiotic resistance, new and exciting methods for treating biofilms have emerged. Ozoneated derivatives, which are produced when ozone reacts with unsaturated substances like oil, offer a wide range of uses because of the high volatility of ozone as a gas (Cao et al. 2017; Song et al. 2018; Ng et al. 2024).

Some studies reported the anti-hemolytic potential of virgin coconut oil (Hmidani et al. 2021; Utarı et al. 2022). In this work ozonized coconut oil had a better anti-hemolytic pattern through prevention of oxidative impact to erythrocytes. It has been reported that the human body's erythrocyte cells are the ones most frequently used in medication transfer. Activated oxygen species is primarily promoted by hemoglobin and polyunsaturated fatty acids, which are oxidant active transporter molecules and erythrocyte-targeting agents. A number of conditions, including, oxidative medications, excess transition metals, and impairments in erythrocyte antioxidant coordination, may contribute to hemolysis, which is caused by oxidative damage to the lipids and proteins that make up the erythrocyte membrane (Hamidi and Tajerzadeh 2003; Ebrahimzadeh et al. 2009; Afsar et al. 2016).

The mental state of humans in areas critical to cognition and behavior—functions weakened in Alzheimer's disease (Tomášková et al. 2016). BChE is primarily expressed in white matter, glia, and different populations of neurons. There is neither a treatment for AD, a neurodegenerative disease that causes dementia. The use of

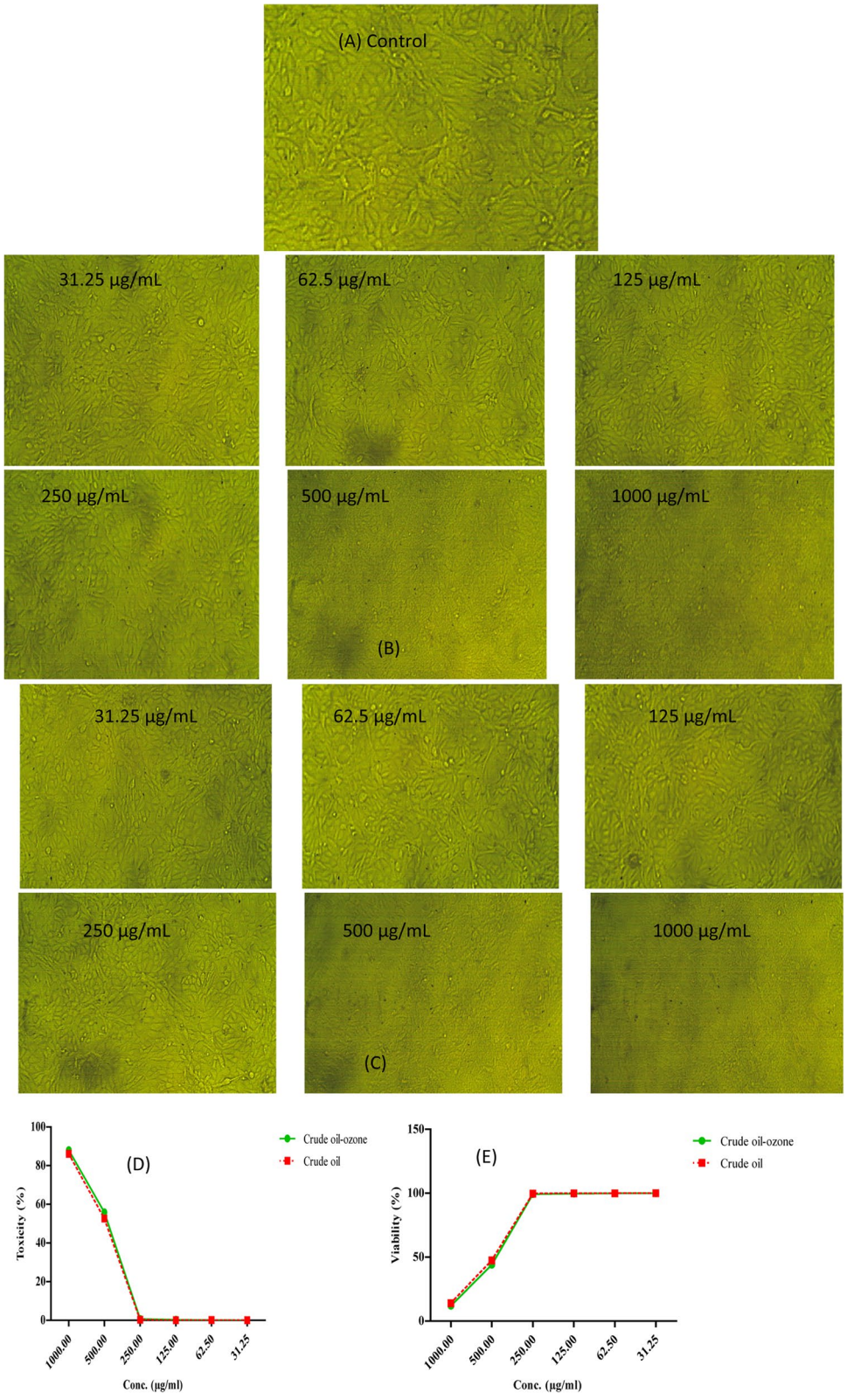

Fig. 7 (**A**) A regular monolayer A431 control cells; (**B**) A431 cells treated by various levels of crude coconut oil and (**C**) A431 cells subjected to different concentrations of ozonized coconut oil. (**D**) Comparing between impact of crude coconut oil and after exposing to O_3 on toxicity % towards A-431 cells at different levels of oils. (**E**) Comparing between impact of crude coconut oil and after exposing to O_3 on viability % of A431- cells at different concentrations of oils

coconut oil to avoid or manage Alzheimer's disease is not well-supported by scientific research. However, some research indicates that include coconut oil in daily food may worsen the symptoms of certain illnesses (Chatterjee et al. 2020; Thawkar and Kaur 2024). The present work illustrated the *in vitro* impact of coconut oil as anti-Alzheimer agent which has been improved upon exposure of the oil to ozone. Many studies showed the anticancer impact of coconut oil (Verma et al. 2019; Alotaibi et al. 2023). In this work the exposing of coconut oil to ozone improve its anticancer activity towards A431 cells. In accordance with a previous report by Al-Rajhi and Abdel Ghany (2023) that showed treatment of oils with ozone enhance its anticancer impact. Finallly this investigation showed that exposing coconut oil to a flow rate of ozone of 0 to 5 L/minute for 3 hours alter its chemical composition and leading to enhance its antibacterial and biofilm capability versus *H. pyloi*, as well as improve its anti-hemolytic activity, anti-Alzheimer impact as well as it cytotoxic effect towards A431 cells to be applied and verification using animal studies. Future studies should focus on seperating active constituents of coconut oil after treatment by ozone along with studying the ultrastructure of *H. pyloi* to understand the action mechanisms of active constituents. Furthermore more than one cancer cell lines must be investigated to confirm the anticancer activity.

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Conceptualization, methodology M.S.A. and M.H.A.; formal analysis, investigation M.K.T., S.K.A., A.A.A., F.A.A., D.A.B, H.M.A.; writing—original draft preparation, writing—review and editing, M.S.W., S.A. All authors have read and agreed to the published version of the manuscript.

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All data that support the findings of this study are available within the article.

Declarations

Conflict of interest
The authors declare no conflicts of interest.

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