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Anti-ulcer effects of cumin (*Cuminum cyminum* L.), thyme (*Thymus vulgaris* L.), and caraway (*Carum carvi* L.) essential oils on peptic ulcer and ulcerative colitis models in rats

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ABSTRACT

Introduction: Essential oils are considered a potential alternative to synthetic drugs in the management of diseases such as peptic ulcer (PU) and ulcerative colitis (UC). This study is concerned with comparing the therapeutic effects of *Cuminum cyminum* L, *Carum carvi* L, and *Thymus vulgaris* L. essential oils on PU and UC models induced by ethanol.

Methods: Rats were divided into 10 groups; control groups were treated with saline and experimental groups with 500 mg/kg body weight of *C. cyminum, C. carvi,* or *T. vulgaris* essential oil. Curative effects were determined by measuring tissue oxidative markers, such as reduced glutathione (GSH), malondialdehyde (MDA), and myeloperoxidase (MPO), as well as the inflammatory marker prostaglandin E2 (PGE2), stomach pepsin (PEP), and colon alkaline phosphatase (ALP). Biochemical and histological examinations were done on stomach and colon tissues.

Results: The current study proved the anti-ulcer effects of *C. cyminum, C. carvi,* and *T. vulgaris* essential oils. They improved the oxidative and inflammatory markers in both stomach and colon tissues and modulated stomach PEP and colon ALP activities. *T. vulgaris* essential oil modulated GSH and MDA levels resulting in a significant elevation in GSH levels by 120.43% and 99.46% and a significant reduction in MDA levels by 20.05% and 24.1% in PU and UC models, respectively. *C. carvi* essential oil was the most effective in restoring PGE2 by 71.51% compared to UC group. Results were confirmed by the morphological and histopathological changes.

Conclusion: *C. cyminum, C. carvi,* or *T. vulgaris* essential oils might be used in the management of acute PU and UC.

Implication for health policy/practice/research/medical education:
Cumin, caraway, and thyme essential oils improve the lesions of peptic ulcer and ulcerative colitis and reduce the related side effects. These essential oils might be recommended to physicians as adjunctive therapy for the treatment of ulcers.

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Introduction

Peptic ulcer (PU) is a chronic inflammatory disorder altering the mucosal integrity of the stomach and/or duodenal lining and affects up to 10% of the world's population (1). It usually occurs when the balance between offensive and protective factors of the mucosa is disturbed (2). The elevation in gastric juice acidity, chronic use of non-steroidal anti-inflammatory drugs, and *Helicobacter pylori* infection are major predisposing factors for mucosal lining injury (3). Different treatment protocols are available based on ulcer etiology, such as administration of antacids, histamine inhibitors, mucosal tissues lining protection, and eradication of *H. pylori* infection (4).

Ulcerative colitis (UC) is a relapsing disease with a high prevalence rate. Management aims to achieve

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rapid resolution of symptoms, mucosal healing, and improvement in a patient's quality of life (5). The etiology of UC involves interactions between the environment, immune system, gut microbiome, or a genetic predisposition to disease (6).

Alcohol consumption is known to be a risk factor for gastrointestinal bleeding and has a positive association with PU (7). It is also associated with a higher risk of relapse in patients with UC. This is correlated to the hypothesis that alcohol modulates the microbiome and facilitates intestinal inflammation (8).

There is an increased need to find a new anti-ulcer drug, and great effort is made for the development of new drug entities from natural origin with a high safety margin and minimum side effects (9). Essential oils have a wide range of pharmacological, biotechnological, and industrial applications; they are incorporated as food additives in many food products as flavoring agents and preservatives (10). Caraway (*Carum carvi* L.) is a biennial plant (Family *Apiaceae*), native to western Asia, Europe, and North Africa commonly used as a spice in foods and beverages. The caraway extract and its essential oil showed anti-diabetic, antioxidant, hepatoprotective, antiulcerogenic, antimicrobial, insecticidal, diuretic, analgesic, renoprotective, molluscicidal, anti-cholinesterase, and immunomodulatory activities (11-14). Caraway fruits contain essential oil, fatty acids, protein, carbohydrate, phenolic acids, flavonoids, tannins, alkaloids, and terpenoids (15).

Cumin (*Cuminum cyminum* L.) (Family *Apiaceae*) is an annual plant and one of the oldest and most cultivated aromatic and herbaceous natural products with numerous medicinal, nutraceutical, and pharmaceutical properties (16,17). The *Cum* essential oil has several pharmacological properties, such as antioxidant, antimicrobial, anti-diabetic, anti-hypertensive, and immunomodulatory effects (18,19).

Thyme (*Thymus vulgaris* L.) is a member of the *Lamiaceae* family cultivated as an aromatic plant (20). It has high antioxidant compounds effective in strengthening the immune system mainly in the treatment of the upper respiratory system (21). *T. vulgaris* essential oil has been used in traditional medicine as an expectorant, anti-inflammatory, antiviral, antibacterial, and antiseptic agent due to its high thymol and carvacrol content (22,23).

Herbs, medicinal plants, spices, and crude drug substances are considered a potential source to control various diseases, including gastric ulcer and UC. The major objective of the current study was to evaluate the therapeutic activities and the chemical profiles of the essential oils of *C. cyminum* L., *T. vulgaris* L. and *Carum carvi* L. on the PU and UC models induced by ethanol and shed light on the potential use of these essential oil as natural anti-ulcer agents with minimum side effects.

Materials and Methods

Chemicals

Ethanol (96%), sodium hydroxide (0.01 N), potassium phosphate buffer, and formalin were purchased from HoldiPharma, El Nasr Pharmaceutical and Chemical Company, Cairo, Egypt.

Plant materials and essential oils extractions

Natural essential oils of *C. cyminum* L., *T. vulgaris* L., and *Carum carvi* L. were purchased from Pharaonic essential oils, PHATRADE company, Cairo, Egypt.

Gas chromatography/Mass spectrometry analyses for plants essential oils

Gas chromatography/Mass spectrometry (GC/MS) analysis was carried out on Shimadzu GCMS-QP 2010 chromatograph (Kyoto, Japan) with Rtx-1MS capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek, USA). The oven temperature was kept at 45°C for 2 minutes (isothermal), programmed to 30°C at 5°C/min, and kept constant at 300°C for 5 minutes (isothermal); injector temperature was 250°C. The carrier gas used was Helium, with a flow rate set at 1.40 mL/min. Diluted samples (1% v/v) were injected with a split ratio of 15:1 and the injected volume was 1 µL. The MS operating parameters were as follows: interface temperature: 280°C, ion source temperature: 200°C, EI mode: 70 eV, scan range: 35–500 amu. Identification of the essential oil constituents was made based on their retention indices, matching their mass spectra with NIST-17 and Wiley library database as well as published the data in the literature. Retention indices (RI) were calculated relative to homologous series of n-alkanes (C8-C30) injected under the same conditions.

4. Animals

Seventy male Sprague-Dawley albino rats weighing between 180 and 200 g were supplied from the oncology unit – (NCI), Cairo University, Egypt. All experiments were conducted following the guidelines for the care and use of experimental animals. Rats were maintained on a standard commercial pellets diet and tap water *ad libitum* (24) and kept individually in stainless cages in constant conditions. Before ulcer induction, the animals were deprived of food for 16 hours with free access to water. Animals were divided into two ulcer models, PU and UC models.

Animal grouping and experimental design

Rats were randomly divided into ten groups (n = 7 per group). In the PU model, a normal control group received a daily oral dose of saline, PU group received an oral dose of 70% ethanol (0.5 mL/100 g body weight) (25) followed by saline for 7 days, PU + *C. cyminum*, PU+ *C. carvi*, and PU+ *T. vulgaris* were administered *C. cyminum*, *C. carvi*, and *T. vulgaris* essential oils, respectively at a dose of (500

mg/ kg body weight) (26) for 7 days after ulcer induction. In the UC model, a normal control group received an intrarectal saline injection, UC group received an intrarectal ethanol injection (0.5 mL/100g body weight) followed by saline for 7 days. UC + *C. cyminum*, UC + *C. carvi*, and UC + *T. vulgaris* were administered *C. cyminum, C. carvi,* or *T. vulgaris* essential oils, respectively, at the dose of 500 mg/kg body weight for 7 days after ulcer induction. Induction of PU or UC was confirmed by visualization of the dissected stomach in the PU and colon in UC positive control groups.

Tissue sampling

After seven days of essential oils, treatment rats were sacrificed. In the PU-induced model, stomachs were dissected, and gastric juice was collected. In the UC-induced model, part of the colon between the ileocecal junction and anus was excised. Excised stomachs and colons were washed with saline for morphological examinations. Part of the stomach and colon were homogenized in potassium phosphate buffer (pH 7.5) and centrifuged at 6000 rpm for 15 minutes at 4°C for biochemical analysis. Specimens from the stomach and colon were kept in 10% formalin and embedded in paraffin for histopathological examinations.

Determination of titratable acidity in gastric juice

The acidic content of the gastric juice, expressed as mEq/L, was determined by titrating 0.2 mL of gastric juice against 0.01 N NaOH solution. Phenol red was used as an indicator (27).

Morphological examination and determination of ulcer score

Stomachs from the PU-induced model and colons from the UC-induced model were placed on a flat plate to visualize the morphological changes induced in each treatment group. Photographs were taken using a digital camera with zooming (10 megapixels 5× zoom). Ulcers were scored (28), as 0 for normal colored stomach/colon, + for red coloration, ++ for spot ulcer, +++ for moderate ulcer with hemorrhagic streaks, and ++++ for severe ulceration.

Assessment of oxidative stress markers

Assessment of reduced glutathione (GSH) and malondialdehyde (MDA), as the measures of lipid peroxidation, were determined in the stomach and colonic tissue samples colorimetrically according to kits instruction provided by PromoKine, USA.

Assessment of myeloperoxidase activity and prostaglandin E2 level

Myeloperoxidase (MPO) activity and prostaglandin E2 (PGE2) levels were assayed in the stomach and colonic tissue samples using ELISA kit provided by CUSABIO, USA (catalog number. CSB-E08722r and CSB-E08920r).

Assessment of pepsin and alkaline phosphatase activities

Pepsin (PEP) activity was assessed in stomach tissues of PU model rats using PEP ELISA kit (CUSABIO, USA – catalog number. CSB-E08920r) and alkaline phosphatase (ALP) activity was measured in colon tissues of UC model rats using ALP ELISA kit (BioVision, USA – catalog number. E4575-100).

Histopathological examination

Samples from the stomach and colon of the rats of different groups in 10% formalin were cleared in xylene and embedded in paraffin at 56°C for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slide microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin stain as well as alizarin red, and examined using light electric microscope (29).

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (SPSS) program, version 17.0. The data were expressed as mean ± standard deviation (SD) of the mean. Statistical differences between groups were performed using one-way analysis of variance (ANOVA). The mean difference was considered significant at $P < 0.05$ level (30)

Results

Gas chromatography/Mass spectrometry analysis of *Cuminum cyminum, Carum carvi*, and *Thymus vulgaris* essential oils

Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oils resulted in the identification and quantification of 20 volatile components in *C. cyminum* essential oil accounting for 97.34% of the total oil composition. Fifteen volatile components in *C. carvi* essential oil represent 91.29% of its total composition. In addition, GC/MS analysis of *T. vulgaris* essential oil resulted in the identification and quantification of 34 volatile components, which represented 92.52% of the total oil composition. Results are summarized in Table 1 and Figure 1. Oxygenated monoterpenes represented the major class of volatile components identified in thyme, cumin, and caraway essential oils (50.99%, 56.01%, and 62.71%, respectively), followed by monoterpenes hydrocarbons (32.88%, 40.94%, and 28.21%, respectively). Furthermore, thyme essential oil contained 5.76% sesquiterpenes hydrocarbons compared to 0.10% in cumin essential oil and 0.20% in caraway essential oil. The major volatile component identified in thyme essential oil was thymol (40.79%), followed by *p*-cymene (18.13%), *γ*-terpinene (8.41%), carvacrol (4.81%), caryophyllene (4.61%), and linalool (2.69%). Whereas cumin aldehyde

Table 1. Chemical profile of thyme, cumin, and caraway essential oils

No.	Retention time	Compound name	RI_{exp} [a]	RI_{rep} [b]	Content%			Molecular formula	Identification[c]
					Thyme	Cumin	Caraway		
1	7.523	α-Thujene	923	923	0.54	0.36	-	$C_{10}H_{16}$	MS, RI
2	7.701	α-Pinene	929	929	0.62	0.85	-	$C_{10}H_{16}$	MS, RI
3	8.054	Camphene	942	942	0.35	-	-	$C_{10}H_{16}$	MS, RI
4	8.802	Sabinen	969	969	0.75	0.58	-	$C_{10}H_{16}$	MS, RI
5	8.865	Sabinene	971	971	0.22	-	-	$C_{10}H_{16}$	MS, RI
6	8.924	β-Pinene	974	974	-	12.16	-	$C_{10}H_{16}$	MS, RI
7	9.355	β-Myrcene	989	990	1.15	0.94	0.5	$C_{10}H_{16}$	MS, RI
8	9.711	α-Phellandrene	1002	1002	0.15	0.86	-	$C_{10}H_{16}$	MS, RI
9	10.118	α-Terpinene	1015	1015	1.39	0.23	-	$C_{10}H_{16}$	MS, RI
10	10.192	o-Cymol	1017	1017	-	-	0.15	$C_{10}H_{14}$	MS, RI
11	10.278	p-Cymene	1020	1019	18.13	7.07	-	$C_{10}H_{14}$	MS, RI
12	10.424	β-Phellandrene	1025	1025	-	0.48	-	$C_{10}H_{16}$	MS, RI
13	10.439	Eucalyptol	1025	1025	0.86	-	-	$C_{10}H_{16}$	MS, RI
14	10.485	$_{D}$-Limonene	1027	1027	0.31	0.32	27.56	$C_{10}H_{16}$	MS, RI
15	11.427	γ-Terpinene	1057	1058	8.41	17.09	-	$C_{10}H_{16}$	MS, RI
16	11.493	4-Thujanol	1059	1040	0.24	-	-	$C_{10}H_{18}O$	MS, RI
17	12.106	Benzene, (2-methylpropenyl)	1079	1072	0.13	-	-	$C_{10}H_{18}O$	MS, RI
18	12.300	Terpinolene	1085	1085	0.15	-	-	$C_{10}H_{16}$	MS, RI
19	12.552	Linalool	1093	1093	2.69	-	-	$C_{10}H_{18}O$	MS, RI
20	13.027	p-Mentha-trans-2,8-dien-1-ol	1109	1110	-	-	0.19	$C_{10}H_{16}O$	MS, RI
21	13.640	Camphor	1128	1128	0.48	-	-	$C_{10}H_{16}O$	MS, RI
22	14.809	4-Carvomenthenol	1166	1166	-	0.31	-	$C_{10}H_{18}O$	MS, RI
23	14.821	(-)-4-Terpineol	1166	1160	0.80	-	-	$C_{10}H_{18}O$	MS, RI
24	14.903	Terpinen-4-ol	1169	1169	0.57	-	-	$C_{10}H_{18}O$	MS, RI
25	15.083	Dihydrocarvone	1175	1182	-	-	1.28	$C_{10}H_{16}O$	MS, RI
26	15.089	3-*p*-Menthen-7-al	1175		-	1.78	-	$C_{10}H_{16}O$	MS
27	15.148	α-Terpineol	1177	1177	0.13	0.12	-	$C_{10}H_{18}O$	MS, RI
28	15.268	*trans*- Dihydrocarvone	1181	1182	-	-	1.60	$C_{10}H_{16}O$	MS, RI
29	16.145	Dihydrocarveol	1210	1208	-	-	0.23	$C_{10}H_{18}O$	MS, RI
30	16.288	cis-Carveol	1215	1216	-	-	0.38	$C_{10}H_{16}O$	MS, RI
31	16.483	Cuminaldehyde	1222	1222	-	21.72	-	$C_{10}H_{18}O$	MS, RI
32	16.519	Thymol methyl ether	1223	1228	1.22	-	-	$C_{11}H_{16}O$	MS, RI
33	16.707	d-Carvone	1229	1228	-	-	49.40	$C_{10}H_{14}O$	MS, RI
34	16.741	Carvone	1230	1236	-	-	4.49	$C_{10}H_{14}O$	MS, RI
35	16.760	Isopiperitenon	1231	1239	-	-	4.47	$C_{10}H_{14}O$	MS, RI
36	16.814	*p*-Cymene-2-ol methyl ether	1233	1228	0.77	-	-	$C_{11}H_{16}O$	MS, RI
37	17.342	Perilla aldehyde	1251	1250	-	-	0.67	$C_{10}H_{14}O$	MS, RI
38	17.392	Nerol	1253	1247	0.20	-	-	$C_{11}H_{14}O$	MS, RI
39	17.764	Terpinen-7-al	1266	1268	-	16.19	-	$C_{10}H_{14}O$	MS, RI
40	17.968	γ-Terpinen-7-al	1273	1274	-	15.05	-	$C_{10}H_{14}O$	MS, RI
41	18.337	Thymol	1286	1286	40.79	-	-	$C_{10}H_{14}O$	MS, RI
42	18.480	Carvacrol	1291	1291	4.81	-	-	$C_{10}H_{14}O$	MS, RI
43	19.057	*p*-Mentha-1,4-dien-7-ol	1311	1315	-	0.84	-	$C_{10}H_{16}O$	MS, RI
44	20.885	Ylangene	1375	1375	0.11	-	-	$C_{15}H_{24}$	MS, RI
45	21.015	α-Copaene	1380	1380	0.14	-	-	$C_{15}H_{24}$	MS, RI
46	21.213	(-)-.β-Bourbonene	1387	1387	0.13	-	-	$C_{15}H_{24}$	MS, RI

Table 1. Continued

No.	Retention time	Compound name	RI_{exp} [a]	RI_{rep} [b]	Content%			Molecular formula	Identification[c]
					Thyme	Cumin	Caraway		
47	21.356	β-Elemene	1392	1392	-	-	0.20	$C_{15}H_{24}$	MS, RI
48	22.143	Caryophyllene	1422	1422	4.61	-	-	$C_{15}H_{24}$	MS, RI
49	22.969	α-Humulene	1454	1454	0.20	-	-	$C_{15}H_{24}$	MS, RI
50	23.019	β- Famesene	1456	1456	-	0.10	-	$C_{15}H_{24}$	MS, RI
51	23.534	γ-Muurolene	1476	1475	0.41	-	-	$C_{15}H_{24}$	MS, RI
52	24.134	α-Muurolene	1499	1502	0.16	-	-	$C_{15}H_{24}$	MS, RI
53	25.976	Caryophyllene oxide	1571	1571	0.74	-	0.11	$C_{15}H_{24}O$	MS, RI
54	26.411	Carotol	1594	1594	-	0.29	-	$C_{15}H_{26}O$	MS, RI
55	27.346	α-Muurolol	1628	1627	0.16	-	-	$C_{15}H_{26}O$	MS, RI
56	27.840	Apiol	1649	1645	-	-	0.06	$C_{12}H_{14}O_4$	MS, RI
Monoterpene hydrocarbons (%)					32.88	40.94	28.21		
Oxygenated monoterpenes (%)					50.99	56.01	62.71		
Sesquiterpene hydrocarbons (%)					5.76	0.10	0.20		
Oxygenated sesquiterpenes (%)					0.90	0.29	0.11		
Others (%)					1.99	-	0.06		
Total identified (%)					92.52	97.34	91.29		

[a] Kovats index determined experimentally on RTX-1 column relative to C8–C30 n-alkanes.
[b] Published Kovats retention indices.
[c] Identification was based on comparison of the compounds mass spectral data (MS) and Kovats retention indices (RI) with those of NIST Mass Spectral Library (2011), Wiley Registry of Mass Spectral Data 8th edition and literature.

was the major identified compound in cumin essential oil representing 21.72% of the total oil composition, followed by *γ*-terpinene (17.09%), terpinen-7-al (16.19%), γ-terpinen-7-al (15.05%), β-pinene (12.16%), and *p*-cymene (7.07%). In caraway essential oil, the major identified compound was carvone (49.40%), followed by, d-limonene (27.56%), d-carvone (4.49%), isopiperitenon (4.47%), and dihydrocarvone (1.28%). The composition of the three essential oils varied, *β*-myrcene and d-limonene were the only common constituents in three essential oils.

Effect of *Cuminum cyminum*, *Carum carvi*, and *Thymus vulgaris* essential oils on ethanol-induced PU model

Morphological examination and detection of ulcer score

In PU model groups (Figure 2), the control group showed mucosa without any lesion or redness (score 0) and PU group showed severe hemorrhagic ulcerated mucosal layer (score + + + +). All treated groups showed improvement

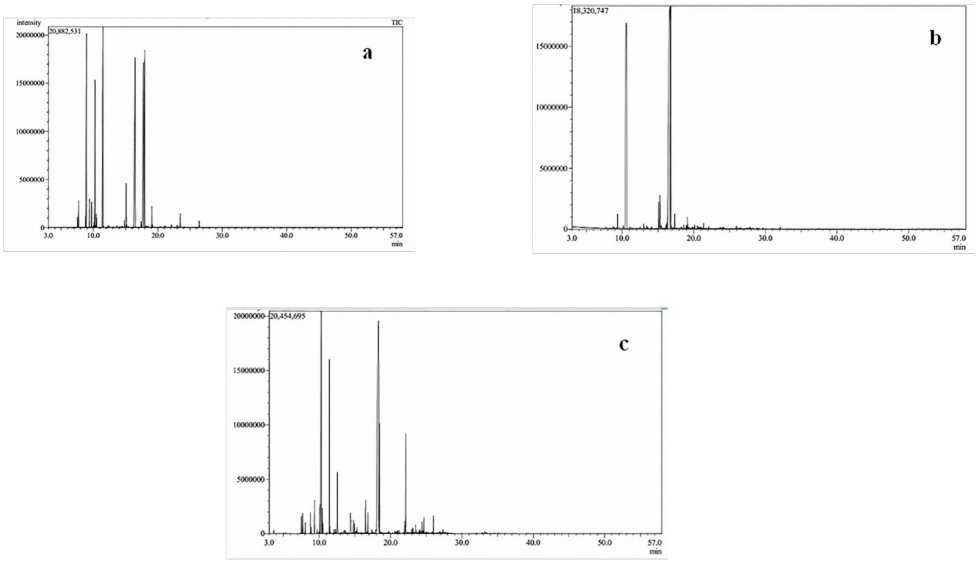

Figure 1. Gas chromatography/Mass spectrometry (GC/MS) chromatogram of essential oils of a) Cumin (Cum), b) Caraway (Car), and c) Thyme (Thy).

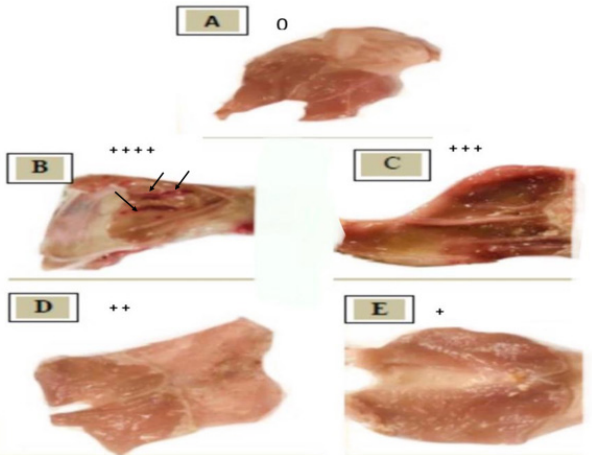

Figure 2. Demonstrative images of stomachs of Peptic ulcer model groups. Control group (A) shows mucosa without any lesion or redness; (B) Peptic ulcer (PU) group; (C) Cumin treated group; (D) Caraway treated group; (E) Thyme treated group.

of stomach tissues morphology with different degrees. *Cum* treated group showed superficial injuries and some redness (score + + +). *C. carvi* treated group effectively normalizes the damaged mucosal layer with a small ulcer spot (score + +). *T. vulgaris* treated group showed some redness and no damage (score +). Morphological examination of the stomach tissues of PU-treated groups showed the powerful effect of *T. vulgaris* essential oil more than the other essential oils on normalizing the damaged mucosal layer very close to the normal control group.

Effect of Cuminum cyminum, Carum carvi, and Thymus vulgaris essential oils on titratable acidity

The result illustrated in (Figure 3c) indicated that ethanol administration resulted in a significant elevation of gastric juice acidity. Treatment by *C. cyminum, C. carvi,* or *T. vulgaris* essential oils significantly reduced the titratable acidity elevation by 15.5%, 53.3%, and 73.3%, respectively.

Effect of Cuminum cyminum, Carum carvi, and Thymus vulgaris essential oils on PU biochemical parameters

The effect of essential oils treatment on oxidative stress markers (S-MDA, S-GSH, and S-MPO), inflammatory marker (S-PGE2), and stomach pepsin (S-PEP) in ethanol-induced PU model is illustrated in Figure 3. Ethanol administration resulted in a significant elevation of S-MDA level and S-MPO activity by 197.16% and 242.31%, respectively, and caused a significant reduction in S-GSH by -73.45% when compared to the normal control group. Treatment with *C. cyminum, C. carvi,* or *T. vulgaris* essential oils reversed the oxidative stress status indicated by a significant reduction in MDA and MPO activity, as well as a significant elevation in S-GSH compared to the PU group. The effect of the three essential oils on MPO activity was comparable; however, *T. vulgaris* essential oil was the most effective in modulating the GSH and MDA levels causing significant elevation of GSH by120.43% combined by a significant reduction in MDA level and MPO activity by -20.05% and -29.48% respectively as compared to PU group ($P<0.05$). Moreover, ethanol administration resulted in a marked reduction of stomach PGE2 level by -55.70% that was reversed by essential oils administration. Both *T. vulgaris* and *C. carvi* groups had reversed the PGE2 level near to normal control that was indicated by non-significant reduction of PGE2 level in both groups as compared to the normal control group ($P<0.05$). A significant elevation in PEP activity in the PU

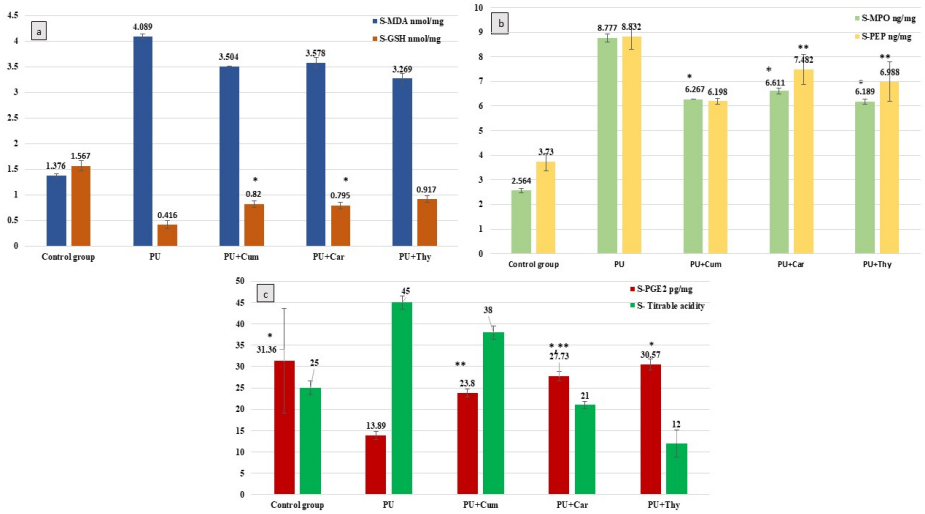

Figure 3. Effect of *Cum*, *Car*, and *Thy* essential oils administration in peptic ulcer model groups. (a) Stomach malondialdehyde (S-MDA) and reduced glutathione (S-GSH) levels, (b) stomach myeloperoxidase (S-MPO) and pepsin (S-PEP) activities and (c) stomach prostaglandin E2 (S-PGE2) level and titratable acidity (S-titratable acidity) in peptic ulcer (PU) model, expressed as mEq/L. Data are expressed as mean ± SD (n = 7). There is no significant difference between means that have the same number of stars at ($P < 0.05$).

group by 87.34% when compared to the normal control was observed. Treatment with any of the essential oils resulted in a significant reduction in PEP activity compared to the PU group ($P < 0.05$). *C. cyminum* essential oil showed the highest improvement of PEP activity by -29.82% when compared to PU group.

Histological examination of Cuminum cyminum, Carum carvi, and Thymus vulgaris essential oils on PU model

Microscopic examination of stomach sections of the normal control group showed no histopathological alteration; normal histological structure of the mucosal epithelial layer, lamina propri, glandular structure and underlying submucosa, muscular, and serosa were observed (Figure 4a). PU induction by ethanol administration caused focal ulceration, necrosis (star), and edema (arrow) with inflammatory cells infiltration in the mucosal lining epithelium and lamina propria, which were extended to submucosa in a few manners as shown in (Figure 4b). Treatment with *C. cyminum* essential oil showed no difference from the PU group (Figure 4c). The *C. carvi* essential oil reversed to a great extent the ethanol-induced damage in the mucosal, submucosa, and muscularis layers; the serosal layer showed mild edema (Figure 4d). Submucosal and muscularis edema was detected in the group treated with *T. vulgaris* essential oil, other layers were intact (Figure 4e).

Effect of *Cuminum cyminum, Carum carvi,* and *Thymus vulgaris* essential oils on ethanol induced UC model

Morphological examination and detection of ulcer

The control group showed intestinal mucosa without any lesion or redness (score 0), while the UC group showed severe hemorrhagic ulcerated mucosal layer (score + + + +). *Cum* treated group showed superficial injuries and hemorrhagic streaks (score + + +). *C. carvi* treated group showed slight redness and no damage (score +), while *T. vulgaris* treated group showed an injured mucosal layer with a small ulcer spot (score + +). Morphological examination of colons indicated that *C. carvi* essential oil was the most effective in restoring the intestinal mucosa integrity similar to the normal intestinal mucosa (Figure 5).

Effect of Cuminum cyminum, Carum carvi, and Thymus vulgaris essential oils on UC biochemical parameters

The current result illustrated the effect of essential oils on colon oxidative stress (C-MDA, C-GSH, and C-MPO) and inflammatory marker (C-PGE2) in addition to colon alkaline phosphatase (C-ALP) activity for ethanol-induced UC model indicating that ethanol intake resulted in a significant increase in C-MDA level by 192.27% and C-MPO activity by 279.68% with a significant reduction in C-GSH level by -73.53% as compared to normal control (Figure 6). Treatment with *C. cyminum, C. carvi,* or *T. vulgaris* oils reversed the oxidative stress status indicated by a significant reduction of C-MDA and C-MPO and significant increase of C-GSH level compared to UC group. There was no significant difference between *C. carvi* and *C. cyminum* essential oils effects on colonic GSH level; *Thy* essential oil had the most significant effect in modulating GSH and MDA levels as *T. vulgaris* increased the GSH level by 99.46% and decrease MDA level by -24.1% as compared to UC group.

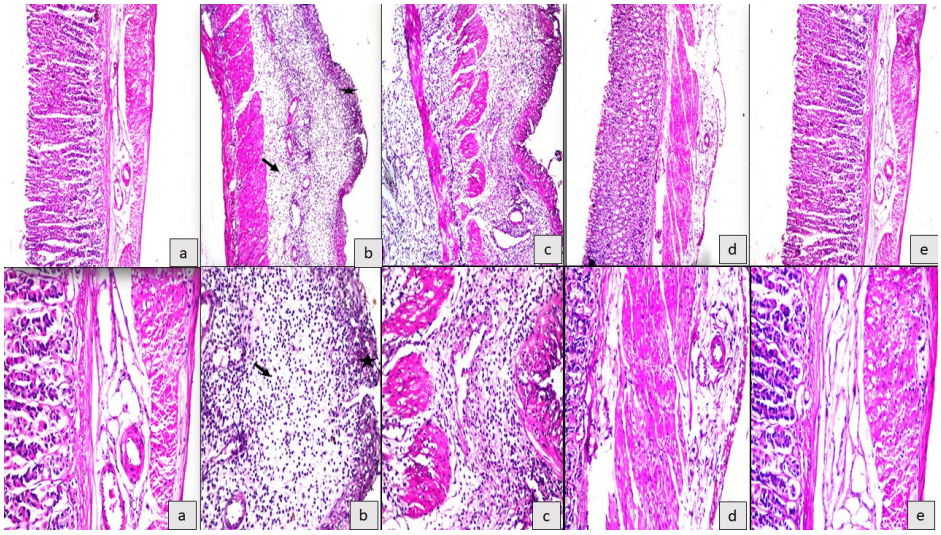

Figure 4. Microscopic examination of stomach tissues in peptic ulcer model groups. (a) Control group showing normal histological structure of the mucosal epithelial layer with lamina propria and glandular structure as well as the underlying submucosa, muscularis and serosa. (b) Peptic ulcer (PU) group showing, focal ulceration and necrosis as well as edema with inflammatory cells infiltration detected in the mucosal lining epithelium and lamina propria extended to the submucosa. (c) Cumin treated group with lamina propria showing focal ulceration and necrosis associated with edema and inflammatory cells infiltration which were extended to muscularis. (d) Caraway treated group with no histopathological alteration in the mucosal, submucosa and muscularis while the serosal layer showed edema. (e) Thyme group stomach showing edema in the submucosa and muscularis while the other layers were intact.

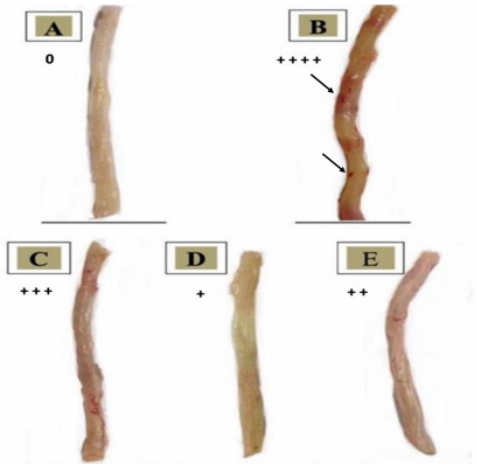

Figure 5. Morphologic changes of the rat's colons in ulcerative colitis model groups: (A) Normal control group showed intestinal mucosa without any lesion or redness (score 0); (B) Ulcerative colitis group showed severe hemorrhagic ulcerated mucosal layer (score + + + +); (C) Cumin treated group showed superficial injuries and hemorrhagic streaks (score + + +); (D) Caraway treated group showed slight redness and no damage (score +); (E) Thyme treated group showed injured mucosal layer with small ulcer spot (score + +).

Moreover, induction of UC by ethanol caused a marked reduction of colon PGE2 level by -66.70% as compared to normal control, whenever treatment with any of the three oils caused a significant increase in C-PGE2 compared to the UC group. *C. cyminum, C. carvi,* and *T. vulgaris* essential oils treated groups had a significant elevation of PEG2 by 71.51%, 37.20%, and 49.15% as compared to UC group indicating that *C. carvi* essential oil was the most effective in restoring the C-PGE2 level toward the normal level.

Regarding colon ALP activity, there was a significant decrease in ALP activity in UC group by -66.70 as compared to normal control. Treatment with any of the three oils caused a significant increase in ALP activity by 28.74%, 49%, and 72.3%, respectively, compared to the UC group and a significant decrease by -57.13%, -50.39%, and -42.63% compared to normal control. Compared to other oils treated groups, *T. vulgaris* essential oil had the greatest improvement of ALP activity.

Histological effects of Cuminum cyminum, Carum carvi, and Thymus vulgaris essential oils on UC model:

Microscopic examination of the intestinal section of UC and treated groups is illustrated in Figure 7. Section of the normal control group showed no histopathological alteration, and normal histological structure of the mucosa, submucosa, and muscularis serosa was recorded in Figure 7a. Ethanol-induced UC caused focal desquamation of the mucosal epithelium with edema (arrow) and inflammatory cells infiltration in the underlying lamina propria (star). The submucosa showed edema with few inflammatory cells infiltration, as well as follicular lymphoid hyperplasia (circle) (Figure 7b). Lining epithelium after treating the UC with *C. cyminum* essential oil showed focal cellular desquamation associated with inflammatory cells infiltration in the underlying lamina propria, while the submucosa had lymphoid follicular hyperplasia (Figure 7c). *C. carvi* essential oil treatment caused focal few inflammatory cells infiltration in the lamina propria of the mucosa as well as in the submucosa associated with edema in the latter (Figure 7d). *T. vulgaris* essential oil effect on the mucosal layer showed focal cellular desquamation associated with edema and few inflammatory cells infiltration in the submucosa (Figure 7e).

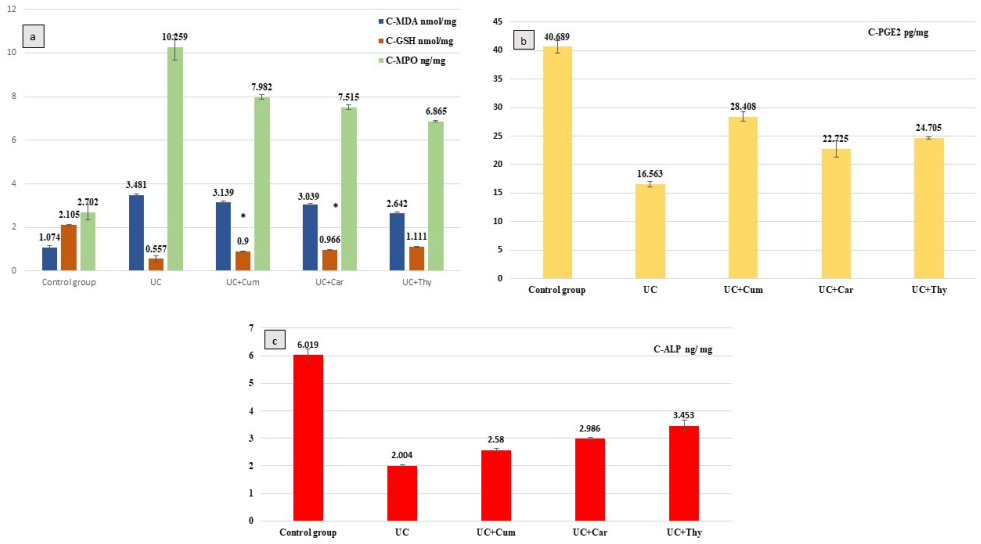

Figure 6. Effect of *Cuminum cyminum, Carum carvi,* and *Thymus vulgaris* oils administration in ulcerative colitis model groups. (a) Colon Malondialdehyde (C-MDA), reduced Glutathione (C-GSH) levels, and colon Myeloperoxidase (C-MPO) activity; (b) colon prostaglandin E2 (C-PGE2) level; and (c) colon alkaline phosphatase (C-ALP) activity. Data are expressed as mean ± SD (n = 7). The same number of stars indicates that there is no significant difference between the means at $P < 0.05$.

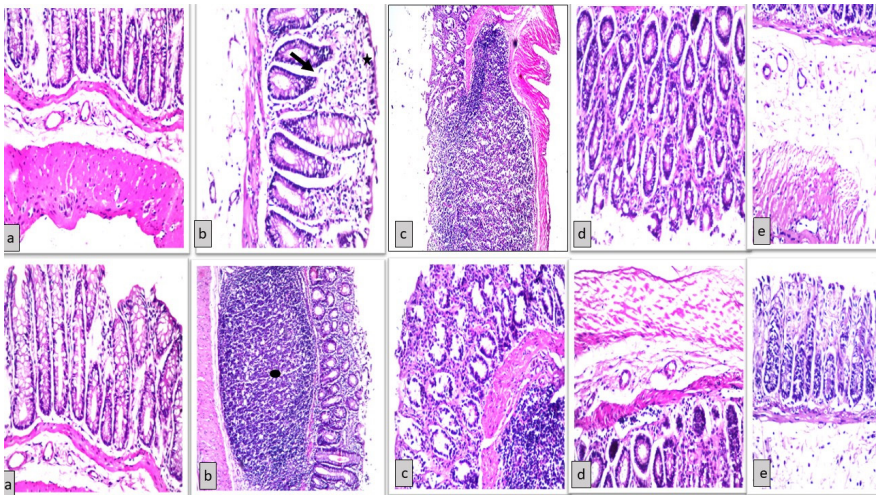

Figure 7. Microscopic examination of intestinal section in ulcerative colitis model groups. (a) Section of normal control intestine recording; no histopathological alteration and normal histological structure of the mucosa, submucosa, muscularis, and serosa. (b) Section of ulcerative colitis (UC) group showing focal desquamation of the lining mucosal epithelium with edema and inflammatory cells infiltration in the underlying lamina propria. The submucosa shows edema with few inflammatory cells infiltration, as well as follicular lymphoid hyperplasia. (c) The lining epithelium of cumin treated group shows focal cellular desquamation associated with inflammatory cells infiltration in the underlying lamina propria, while the submucosa has lymphoid follicular hyperplasia. d) Focal few inflammatory cells infiltration is detected in caraway treated group lamina propria of the mucosa as well as in the submucosa associated with edema in the later. e) Section of thyme treated group mucosal layer showing focal cellular desquamation associated with edema and few inflammatory cells infiltration in the submucosa.

Discussion

The current investigation evaluated the antiulcerogenic activity of *C. cyminum, C. carvi,* and *T. vulgaris* essential oils on the ethanol-induced PU and UC models. The mechanism of action of the essential oils was evaluated by measuring oxidative stress, inflammatory markers, certain enzyme activities and histopathological changes.

Peptic ulcers result from increased reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl radicals. These radicals, in turn, increase gastric oxidative stress leading to gastric hemorrhage and ulcer formation. Reactive oxygen species formation can lead to an increase in lipid peroxidation (MDA), with a reduction in reduced GSH level in gastric tissue (23). In the present study, ethanol significantly reduced the GSH levels and elevated MDA levels. Oxidative stress subsequently damaged the integrity of the stomach tissue confirmed by histological examination. Excessive ethanol consumption is considered one of the risk factors for gastric ulcers in humans; hence, ethanol-induced gastric ulcers have been widely used for the evaluation of gastroprotective activity (29). Ethanol induces depletion of non-enzymatic defenses and inhibits the antioxidant enzyme catalase and superoxide dismutase (30). Another action of ethanol is the reduction of PGE2 levels inducing peptic cell damage. This damage starts with severe disruption to the surface epithelium and necrotic lesions penetrating deeply into the mucosa, leading to cell lysis observed in microscopic lesions and reflected on the stomach function and PEP activity that was significantly elevated. The activity of prostaglandins (PGs) as anti-ulcer agents is well known; PGs level can protect the damaged gastric mucosal barrier, increase gastric blood circulation, and enhance gastric mucus and bicarbonate secretion (31). One of those PGs is PGE2, which is involved in the increase of mucus secretion, decrease in acid production, and promotion of gastric blood flow, thus initiating the gastroprotective effect. In order to promote tissue repair, induction of angiogenesis is extremely important as PGE2; angiogenic growth factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor are actively involved in angiogenesis-induced tissue repair (23). Ethanol-induced gastric damage by inhibiting mucosal PGE2 (31) causes mucosal necrosis, edema, congestion and inflammatory process, which is characterized by neutrophils infiltration, as demonstrated by the histopathological examination (30).

Our results confirmed that ethanol causes gastric damage at the microscopic level by reduction of PGE2. On the other hand, the principal mechanisms involved in the destruction of colon structure and mucosa barrier by chemical injury of ethanol are enhanced vessel permeability, inflammatory mediator levels, and disturbance of the cruor process. In the present investigation, intra-rectal injection of ethanol to rats elicited clinical symptoms of colitis, whereas it significantly increased the oxidative stress index and markedly decreased the colon proinflammatory index. These observations clearly indicated that ethanol administration exacerbated the clinical manifestation of colitis in the treated rats and caused a reduction in colonic ALP activities. In the light microscopic examination of the colon, using H&E staining, there was severe mucosal

ulceration with crypt and goblet cell distortion, and erosion of submucosa, muscularis mucosa, and serosa.

The oxidation of ethanol is an integral component of alcoholic metabolism where excessive ROS generation occurs, leading to cellular damage in the colon during ethanol consumption. Ethanol significantly decreases total thiol and GSH levels leading to the destruction of cellular lipid components, consequently inducing oxidative damage in the colon, as shown by elevated MDA levels (32). Additionally, there was significant elevation in MPO activity in the colon of rats that received ethanol, indicating induction of inflammation in these tissues. MPO possesses cytokine-like properties and is known to activate neutrophils. In addition, MPO uses H_2O_2 to generate hypochlorite via a process associated with ROS production and consequent tissue injury (33).

Interestingly, treatment with any of the 3 essential oils modulated the damaging effect induced by ethanol in both PU and UC models. This was indicated by improvement in oxidative and inflammatory status. Moreover, the histopathological changes triggered by ethanol were significantly diminished. The gastric mucosa showed a more regular architecture and less hemorrhaging and submucosal edema in the PU model. Also, the rats with colitis described a substantial reduction in colonic mucosal injury.

The medicinal and health potential of cumin is mainly attributed to its antioxidant, antibacterial, antifungal, anti-inflammatory, antidiabetic, insecticide, and immunomodulatory properties. Treatments supplemented with *C. cyminum* have profound effects on several inflammatory biomarkers, such as adiponectin, high sensitivity C-reactive protein, and tumor necrosis factor-a (14). The anti-inflammatory activity of *C. cyminum* has also been reported. Flavonoids present in *C. cyminum* fruits are recognized to have antioxidant activity improving the antioxidant system (15). Moreover, *C. cyminum* essential oil may contribute to pain and inflammation reduction by inhibiting PG production through inhibiting cyclooxygenase enzyme activity (34).

Cumin and caraway products (essential oils as well as their aqueous and solvent-derived extracts) have shown significant antioxidant activity in several test methods. These effects are documented as their ability to prominently quench hydroxyl radicals and lipid peroxides. The antiradical profile of cumin and caraway has been proposed as the underlying mechanism for their multifaceted pharmacological properties (35).

Ethanolic extracts of *C. carvi* significantly reversed the elevated levels of aspartate aminotransferase (AST), alanine aminotransferase (AST), ALP, and lipid peroxidation and significantly increased the reduced level of GSH in a rat hepatotoxicity model induced by ethanol (9). The depletion of gastric mucosa was significantly ($P < 0.05$) replenished with the pretreatment of aqueous extract of *C. carvi* (500 mg/kg, body weight) as compared to the ethanol (80%) induced decrease in gastric mucosal in the gastric tissue. Caraway essential oil also completely protects the different histopathological changes (hemorrhage, inflammatory, erosions, and ulceration) caused in the gastric mucosa of ethanol-treated rats (36). Caraway extract produced a dose-dependent antiulcerogenic effect against indomethacin-induced gastric ulcers, accompanied by a reduction in acid and leukotrienes' output, and increased mucin secretion and PGE2 release (37).

Thymus vulgaris extract protects hepatocytes from damage by alcohol reflecting improvement on liver performance and inhibition of oxidative stress status of the liver (24). GSH concentration was significantly augmented when rats co-administrated *T. vulgaris* extract. Meanwhile, *T. vulgaris* extract is able to protect the liver from lipid peroxidation represented as a significant reduction in MDA, decreasing the elevated ALP activity in alcohol-induced hepatotoxicity in rats. In a previous study, the reduction of ALP activity in *T. vulgaris* essential oil-treated groups rendered ALP to its normal level by mediating the impacts of oxidized fats (38).

Previously *C. cyminum* and *C. carvi* essential oils were approved as protective oils against PU or UC. Additionally, the present work indicated that these essential oils also have a modulating effect after PU or UC incidence. Surprisingly, *T. vulgaris* essential oil contains high levels of antioxidants and anti-inflammatory components more than those in *C. cyminum* or *C. carvi* essential oils that give *T. vulgaris* an advanced effect more than the other essential oils as an anti-ulcerative agent.

Conclusion

The present study indicated that *C. cyminum, C. carvi,* or *T. vulgaris* essential oils against acute PU and UC in rats significantly enhance gastric and colonic PGs synthesis, exhibit downregulation of gastric and colonic MPO activity, and improve oxidative stress index compared to ulcer control groups. So, it could be said that PGs effect on inflammatory cascades and amelioration of oxidative status may be contributed to controlling the progression of gastric damage induced by ethanol. Generally, the results pointed out that *T. vulgaris* essential oil exerted the highest effect more that *C. cyminum* or *C. carvi* essential oils on both ulcer models. Meanwhile, *C. carvi* essential oil showed some signs of improvement on colon inflammatory marker more than *T. vulgaris* oil. *C. cyminum* essential oil treatment was less effective than the other two oils.

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Authors' contributions

NNHS and RWM designed and performed all experiments and biochemical analyses. NNHS and RWM prepared and wrote the whole manuscript, interpreted and discussed the results. ANBS and NMF were responsible for the analysis and interpretation of the GC/MS of the essential oils. NMF wrote the GC/MS part of the manuscript. All authors read and approved the final manuscript.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

All authors declare that principles of laboratory animal care (National Institute of health guide for care and use of laboratory animal) (NIH Publication No. 85-23 received 1985) were followed. All experiments have been examined and approved by the appropriate ethics committee, Cairo University (IACUC NO. 1612: on 24/11/2020).

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