

## Oregano (*Origanium Vulgare*) Extract for Food Preservation and Improving Gastrointestinal Health

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### Abstract

The Mediterranean diet has long been known to provide a variety of health benefits including cardiovascular protection, cancer prevention, and lowering gastrointestinal inflammation. Oregano (*Origanium vulgare*) is an herb prominent in the Mediterranean diet, and has been shown to possess several bioactive properties including anti-oxidant, anti-microbial, anti-inflammatory, and analgesic properties. The anti-oxidant and anti-microbial properties of oregano also make it a strong candidate as a natural food preservative. Because of the recent public concern with synthetic food preservatives, natural alternatives are increasingly being evaluated for effective food preservation. Oregano extract (OE) and essential oil (OEO) are two such agents that have shown promise as natural food preservatives. Additionally, oregano is being evaluated for its positive effect on gastrointestinal health, suggesting an additional benefit of food preservation with oregano. This review will describe in vitro studies related to the anti-microbial and anti-oxidant properties of oregano along with food preservation studies with oregano in various model food matrices. The major phytochemical content reported for OE and OEO will also be outlined to highlight the importance of characterizing the extract that is used, since the extraction process can have a significant effect on the phytochemicals therein. Finally, *in vivo* studies that investigate the gastrointestinal health benefits of oregano, specifically against inflammation, will be addressed to describe the role of oregano on gastrointestinal health.

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## Introduction

Approximately 60 plant species and 17 genera share a similar flavor and color often being labelled as "oregano"<sup>1</sup>. In traditional medicine, oregano has been used for respiratory conditions (i.e. asthma, bronchitis, cough), gastrointestinal (i.e. diarrhea, indigestions, stomachache), anti-bacterial, anti-inflammatory, menstrual disorders, and diabetes<sup>2-4</sup>. Greek oregano (*Origanum vulgare*) throughout the world is the most recognized herb as being authentic or original "oregano"<sup>5</sup>. This variety is often reported to be elevated in rosmarinic acid, a phytochemical first isolated from rosemary (*Rosmarinus officinalis*) in 1958 by the Italian Chemists Scarpati and Oreinte<sup>6</sup>. The benefits of oregano on human health have been associated and attributed to the phytochemicals found therein. Phytochemicals isolated from oregano represent a heterogeneous class of compounds generated during secondary metabolism with most not appearing to participate in essential metabolic functions<sup>7</sup>.

The Mediterranean diet has been recognized for its health-promoting benefits related to cardiovascular disease, stroke reduction, and cancer reduction<sup>8-11</sup>. In the Mediterranean region the diet can vary, however, it typically consists of fruits, vegetables, nuts, legumes, moderate fish and poultry consumption, high polyunsaturated fats, and low consumption of red meat<sup>12,13</sup>. Another important component of the Mediterranean diet includes the herb oregano (*Origanium vulgare*). This perennial herb has been suggested to have a variety of health promoting properties ranging from anti-oxidant, anti-inflammatory, analgesic, and anti-microbial<sup>14-16</sup>. Interestingly, two attributes that have received significant attention include the antioxidant activity and anti-microbial activity of oregano with regard to enhancing food stability<sup>17, 18</sup>.

There is growing public concern among consumers regarding the addition of chemical additives to foods<sup>19</sup>. Specific examples include the use of synthetic antioxidants that have been routinely used to enhance shelf life of various foods. In the USA, the meat and poultry industry has relied heavily on synthetic antioxidants including butylated hydroxyanisole, butylated hydroxytoluene, tert-butylhydroquinone, and propyl gallate, as well as tocopherols to prevent lipid

and protein oxidation<sup>20-22</sup>. Consequently, researchers and food manufacturers are starting to shift from synthetic antioxidants to natural antioxidants. Oregano is one such herb that has been approved in the United States as a spice and natural flavor that can reduce oxidation. A recent study reported that oregano extract can maintain the physicochemical, sensory acceptance, reduce lipid and protein oxidation of lamb meat after frozen storage after 120 days<sup>23</sup>. In addition to maintaining the physical and chemical properties of the meat there was also good sensory acceptance by consumers. These results suggest that that oregano prepared using a variety of extraction methods may be a promising alternative to the synthetic food preservatives.

In addition to the food preservation properties of oregano, there is compelling evidence that these phytochemicals display health promoting properties related to the gastrointestinal tract<sup>1</sup>. Different extraction methods and solvents for each plant are detailed and the most abundant phytochemicals from each extract are outlined in this report. The anti-oxidant and anti-microbial properties of each herb are described along with specific food preservation studies. Finally, *in vivo* studies that show the impact of herb extracts on gastrointestinal health are outlined with significant findings. The goal of this review is to highlight oregano as a natural food preservative and to describe the potential benefits related to gastrointestinal health.

### *Oregano (Origanium vulgare)*

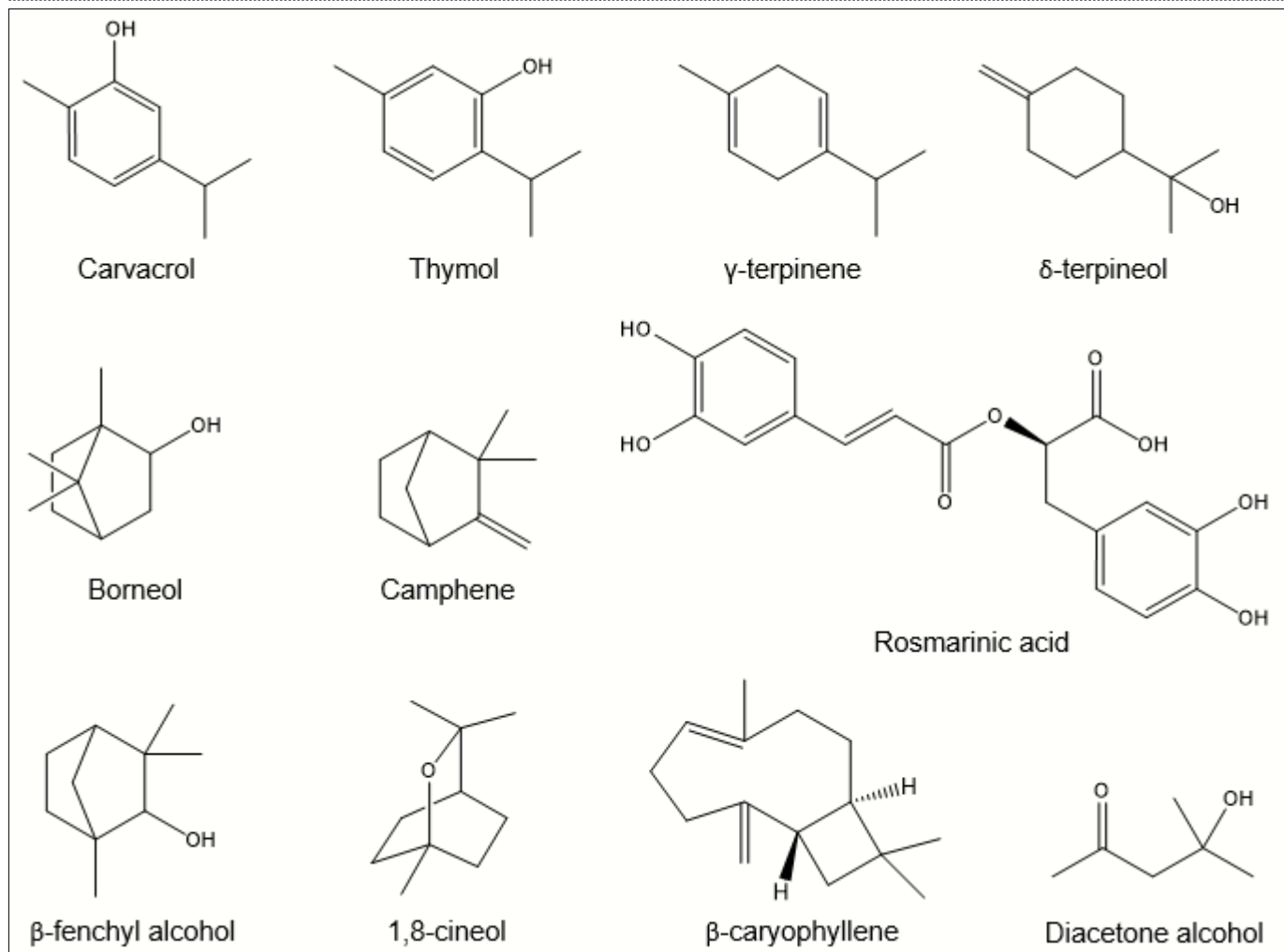
#### *Phytochemicals*

Oregano extract (OE) and oregano essential oil (OEO) have been studied for their bioactive properties, and an important step to understanding their mechanisms of action is identification of major components (Table 1). The volatile aroma compounds in oregano OEO has been well characterized, with the majority of published articles on oregano focusing on the essential oil. The most abundant compounds present in OEO include the diterpenes carvacrol and thymol, which have been extensively studied for their anti-bacterial and anti-oxidant properties. Studies have identified these compounds as comprising up to 92% of the total essential oil content<sup>24-26</sup>. Oregano has been extracted various methods creating a variety of extracts that can vary in phytochemical composition (Figure 1). In a

Table 1. Most abundant phytochemicals characterized in oregano extract prepared by different extraction methods

Extraction method or essential oil	Major phytochemicals	Citation
Essential oil	Carvacrol (14.5% total oil composition) β-fenchyl alcohol (12.8%) Thymol (12.6%) γ-terpinene (11.6%) δ-terpineol (7.5%)	14
Essential oil	Carvacrol (61.3% total oil composition) Thymol (13.9%) γ-terpinene (3.1%) 1,8-cineole (1.6%) Borneol (1.3%)	25
Essential oil	Carvacrol (not quantified) γ-terpinene Camphene 1-octen-3-ol β-caryophyllene	28
Supercritical CO <sub>2</sub>	Carvacrol (not quantified) <i>Cis</i> -sabinene hydrate Linalyl acetate <i>Trans</i> -sabinene hydrate Thymol	28
Hydroalcoholic (methanol:water 80:20)	Luteolin 7- <i>O</i> -glucoside (20.88 mg/g extract) Rosmarinic acid (14.62 mg/g) Luteolin <i>O</i> -glucuronide (12.48 mg/g) Apigenin 7- <i>O</i> -glucuronide (5.78 mg/g) Quercetin 3- <i>O</i> -rutinoside (3.71 mg/g)	27
Infusion (aqueous)	Luteolin <i>O</i> -glucuronide (26.50 mg/g extract) Luteolin 7- <i>O</i> -glucoside (22.93 mg/g) Rosmarinic acid (15.91 mg/g) Apigenin 7- <i>O</i> -glucuronide (8.24 mg/g) Kaempferide <i>O</i> -glucuronide (3.99 mg/g)	27
Decoction (aqueous)	Luteolin <i>O</i> -glucuronide (28.27 mg/g extract) Luteolin 7- <i>O</i> -glucoside (25.26 mg/g) Rosmarinic acid (15.42 mg/g) Apigenin 7- <i>O</i> -glucuronide (8.63 mg/g) Kaempferide <i>O</i> -glucuronide (3.97 mg/g)	27
Ethanol (70%)	Diacetone alcohol (32.18 mg/g dry weight) Rosmarinic acid (25.02 mg/g) Thymol (24.36 mg/g) n-heptanoic acid (17.06 mg/g) Luteolin 7- <i>O</i> -glucoside (10.08 mg/g)	29
Methanol (70%)	Diacetone alcohol (28.87 mg/g dry weight) Rosmarinic acid (28.42 mg/g) Thymol (16.29 mg/g) Nitro-L-arginine (15.93 mg/g) Luteolin 7- <i>O</i> -glucoside (13.53 mg/g)	29

Figure 1. Several compounds found in OE or OEO that have been reported in the literature



hydroalcoholic (80:20 methanol:water) extract, Martins et al. reported the major components to be luteolin 7-*O*-glucoside (20.88%), rosmarinic acid (14.62%), luteolin *O*-glucuronide (12.48%), and apigenin-7-*O*-glucuronide (5.78%)<sup>27</sup>. In sub- and supercritical CO<sub>2</sub> extraction, the major phytochemicals identified include carvacrol, linalyl acetate, thymol, and cis-sabinene hydrate<sup>28</sup>. Other compounds identified in an ethanolic extract of oregano are 4-hydroxy-4-methyl-2-pentanone, rosmarinic acid, thymol, luteolin 7-*O*-glucoside, and caffeic acid<sup>29</sup>.

#### Applications of Oregano as a Food Preservative

**Anti-oxidant activity:** Teixeira et al. compared the anti-oxidant activities of OEO and three types of OE (i.e. hot water, cold water, and ethanolic) using three different assays – ferric reducing anti-oxidant power (FRAP), reducing power of Fe<sup>3+</sup> to Fe<sup>2+</sup>, and DPPH

assay<sup>14</sup>. The chemical constituents of the OEO were characterized by GC-MS and reported the most abundant compounds being carvacrol (14.5%),  $\beta$ -fenchyl alcohol (12.8%), and thymol (12.6%). However, the chemical compositions of the extracts were not reported. FRAP analysis showed OEO (38.5  $\mu\text{mol Fe}^{2+}/\text{g}$ ) and cold water OE (37.7  $\mu\text{mol Fe}^{2+}/\text{g}$ ) having the highest anti-oxidant power, followed by hot water (27.9  $\mu\text{mol Fe}^{2+}/\text{g}$ ) and ethanolic (12.6  $\mu\text{mol Fe}^{2+}/\text{g}$ ). In terms of reducing power, hot water OE (621.7  $\mu\text{mol ascorbic acid}/\text{g}$ ) was the highest, followed by ethanolic (232.7  $\mu\text{mol ascorbic acid}/\text{g}$ ), cold water (203.3  $\mu\text{mol ascorbic acid}/\text{g}$ ), and OEO (74.5  $\mu\text{mol ascorbic acid}/\text{g}$ ). DPPH assay followed the same trend as the reducing power analysis with EC<sub>50</sub> being 25.1  $\mu\text{g}/\text{mL}$  for hot water, 64.1  $\mu\text{g}/\text{mL}$  for ethanolic, 144.3  $\mu\text{g}/\text{mL}$  for cold water, and 1509.1  $\mu\text{g}/\text{mL}$  for OEO. The researchers concluded that hot water OE had the

highest anti-oxidant activity, followed by ethanolic, cold water, and OEO, respectively. Interestingly, the results of this study did not correlate total phenolic content with anti-oxidant activity, suggesting that other phytochemicals were contributing to the anti-oxidant activity of oregano.

**Anti-microbial activity:** In the same study, Teixeira et al. also tested the anti-bacterial activity of hot water, cold water, and ethanolic OE and OEO against *Brochothrix thermosphacta*, *Escherichia coli*, *L. innocua*, *Listeria monocytogenes*, *Pseudomonas putida*, *Salmonella typhimurium*, and *Shewanella putrefaciens*<sup>14</sup>. Anti-bacterial activity was tested by paper disc diffusion test and growth in liquid medium. Neither the hot or cold water extracts had any significant inhibitory properties against the growth of any bacteria. Ethanolic OE demonstrated moderate activity against *S. typhimurium*, *E. coli*, *L. innocua*, and *L. monocytogenes*, resulting in MICs of 13.9 mg/mL, 6.9 mg/mL, 13.9 mg/mL, and 6.9 mg/mL, respectively. OEO displayed strong activity against all bacterial strains, with the strongest against *P. putida* at 0.4 mg/mL and the weakest against *S. putrefaciens*. Ethanolic OE and OEO at the calculated MICs reduced bacterial growth in liquid broth dilution of the species susceptible to treatment in the disc diffusion experiment. Coccimiglio et al. tested the anti-bacterial activity of an ethanolic OE against a panel of various gram-negative and gram-positive bacteria using the agar dilution method<sup>30</sup>. The researchers calculated MICs for OE against all strains of bacteria, and the study noted that OE had the strongest effect against non-mucoid isolates of *Pseudomonas aeruginosa* (MIC = 6.3 µg/mL), that are more susceptible to immune responses and drug treatment than mucoid isolates. Hernández-Hernández et al. tested the anti-bacterial activity of both free and microencapsulated OEO by disc diffusion method<sup>31</sup>. Microencapsulation was performed by adding OEO to a modified starch dispersion and homogenizing by ultra-centrifugation. The oils were tested against *Bronchothrix thermosphacta*, *Lactobacillus plantarum*, *Pseudomonas fragi*, *Salmonella* sp., and *Micrococcus luteus*. The study showed that free OEO at 25% w/v possessed activity against all species except for *L. plantarum*, while microencapsulated OEO showed no activity against *L. plantarum* and *Salmonella* sp. and was less effective than free OEO against all other

species.

#### *Food Preservation:*

Oregano has been evaluated for its anti-oxidant and anti-microbial properties with special application to food preservation. One application of oregano in food preservation is Cypriot pastrami, a type of sun-dried meat product, also known as samarella<sup>32</sup>. The more modern way of making samarella is to take the meat of a goat or sheep and dry the meat using the sun. Once dry, the meat is washed and sprinkled with oregano. A second application of OEO as a food preservative includes that use of additive-free meats that have been dried and cured. This approach has been recognized in the European Union where essential oils are considered safe food additives at concentrations <2 mg/kg body weight/day<sup>33</sup>.

Several studies evaluated the effectiveness of food preservation using OE and OEO. Aqueous OE was shown to significantly reduce microbial growth and TBARS values in raw beef samples over a 15 day period compared to controls, although the strongest effect was observed in both experiments when combined with cinnamon and clove extracts<sup>34</sup>. In a study of anti-oxidant herbs added to animal fat, methanolic OE was determined to have anti-oxidant capacity of 13.59 ± 0.87 nmol equivalent of Vitamin E per gram of fat at a concentration of 5 µg extract per g fat, which was more than double the control group<sup>35</sup>. The major phytochemicals detected in this study were rosmarinic acid (30.03 mg/g) and carvacrol (16.29 mg/g). OEO prepared by an oil-in-water nanoemulsion inhibited growth of *L. monocytogenes*, *S. Typhimurium*, and *E. coli* on commercial iceberg and romaine lettuce leaves at concentrations of 0.05% and 0.1%, with the latter being the most effective<sup>36</sup>.

Another strategy of food preservation is to incorporate a combination of extracts to enhance preservation capabilities. Oregano tested in combination with other natural extracts has been reported to enhance its preservative qualities. Chitosan (1%) was found to be effective with OEO (2-4%) in pork meat at inhibiting bacterial growth, protecting from lipid oxidation, and extending shelf-life with minimal effect on sensory qualities<sup>37</sup>. OEO was tested with caprylic acid at 0.2% and 0.5%, respectively, and found to have a

Table 2. *In vivo* models evaluating oregano extracts and essential oil.

Model	Experimental conditions	Significant findings	Citation
<i>P. acnes</i> -induced mouse ear edema	8-week old ICR mice Groups (5 mice each): Ethanollic OE (2 mg/10 µL) in 5% DMSO Vehicle control (5% DMSO) <i>Propionibacterium acnes</i> (6 x 10 <sup>7</sup> CFU/10 µL PBS) was inoculated in right ear by intradermal injection and PBS (10 µL) was injected in right ear Ethanollic OE or vehicle (5% DMSO) was injected in both ears directly after <i>P. acnes</i> 24 hours after <i>P. acnes</i> injection, mouse ear swelling was measured and mice were euthanized	<i>P. acnes</i> injection increased ear thickness by 2.5-fold and ear weight by 2.3-fold OEO reduced <i>P. acnes</i> -induced ear thickness by 32% and ear weight by 37%	41
Streptozotocin-induced type 1 diabetes mice	8-12 week old C57BL/6 mice Groups (≥7 mice each): Streptozotocin (40 mg/kg/day) + OE (2 mg/day) Streptozotocin only Streptozotocin (40 mg/kg/d) was administered through intraperitoneal (IP) injection at multiple low doses (MLDS) for 5 consecutive days Ethyl acetate OE administered by IP at 2 mg doses for 10 consecutive days, starting on same day as Streptozotocin dosing	Streptozotocin administration alone resulted in 80% development of hyperglycemia in mice, and ethyl acetate OE administration reduced hyperglycemic development to 15% of mice Ethyl acetate OE reduced the number of F4/80* macrophages and concentrations of IL-1β and IL-6, while also increasing concentrations of TNF and IL-10 Th1 and Th17 cell counts decreased with ethyl acetate administration, along with concentrations of associated cytokines IFN-γ and IL-17	4
TNBS-induced colitis mice	7-week old Balb/c mice Groups (16 mice each): Group A: TNBS + 0.4% thyme oil + 0.2% oregano oil Group B: TNBS + 0.2% thyme oil + 0.1% oregano oil Group C: TNBS + 0.1% thyme oil + 0.05% oregano oil Group D: TNBS only Group E: Sham Colitis induced by intrarectal administration of 120 mg/kg TNBS in 50% ethanol Thyme and oregano oil combinations were administered for 7 consecutive days	Mice in Group B recovered body weight significantly compared to TNBS-only mice Mice in Group B had a significantly lower colonic damage score compared to TNBS-only mice The mRNA and protein levels of IL-1β and IL-6 cytokines was significantly reduced in Group B mice	42

greater anti-microbial effect than either compound alone in minced beef<sup>38</sup>. A combination of OEO and REO inhibited growth of naturally occurring fungi in table grapes and preserved the sensory and physical (appearance, firmness) qualities during storage<sup>39</sup>. In a study using cooked chicken, OEO in combination with sage essential oil and honey reduced the amount of lipid oxidation after 48 and 96h as measured by TBARS<sup>40</sup>. These studies suggest that oregano can be used alone, however, some evidence suggests a combination of other herbs may enhanced food preservation properties.

#### *In vivo GI Health Benefits*

Another benefit of phytochemicals in food preservation is that they may also improve gastrointestinal health (Table 2). Both OE and OEO have been shown to exhibit anti-inflammatory effects *in vivo* by suppression of TNF- $\alpha$  expression<sup>15, 41</sup>. In mice with streptozotocin-induced diabetes, an ethyl acetate OE compared to control mice. This study found that OE reduced the number of TH1 and TH17 cells and activity measured by expression of the cytokines IFN- $\gamma$  and IL-17, and both of these results reached statistical significance ( $P < 0.05$ )<sup>4</sup>. Treated mice also had a lower population of pro-inflammatory macrophage F4/80<sup>+</sup> cells in the peritoneum while also decreasing the expression of IL-6 and IL-1 $\beta$  ( $P < 0.05$ ), further suggesting that the ethyl acetate OE has anti-inflammatory properties *in vivo*.

A study by Bukovská et al. investigated the effects combined oregano and thyme essential oils in a colitis mouse model induced by trinitrobenzene sulphonic acid (TNBS) (120 mg/kg in 50% ethanol)<sup>42</sup>. The oregano and thyme oils were combined at different concentrations and given in the feed for 6 days prior to TNBS administration. The 0.1% oregano and 0.2% thyme combination treatment also had the highest prevention of macroscopic colonic damage ( $P < 0.05$ ) and also the most recovery in body weight (not statistically significant). This dose also decreased both the mRNA and protein the expression of pro-inflammatory cytokines IL-6 and IL-1 $\beta$  ( $P < 0.01$ ). The investigators found that oregano and thyme at 0.1% and 0.2%, respectively, had the highest decrease of mortality rate at 33.3% compared to 53.3% in the TNBS-only group, although this result was not

statistically significant. These studies suggest that oregano may decrease inflammatory markers *in vivo* while improving gastrointestinal health.

#### **Conclusion**

This review summarizes the biological activities of different oregano extracts related to their natural food preservation properties and health promoting properties related to gastrointestinal health. The public interest for clean-labeling of food products and alternatives to synthetic food preservatives provide the demand natural alternatives. Oregano extract and essential oil present promising methods of natural food preservation due to their bioactivities that prevent many types of food spoilage and microbial growth. Defining the phytochemical content is essential in understanding the advantages of different forms tested for anti-oxidant and anti-microbial properties. In addition to using a single herb such as oregano, a combination of multiple extracts or essential oils may produce an even greater effect on food preservation. Beyond food preservation, however, these herbs have also been shown to promote GI health. Studies performed in mice have shown positive effects of lowering GI inflammation and lessening the symptoms of TNBS exposure. Future studies will be needed to determine the full potential and health benefits of oregano extract as an alternative to synthetic food preservatives.

#### **Conflicts of Interest**

The authors do not declare any conflicts of interest.

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