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Original Research Article

Screening of Bioactive Natural Compounds of Ethanolic Leaves Extract *Origanum vulgare* Using GCMS Technique and Evaluation of its Antioxidant (Singlet Oxygen Scavenging and Hypochlorous Acid Scavenging) Activity

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Abstract: Scientists consider Oregano (Origanum vulgare L.) as the leading spice plant for antioxidant production and its antioxidant amounts show noteworthy diversity among various plant variations. The research examined in vitro antioxidant capacities of oregano plants (Origanum vulgare) along with their chemical substance profile determination. Researchers at 4 °C macerated 10 g of aerial-dried flowered parts in a 250 mL solution containing 50% ethanol by volume mixed with water until the extraction process reached 24 hours before gravity filtration. The extractive process was conducted four times to obtain maximum compounds before rotary-evaporation condensed the solution. This work identified seventeen metabolites by (GC-MS) beta-Terpinene, (+)-piperitol, 5-Isopropyl-2-methylphenol, Limonene, 2-Isopropyl-5-methylphenol, Linalool, beta-Ocimene, 1-octanol, Geraniol, Thujene, Thymol methyl ether, Sabinen, beta-Myrcene and 1-p-Menthene-8-thiol. Antioxidant activity (Singlet oxygen scavenging) of Leaves extract (Ethyl acetate, Ethanol and Gallic acid (standard)) of *Origanum vulgare* recorded 74.82 ± 3.70, 66.31 ± 3.68, 58.18 ± 3.40, 52.67 ± 3.32, 57.04 ± 3.37, and 51.84 ± 2.91 respectively. While recorded 114.55 ± 5.41, 125.06 ± 6.08, 112.44 ± 5.97, 140.05 ± 5.42, 150.17 ± 6.27, and 215.72 ± 8.15 respectively. Even after advancements in pharmaceutical sciences, medicinal plants are used to prevent and cure a broad range of diseases due to their exceptional nutraceutical and therapeutic properties. *Origanum vulgare* is a medicinal plant with numerous therapeutic benefits.

Keywords: Origanum vulgare, Natural Compounds, GCMS Technique, Singlet oxygen scavenging, Hypochlorous acid scavenging.

INTRODUCTION

Traditional applications of Origanum vulgare L. include its use as both a condiment in food industry and cooking and a stimulant for digestion and circulatory systems. People employ essential oil from this plant for aromatherapy purposes to create perfumes as well as soaps. Traditionally O. vulgare found use in medical treatments because the essential plant components offer antispasmodic and calmative and carminative and expectorant stomachic and tonic qualities which explain its application in making infusions to cure digestive problems and headaches along with sore throats while helping with colds [1]. Scientific research demonstrates that the various components from O. vulgare display antioxidant effectiveness by decreasing free radicals and showing antioxidant and protective functions when studied in vivo. Research identifies free radical buildup as a potential aging disease factor so scientists actively search for substances that reduce their levels within the body. The way through which a drug enters the body influences its ability to impact pharmacological functions [2-4]. Patients prefer oral administration above other delivery methods because it provides them with maximum comfort. Medical plant extracts within digestive conditions present heterogeneous sizes together with multiple key compounds for their function that experience alterations from digestive enzymes and pH changes. The pharmaceutical oral

formulation plays a vital role in determining pharmacokinetic and pharmacodynamic outcomes of these products because of these factors. The laboratory enables simulation of gastrointestinal processes by an in vitro model to assess digestive effects on extract components followed by in vitro and in vivo pharmacological activity assessment. The analysis of DPPH% radical scavenging activity serves as the most commonly employed method for in vitro antioxidant activity quantification among all such techniques. Caenorhabditis elegans represents an efficient in vivo research model which scientists prefer because it costs little yet remains both cheap and easy to reproduce and similar to human molecules [1, 5]. The analysis of natural sources for their antimicrobial and antioxidant properties interests researchers because these substances may serve future applications in food and pharmaceutical industries. Scientific research has identified essential oils extracted from aromatic and medicinal plants as they contain biological active properties. Oregano (Origanum vulgare) possesses aromatic characteristics since it naturally grows across Mediterranean territories and Asian regions. Oregano as a European native herb expanded its cultivation to the Chilean territory during more than a century ago. The arid geographical environment produces this very aromatic spice which maintains distinct qualities that stem from its surroundings [6-8]. Oregano functions as an ethnopharmacological medicinal herb to address various diseases including respiratory conditions as well as dyspepsia and painful menstruation and rheumatoid arthritis and scrofulosis and urinary tract disorders. The high level of volatile essential oils gives the spice both antimicrobial properties and aroma together with flavor. The essential oil components of Origanum vulgare along with its equivalents exhibit extensive chemical variety aside from significant changes in both quantitative and qualitative aspects of their chemical makeup [9, 10]. Scientists have documented that oregano essential oil demonstrates food preserving properties which maintain the quality of both salmon and seaweed burgers alongside fish and meat items. Researchers have conducted various studies to determine O. vulgare essential oil components from various parts of the world. The objective of this research involved detecting O. vulgare essential oil chemistry components as well as assessing its antioxidant capabilities.

MATERIALS AND METHODS

Preparation of *Origanum vulgare*

Plant material, extraction and pharmaceutical form preparation Flowered aerial parts from Origanum vulgare L. The procedure involved extracting 10 grams of flowered aerial-dried parts using 250 milliliters of ethanol-water mixture (50% v/v) at 4 °C for a time duration of 24 hours followed by gravity filtration. The extraction process of maceration was executed four consecutive times before using a rotary-evaporator to concentrate the obtained components.

Gas Chromatography–Mass Spectrometry Analysis

The GC-MS analysis followed the method described by Teixeira et al. although researchers made some adaptations to the procedure. The researchers performed analysis of essential oil on an Agilent 5975 gas chromatograph which integrated with an Agilent 5973N mass-selective detector obtained from Agilent Technologies based in Palo Alto CA USA. The analytical instrument operated with an HP-5MS capillary column designed at 30 meters in length and 0.32 millimeters in diameter featuring an additional 0.25 micrometers of film thickness. The thermal program for the oven consisted of holding 45 ◦C for 1 min while increasing to 250 ◦C at 5 ◦C min−1 then maintaining 250 ◦C for 5 min. The gas carrier used helium at 30 cm s−1 with an injection volume of 1 µL[11-13]. Each identified compound received its identity based on the test with n-alkanes standards and evaluation of mass spectra features from the Wiley data bank (Wiley 7N Edition [Agilent Part No. G1035B].

Antioxidant (Singlet oxygen scavenging) Activity

The procedure for measuring singlet oxygen involved monitoring the bleaching of N, N-dimethyl-4-nitrosoaniline (RNO) following a previously documented spectrophotometric method. The generation of singlet oxygen through NaOCl and H2O2 reaction enabled researchers to measure RNO bleaching at wavelength 440 nm. The 2 ml reaction solution incorporated 45 mM phosphate buffer (adjusted to pH 7.1) together with 50 mM NaOCl, 50 mM H2O2, 50 mM histidine, 10 µM RNO, the variable sample concentrations (0–200 µg/ml) and ended up with a final reaction volume of 2 ml. The solution became incubated at 30°C for 40 minutes before measuring the decreased RNO absorbance at 440 nm wavelength. The research examined the scavenging power of the sample solution against lipoic acid reference drug. The experiments were carried out six separate times.

Antioxidant (Hypochlorous acid scavenging) Activity

Previous creation of a 10 % (v/v) sodium hypochlorite solution NaOCl received titration with 0.6 M H2SO4 until pH reached 6.2 just prior to testing to produce hypochlorous acid HOCl. The measurement was obtained using 100 M cm-1 molar absorptivity values at 235 nm wavelength position. A modified version of this experiment followed the experimental protocol described by Aruoma and Halliwell. The researchers monitored the activity by following the 404 nm wavelength absorbance changes that measured the reducing catalase activity. With 8.4 millimolar hydrochloric acid and 7.2 microgram of catalase combined in one milliliter of 50 millimolar phosphate buffer (pH 6.8) one can prepare the reaction mixture alongside plant extract concentrations ranging from 0 to 100 microgram per millile in dependent order [14]. The system was read with a suitable blank after the test solution allowed to stand at 25°C for 20 minutes. Every test was run six times. References listed ascorbic acid since it acts as an effective HOCl blocking agent.

Statistical Analysis

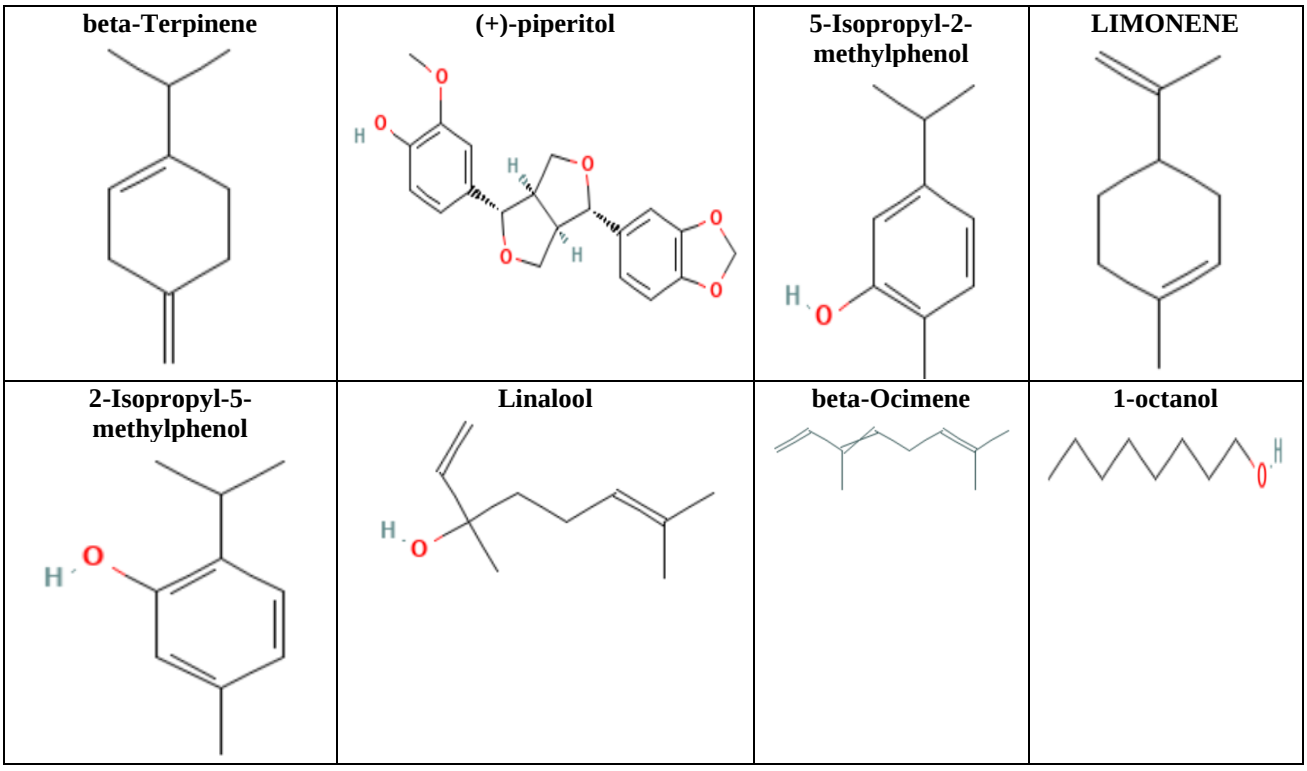
The measured data obtained from three repeated trials enabled the calculation of results by using their average values and standard deviation values. Data analysis proceeded using one-way analysis of variance (ANOVA). Significant differences between the means were identified by Tukey's multiple range test at a 95% confidence level.

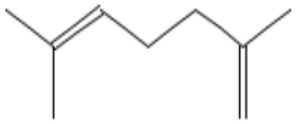
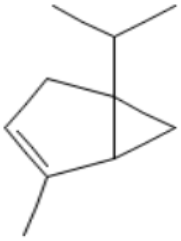
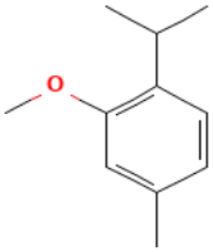
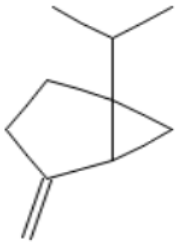
RESULTS AND DISCUSSION

The metabolomics method featuring Gas chromatography coupled to electron ionization (EI) quadrupole mass spectrometry (GC-MS) stands as one of the most advanced and robust technologies today. This method enables the parallel detection of many various chemical compounds which encompass organic acids, amino acids, sugars, sugar alcohols, aromatic amines and fatty acids. The identification process of metabolites during untargeted GC-MS testing under full scan data acquisition methods needs complicated data processing along with some uncertainties when identifying target molecules. Targeted analysis using GC-MS/MS can provide better specificity, increase sensitivity, and simplify data processing and compound identification but wider application of targeted GC-MS/MS approach in metabolomics is hampered by the lack of extensive databases of MRM transitions for non-derivatized and derivatized endogenous metabolites. beta-Terpinene, (+)-piperitol, 5-Isopropyl-2-methylphenol, Limonene, 2-Isopropyl-5-methylphenol, Linalool, beta-Ocimene, 1-octanol, Geraniol, Thujene, Thymol methyl ether, Sabinen, beta-Myrcene and 1-p-Menthene-8-thiol.

Table 1: Bioactive Natural Compounds of Ethanolic Leaves Extract *Origanum vulgare* Using GCMS Technique

No.	Compound	Molecular Formula	Molecular Weight
1.	beta-Terpinene	$C_{10}H_{16}$	136.23 g/mol
2.	(+)-piperitol	$C_{20}H_{20}O_6$	356.4 g/mol
3.	5-Isopropyl-2-methylphenol	$C_{10}H_{14}O$	150.22 g/mol
4.	Limonene	$C_{10}H_{16}$	C10H16
5.	2-Isopropyl-5-methylphenol	$C_{10}H_{14}O$	150.22 g/mol
6.	Linalool	$C_{10}H_{18}O$	154.25 g/mol
7.	beta-Ocimene	$C_{10}H_{16}$	136.23 g/mol
8.	1-octanol	$C_8H_{18}O$	130.23 g/mol
9.	Geraniol	C_9H_{16}	154.25 g/mol
10.	Thujene	$C_{10}H_{16}$	136.23 g/mol
11.	Thymol methyl ether	$C_{11}H_{16}O$	164.24 g/mol
12.	Sabinen	$C_{10}H_{16}$	136.23 g/mol
13.	beta-Myrcene	$C_{10}H_{16}$	136.23 g/mol
14.	1-p-Menthene-8-thiol	$C_{10}H_{18}S$	170.32 g/mol



Geraniol	Thujene	Thymol methyl ether	Sabinen
			
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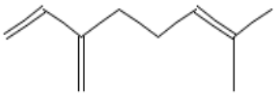			

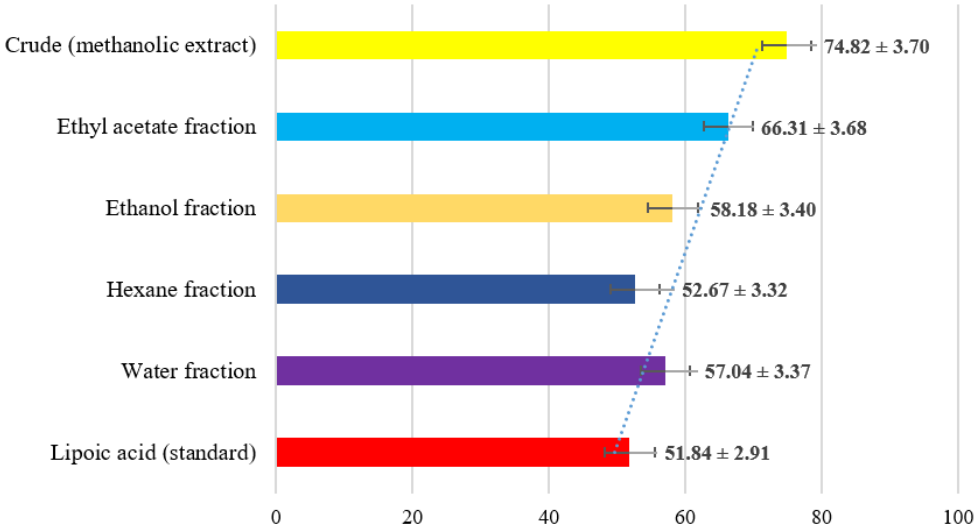

Figure 1. Antioxidant activity (Singlet oxygen scavenging) of Leaves extract (Ethyl acetate, Ethanol and Gallic acid (standard)) of *Origanum vulgare*

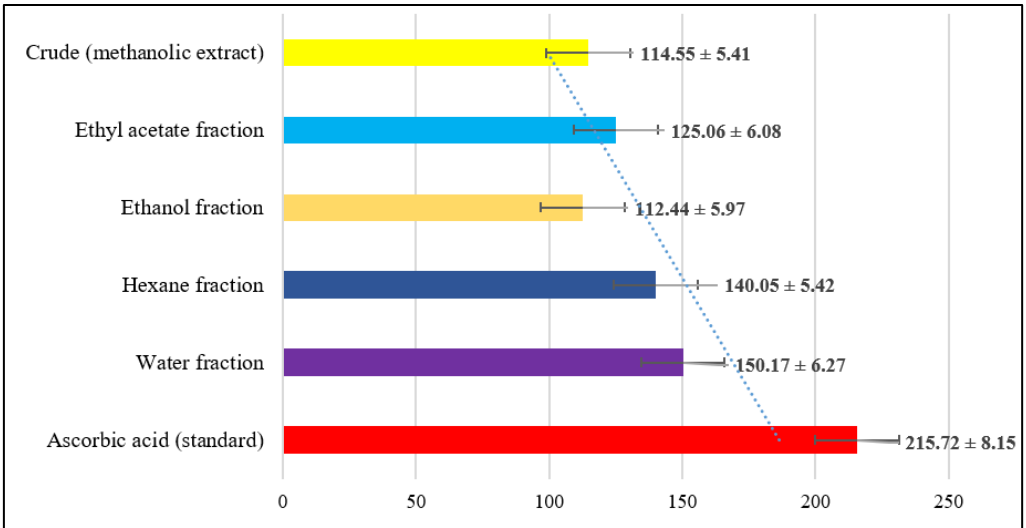

Figure 2. Antioxidant activity (Hypochlorous acid scavenging) of Leaves extract (Ethyl acetate, Ethanol and Gallic acid (standard)) of *Origanum vulgare*

Antioxidant activity (Singlet oxygen scavenging) of Leaves extract (Ethyl acetate, Ethanol and Gallic acid (standard)) of *Origanum vulgare* recorded 74.82 ± 3.70, 66.31 ± 3.68, 58.18 ± 3.40, 52.67 ± 3.32, 57.04 ± 3.37, and 51.84 ± 2.91 respectively. While recorded 114.55 ± 5.41, 125.06 ± 6.08, 112.44 ± 5.97, 140.05 ± 5.42, 150.17 ± 6.27, and 215.72 ± 8.15 respectively. Two different tests measured the antioxidant capabilities within Origanum vulgare essential oil. The major substances in oregano oils produce distinct free radical scavenging capacities between them. A significant indicator of possible antioxidant behavior in essential oils is their reducing power. The Folin–Ciocalteu method detects all reducing substances within a matrix instead of polyphenolics which leads to the lack of correlation between total polyphenol content and antioxidant capacity. non-phenolic substances could exist in the examined samples. The pathologies connected to normal aging appear to develop because of reactive oxygen species (ROS) becoming unbalanced. ROS act as critical signaling elements for maintaining homeostasis across the gastrointestinal tract so potential oral treatments could involve substances that control unnecessary ROS formation while acting to neutralize existing ROS. The antioxidant properties shown by herbal preparations in test tubes disappear during in-vivo testing despite initial laboratory results. The lack of pharmacological information coupled with various factors controls the stability of these extracts' chemical composition. Research findings demonstrate that the solvent selection strongly affects extract stability levels. The chemical stability reaches peak levels when using ethanol as a solvent followed by methanol then DMSO in second place and third place respectively [13-16]. Multiple scientific studies have proved that water plays an essential role in causing extract instability. Hydroxyl radicals along with hydrogen peroxide emerge during redox reactions initiated by water solution which exhibit oxidative effects. Water helps enable enzymatic reactions that primarily damage extracts when they exist as liquids. When combined with temperature changes solvent physicochemical properties can modify both the extracted chemical compounds and pharmacological actions from plant matter. Organic solvents show stronger extraction capabilities for polyphenols while these compounds serve as the main antioxidants in the solution. The selected sample for this research was a lyophilized 50 % ethanolic cold extract with rosmarinic acid content at 33 %. An antioxidant activity of O. vulgare likely explains various pharmacological effects of this plant. The evaluation of O. vulgare antioxidant activity through both in vitro and in vivo analysis occurred with or without in vitro gastrointestinal digestion of the extract [17, 18]. This study focuses on understanding how gastric enzymes and stomach pH condition the activity of this plant extract after oral delivery because this information can serve as a guide for maximizing the effectiveness of oral medicine using this plant [19-21]. Water-based oral medications present dual problems because they can become contaminated by microorganisms and they restrict the ability to dissolve some crucial drugs while needing additional substances to improve their palatable properties. Liquid oral pharmaceutical forms remain costlier than other options because they present several practical and safety issues associated with their storage properties of limited transportation capabilities and significant volume requirements and delicate packaging [22-25]. The advantages of solid oral dosage forms over liquid forms include stable chemical properties that grant accurate measurements with superior storage capacity and reduced transport challenges. The chosen solid oral single-dose pharmaceutical preparation consists of gelatine capsules filled with divided powder. The encasement preparation method was selected because of its straightforward production process which maintains the bioactive chemical compounds of raw substances. Gelatine exists as a gelling protein which industries apply it for both food and pharmaceutical usage. Due to their easy swallowability and their absence of smell and taste hard gelatine capsules effectively conceal unpleasant medication flavors. We included silicon dioxide as an antiaglomerant to overcome issues of hygroscopy during industrial manufacturing and drug delivery processes and user ingestion. Our choice focuses on capsule size 2 because it contains 0.36 mL of content without limiting the available dose but avoids swallowing difficulty. Powder acts as the second formulation because it has no physical constraints regarding dosage. The formulation exists in two formats: unidose which consists of divided powder and multidose presents as bulk powder. The unidose powder system already comes prepared for use yet bulk powder requires an additional dispensing tool such as a measuring spoon. Regardless of its packaging type the formulation consists of solid dry loose particles that dissolve in water as a method to allow patients who struggle with swallowing to avoid discomfort. The formulation requires additional coloring additives as well as flavoring agents based on the compounds present. Each daily dose of the powder medicine dissolves easily to a concentration of 40 mg/mL when mixed in a glass of water for administration. The developed drug formulations presented suitable ex corpore characteristics for administration in an experimental biological setting [26-29]. The first fundamental element of pharmacokinetics (LADME system) is drug release since small distinctions can explain the variations in the pharmacological activities of the product. We have used a straightforward and dependable approach developed recently to measure internal antioxidant activity in this organism even though oxidative activity assessment can proceed through simultaneous protein and gene expression measurements [30, 31]. We confirmed through this assay that antioxidant properties detected in vitro for the different samples operate in the living organism. The main compound rosmarinic acid located in Origanum vulgare extracts demonstrates antioxidant properties both before and after digesting processes. The encapsulated extract performed better when measuring antioxidant activity within worms because of its different flavonoids concentration level when compared to the powder. Scientific data confirmed that non-digested extract demonstrated elevated antioxidant activity in living organisms. Surprisingly the antioxidant properties surpass those of undigested pure extract even though the encapsulated extract undergone digestion processes. The findings between in vitro and in vivo tests match despite the measurement variability being stronger in the in vivo experiments. Since the crude extract received no digestion treatment it should display antioxidant properties like those observed in the encapsulated extract. The maximum antioxidant activity appeared in the crude extract during in vitro examination yet in vivo studies require exposure to live

worm physiological settings. Data collected at baseline in the tests conducted with worms allowed researchers to establish connections with antioxidative processes activated within those worms. Scientists use time-based antioxidant activity measurements for stability evaluations [32]. The reaction speed depends on whether stability reaches before 30 minutes because that makes it fast but it becomes intermediate if stability occurs between 30 to 45 minutes and slow when stability is detected after sixty minutes. The antioxidant properties of rosmarinic acid after enzymatic digestion appear as intermediate so that it maintains its protective capabilities for over thirty minutes in both test tubes and animal models (Figures 3 and 4). However, rosmarinic acid before digestion functions as a fast antioxidant in test tubes and as a slow antioxidant inside animal models reaching its maximum protection points at fifteen minutes and sixty minutes respectively. When using non-digested extract as basal measurement for kinetic assays the digestion process produces a material with antioxidant properties matching those of rosmarinic acid yet taking an additional 15 minutes to reach stability in both lab and rat samples [33]. The kinetic classification shows no sample differences according to its analysis rules. The ethanolic extract follows a similar pattern for antioxidant properties both during digestion and before it both in tester solutions and living subjects for both treatments. The reaction stability does not determine the noticeable differences which exist between digested powder and encapsulated extract.

CONCLUSIONS

The oral pharmaceutical form serves as an essential component for pharmacological actions in medicinal plant extracts. Herba Extract of Origani vulgaris offers manufacturers a solution for refrigerated beef preservation thus leading to diminished use of food additives. The findings of this study support the justification of the antimicrobial functions in addition to antioxidant capabilities of oregano extract. The ingredient supports stability of color indexes positively thus enhancing the product's storage characteristics through its multifunctional character. More studies need to identify the precise dosage amount of oregano extract while considering its antioxidant function is dose-dependent for different food products.

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