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Original Research Article

Antioxidant Properties [Singlet Oxygen Scavenging and Nitric Oxide Radical Scavenging] of *Thymus vulgaris* and *Piper nigrum* and Screening of Its Main Constitutes Using Fourier Transform Infrared Spectroscopy Technique

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Abstract: Several types of medical substances derive from natural origins. The present pharmaceutical market contains fifty percent of drugs which derive from plants or utilize natural substances as their active components. Natural approaches became more widespread through the urgent need for new medical solutions to health problems. The selectivity levels proved superior when natural chemicals were used to target antibiotic-resistant bacteria and cancer cells when compared to artificial chemicals. The research investigated two aspects of antioxidant properties that included Singlet Oxygen Scavenging and Nitric Oxide Radical Scavenging through examination of Thymus vulgaris and Piper nigrum. Screening of Its Main Constitutes Using Fourier Transform Infrared Spectroscopy Technique. FT-IR peak values of solid analysis of *Thymus vulgaris* [669.30 and 684.73 Strong, C-Cl, alkyl halides], [827.46, 873.75, 927.76 Strong, =C–H, Alkenes], [1010.70, 1236.37, 1313.52 Strong, C-F, alkyl halides], [1417.68, Medium, C=C, Stretch, Aromatic], [1604.77, Bending, N-H, Stretch, Amide], [2918.30, Strong, C-H, Stretch, Alkane], [3269.34, Bending, N-H, Stretch, Amide]. FT-IR peak values of solid analysis of *Piper nigrum* [667.37, Strong, C-Cl, alkyl halides], [873.75, 921.97, Strong, =C–H, Alkenes], [1026.13, 1026.13, 1139.93, 1234.44, 1317.38, 1379.10 Strong, C-F, alkyl halides], [1415.75, 1519.91, 1598.99 Medium, C=C, Aromatic], [1740.72, Strong, C=O, Aldehyde], [2852.72, Strong, C-H, Alkane]. Antioxidant Properties [Singlet Radical Scavenging] of Crude (methanolic extract), Ethanol, Water and Lipoic acid (standard) of Thymus vulgaris recorded 62.05 ± 4.49, 48.17 ± 2.99, 59.08 ± 3.97 and 39.00 ± 1.26 respectively and recorded 58.96 ± 4.05, 45.00 ± 2.08, 55.97 ± 3.00 and 34.81 ± 1.09 for Piper nigrum while Antioxidant activity [Nitric oxide radical scavenging] of Crude (methanolic extract), Ethanol, Water and curcumin (standard) of Thymus vulgaris recorded 41.97 ± 2.04, 37.16 ± 2.03, 54.00 ± 2.99 and 89.17 ± 7.22 respectively and recorded 45.00 ± 2.19, 39.08 ± 2.14, 60.27 ± 3.89 and 96.13 ± 7.81 respectively for Piper nigrum. Thorough scientific studies should explore the medical and pharmaceutical characteristics of Thymus vulgaris and Piper nigrum since the plant demonstrates multiple treatment applications in medicine.

Keywords: Antioxidant, *Thymus vulgaris*, *Piper nigrum*, Constitutes, Fourier Transform Infrared Spectroscopy Technique.

INTRODUCTION

The past decades have demonstrated the use of multiple natural products which function as chemopreventive agents that trigger cancer cell apoptosis without generating severe side effects. Scientists have documented multiple observations regarding how plants affect the clinical landscape. Several research groups maintain their focus on assessing different species from Thymus genus [1, 2]. The plant species Thymus Vulgaris L. receives extensive research because it

constitutes one of the primary species within the genus. Thymus Vulgaris L. grows in the western Mediterranean region of Europe as a member of the family Lamiaceae. Thyme stands as its scientific name. Scientific investigations show that this species contains multiple constituents while human beings have practiced its utilization for a long period. Thyme along with every genus species is commonly found in herbal tea products and functions as both spice and medicinal herbs. Folk medical practitioners frequently utilize thyme because it demonstrates expectorant and antitussive actions as well as antispasmodic antibroncholitic and carminative diuretic anthelmintic properties [3]. The medicinal use of Thyme can be done through infusions or surface baths to treat both skin ailments and rheumatic conditions. Many scientific studies documented the beneficial activities of thyme in literature. Essential oils derived from Thymus species reveal various biological activities which match many of the established activities of Thyme oil including antimicrobial, antibacterial, antitussive, expectorant, antispasmodic and antioxidant properties. Several scientific investigations have centred on Thymus oils because of these reasons [4-6]. Scientific studies investigating the antioxidant and antifungal effects as well as pesticide and antibacterial capabilities of essential oils from these plants identify their potential use within the food industry. The worldwide food preservation market includes thyme oil among its ten leading essential oils recently. The essential plant oils extracted from thyme plants serve pharmaceutical businesses as well as natural therapies and alternative medicine systems. The biological actions emanating from Thymus vulgaris EOs result from their presence of monoterpene compounds. Essential oil composition from this plant contains two phenolic terpene molecules including thymol and its isomer carvacrol together with precursors p-cymene and γ-terpinene. The presence of distinct thyme chemotypes varies according to the primary monoterpene produced in leaf surface glandular trichomes. Research done by both [7, 8] validated this conclusion. The quantity of main monoterpene compounds exist in phenolic chemotypes at a lower amount than nonphenolic chemotypes because in phenolic types both p-cymene and γ-terpinene serve as oil components that demonstrate incomplete precursor conversion [9]. The diverse chemical makeup of thyme EOs determines their different pharmacological responses observed in pharmacological evaluations. Numerous studies throughout recent years established that thyme oil produces cytotoxic effects on various tumor cell lines extending from lung to breast and cervical tumors as well as colon tumors and gastric cancer cells. The existence of monoterpene composition in thyme oil provides antitumor effects which may function through monoterpene-dependent reduction of oxidative stress. Reactive oxygen species trigger DNA-damage within cancer development as they initiate oxidation and inflammatory processes. The Campania farming programs introduced initiatives to promote medicinal plant cultivation including this particular species. Laboratory examination of the EO utilized gas-chromatography and mass spectrometry (GC-MS) as its semi-quantitative analytical method [10-13]. FTIR spectroscopy determined the presence of important functional groups found in the compounds present in the oil. We studied the anti-proliferative activity in vitro because the EO contained biologically active compounds with dominant thymol, carvacrol and p-cymene concentrations. The research goals involved undertaking two assessments: S.O. Scavenging and NO. Radical Scavenging as antioxidants from the extracts of Thymus vulgaris and Piper nigrum. Additionally, the study performed FTIR analysis to detect the primary chemical structures of these plant extracts.

MATERIALS AND METHODS

Plant Collection and Preparation

The Botany Department of the University of Bbylon performed verification on the Thymus vulgaris plant that the room temperature. The fresh plant of Thymus vulgaris was obtained from Al-Hillah market in Iraq. Thymus vulgaris plants received fresh water washing before drying at room temperature for three days. The whole plant was processed into a fine powder amounting to 500 grams after completing the drying stage. Thymus vulgaris powder received plastic bottle containers to protect it until the extraction process commenced. Rotary evaporators played a role during extraction followed by vaporization to create amorphous solid masses.

Plant Material and Preparation of Extracts *Piper nigrum*

The dried plants P. nigrum were obtained from the Hilla city local markets in Iraq. Foreign materials were removed before storing the fruits inside an airtight container under room temperature for future use. A mixture of 25 g powdered plant material was placed in 100 mL methanol and shaken for 16 h using a rotatory shaker. The separation of extract from the plant utilized Whatman No.1 filter paper. The subsequent phytochemical analysis relied on the filtrate obtained from the separation. The extract of plant went through filtration with sodium sulphate to eliminate water remnants

Fourier-Transform Infrared Spectroscopy (FTIR)

The methanolic extract Thymus vulgaris underwent mixing with KBr salt before receiving compression into a thin pellet. The Perkin Elmer Spectrum two FTIR spectrometer conducted spectroscopy tests of the samples through a scan range from 4000 to 500 cm−1.

Preparation of KBr Pellet for IR Analysis

1. Dry IR-grade KBr in a drying oven for at least 1 hour.
2. Use an agate mortar and pestle to grind the 15 mg substance of piperine needles for 20 minutes. The crystal drying procedure should be combined with a grinding step to remove reflective surfaces and minimize particle dimensions while keeping the samples dry.
3. Set the KBr to cool beneath desiccant conditions. Feeding the stainless-steel mixing vial a 300 mg weight requires a stainless steel mixing ball.
4. The vial containing KBr should hold 3 mg of the finely ground piperine.
5. The Wig-L-bug shaking device requires 60 seconds of operation to mix all components properly.
6. The KBr-piperine mixture should go into an evacuable die installed on a hydraulic laboratory press. A vacuum pressure of 15,000 pounds acts on the die for 6 minutes during the pressing operation.
7. The press operation should stop and the operator must detach the die then disassemble the components and extract the KBr pellet. The KBr pellet gets placed into a holder before insertion into the sample beam of an IR spectrophotometer.
8. Measure the spectrum of the pellet between 4,000 and 600 centimeters negative one.
9. Locate the spectral bands for piperine on its IR spectrum by referring to the provided table of expected absorption bands.

Singlet oxygen scavenging

The spectrophotometric method described by the previous report measured singlet oxygen production through N, N-dimethyl-4-nitrosoaniline (RNO) bleaching. The generation of singlet oxygen occurred through NaOCl and H2O2 reaction while researchers measured RNO bleaching at a wavelength of 440 nm. The reaction mixture consisting of 2 ml volume contained 45 mM phosphate buffer at pH 7.1, 50 mM NaOCl, 50 mM H2O2, 50 mM histidine and 10 µM RNO, while different sample concentrations from 0 to 200 µg/ml were used. The reaction mixture which contained 45 mM phosphate buffer (pH 7.1), 50 mM NaOCl, 50 mM H2O2, 50 mM histidine, 10 µM RNO and various concentrations (0–200 µg/ml) of sample in a final volume of 2 ml was incubated at 30°C for 40 min before measuring the reduction in RNO absorbance at 440 nm [14]. Testing received a comparison of its scavenging properties against the reference compound which used lipoic acid. Each test was conducted six repetitions.

Nitric oxide radical scavenging

The generated nitric oxide at physiological pH from aqueous sodium nitroprusside (SNP) solution forms nitrite ions through oxygen consumption that can be measured by Griess Illosvoy reaction. The reaction mix used 10 mM SNP with phosphate buffered saline at pH 7.4 and different test solution concentrations from 0 to 70 µg/ml in a total volume of 3 ml. A mixture of 1 ml sulfanilamide (0.33% in 20% glacial acetic acid) with 0.5 ml incubated solution was allowed to stand for 5 minutes at 25°C [15, 16]. The combination of NED (0.1% w/v) at 1 ml volume was added to the mixture which received temperature-controlled incubation of 30 minutes at 25°C. The spectrophotometer measured pink chromophore production at 540 nm using NED to couple with nitrite ions diazotized by sulphanilamide against a blank test. The tests were carried out six times for each experiment. The scientists depended on curcumin as their benchmark compound.

RESULTS AND DISCUSION

Medical plants serve as vital sources to produce new pharmaceutical compounds which demonstrate strong effectiveness and affordability while eliminating all negative side effects. Studies from the World Health Organization confirm that medical herbs form 80 percent of treatment practices for people worldwide. FT-IR peak values of solid analysis of *Thymus vulgaris* [669.30 and 684.73 Strong, C-Cl, alkyl halides], [827.46, 873.75, 927.76 Strong, =C–H, Alkenes], [1010.70, 1236.37, 1313.52 Strong, C-F, alkyl halides], [1417.68, Medium, C=C, Stretch, Aromatic], [1604.77, Bending, N-H, Stretch, Amide], [2918.30, Strong, C-H, Stretch, Alkane], [3269.34, Bending, N-H, Stretch, Amide]. FT-IR peak values of solid analysis of *Piper nigrum* [667.37, Strong, C-Cl, alkyl halides], [873.75, 921.97, Strong, =C–H, Alkenes], [1026.13, 1026.13, 1139.93, 1234.44, 1317.38, 1379.10 Strong, C-F, alkyl halides], [1415.75, 1519.91, 1598.99 Medium, C=C, Aromatic], [1740.72, Strong, C=O, Aldehyde], [2852.72, Strong, C-H, Alkane]. The 3460 cm−1 absorption occurs due to O–H bonds stretching within hydration water molecules and between alcohol and phenol groups. The three intense bands at 2956, 2927, 2872 cm−1 derive from aliphatic –CH3 groups' symmetrical and asymmetrical C–H stretching modes. The signal within 1458 to 1380 cm−1 results from asymmetrical and symmetrical bending modes of this compound. Two main strong bands between 1618 cm−1 and 1581 cm−1 (C=C–C stretching) together with multiple peaks from 1228 cm−1 to 943 cm−1 and 860 cm−1 to 810 cm−1 which signify aromatic C–H in-plane and out-of-plane bending confirm that aromatic rings are present. The phenolic function in thymol is verified through specific O-H bending at 1340–1255 cm−1 as well as C-OH stretching at 1155 and 1056 cm−1 and O-H out-of-plane bending between 736–590 cm−1. Secondary metabolites from Thymus vulgaris extract derive from the source plant and include phenols and tannins along with glycosides and flavonoids and these compounds produce various biological effects. The phytochemical components in T. vulgaris methanolic extract were evaluated by GC-MS and HPLC analysis of its ethyl acetate and n-butanol fractions. In addition to chemical genomic screen evaluations of gastric cancer-causing genes and in vitro and in vivo biological testing

such as anti-inflammatory effects and antioxidant capabilities as well as hemolytic and thrombolytic activities and pyretic reactions and antidiabetic measurements the plant extract underwent analysis [17, 18]. The current study identifies strong plant components which function as essential therapeutic agents for medical treatments.

Antioxidant Properties [Singlet Radical Scavenging] of Crude (methanolic extract), Ethanol, Water and Lipoic acid (standard) of Thymus vulgaris recorded 62.05 ± 4.49, 48.17 ± 2.99, 59.08 ± 3.97 and 39.00 ± 1.26 respectively and recorded 58.96 ± 4.05, 45.00 ± 2.08, 55.97 ± 3.00 and 34.81 ± 1.09 for Piper nigrum while Antioxidant activity [Nitric oxide radical scavenging] of Crude (methanolic extract), Ethanol, Water and curcumin (standard) of Thymus vulgaris recorded 41.97 ± 2.04, 37.16 ± 2.03, 54.00 ± 2.99 and 89.17 ± 7.22 respectively and recorded 45.00 ± 2.19, 39.08 ± 2.14, 60.27 ± 3.89 and 96.13 ± 7.81 respectively for Piper nigrum. The first evidence of human use of plants for 60000 years comes from analysis of fossil fuels. Research studies demonstrate that medicinal plant-derived phytoconstituents determine biological actions including anti-inflammatory properties along with antibacterial effects and antifungal action besides antimalarial ability and anticancer effects and antioxidant properties and other potential activities. Reactive oxygen species (ROS) develop as a result of exogenous factors that include drugs, chemicals, smoke and environmental stress conditions. Medical evidence has established that external factors produce diseases including atherosclerosis, inflammation, neurodegenerative ailments, cardiovascular illnesses and cancer [19-22]. Cell damage occurs because of excessive human body free radical production which includes OH+ and O2− among other radicals. Such damage extends to essential biological molecules consisting of lipid and deoxyribonucleic acid (DNA) and protein. Phenolic compounds in nature defend the human body against illnesses caused by free radicals [23, 24]. The phenolic compounds within natural antioxidants include curcuminoids and phenolics as well as lignans and tannins and coumarins and flavonoids [25-28]. Secondary metabolites of natural origin capture significant interest from health-based and food industries as a result of increased attention. Natural antioxidants protect human bodies by scavenging harmful reactive oxygen species known as ROS. Tissue injury along with chemical irritation or any viral or pathogenic infection triggers biological inflammation [29-32]. The innate immune system receptors activate this response when germs produce the symptoms of pain and redness combined with warmth and swelling. The medical field distinguishes between two inflammatory categories which are chronic and acute. When faced with possible harm the body creates an immediate inflammation response. Pain together with loss of function and swelling are the main symptoms that occur. Human bodies create the pain-causing substances prostacyclins along with prostaglandins and thromboxane under cyclooxygenase (COX) enzymatic activity that leads to inflammation and platelet aggregation. Three inflammatory proteins COX1 and COX2 together with proinflammatory arachidonic acid metabolites show increased expression throughout various pathological conditions that include cardiovascular disease, inflammation together with cancer.

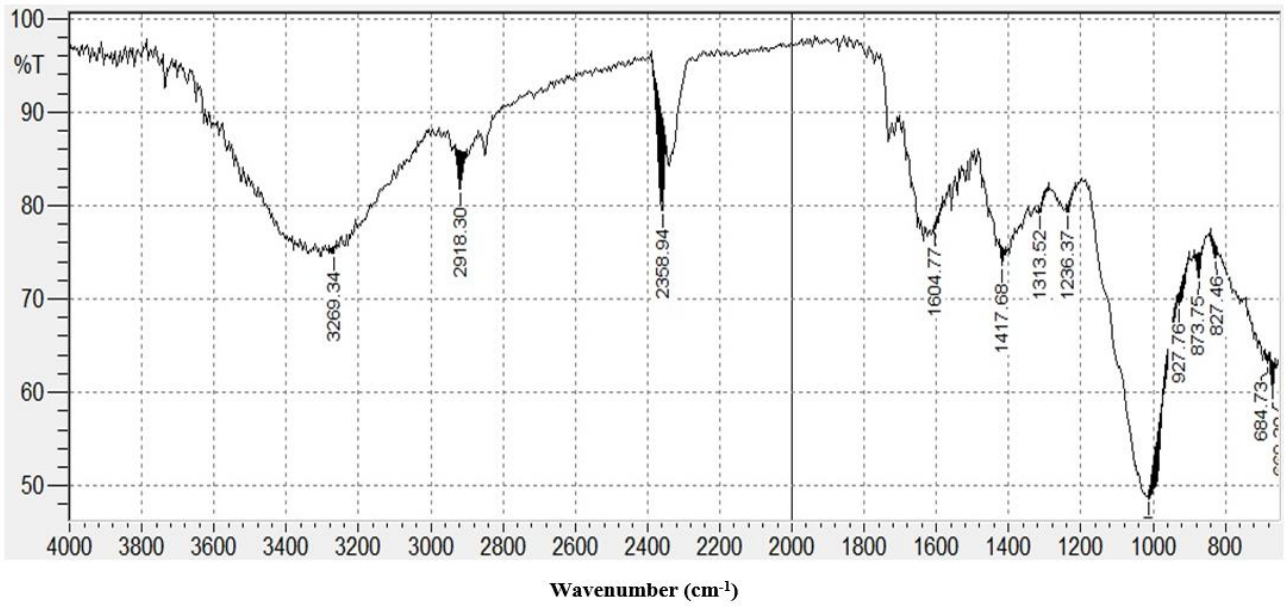

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Thymus vulgaris*

Table 1. FT-IR peak values of solid analysis of *Thymus vulgaris*.

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	669.30	59.416	3.865	677.01	663.51	2.859	0.176	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	684.73	63.115	0.860	690.52	678.94	2.285	0.038	Strong	C-Cl	Stretch	alkyl halides	600–800
3.	827.46	74.505	1.152	840.96	821.68	2.387	0.081	Strong	=C–H	Bending	Alkenes	650-1000
4.	873.75	72.300	2.593	885.33	866.04	2.555	0.131	Strong	=C–H	Bending	Alkenes	650-1000
5.	927.76	69.360	0.661	931.62	900.76	4.511	0.180	Strong	=C–H	Bending	Alkenes	650-1000
6.	1010. 70	48.730	0.709	1012.63	933.55	18.575	0.677	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1236.37	79.328	0.352	1240.23	1217.08	2.182	0.052	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1313.52	79.285	1.798	1315.45	1290.38	2.296	0.008	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1417.68	73.681	0.430	1425.40	1408.04	2.204	0.073	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1604.77	77.448	9.752	1608.63	1581.63	2.817	0.060	Bending	N-H	Stretch	Amide	1550-1640
11.	2358.94	79. 466	3.998	2389.80	2349.30	2.488	0.880	Unknown	-	-	-	-
12.	2918.30	81.850	1.636	2931.80	2899.01	2.468	0.286	Strong	C-H	Stretch	Alkane	2850-3000
13.	3269.34	74.844	0.747	3280.92	3261.63	2.381	0.034	Bending	N-H	Stretch	Amide	3100-3500

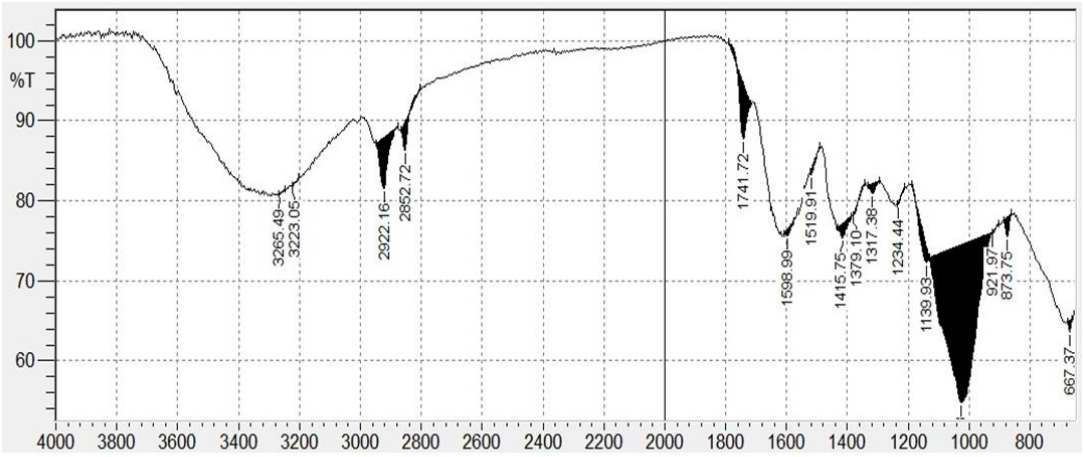

Figure 2. Fourier-transform infrared spectroscopic profile solid analysis of *Piper nigrum*.

Table 2. FT-IR peak values of solid analysis of *Piper nigrum*.

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	667.37	63.602	1.458	677.01	661.58	2.947	0.064	Strong	C-Cl	Stretch	alkyl halides	600–800
2.	873.75	75.464	2.486	885.33	860.25	2.864	0.161	Strong	=C–H	Bending	Alkenes	650-1000
3.	921.97	76.037	0.115	923.90	902.69	2.459	0.006	Strong	=C–H	Bending	Alkenes	650-1000
4.	1026.13	54.832	19.670	1130.29	923.90	40.600	14.196	Strong	C-F	Stretch	alkyl halides	1000-1400
5.	1139.93	72.360	1.874	1188.15	1132.21	6.633	0.381	Strong	C-F	Stretch	alkyl halides	1000-1400
6.	1234.44	79.518	0.208	1236.37	1211.30	2.346	0.015	Strong	C-F	Stretch	alkyl halides	1000-1400
7.	1317.38	80.927	1.224	1332.81	1296.16	3.234	0.116	Strong	C-F	Stretch	alkyl halides	1000-1400
8.	1379.10	78.194	0.230	1381.03	1342.46	3.677	0.026	Strong	C-F	Stretch	alkyl halides	1000-1400
9.	1415.75	75.384	1.784	1431.18	1388.75	4.948	0.223	Medium	C=C	Stretch	Aromatic	1400-1600
10.	1519.91	83.275	0.580	1521.84	1490.97	2.191	0.065	Medium	C=C	Stretch	Aromatic	1400-1600
11.	1598.99	75.718	0.635	1608.63	1577.77	3.642	0.085	Medium	C=C	Stretch	Aromatic	1400-1600
12.	1740.72	87.747	6.874	1789.94	1718.58	2.000	0.743	Strong	C=O	Stretch	Aldehyde	1720-1740
13.	2852.72	86.395	3.584	2866.22	2802.57	2.649	0.244	Strong	C-H	Stretch	Alkane	2850-3000
14.	2922.16	81.542	6.382	2947.23	2877.79	4.668	0.893	Strong	C-H	Stretch	Alkane	2850-3000
15.	3223.05	81.889	0.120	3224.98	3201.83	1.955	0.016	Bending	N-H	Stretch	Amide	3100-3500
16.	3265.49	80.746	0.173	3269.34	3248.13	1.934	0.010	Bending	N-H	Stretch	Amide	3100-3500

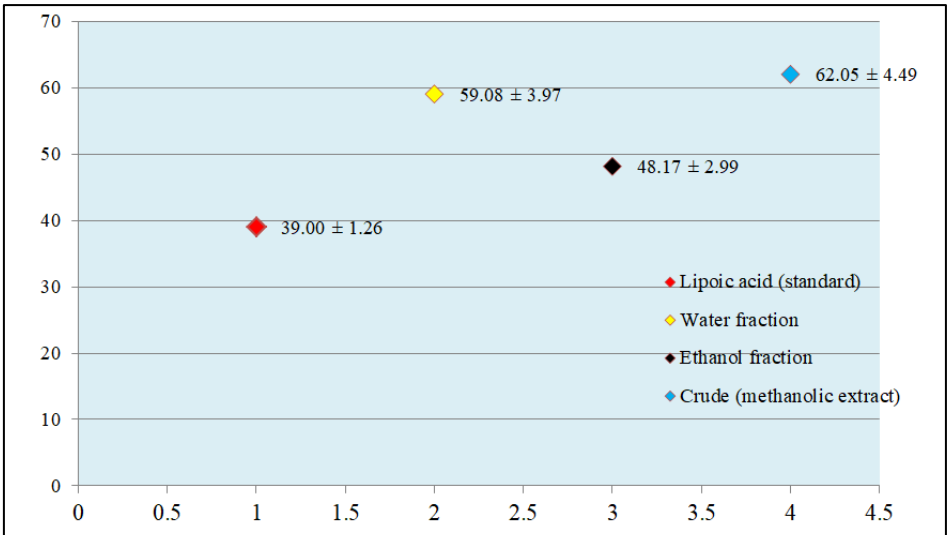

Figure 3. Antioxidant [Singlet Oxygen Scavenging] Properties of Crude (methanolic extract), Ethanol, Water and Lipoic acid (standard) of *Thymus vulgaris*.

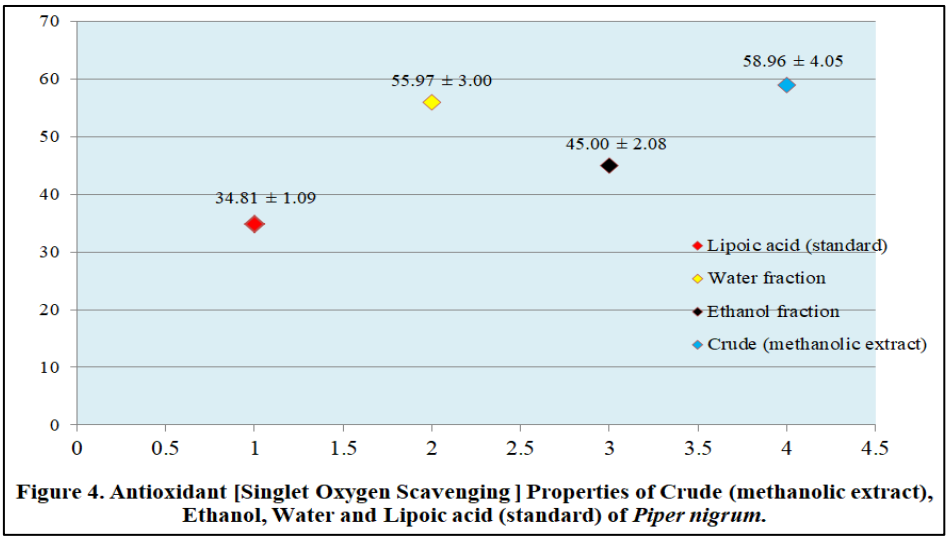

Figure 4. Antioxidant [Singlet Oxygen Scavenging] Properties of Crude (methanolic extract), Ethanol, Water and Lipoic acid (standard) of *Piper nigrum*.

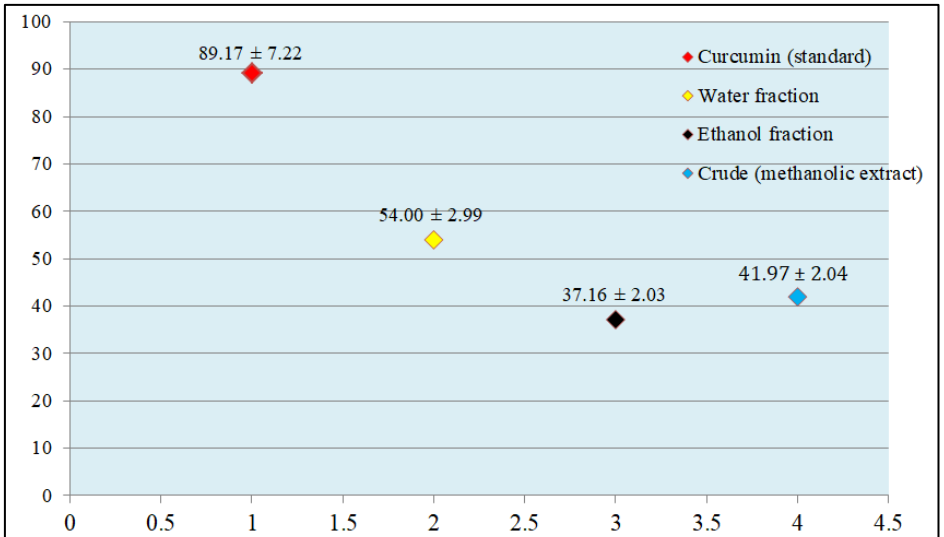

Figure 5. Antioxidant [Nitric Oxide Radical Scavenging] Properties of Crude (methanolic extract), Ethanol, Water fraction and Curcumin (standard) of *Thymus vulgaris*.

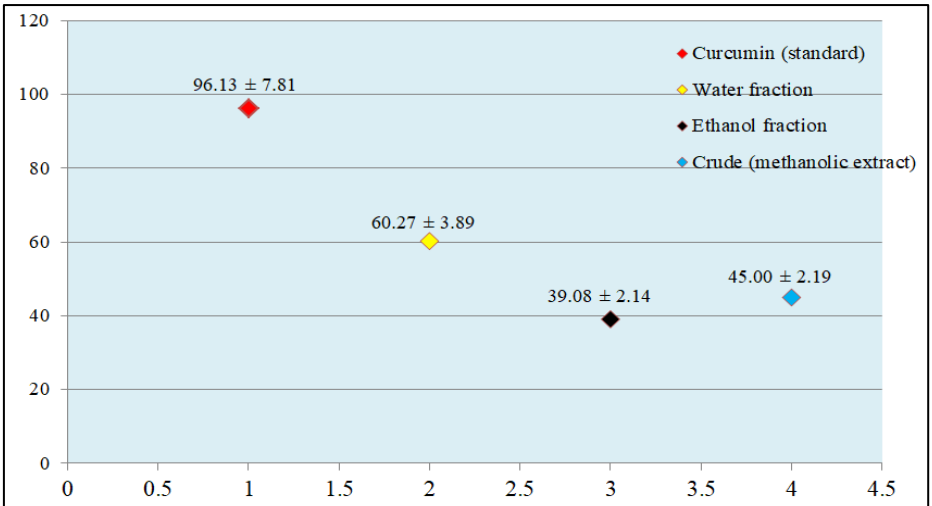

Figure 6. Antioxidant [Nitric Oxide Radical Scavenging] Properties of Crude (methanolic extract), Ethanol, Water fraction and Curcumin (standard) of *Piper nigrum*

The infrared area of electromagnetic radiation spans between 0.75 µm at the high end of visible light to 400 µm within microwave frequencies. The chemical information which proves most beneficial comes from the spectral region between 2.5-16 µm (or wave numbers 4,000–625 cm-1). This part of the instrument uses basic but affordable technology for construction. Molecules accept IR radiation energy which leads to bond structural modifications that perform either bond stretching or bond bending actions. The bond axis frequency vibration changes through excitation during stretching motions and bending motions result from atoms moving perpendicular to the bond axis. The measurement of stretching and bending energy needs both knowledge about atomic or molecular mass and information about atomic hybridization within bonds. The vibration frequencies of OH and NH and CH chemical bonds exist between 3,600–2,800 cm-1. SH bonds and carbonyls alongside amino acids and triple bonds along with cumulative double bonds produce VCD absorption between 2,850 and 1,850 cm-1. The group frequency region extends from 1,500 cm-1 to 650 cm-1. The spectral complexity in this section is high which makes complete band identification impossible because numerous bond deformation combinations and group vibrations appear simultaneously. The absorption bands in the particular molecule are more distinctive than the group frequency region bands. The direct analysis of two spectra in this zone leads to positive correct structural determination. Organic chemists throughout time developed recorded documentation of IR absorption patterns emitted by numerous chemical bonds in various molecular environments [33, 34]. Researchers can easily obtain IR absorption information from tables which they use for spectrum comparison purposes. The bond order and types of atoms connected by a bond represent the two key elements which determine which absorption areas a chemical bond will select. The combination of chemical bond conjugation with adjacent atoms results in minor shifts of absorption frequencies. The identical or comparable functional groups across molecules absorb light of matching precise frequency bands. Different versions of IR absorption tables use functional groups as an organizational structure with some including additional subdivisions for advanced identification. When examining an ordinary infrared spectrum it will naturally break into two distinct sections. The left part extending above 2000 cm-1 presents minimal peaks even though it contains vital diagnostic signals. The presence of saturated carbons shows as C-H absorbance signals below 3000 cm-1 and unsaturated carbons appear as signals above 3000 cm-1. Exchangeable protons located in alcohol, amine, amide or carboxylic acid groups produce a wide band which spans from 3100 to 3600 cm-1 [32, 33]. The absorptions active between 2800 to 2000 cm-1 remain blank areas hence detecting alkyne or nitrile groups becomes straightforward. Many peaks exist in the right half of the spectrum which extends below 2000 cm-1 yet most signals remain unidentified by standard interpretation. A very strong peak occurs near 1700 cm-1 resulting from carbonyl groups while the C-O bond yields one or two strong peaks between 1200 cm-1. The complex lower section of the IR spectrum carries the "fingerprint region" name since this part displays distinctive patterns for each organic substance thus spectral comparison leads to identity confirmation [34]. IR absorption frequency of chemical bonds depends heavily on the characteristics of the bonded atoms as explained earlier in the discussion. The precise requirement in this case is to focus on the atomic masses of these two atoms. The larger the mass of specific atoms attached to a bond determines the lower frequency band in which absorption occurs during IR analysis. The key differences between chloroform and deuterochloroform spectra consist of (1) the extinction of C-H stretching (3020 cm-1) and bending (1220 cm-1) along with (2) an absorption peak move to 20 cm-1 rightward compared to CHCl3. The lack of C-H bonds in CDCl3 explains the first spectrum change. When deuterium atoms substitute hydrogen atoms in a molecule the attached bonds shift their absorption frequencies to lower values. People readily identify a broad O-H absorption band in IR spectra because of its signature appearance which comes specifically from alcohol and phenolic functional groups. Evaluation of peak broadening needs an explanation of its mechanisms and analysis of situations that deviate from typical behavior. A significant amount of compound contains a very vast number of molecules which exhibit

different extents of hydrogen bonding interactions [35, 36]. The acquisition of an IR spectrum results in bond absorptions appearing at respective frequencies for all present bonds. Through the averaging process the IR peak shows broadening since it results from the summation of multiple similar but distinct absorptions.

The research measured peroxidase (PX), catalase (CAT) and superoxide dismutase (SOD) enzyme activities to determine if EOs triggered antioxidant enzyme actions. The addition of thymol to LPS-treated cells enabled higher PX and SOD activities at the same time it reduced CAT activity. The administration of thymol prior to treatment caused an increase in the enzyme activities of PX, SOD and CAT simultaneously. TEO's action in initiating flowering shared identical effects with those of thymol as an EO. The combination of TEO/end of flowering delivered poor effects on PX activity yet it proved best at enhancing CAT activity when used as LPS pretreatment. The combination of EO pre-treatment followed by LPS treatment resulted in similar or lower effects on CAT and SOD activities than both thymol and TEO when used at the beginning of flowering. EO effectiveness compared to thymol probably differs because the main compound concentration reaches 1182 ng/ml.

The thymol content reached 1071.55 ng/mL within TEO/beginning of flowering and it declined to 1040.83 ng/mL throughout TEO/end of flowering. The dissimilar effects between TEOs on antioxidant enzymes result from the different concentrations of ingredients since both active compounds may either boost each other or block the mechanism of action. The p-cymene together with linalool and carvacrol and (E)-caryophyllene appeared at elevated concentrations in TEO/end of flowering. The antioxidant properties appear in these chemical compounds along with other functions. TEO/beginning of flowering contained higher quantities of three antioxidant-active compounds comprising myrcene-, α-terpinene, and γ-terpinene. Higher components' levels appear to produce stronger effects of TEO/beginning of flowering when used before EO treatment. Intracellular total antioxidant capacity (TAC) testing indicates that TEOs demonstrate ROS-scavenging abilities which also affect antioxidant enzyme functioning. TEO/beginning of flowering demonstrated excellent ROS scavenging properties since it resulted in the highest TAC measurement among THP-1 cells following LPS treatment. Long-term LPS pretreatment revealed equal protective effects of all three EOs which implies their dual functions between ROS scavenging and enzyme-triggering actions. The combination of pretreatment with TEO at end of flowering and long-term LPS treatment suggests that it possibly strengthens both enzymatic and nonenzymatic antioxidant functions including glutathione. The extraction method often selects ethanol because it presents reduced toxic effects on antioxidant compounds [37, 38]. Antioxidative functions of different compounds in solution might depend on fundamental molecular features including phenolic hydroxyl and methoxyl groups and flavone hydroxyl groups together with keto groups and free carboxylic group composition. Scientists examined the antioxidant capacities of Piper species because these plants are widely utilized in diet-based healthcare practices. Piper nigrum Linn. possessed maximum levels of glutathione peroxidase and glucose-6-phosphate dehydrogenase enzymes whereas green pepper exhibited the most peroxidase and vitamin C activities yet Piper longum Linn. and Piper nigrum Linn. showed higher levels of vitamin E. Piper brachystachyum Linn. and Piper longum Linn. Among the tested samples vitamin A occurred most abundantly. Research studies have extensively documented the effective antioxidant functions and radical absorption abilities of black pepper (Piper nigrum Linn.) seeds. The antioxidant capabilities of water extract and ethanol extract from black pepper extracts were shown to be strong.

CONCLUSION

Fourier transform infrared spectroscopy confirms the existence of functional groups which verifies the phytochemical identification performed through GC-MS. Ethanol exhibits powerful antioxidant properties in biological activities since its compounds perform effective hydrogen donation. Independent testing of the compounds thymol and carvacrol with p-Cymene and eugenol displayed strong binding energy thus indicating their combined ability to block COX1 and COX2 and counteract inflammation. The medicinal and pharmacological properties of Thymus vulgaris and Piper nigrum indicate they have excellent therapeutic potential in medicine which requires additional investigation.

REFERENCES

1. Hemmalakshmi, S.; Priyanga, S.; Devaki, K. Fourier Transform Infra-Red Spectroscopy Analysis of Erythrina variegata L. J. Pharm. Sci. Res. 2017, 9, 2062–2067.
2. Mahrye; Anwar, F.; Mehmood, T.; Qadir, R.; Riaz, M. Phenolics profiling and biological activities of different solvent extracts from aerial parts of wild thyme (Thymus vulgaris L.). J. Food Meas. Charact. 2022, 16, 610–618.
3. Asif, M.; Yehya, A.H.S.; Al-Mansoub, M.A.; Revadigar, V.; Ezzat, M.O.; Ahamed, M.B.K.; Oon, C.E.; Murugaiyah, V.; Majid, A.S.A.; Majid, A.M.S.A. Anticancer attributes of Illicium verum essential oils against colon cancer. South Afr. J. Bot. 2016, 103, 156–161.
4. M.G. Miguel, C. Gago, M.D. Antunes, C. Megias, I. Cortés-Giraldo, J. Vioque, A.S. Lima and A.C. Figueiredo, Antioxidant and antiproliferative activities of the essential oils from Thymbra capitata and Thymus species grown in Portugal. Evidence-Based Complementary and Alternative Medicine, 2015, 8 (2015).

5. Q.H. Yin, F.X. Yan, X.Y. Zu, Y.H. Wu, X.P. Wu, M.C. Liao, S.W. Deng, L.L. Yin and Y.Z. Zhuang, Anti-proliferative and pro-apoptotic effect of carvacrol on human hepatocellular carcinoma cell line HepG-2. Cytotechnology, 64, 43–51 (2012).
6. Y. Zu, H. Yu, L. Liang, Y. Fu, T. Efferth, X. Liu and N. Wu, Activities of ten essential oils towards Propionibacterium acnes and PC-3, A-549 and MCF-7 cancer cells. Molecules, 15, 3200–10 (2010).
7. R.P. Adams, Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured Pub. Corp, Carol Stream, IL (2007).
8. l. Imperial Chemical Industries and C. Mass Spectrometry Data, Eight peak index of mass spectra: The eight most abundant ions in 66,720 mass spectra, indexed by molecular weight, elemental composition and most abundant ions. Mass Spectometry Data Centre, Royal Society of Chemistry, Nottingham (1986).
9. W. Jennings and T. Shibamoto, Qualitative analysis of flavor and fragrance volatiles by glass capillary gas chromatography. Academic Press, New York (1980).
10. M.d.S. Gomide, F.d.O. Lemos, M.T.P. Lopes, T.M.d.A. Alves, L.F. Viccini and C.M. Coelho, The effect of the essential oils from five different Lippia species on the viability of tumor cell lines. Revista Brasileira de Farmacognosia, 23, 895–902 (2013)
11. Dessalegn, E.; Bultosa, G.; Haki, G.D.; Rupasinghe, H.P.V. Evaluation of In vitro Antidiabetic Potential of Thymus schimperi R. and Thymus vulgaris L. J. Health Med. Nurs. 2019, 69.
12. Perumalsamy, R.; Krishnadhas, L. Anti-Diabetic Activity of Silver Nanoparticles Synthesized from the Hydroethanolic Extract of Myristica fragrans Seeds. Appl. Biochem. Biotechnol. 2022, 194, 1136–1148.
13. Williams, L.A.D.; O'Connar, A.; Latore, L.; Dennis, O.; Ringer, S.; A Whittaker, J.; Conrad, J.; Vogler, B.; Rosner, H.; Kraus, W. The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. West Indian Med. J. 2008, 57, 327–331.
14. Chakraborty N, Tripathy BC: Involvement of singlet oxygen in 5-aminolevulinic acid-induced photodynamic damage of cucumber (Cucumbis sativus L.) chloroplasts. Plant Physiol. 1992, 98: 7-11.
15. Miller MJ, Sadowska-Krowicka H, Chotinaruemol S, Kakkis JL, Clark DA: Amelioration of chronic ileitis by nitric oxide synthase inhibition. J Pharmacol Exp Ther. 1993, 264 (1): 11-16.
16. Balavoine GG, Geletti YV: Peroxynitrite scavenging by different antioxidants. Part 1: convenient study. Nitric oxide. 1999, 3: 40-54.
17. Adedapo, A.A.; Falayi, O.O.; Oyagbemi, A.A. Evaluation of the analgesic, anti-inflammatory, anti-oxidant, phytochemical and toxicological properties of the methanolic leaf extract of commercially processed Moringa oleifera in some laboratory animals. J. Basic Clin. Physiol. Pharmacol. 2015, 26, 491–499.
18. Schneidman-Duhovny, D.; Inbar, Y.; Nussinov, R.; Wolfson, H.J. Geometry-based flexible and symmetric protein docking. Proteins 2005, 60, 224–231.
19. El-Newary, S.A.; Shaffie, N.M.; Omer, E. The protection of Thymus vulgaris leaves alcoholic extract against hepatotoxicity of alcohol in rats. Asian Pac. J. Trop. Med. 2017, 10, 361–371.
20. Perveen, N.; Ahmed, K.Z.; Irshad, T.; Latif, N.; Kaleem, Z.; Naseer, F. Evaluation of acute and sub acute hepatotoxic activity of Trichodesma indicum aqueous methanolic extract in mice. Res. Pharm. Health Sci. 2015, 2, 32–35.
21. Tang, M.; Xie, X.; Yi, P.; Kang, J.; Liao, J.; Li, W.; Li, F. Integrating Network Pharmacology with Molecular Docking to Unravel the Active Compounds and Potential Mechanism of Simiao Pill Treating Rheumatoid Arthritis. Evid.-Based Complement. Altern. Med. 2020, 2020, 5786053.
22. Kumar, G.; Karthik, L.; Rao, K.V.B. Hemolytic activity of Indian medicinal plants towards human erythrocytes: An in vitro study. Elixir. Appl. Botany 2011, 40, e5537.
23. Ramjan, A.; Hossain, M.; Runa, J.F.; Hasanuzzaman; Mahmodul, I. Evaluation of thrombolytic potential of three medicinal plants available in Bangladesh, as a potent source of thrombolytic compounds. Avicenna J. Phytomedicine 2014, 4, 430.
24. Muhammad, I.; Xiao, Y.Z.; Hassan, S.S.U.; Xiao, X.; Yan, S.K.; Guo, Y.Q.; Ma, X.P.; Jin, H.Z. Three new guaiane-type sesquiterpenoids and a monoterpenoid from Litsea lancilimba Merr. Nat. Prod. Res. 2022, 36, 3271–3279.
25. Hassan, S.S.U.; Ali, F.; Ishaq, M.; Bano, I.; Hassan, M.; Jin, H.-Z.; Bungau, S. A Comprehensive In Silico Exploration of Pharmacological Properties, Bioactivities, Molecular docking, and Anticancer Potential of Vieloplain F from Xylopia vielana Targeting B-Raf Kinase. Molecules 2022, 27, 917.
26. Naveed, M.; Bukhari, B.; Aziz, T.; Zaib, S.; Mansoor, M.A.; Khan, A.A.; Shahzad, M.; Dablool, A.S.; Alruways, M.W.; Almalki, A.A.; et al. Green Synthesis of Silver Nanoparticles Using the Plant Extract of Acer oblongifolium and Study of Its Antibacterial and Antiproliferative Activity via Mathematical Approaches. Molecules 2022, 27, 4226.
27. Muhammad, I.; Hassan, S.S.U.; Cheung, S.; Li, X.; Wang, R.; Zhang, W.-D.; Yan, S.-K.; Zhang, Y.; Jin, H.-Z. Phytochemical study of Ligularia subspicata and valuation of its anti-inflammatory activity. Fitoterapia 2021, 148, 104800

28. Yosr, Z.; Imen, B.H.Y.; Rym, J.; Chokri, M.; Mohamed, B. Sex-Related Differences in Essential Oil Composition, Phenol Contents and Antioxidant Activity of Aerial Parts in Pistacia lentiscus L. during Seasons. Ind. Crops Prod. 2018, 121, 151–159.
29. Pandur, E.; Balatinácz, A.; Micalizzi, G.; Mondello, L.; Horváth, A.; Sipos, K.; Horváth, G. Anti-Inflammatory Effect of Lavender (Lavandula angustifolia Mill.) Essential Oil Prepared during Different Plant Phenophases on THP-1 Macrophages. BMC Complement. Med. Ther. 2021, 21, 287.
30. Cazella, L.N.; Glamoclija, J.; Sokovi´c, M.; Gonçalves, J.E.; Linde, G.A.; Colauto, N.B.; Gazim, Z.C. Antimicrobial Activity of Essential Oil of Baccharis dracunculifolia DC (Asteraceae) Aerial Parts at Flowering Period. Front. Plant Sci. 2019, 10, e00027.
31. Galovi˘cová, L.; Borotová, P.; Valková, V.; Vukovic, N.L.; Vukic, M.; Štefániková, J.; ˘Dúranová, H.; Kowalczewski, P.Ł.; ˘Cmiková, N.; Ka˘cániová, M. Thymus vulgaris Essential Oil and Its Biological Activity. Plants 2021, 10, 1959.
32. Gellatly, S.L.; Hancock, R.E.W. Pseudomonas aeruginosa: New Insights into Pathogenesis and Host Defenses. Pathog. Dis. 2013, 67, 159–173.
33. Chai, Y.H.; Xu, J.F. How Does Pseudomonas aeruginosa Affect the Progression of Bronchiectasis? Clin. Microbiol. Infect. 2020, 26, 313–318. [CrossRef] [PubMed]
34. Rodrigo-Troyano, A.; Suarez-Cuartin, G.; Peiró, M.; Barril, S.; Castillo, D.; Sanchez-Reus, F.; Plaza, V.; Restrepo, M.I.; Chalmers, J.D.; Sibila, O. Pseudomonas aeruginosa Resistance Patterns and Clinical Outcomes in Hospitalized Exacerbations of COPD. Respirology 2016, 21, 1235–1242.
35. Yum, H.K.; Park, I.N.; Shin, B.M.; Choi, S.J. Recurrent Pseudomonas aeruginosa Infection in Chronic Lung Diseases: Relapse or Reinfection? Tuberc. Respir. Dis. 2014, 77, 172–177.
36. Nguyen, L.; Garcia, J.; Gruenberg, K.; MacDougall, C. Multidrug-Resistant Pseudomonas Infections: Hard to Treat, But Hope on the Horizon? Curr. Infect. Dis. Rep. 2018, 20, 23.
37. Wang, S.; Xiang, D.; Tian, F.; Ni, M. Lipopolysaccharide from Biofilm-Forming Pseudomonas aeruginosa PAO1 Induces Macrophage Hyperinflammatory Responses. J. Med. Microbiol. 2021, 70, 001352.
38. Phuong, M.S.; Hernandez, R.E.; Wolter, D.J.; Hoffman, L.R.; Sad, S. Impairment in Inflammasome Signaling by the Chronic Pseudomonas aeruginosa Isolates from Cystic Fibrosis Patients Results in an Increase in Inflammatory Response. Cell Death Dis. 2021, 12, 241.