ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Characterizations of Lavender Flowers Extracts and Study of their Effects on Peroxidase Activity and Some Microorganisms

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Abstract

Herbal medicinal plants have been used traditionally to treat diseases in addition to commercial use. Therefore, this study is designed to extract the most important components of lavender flowers herbal and study their biological activities against some important species of bacteria and fungi as well as against peroxidase activity. The study also includes a description of Lavender flower extract using UV-Vis and GC-MS techniques. In this study, three extracts (ethanolic, cold aqueous and hot aqueous extracts) were prepared by maceration (1gm/50ml solvent). UV-Vis spectroscopy was revealed peaks embedded in a significant functional range (200-400) nm, which may be attributed to unsaturated functional groups and hetero atoms (S, N, O). The results showed an inhibitory effect of the cold and hot aqueous extracts on the activity of peroxidase. Kinetic study demonstrated that the cold extract caused a non-competitive inhibition while hot extract caused a mixed type inhibition. The kinetic parameters obtained by Lineweaver-Burk plot, in the presence and absence of lavender extracts respectively, indicated that the Km values were (0.0007, 0.001) mM while Vmax values were (95.23, 125) U/L for cold aqueous extract, while the Km values were (0.0028, 0.003) mM, and Vmax values were (80.64, 112.5) U/L for hot aqueous extract. Antibacterial and antifungal effects against isolates in our study showed that ethanolic extract is more potent than aqueous extracts which could be predominantly the result of highly account of active phenolic compounds that presence in Lavender herbal ethanolic extract. The results of the current work support the traditional use of the studied plant and suggest that some botanical extracts, particularly ethanolic extract, have compounds with antibacterial properties that can be used as antibacterial agents in new drugs to treat infectious diseases caused by pathogens. The most effective extracts can be subjected to therapeutic antibacterial isolation and further medical evaluation.

Keywords: *Lavender; GC-MS; UV-Vis; Peroxidase; Antibacterial; Antifungal.*

Introduction

Peroxidases are a group of enzymes that catalyze oxido-reduction reactions. Peroxidases are widely found in plants, animal tissues and micro-organisms. These types of enzymes carry out a variety of functions with the consumption of hydrogen peroxide compound as oxidant.

Also, they are responsible for protecting cells against reactive peroxide compounds, which form as side-products throughout oxygen metabolism [1, 2]. Plant peroxidase [EC 1. 1 1.1.7] is a heme-containing metallo enzyme, which catalyzes the oxidation of a wide range of inorganic and organic substrates by using hydrogen peroxide as an electron acceptor [3-6].

Oxidation of aromatic substrates by one-electron catalyzed by peroxidases is depicted by the Chance-George mechanism [7]. In this mechanism, the native pure enzyme is oxidized by a substrate H_2O_2 and subsequent oxidized other compounds. It oxidized a biomolecule as organic compound to free

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radical, then terminally enzyme returns and back to its native state [8].

Lavender (also known Lavandula) is a wonderful flowering plant of the mint family Lamiaceae. The original name lavender derived from the Latin world *lavar,* which mean to wash. In ancient Greece, Rome and Persia, lavender was used as a perfume for laundry and baths, and also used as an antiseptic. In India, lavender was renowned as a healing agent.

While in Tibetans Buddhist medicine, lavender was used as a treatment for psychotics. At present, this herbal is widely used commercially as an anxiolytics, cosmetically, detergents, jelly, in massage (especially lavender oil), shampoo, perfumery and soaps [9]. Many therapeutic uses have been revealed for lavender herbs particularly in angina pectoris, acne, pain, colonic spasmosing and convulsion. It also can be used as anti-flatulent, anti-inflammatory and anti-fungal [10, 11].

Furthermore, lavender can used as antioxidant, antiseptic, antipyretic, stimulant of appetite, as treatment for asthma, chronic bronchitis and diabetes. It has been found that lavender extracts could be used in treatment of infertility, hypertension and migraine [12, 16]. In fact, lavender is rich in volatile oils and currently used as an aroma therapeutic and hypnotic [17].

Lavender flowers is comprised of over 95 important constituents such as perillyl alcohol, limonene, linalyl, linalool, camphor acetate, tannins, coumarins, triterpene, cineole and flavonoids [18]. Herbal medicinal plants have been used extensively to treat diseases traditionally. The importance of medicinal plants lies in their basic components, where the therapeutic properties of plants are attributed to these components.

Therefore, the present study was conducted to highlight an effective and accurate process to extract the most important components of lavender flowers and study their biological activities against some important types of bacteria and fungi as well as against the activity of peroxidase. The study also includes descriptions of lavender flower extracts using UV-Vis and GC-MS techniques.

Material and Methods

Lavender Flowers Extractions and Characterizations

In order to prepare extracted stock solutions, lavender flowers were purchased from the trading market. These flowers were used to prepare three extracts; ethanol extract was prepared by weighted 1gm of the flowers and macerated in 50 ml of absolute ethanol for 22 hours. The same method was applied with distilled water to prepare an aqueous extract. Third extract was prepared by heating 1gm of this flower for a half hour with 50 ml of distilled water and then leave to cool on punch. Then these mixtures in three prepared extracts were shaken and filtered by two steps; the first step by medicinal lint and the second step by filter paper. Two extracts (cold and hot aqueous extracts) were used to study their effect on the serum peroxidase activity in vitro, while all three extracts were tested on microorganisms.

Characterization

UV-Vis Spectroscopy Analysis

UV-Vis spectra of the aqueous and ethanolic extracts were analyzed and recorded using Cecil Aquarius CE 7200 Double Beam Spectrophotometer, UK. The three prepared extracts were presented for UV-Vis spectroscopy scanning at range (900-190) nm independently. The aqueous and ethanolic extracts solvents were used as a blank for each extract. The data obtained were recorded and presented.

GC-Ms Analysis

GC-MS identification was carried out using Shimadzu GCMS-QP2010Ultra, and column (5 Ms). The GC was operated under the following conditions: temperature programmed (60, 180, 300)°C, at rate (0, 15, 10) with hold time 1 min and column oven temp 60 °C. Injection mode split less with total flow 20.7 ml/min and pressure 100 Kpa.

Peroxidase activity Assay and Kinetic Study

In order to study the effect of lavender extracts on peroxidase activity, the enzyme activity were measured in sera of healthy volunteers. The assay was achieved using manual colorimetric method as follows: 1.4 ml of phenol were added to four test tubes, then 1.5 ml of different concentrations of

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hydrogen peroxide (0.0018, 0.01, 0.015, and 0.017) mM were added to the four test tubes respectively.

The tubes were incubated for 3 minutes, then 100 µl of serum and 20 µl of distilled water were added to each tube and mixed well. The absorption at 510 nm was read against the reagent blank for zero time and 5 minutes and the difference between the two readings was calculated (ΔA) [19]. The same procedure was performed by replacing the distilled water with an aqueous extract of lavender to evaluate the effect of lavender extract on enzyme activity. Furthermore, kinetic study was performed using different volumes of cold and hot aqueous lavender extracts (2, 5, 10 and 20) µl at constant substrate concentration 0.017 mM, to estimate (K_m, V_{max}) values and type of inhibition. Peroxidase activity and inhibition percentage was calculated according to the following equations.

$$Activity\,(U/L) = \frac{\Delta A/min}{€\,Vs} \times Vt \times 10^6.$$

(Vt = total volume 3.1 ml), (Vs = sample volume 100 µl), (€ = 6580).

$$(\%Inhibition = 100 - \frac{activity\ with\ extract}{activity\ witout\ extract}, \times 100)$$

Agar Disk Diffusion Test and Target of Microorganisms

Antibacterial activity was examined for the three prepared extracts of lavender. Microorganisms were obtained from bacteria dynasty that preservative and available in the laboratory of Biology Department, College of Sciences, Mustansiriyah University. The studied microorganisms included; gram positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram negative (*Escherichia coli* and *Klebsiella sp*).

The studies also included examine the antifungal activity of extracts against *Candida albicans*. The components of media that used in this study were Eozin methylene blue (EMB), nutrient agar media and Muller Hinton agar media to breed development of different microorganisms [20, 21]. Disk diffusion test of lavender extracts was carried out on the cultured bacteria and fungi for 24 hours at 37℃ in typically agar when add 100 µl of each extracts. The growth of bacterial was determined by measuring the inhibition zone diameter.

Results and Discussion

A lavender flower is one of the important herbal plants that have many advantages and multi commercial and medical uses. It was therefore considered more reliable to evaluate the efficacy of its components in biochemical and biomedical applications. Three lander extracts were prepared and characterized by UV-Vis and GC-MS techniques. UV-Vis spectra were recorded for all lavender extracts in the survey range (190-900) nm. The results provided important spectral information as shown in Figures 1, 2 and 3. The number of peaks obtained from the ethanol extract spectrum is four distinct absorption peaks (666, 368, 312, 296) nm, where one peak for each cold aqueous extract and a hot aqueous extract (372, 386) nm, respectively.

These differences in the number of peaks presented in Figures 1, 2 and 3 reflect the nature of the compounds that can be extracted with organic solvent rather than the aqueous solvent. In fact, the strong absorption bands that appeared in the spectra of lavender extracts may be attributed to the presence of phenolic compounds. The band 296 nm in ethanolic extract indicated to characteristic nearly of CΞN chromophore that represent exactly at region 272 nm [22].

This result is consistent with the results of other plant components such as Cissampelos pareira herbal [23]. The peaks range in the region (200-400) nm was very clear in all three extracts indicating that there were unsaturated functional groups and heterozygous atoms such as S, N, and O [24].

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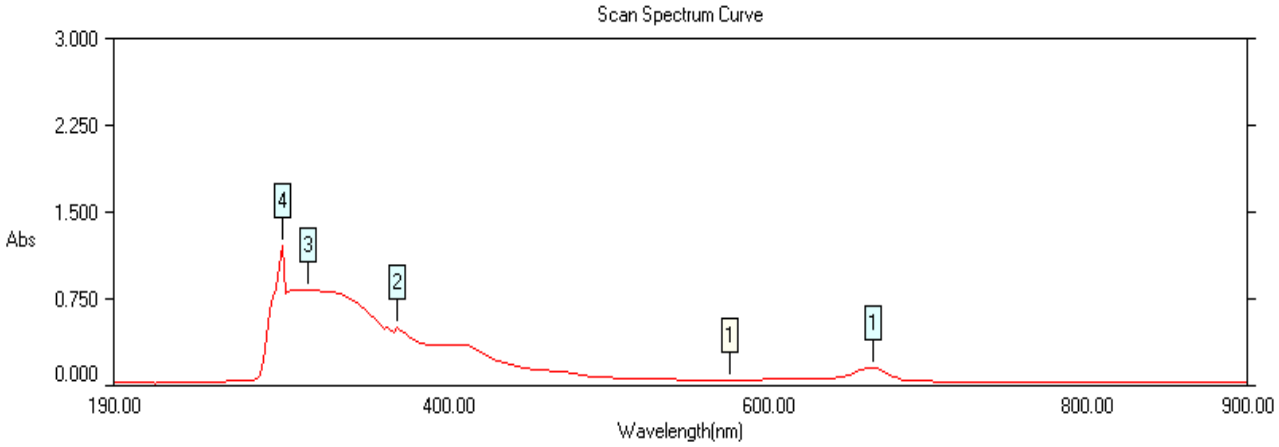

Figure 1: UV-Vis spectra of Ethanolic lavender extract from stock solution (1gm/50ml Ethanol)

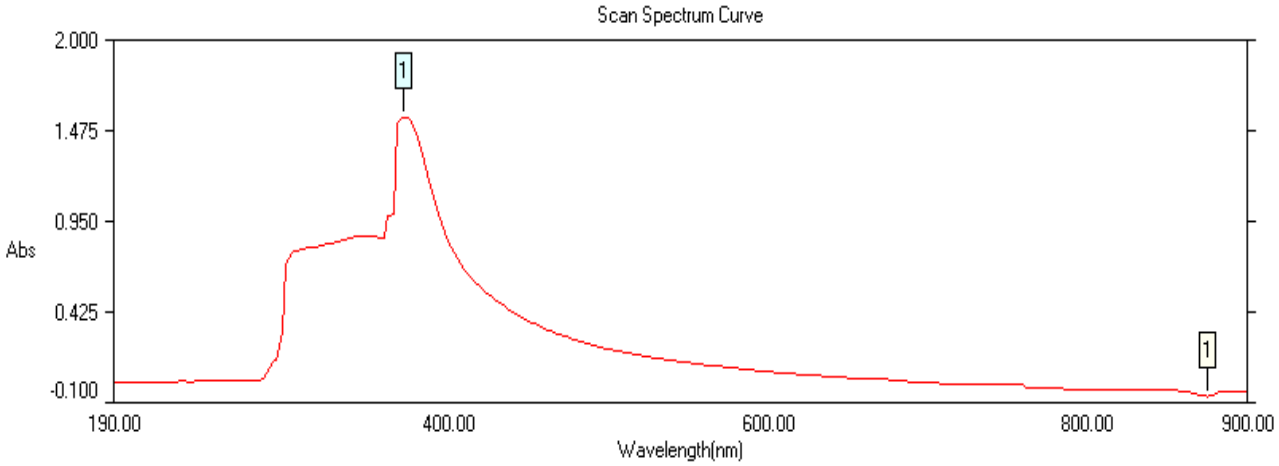

Figure 2: UV-Vis spectra of cold aqueous lavender extract from stock solution (1gm/50ml distilled water)

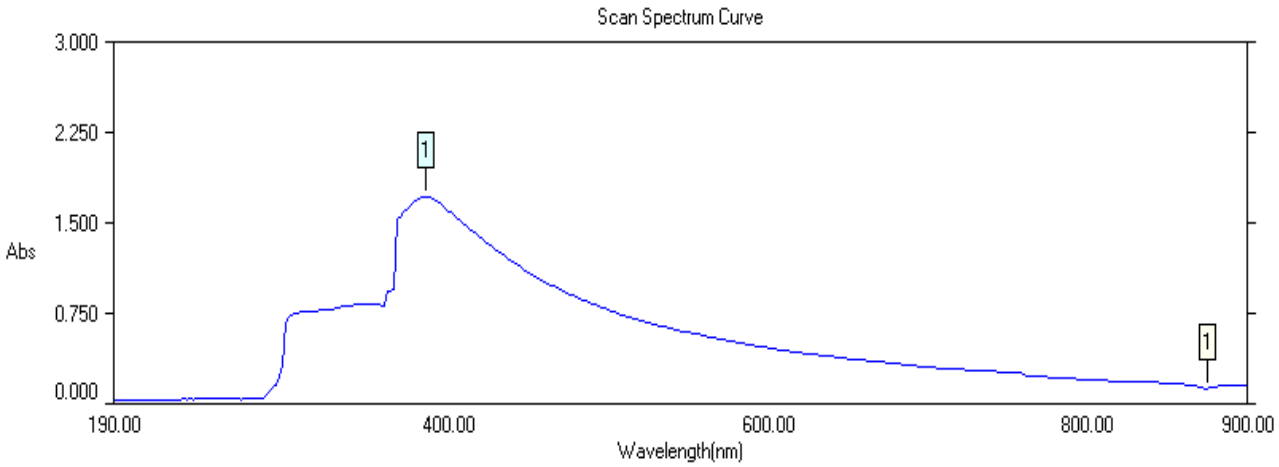

Figure 3: UV-Vis spectra of hot aqueous lavender extract from stock solution (1gm/50ml hot distilled water)

GC-MS analysis identification was performed by Shimadzu GCMS-QP2010 Ultra with column (5Ms). GC was operated under the following conditions: temperature programmed (60, 180, 300) °C, at rate (0, 15, 10) with hold time 1 min and column oven temp 60 °C. Injection mode splitless with total flow 20.7 ml/min and pressure 100 Kpa. GC-MS technique was carried out on the lavender flower extract for qualitative characterization of its constituents. The results obtained based on the potential compounds from the library contain 40 compounds, as shown in Table 1, that is based on the chromatographic chart in Figure 4. The GC-MS library has large number of compounds (approx. ninety compounds). Lavender ingredients were demonstrated during this analysis as a comparison of the peak in the chromatogram obtained with the library. These peaks indicate the identity of the components that have been demonstrated previously in other studies, where 95% of total mass was

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identified by this technique [25]. The programmed temperature used in our work may yield different results from other studies. Because the low-temperature isothermal program is given no more than 1% terpenoids [26].Also our results were slightly differing from other studies according to the nature of solvent mixtures used in extraction.

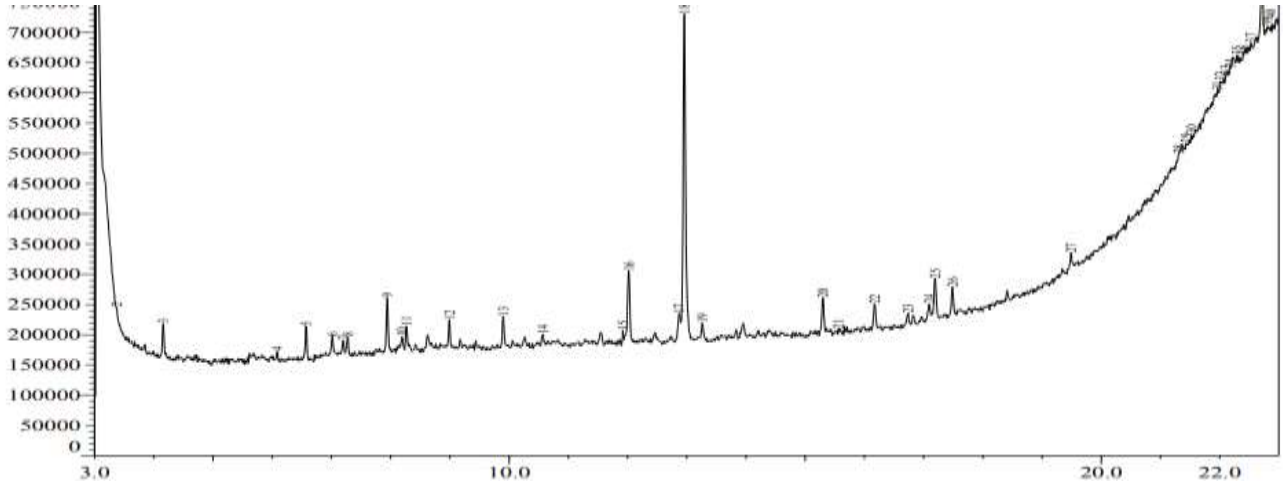

Figure 4: TIC chromatogram for lavender flower, extracted by (1:1 Hexane and Ethyl acetate)

Table 1: Lavender flowers components with data that identified by GC-MS analysis

Peak	R.Time	Area	Area%	Height	Height%	Name
1	3.04	7057760	57.17	838539	30.97	1-Hexacosene
2	3.38	112734	0.91	56825	2.10	l-Methionine, N-(2-chloroethoxycarbonyl)-, heptyl ester
3	4.16	90668	0.73	51196	1.89	2-Hexanol, 2-methyl-
4	6.08	21084	0.17	14895	0.55	Carbonic acid, butyl tridecyl ester
5	6.57	111721	0.90	55178	2.04	Eucalyptol
6	7.02	59336	0.48	28562	1.05	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol
7	7.20	34783	0.28	20303	0.75	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol
8	7.27	43444	0.35	25102	0.93	1,6-Octadien-3-ol, 3,7-dimethyl-
9	7.94	188753	1.53	88446	3.27	Camphor
10	8.19	40099	0.32	19691	0.73	Borneol
11	8.27	75390	0.61	37521	1.39	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
12	8.99	80376	0.65	45391	1.68	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
13	9.90	97597	0.79	48386	1.79	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
14	10.57	29253	0.24	14357	0.53	2-Butenedioic acid (Z)-, monododecyl ester
15	11.92	24657	0.20	23651	0.87	Acetamide, N-[2-[[2-[2-(2-nitrophenyl)ethenyl]phenyl]azo]phenyl]-
16	12.02	356251	2.89	118896	4.39	Phenol, 2,4-bis(1,1-dimethylethyl)-
17	12.87	131907	1.07	42791	1.58	Diethyl Phthalate
18	12.96	1607611	13.02	538684	19.89	Diethyl Phthalate
19	13.26	53269	0.43	25059	0.93	Caryophyllene oxide
20	15.30	128972	1.04	56101	2.07	n-Pentadecanol
21	15.56	36655	0.30	10407	0.38	L-Aspartic acid, N-methyl-N-(trifluoroacetyl)-, bis(1-methylpropyl) ester
22	16.17	100969	0.82	40483	1.49	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
23	16.74	33290	0.27	16823	0.62	Heptacosanoic acid, 25-methyl-, methyl ester
24	17.09	65508	0.53	25937	0.96	l-(+)-Ascorbic acid 2,6-dihexadecanoate
25	17.19	196396	1.59	67527	2.49	Dibutyl phthalate
26	17.48	98023	0.79	45167	1.67	1-Nonadecene
27	19.49	52239	0.42	26304	0.97	1-Docosene
28	21.31	73859	0.60	9895	0.37	1-Bromo-11-iodoundecane
29	21.43	26934	0.22	10443	0.39	Cyclotetrasiloxane, (iodomethyl)heptamethyl-
30	21.52	71115	0.58	12878	0.48	1,2-Bis(trimethylsilyl)benzene
31	21.96	15813	0.13	9373	0.35	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
32	22.01	75734	0.61	19507	0.72	Silicic acid, diethyl bis(trimethylsilyl) ester
33	22.10	66844	0.54	19962	0.74	Silicic acid, diethyl bis(trimethylsilyl) ester
34	22.18	52082	0.42	22007	0.81	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
35	22.29	213896	1.73	30667	1.13	1,2-Benzisothiazol-3-amine tbdms
36	22.40	188062	1.52	19577	0.72	1,4-Benzenediol, 2,5-bis(1,1-dimethylethyl)-
37	22.53	152924	1.24	24819	0.92	Silicic acid, diethyl bis(trimethylsilyl) ester
38	22.71	338793	2.74	114825	4.24	1,2-Benzenedicarboxylic acid, diisooctyl ester
39	22.80	117742	0.95	19820	0.73	Silicic acid, diethyl bis(trimethylsilyl) ester
40	22.96	23601	0.19	12004	0.44	Cyclotrisiloxane, hexamethyl-
		12346144	100.00	2707999	100.00	

Peroxidase activity was studied in the presence and absence of hot and cold aqueous lavender extracts. The results obtained showed an increase in enzyme activity with increased substrate concentration, but with the presence of 20 µl of lavender extract the enzyme activity was clearly decreased for the same concentration of substrate. The current results indicated the inhibition effects of hot and cold aqueous extracts on peroxidase at a fixed volume of extract (20µl of stock 1gm / 50ml) with different concentration of substrate as shown in Figures 5 and 6. Furthermore, different volumes of extracts were studied to test the effect of extract concentration on enzyme activity where the results showed a clear increased in the inhibition ratio with increasing extract volume as shown in Figures 7 and 8, and Tables 2 and 3 for two aqueous extracts.

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The kinetic study was also performed for the two aqueous lavender extracts using Lineweaver-Burk plot as shown in Figures 9 and 10. The kinetic parameters were calculated from the plots and presented in Table 4. The calculated data, in the presence and absence of lavender extracts respectively, indicated that the K_m values were (0.0007, 0.001) mM while *Vmax* values were (95.23, 125) U/L for cold aqueous extract, while the K_m values were (0.0028, 0.003) mM, and *Vmax* values were (80.64, 112.5) U/L for hot aqueous extract.

In addition, cold aqueous extract exhibited uncompetitive inhibition while hot aqueous extract exhibited mixed type inhibition. In this study, peroxidase activity inhibition may be due to the reaction of one or more components of the lavender extract. Lavender contains various constituents such as phenolic compounds that have more biological activity than other components. It has been reported that the phenolic compounds in lavender of Bulgarian origin is 51.9% linalol and 9.5% linalyl acetate [27]. The action of inhibitors is to prevent enzymes from their catalytic activity, throughout interaction with some steps of catalytic cycle [28]. The action of phenolic compounds with biological systems showed different mechanisms, including interaction with the enzymes [29].

Several reports have recently been recorded, describing the inhibitory action of phenolic compounds versus human enzymes, antioxidants, anticancer and antimicrobial [30]. The structure of peroxidase included iron metal, where it involved in enzyme activation. Catalyzes action of this enzyme is take place via formation of iron-substrate active complex and the reaction is continuous when H_2O_2 is added to initiate the reaction [31].

Of our results, we can assume that the inhibition effect is result from the reaction of the compounds, in the lavender extract, with the active center of the enzyme that is represented in iron metal. These reactions are attributed to one of the large ingredients that are composed in Lavender extract such as salt acetate, flavonoids, terpenes, tannins and phenols. Therefore they prevent iron-substrate active complex formations, and ultimately lead to inhibit of enzyme activity.

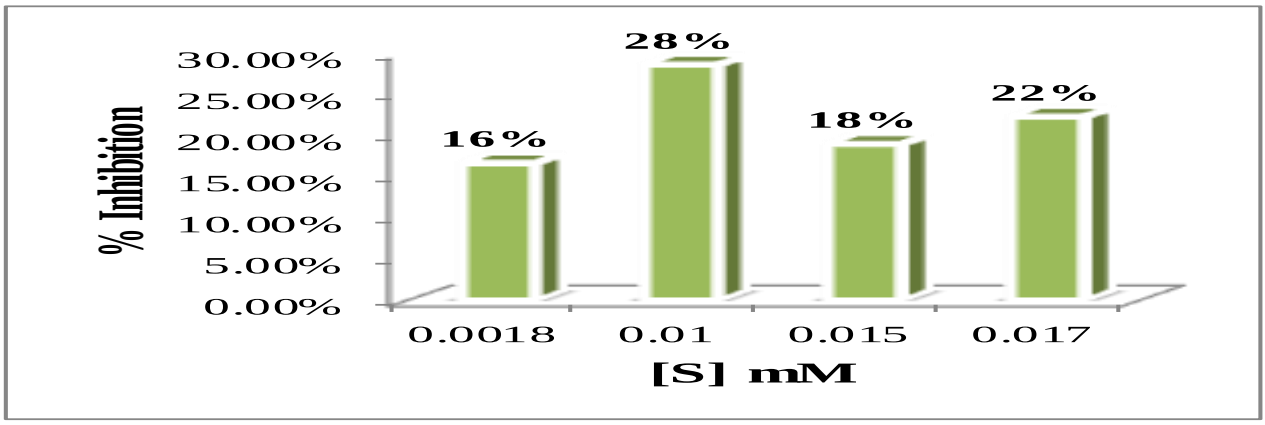

Figure 5: % inhibition of peroxidase by different concentration of substrate at constant extract volume (20 µl of cold aqueous extract)

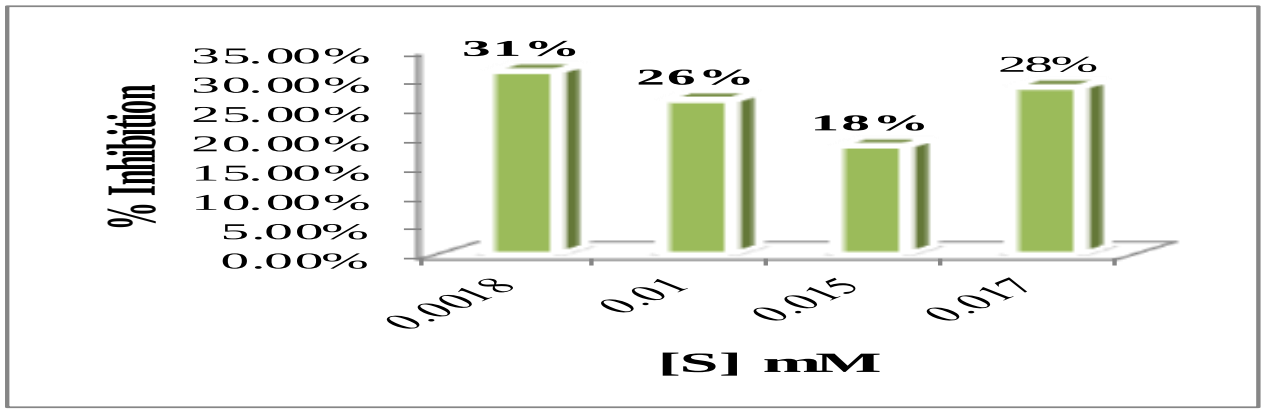

Figure 6: % inhibition of peroxidase by different concentration of substrate at constant extract volume (20 µl of hot aqueous extract)

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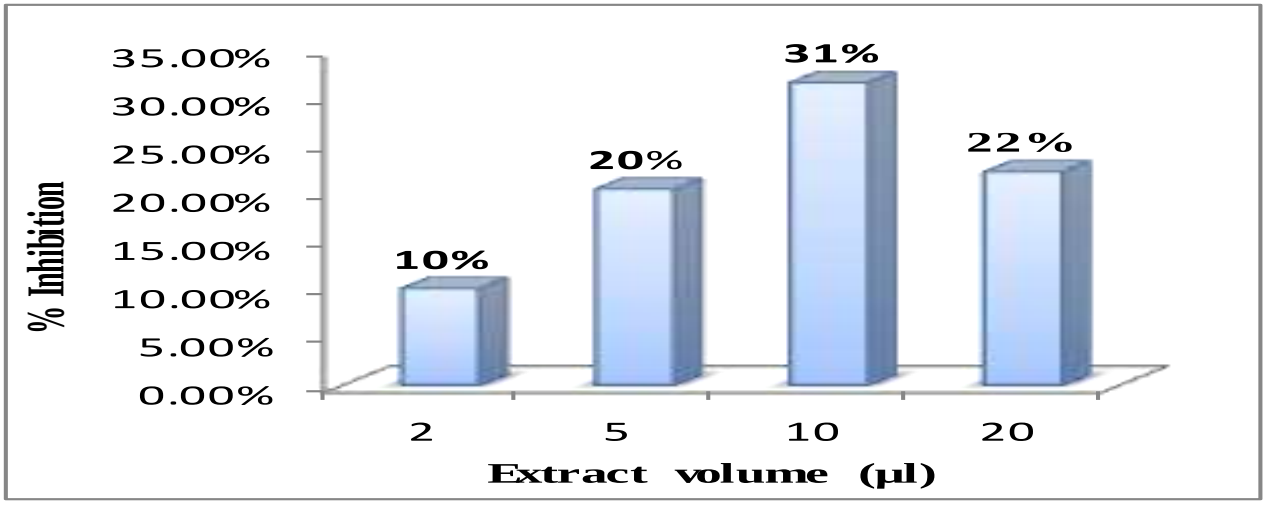

Figure 7: % Inhibition of peroxidase by different volumes of cold aqueous extract at constant substrate conc. 0.017 mM

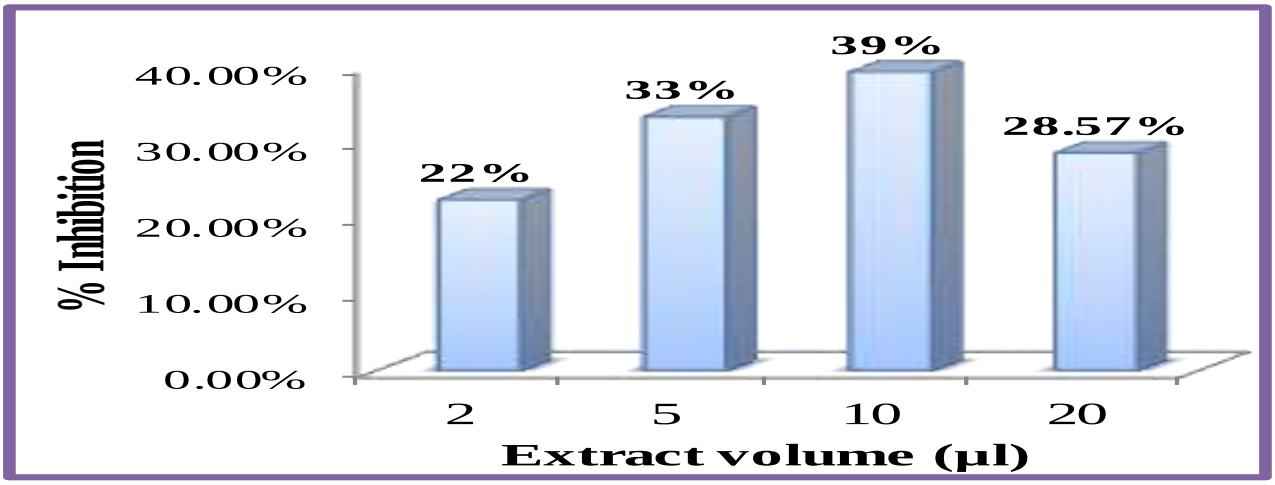

Figure 8: % Inhibition of peroxidase by different volumes of hot aqueous extract at constant substrate conc. 0.017 mM

Table 2: Activity and inhibition percentage by different volumes of lavender cold aqueous extract

Lavender extract (µl)	*Peroxidase activity (U/L)*	*% Inhibition*
Nil	142.8	-
2	128.46	10.04
5	113.65	20.4
10	97.84	31.48
20	111	22.2

Table 3: Activity and inhibition percentage by different volumes of lavender hot aqueous extract

Lavender extract (µl)	*Peroxidase activity(U/L)*	*% Inhibition*
Nil	140	-
2	108.62	22.4
5	93.34	33.32
10	85.11	39.2
20	100	28.57

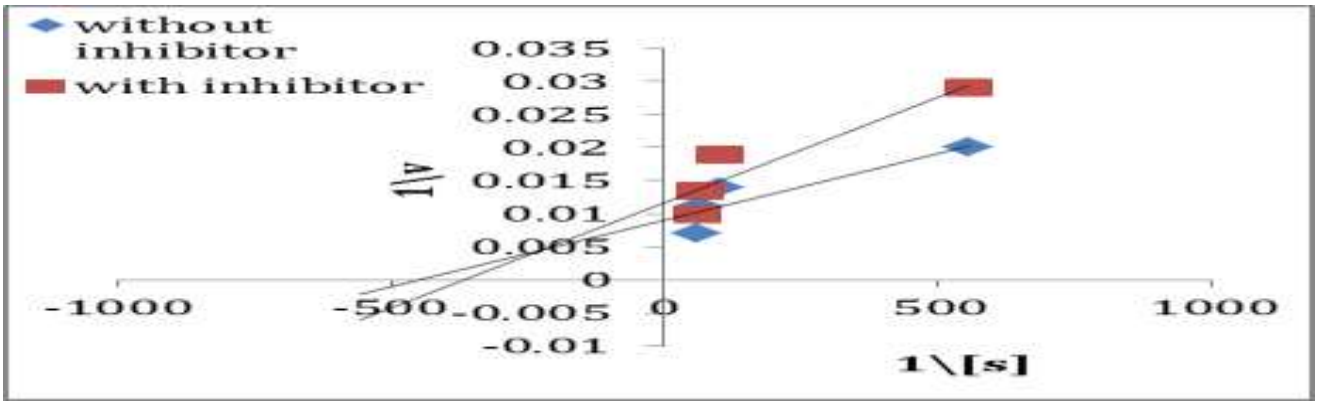

Figure 9: Line weaver-Burk plot of peroxidase with 20µl and without cold aqueous extract

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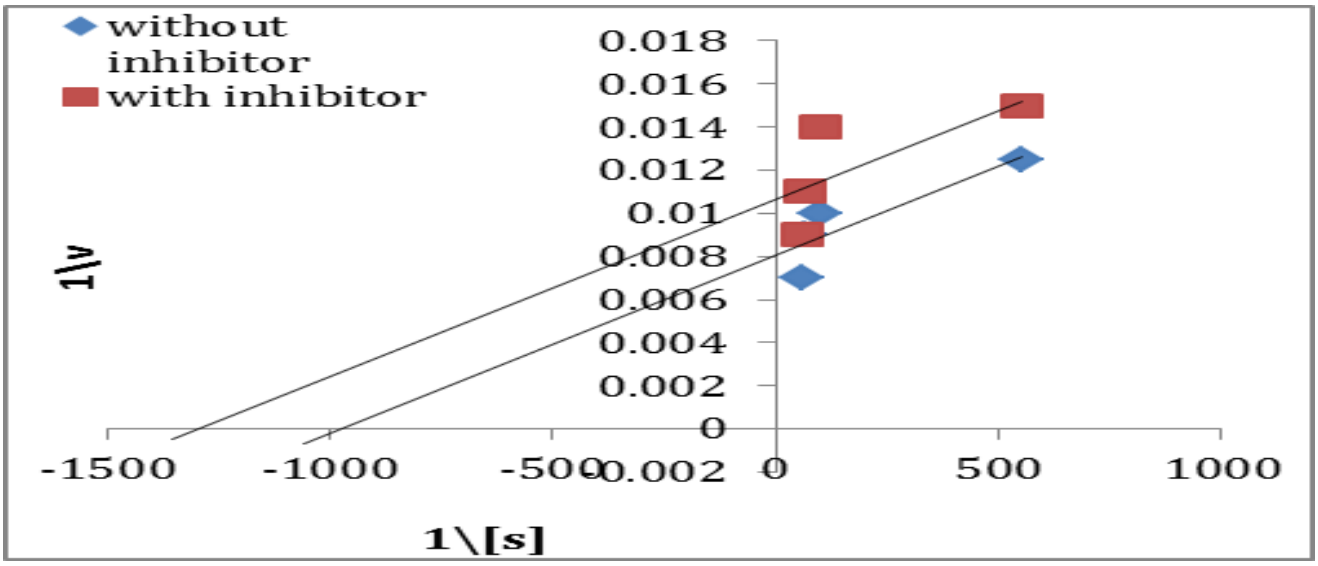

Figure 10: Line weaver-Burk plot of peroxidase with 20µl and without hot aqueous extracts

Table 4: Kinetic properties of peroxidase with and without cold and hot aqueous extracts

Nature of extract	V_{max} (U/L)		K_m (mM)		Inhibition type
	With	without	with	without	
Cold aqueous	95.23	125	0.0007	0.001	Un-competitive
Hot aqueous	80.64	112.5	0.0028	0.0023	Mixed

In the present study, disk diffusion test results of lavender extracts against studied bacteria and fungi are shown in Table 5. The results of lavender extracts, incubated with isolates, show variable effects as anti-bacterial and antifungal agents. These effects can be measured by the diameter of the inhibition zone, which showed that the highest effects were detected of the ethanolic extract rather than the cold and hot extracts.

According to the results obtained, ethanolic extract showed an antifungal activity against *Candida albicans* with an inhibition zone 17 mm. However, the same extract showed less efficacy against bacteria, compared to fungi, with inhibitory zones (12, 10, 11, and 12) mm for *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli and Klebsiella sp*, respectively. Antibiotics are often synthesized against microorganisms, but some isolates have shown resistance to these drugs to be less effective on microorganisms. Recent research has therefore used the sources of alternative natural medicines, as well as obtained from plants or phytochemicals [32].

One of the important targets, of the present work, was examined the efficacy of lavender extracts against microbial activities. Antimicrobials and antifungal were observed within limits volume of extracts, which were equal to 100 µl for 24 hours. These results are consistent with many previous studies of lavender extracts on different isolates, such as inhibiting the growth of *A. hydrophila*, *C. freundiindii* and *Pet turtle-borne* pathogenic bacteria [33, 34]. Antimicrobial effect against isolates in our study, could be predominantly the result of highly account of active phenolic compounds that presence in Lavender herbal, such as linalyl acetate and linalool that previously exhibited strong antimicrobial properties [35, 36]. In fact, most components of lavender, such as organic compounds, can be extracted in larger amounts of organic solvents.

Therefore, the effects of ethanolic extracts against isolates were very clear in our study, compared with cold and hot water solvents. This fact was demonstrated by measuring the inhibition zone observed in the images as shown in Figure 11. The efficiency of these lavender components as an inhibitor for isolation growth can be explained by their ability to disrupt the permeability barrier structure of the cell membrane [37].

The results of the current work support the traditional use of the studied plant and suggest that some botanical extracts, particularly phenolic extract, have compounds with antibacterial properties that can be used as antibacterial agents in new drugs to treat infectious diseases caused by pathogens. The most effective extracts can be subjected to therapeutic antibacterial isolation and further medical evaluation.

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Table 5: Inhibition zones diameter by different extracts.

No	bacteria species and fungi	Inhibition zone (mm) with 100 µl Ethanolic extract	Inhibition zone (mm) with 100 µl cold aqueous extract	Inhibition zone (mm) with 100 µl hot aqueous extract
1	Staphylococcus aureus	12	1	0
2	Staphylococcus epidermidis	10	3	5
3	Escherichia coli	11	2	1
4	Klebsiella sp	12	2	2
5	Candida albicans	17	1	0

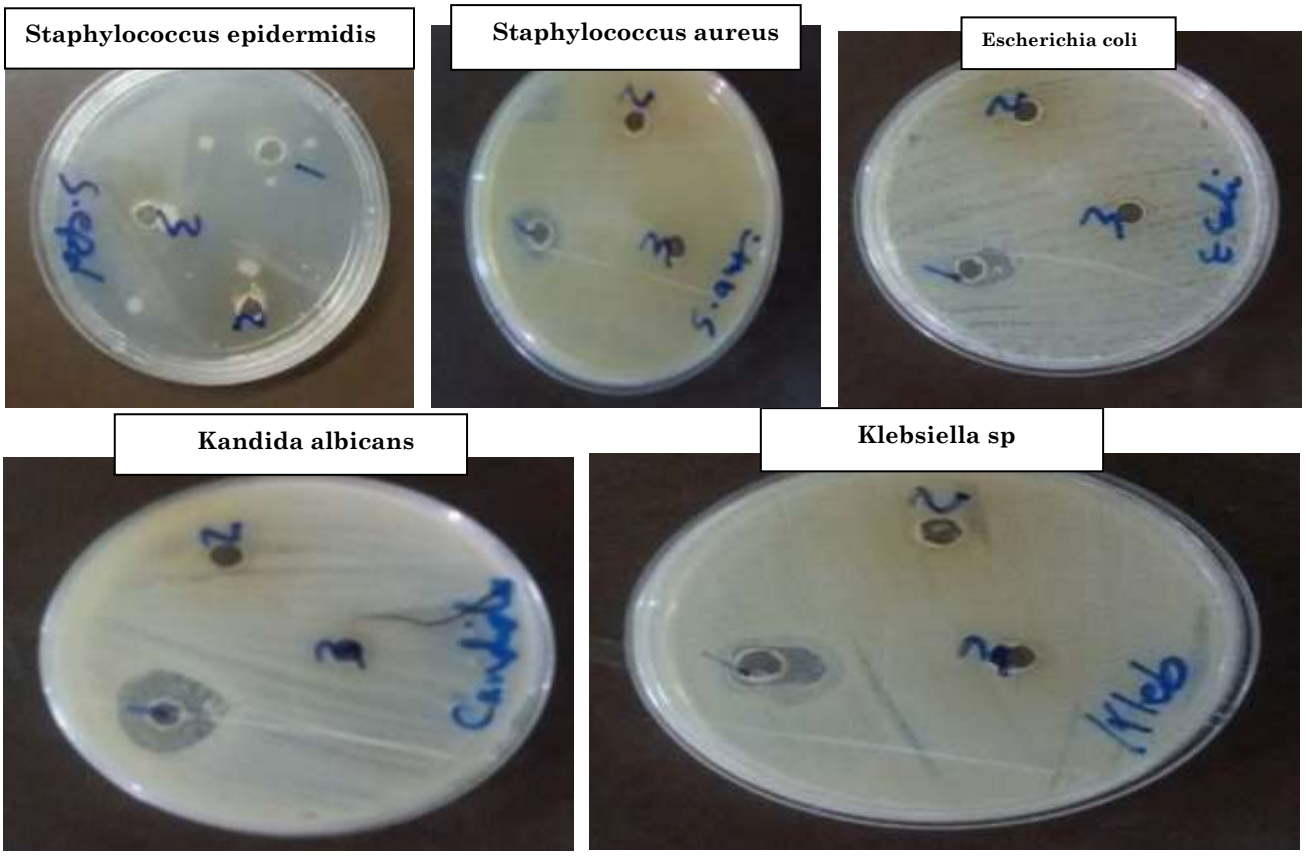

Figure 11: The efficacy of antibiotics with 100 µl extracts, the numbers on the diffusion disk refer to: (1) an ethanolic extract, (2) a cold aqueous extract (3) a hot aqueous extract

Conclusion

According to the findings of the present work, it can be concluded that the success in extracting potential compounds from plant materials depends, to a large extent, on the type of solvent extraction procedures. This study clearly indicates that ethanol extraction is the best and most effective method for lavender flowers than aqueous extraction. This can be due to the perfect solubility of the lavender constituents in organic solvent.

The results of the current work support the traditional use of the studied plant and suggest that some botanical extracts, particularly phenolic extract, have compounds with antibacterial properties that can be used as antibacterial agents in new drugs to treat infectious diseases caused by pathogens. The most effective extracts could be subjected to therapeutic antibacterial isolation and further medical evaluation. Also, the current study highlighted the biological activities of lavender extracts for research and commercial use through the study of inhibitory effect of lavender extract on peroxidase.

Further study is required to focus on determining the efficacy of lavender oil against pathogenic bacteria to verify of its important in biological activities. Moreover, according to their biological roles [38] more work is needed to verify the trace elements content in the lavender flowers.

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Acknowledgment

The authors would like to thank Mustansiriyah University. (www.uomustansiriyah.edu.iq). Baghdad, Iraq, for its support in the present work.

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