Kinetic and Thermodynamic Studies of Antioxidant and Antimicrobial Activities of Essential Oil of *Lavendula steochs*

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Abstract: Antioxidant, antimicrobial activities of essential oil from plant *Lavendula steochs* were studies by kinetics and thermodynamic approach. Hydro-distillation was used for the extraction of oil from the flowers of *Lavendula steochs*. - Antioxidant activity was performed using DPPH method, in which the IC_{50} showed that essential oil has good antioxidant activity. Antimicrobial activity has been analyzed against Methicillin-resistant *Staphlococcus aureus* (MRSA) and Vancomycin-resistant *Staphlococcus aureus* (VRSA) which shows that *Lavendula steochs* is found to be effective against MRSA and VRSA. Chemical composition of essential oils were measured by GC-MS and FT-IR techniques and the kinetic & thermodynamic parameters were used for the characterization of essential oils.

Keywords: *Lavendula steochs*, Antioxidant, Antimicrobial, FT-IR, GC-MS.

INTRODUCTION

The generation of reactive oxygen from several biochemical reactions in human body cause several diseases by damaging important bio molecules. This harmful reaction can be stopped by antioxidants which scavenge the free radicals present in the human body. Therefore, the presence of antioxidant compounds in food plays an important role as a health protecting factor. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties [1]. Considering the vast potentiality of plants as sources for antimicrobial drugs and as an antioxidant agent, a systematic investigation was undertaken to screen the antimicrobial and antioxidant activities of *Lavendula steochs* oil.

Oils and fats are essential constituents of all forms of plant and animals life. Essential oils are the subtle, natural, aromatic and volatile compounds and have been extracted from the flowers of herbs [2]. *Lavendula steochs*, known also as French Lavender belongs to the Lamiaceae family. It is an herb, and inhabitant of the coast, but only occurs on sand or other crystalline rocks, and never on limestone [3]. The flower of lavender is used to make medicine. It is used for restlessness, insomnia, nervousness, and depression, and in a variety of digestive complaints including meteorism (abdominal swelling from gas in the intestinal or peritoneal cavity), loss of appetite, vomiting, nausea, intestinal gas (flatulence), and upset stomach. Some people use lavender for painful conditions including migraine headaches, toothaches, sprains, nerve pain, sores, and joint pain [4]. The essential oil of lavender flowers is pale yellow, yellowish-green or nearly colorless, with the fragrant odor of the flowers and a pungent, bitter taste. The major constituents of the oil are linalool, linalyl acetate, cineol, pinene, limonene, geranial, borneol and some tannin [5].

The aim of the present study was to assess the physicochemical properties of essential oil with their antioxidant and antimicrobial activities and to determine the kinetics and thermodynamics parameters for the characterization of oil in order to understand the usefulness of this plant as a food and as medicine.

MATERIAL AND METHODS

Sample Collection

Flowers of *Lavendula steochs* were taken from local market and identified by the taxonomist of Department of Botany, University of Karachi

Hydro-Distillation Method

The hydro distillation method was used for the extraction of the essential oil from the flowers of *Lavendula steochs*. A total of 120g flowers were soaked into 500 mL round-bottom flask containing deionized water (200 mL). To study the hydro-

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ISSN: 1814-8085 / E-ISSN: 1927-5129/15

distillation rate, the mixture was subsequently heated using a heating mantle by manipulating the power. Next, the extract was concentrated using a rotary evaporator at 60°C. The concentrated essential oil obtained was weighed. [5]

Physicochemical Properties

Acid Value (AV)

0.1gm of oil in 250 mL conical flask, add 50ml of neutral alcohol and heat on steam bath till the oil gets dissolves, cool for a while and and add 4drops of phenolphthalein indicator, then with constant shaking titrate the contents in the flask against the 0.1 N NaOH solution from the burette till the pink color persists.

Acid value is calculated from following formula:

$$AV = 40 \times V \times C / m \quad (a)$$

Where:

40 is equivalent weight of NaOH, V is the volume in ml of standard volumetric KOH solution used, C is the exact concentration in NaOH solution used (0.1 N), m is the mass in grams of the test portion. [6]

Peroxide Value (PV)

A known weight of oil sample of 0.3g was dissolved in glacial acetic acid 7.5 mL and chloroform (5.0ml) then saturated solution of KI 0.25 mL was added. The mixture was kept in dark for 15 min. After the addition of distilled water 12.5 mL the mixture was titrated against sodium thiosulphate of 0.02M using starch as an indicator. A blank titration was done parallel to treated and peroxide value meq of oxygen/kg was calculated.

$$PV = (V1-V2) \times 10/m \quad (b)$$

Where:

V1-volume of thiosulfate solution required to titrate the sample (mL), V2-volume of thiosulfate solution required titrating the blank determination (mL) m-mass of sample (g). [AOAC, 1984]

Iodine Value (IV)

0.3 g of oil sample in a conical flask and Wij's solution 3.8 mL was added into it. The flask was allowed to stand in a dark for 30 min. The mixture was shaking occasionally then KI 0.15g and water 15 mL were added then the solution was titrated against a 0.1 N standard sodium thiosulfate solution The flask was shake vigorously towards the end of titration by using starch as an indicate. The blank was run under the experimental condition omitting the sample.

$$IV = 12.69 \times N \times (B-S) / m. \quad (c)$$

Where:

N is the normality of sodium thiosulfate,

B is the volume of sodium thiosulfate required to titrate the blank (mL), S is the volume of sodium thiosulfate required to titrate the sample (ml), m is the mass of sample (g) [7,8].

Viscosity

The viscosity of oil sample was measured at two temperatures, 40 and 100^{o}C by an Ostwald Viscometer techniconominal constant 0.05 Cs/c, ASTMAD 445 England. The flow time of oil sam-ples were recorded with a stop watch (Japan, CBM, and Corp QSQ) least count ±0.01 s.

Antioxidant Activity with the 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

0.1g essential oil was diluted in 5mL pure methanol than 0.0025g DPPH was prepared in 100mL pure methanol. 2mL of DPPH were added in 120,140,160,180 and 200µL of essential oil respectively, Mixture was shaken vigorously and kept in incubator for 30min. Same process was done with the ascorbic acid (taken as standard). The UV absorbance of the resulting solution was then measured at 517nm after 30min on spectrophotometer. The antiradical activity was expressed as IC_{50} (µg/mL). The antiradical dose required to cause a 50% inhibition. The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(Abs0-Abs1)/Abs0] \times 100 \quad (e)$$

Kinetic study of antioxidant activity after incubation absorbance was noted at 0, 30, 60, 90, 120 and at 150 min was calculated by using

$$\frac{dY}{dt} = kY^{n} \quad (f)$$

Where Y is the antioxidant activity; t is the time of extraction (min); k is the extraction constant; and n is the reaction order.

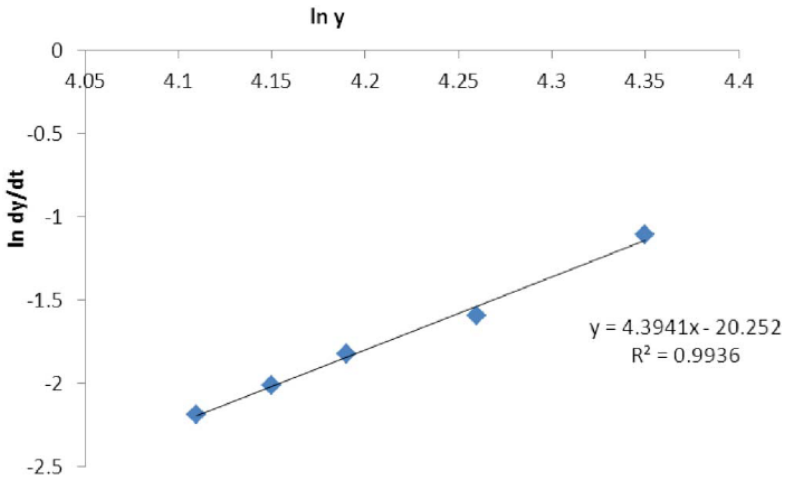

Figure 1: Kinetic study of Antioxidant activity at 20 °C.

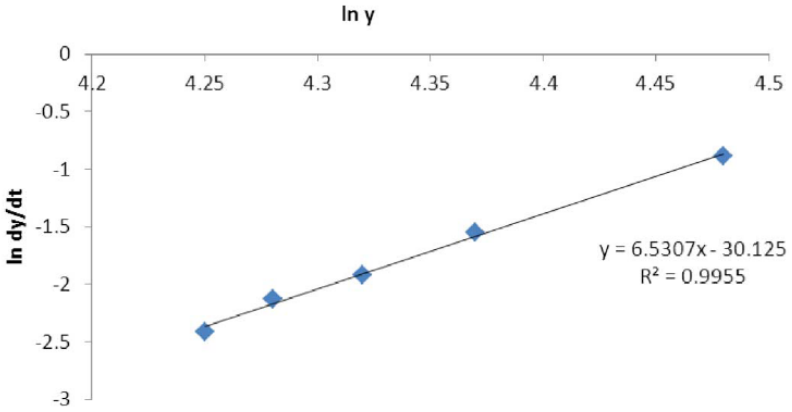

Figure 2: Kinetic study of Antioxidant activity at 35 °C.

Where;

Abs0 = Absorbance of control

Abs1 = Absorbance of sample/Standard [9].

Analysis of Antimicrobial

To investigate antimicrobial activity of different extracts of *Lavendula steochs* and Pearl Millet plants, clinical isolates of *Staphylococcus aureus* (KIBGE: MBSA-01 to MBSA-43), strains of *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Bacillus cereus* (ATCC 11778), *Salmonella typhi* (ATCC 3632) and *Pseudomonas aeruginosa* (KIBGE: IB-67) were used. Disc diffusion method was adopted to determine the antimicrobial activity using 6mm sterile filter disc.

Solutions were prepared for each extract in chloroform (Merck, Germany), concentrations ranging from 7.5 µg to 350 µg per disc. As positive control antimicrobial susceptibility discs of 30 µg vancomycin, oxcillin 1 µg and ciprofloxacin 5 µg (Oxoid, UK) were used, whereas chloroform (Merck, Germany) disc were used as negative control.

Microorganisms were culture in Luria Broth (Oxoid, UK) at 35ºC, overnight. 1.5 x 10 6cells/mL (0.5 McFarland index) were inoculated on Mueller Hinton Agar (Oxoid, UK) [10, 11]. On each plate filter disc with plant extract, positive control discs and negative control discs were placed. After an incubation period of 24h at 35ºC, zone of inhibition (in mm) were measured under bright light and on non-reflecting background.

For the chemical composition of oil the GC-MS (Shimadzu GC-9 A) and FT-IR -8900 (Shimadzu Japan) were used.

RESULT AND DISCUSSION

Physicochemical properties of essential oil of *Lavendula steochs* at room temperature (35°C) are presented in Table **1**. Essential oils were soluble in

alcohol, chloroform, carbon tetrachloride and hexane. Acid value 3.23 mg/KOH/g indicates the lowest amount of free fatty acids presents in the essential oil. It is an important index of physicochemical properties, being indicative of age, quality, edibility and suitability of oils. The high acid value is indicative of oil becoming rancid under improper conditions or adulteration there in. The peroxide value is used for identifying the onset of oxidative change in fats and oils, during which the oxygen molecules penetrates the fat molecules in the form of peroxide group which eventually affect odor, flavor and quality. Results showed 1.36 meq/kg of Peroxide value. Lower the peroxide value, the fresher the oil would be. In general, peroxide levels higher than 10.0 means less stable oil with a shorter shelf life [12]. Iodine value (g/g) indicates the number of double bonds present and, therefore, the degree of unsaturation. The higher the iodine value, the more the double bonds in the molecule as also the oil being more prone to rancidity. On the other hand, the low acid value and the low peroxide value of the essential oils are indicative of their resistance toward lipolytic hydrolysis and oxidative deterioration. *Lavendula steochs* showed low value of Iodine value 29.46 g.

Table 1: Physicochemical Characterization of Essential Oil at Room Temperature 35 ° C

Parameters	oil
Acid Value (mg/KOH/g)	3.23
Peroxide Value (meq/kg)	1.36
Iodine Value (g)	29.46

Medicinal plants are a vast unrevealed source to cure several diseases many of them have shown antimicrobial activity thus having enormous therapeutic potential for bacterial, fungal and viral infections. These plants and/or their special parts may contribute towards the emerging problem of multiple drug resistance in human pathogens, as bacteria show high genetic adaptability to acquire and transmit resistivity against anti-microbial therapeutic agents. In this study antimicrobial activities against Methicillin-resistant *Staphlococcus aureus* (MRSA) and Vancomycin-resistant *Staphlococcus aureus* (VRSA) were measured. The activities of obtained extracts were evaluated by disc diffusion method. LS fraction provided some promising activity against VRSA (40) and MRSA (40%) with an activity zone of 2 to 8mm in case of VRSA and MRSA Fraction of oil provided some promising activity against VRSA (40) and MRSA (40%) with an activity zone of 2 to 8mm in case of VRSA and MRSA clinical isolates as shown in Table **2**.

The oil composition and their relative characteristic vibrational mode of each molecular group causes the appearance of bands in the infrared spectrum at a specific frequency were recorded in Table **3-4** by using GC-MS and FT-IR respectively. The FT- IR results showed the assignment for the most characteristic bands of the essential oil as well as the compounds of the oil by using GC-MS.

The antioxidant potential of given oil samples was determined by DPPH-free radical scavenging activity as shown in Table **5**. DPPH is a molecule containing a stable free radical. The test provides information on the ability of a compound to donate a hydrogen atom. The IC_{50} value of essential oil comes out to be 22.32 which are nearly same as for ascorbic acid 24.97 as standard shows good antioxidant activity. The kinetics of essential oil was studied by using antioxidant property which shows that the kinetics for essential oil was first order. The antioxidant Table **5** at various times shows the first order kinetics in *Lavendula steochs.*

Table 2: Antimicrobial Activity of Essential Oil *of Lavendula steochs*

Parameters	ACVTIVITY AGAINST VRSA (n=10)	ACVTIVITY AGAINST MRSA (n=15)
ZONE OF ACTIVITY (mm) Maximum	2	2
ZONE OF ACTIVITY (mm) Minimum	8	8
NO ACTIVITY (Number of Strains)	6	9
ACTIVITY (Number of Strains)	4	6
Percentage %	40	40

Table 3: Chemical Composition of Essential Oil of *Lavendula steochs* by GC-MS

S. No.	Name of Compound	Retention time	Molecular Formula	Molecular Weight	Relative Concentration %
1.	(S)-Camphor	7.73	$C_{10}H_{16}O$	152	25.64%
2	Vanillin	9.58	$C_8H_8O_3$	152	4.10%
3	Benzyl ether	10.03	$C_9H_{12}O$	136	3.72%
4	Vanillin	11.03	$C_8H_8O_3$	152	3.97%
5	p-Cymen-7-ol	11.65	$C_{10}H_{14}O$	150	5.64%
6	Coumarin	15.38	$C_9H_6O_2$	146	4.61%
7	Hexadecanoic acid	27.4	$C_{16}H_{32}O_2$	256	3.72%
8	(Z)-11 Octadecanoic acid	29.52	$C_{18}H_{34}O_2$	282	8.72
9	Methyl ester	29.67	$C_{18}H_{38}O_2$	284	3.72%
10	(Z)-9-Octadecanoic acid	31.4	$C_{18}H_{35}NO$	281	5.13%

Table 4: Assignment for the Most Characteristic FT- IR Bands of the Essential Oil

Region of H Stretching (cm^-)	-OH asymmetric and symmetric stretching vibration	3458.7
	Aliphatic CH_2	2929.9
	-C=O ester carbonyl of triglyceride	2873.2
Region of double bond Stretching (cm^-)	-C=C- stretching vibrations of cis olefins	1740.3
	-C-H bending vibration of the CH_2 and CH_3 aliphatic groups	1680.6
Region of other deformation and bending (cm^-)	-C-H bending vibration of CH_2	1451.6
	Stretching vibration of –C-O ester group	1374.0
Finger Print Region (cm^-)	$-(CH_2)-$, -CH=CH- overlapping of CH_2 rocking vibration and the out- of-plane vibration of cis-disubstituted olefins	1258.3 1173.4
	$-(CH_2)-$, -CH=CH- overlapping of CH_2 rocking vibration and the out- of-plane vibration of cis-disubstituted olefins	753.5

Table 5: Kinetic Study of Antioxidant Activity with the 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method

Time (min.)	At temperature 20 ºC			At temperature 35 ºC		
	% DPPH Scavenging Activity (y)	ln (dy/dt)	ln y	% DPPH Scavenging Activity (y)	ln (dy/dt)	ln y
0	61.12	-2.19	4.11	70.23	-2.42	4.25
20	63.36	-2.01	4.15	72.01	-2.13	4.28
40	66.48	-1.82	4.19	74.98	-1.92	4.32
60	70.84	-1.59	4.26	79.03	-1.55	4.37
800	77.43	-1.11	4.35	87.18	-0.89	4.48

Thermodynamic study of oil was done at two different temperatures from which activation energy (Ea.), Arrhenius constant (A), enthalpy (ΔH), entropy (ΔS), and Gibbs free energy (ΔG) were calculated as shown in Table **6**. The positive value of enthalpy (ΔH) shows that the reaction is endothermic and the negative value of entropy (ΔS) shows the system becomes more disordered through the course of the reaction and positive value of Gibbs free energy (ΔG) indicates that the reaction is non-spontaneous. Furthermore, by

Table 6: Thermodynamics Parameters of Essential Oil

Temperature (K)	ΔS J/mol	ΔH J/mol	ΔG J/mol
303	-165.22	17383.49	65672.82
328	-164.81	17258.79	68146.55

increasing temperature, value of enthalpy (ΔH) decreases, whereas Gibbs free energy (ΔG) and entropy (ΔS) increases.

CONCLUSION

The study shows that the essential oil extracted from *Lavendula steoch* could be a good source of antioxidant and antimicrobial activity.

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Received on 18-02-2015 Accepted on 26-02-2015 Published on 05-03-2015

http://dx.doi.org/10.6000/1927-5129.2015.11.31