ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research, Volume 3, Issue 4, 2014, 30-40

RESEARCH ARTICLE

# PHYTOCHEMICAL STUDIES AND *IN VIVO* ANTIOXIDANT ACTIVITY OF TWO *LAVANDULA* SPECIES (LAMIACEAE) AGAINST STREPTOZOTOCIN INDUCED OXIDATIVE STRESS IN ALBINO RATS

**[†1]Nevein M. Abdel-Hady, [2]Gamil M. Abdallah and [3]Nagi F. Idris**

Departments of [1]Pharmacognosy and [3]Pharmacology, Faculty of Pharmacy, Omar El-Mokhtar University, Libya, [2]Biochemistry, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

**Received 21 June 2014; Accepted 7 July 2014**

**ABSTRACT**

Phytochemical studies of two *Lavandula* species namely *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae) cultivated in Egypt was carried out to subsist positive diagnostic indices for the research of their monographs as well as GC/MS analysis of their volatile oils and quantitative estimation of total phenol and flavonoid contents.

The gained results revealed both qualitative and quantitative variation in the chemical composition of the investigated species where twenty-six and thirty-one compounds were identified in the volatile oils of *L. dentata* L. and *L. angustifolia* Mill. representing 96.43 % and 98.56 % respectively, the major chemical constituents in the volatile oil of *L. dentata* were menthe-1,5-dien-8-ol <*Para*> (26.80%), caryophyllene oxide (16.40%) and guiaol<*Alpha*> (15.36%), while in *L. angustifolia* were linalyl acetate (18.99%), citronellol<*Alpha*> (17.36%) and menthe-1,5-dien-8-ol<*Para*> (16.21%), moreover, the calculated values of total phenol and flavonoid contents were 188.50±2.07 mg GAE $g^{-1}$, 90.40±1.57 mg QE $g^{-1}$, 194.95±2.55 mg GAE $g^{-1}$, 98.83±2.68 mg QE $g^{-1}$and 167.10±2.30 mg GAE $g^{-1}$, 116.15±1.91QE $g^{-1}$,152.50±2.21mg GAE $g^{-1}$, 119.95±1.87 mg QE $g^{-1}$ for their aqueous and methanol extracts respectively.

Investigation of their antioxidants impact against Streptozotocin-induced oxidative stress in liver and kidney tissues of albino rats revealed that the methanol extract of *L. angustifolia* exhibited the highest antioxidant potential followed by, that of *L. dentata* and volatile oil *L. angustifolia* in dose dependent manner respectively which afford useful results for developing new natural antioxidant agents.

**Key words:** *Lavandula*, Volatile Oils, Phenols, Flavonoids, Antioxidant, Streptozotocin, Glibenclamide and Oxidative Stress.

## INTRODUCTION:

Genus *Lavandula* (Lamiaceae), consist of about 39 species, dozens of subspecies, hundreds of hybrids and cultivars those are widely distributed in the archipelagoes of the Atlantic Ocean and Mediterranean region, it is divided into four main categories*;* *L. angustifolia*, *L. dentata*, *L. latifolia* and *L. intermedia* (**1** and **2**), in Egypt, there are two *Lavandula* species; *L. dentata* L. and *L. angustifolia* Mill. (**3**); *L. dentata* commonly known as French lavender is a large plant with greenish-grey foliage late blooming and with characteristic very strong odour (**4**) while *L. angustifolia* commonly known as English lavender is a frost hardy species that has many cultivars, habitats and blossom colours (**5**).

The advantageous value of *Lavandula* species can be referred to the virtue of its versatile therapeutic potentials those can be attributed to the high content of their uniquely constituted volatile oil as well as phenolic content (**1, 2** and **6**); several researches reported isolation of over 150 compounds from the volatile oil of *L. dentata* (**7**) while that of *L. angustifolia* contained at least 38 different compounds, the chemical compositions of these volatile oils are complex and variable as reported in several investigations for cultivated species in Saudi Arabia, Spain, Morocco, Algeria and Canada (**4, 8-20**), table, 1.

The most prominent chemical constituents in the volatile oil *L. dentata* (**8**) are1,8-cineole, α-pinene, β-pinene and p-cymene which exhibit antifungal and antibacterial activities (**21** and **8**), α-terpineol, terpenen-4-ol and camphene have anti-lice activity (**22**), whereas those in the volatile oil of *L. angustifolia* (**7** and **23**) are linalyl acetate and linalool which have sedative (**24** and **25**) and local anesthetic effects (**26**); linalool also exhibited antibacterial (**5** and **27-29**), fungistatic (**21, 27** and **30-32**) and insecticidal (**22**) effects.



*Corresponding author: Nevein M. Abdel-Hady | E-mail: nevein.abdelhady@yahoo.com

**Table 1: The reported major constituents and biological activities of the volatile oils isolated from *Lavandula spp.* under investigation:**

| Spp. | Major constituents | Uses | Country | Reference(s) |
|---|---|---|---|---|
| *Lavandula dentata* L. | Camphor, *trans*-pinocarveol, β-eudesmol and α-guiaol. | Antimicrobial and antioxidant | Yemen | **9** |
| | β-pinine, pinocarveol, myrtenal , α-pinene and 1, 8 cineol. | Cytotoxic and antioxidant | Morocco | **10** |
| | 1, 8 cineol, sabinene, bicycle [3.1.0] hex-an-3-ol,4-methylene-1-(1-methylethyl), myrtenal and α-pinene. | Antimicrobial | Morocco | **4** |
| | 1, 8-cineole, cis-verbenol , p-cymen-8-ol, fenchone and myrtenal . | --- | Algeria | **11and 12** |
| | β-pinine, pinocarveol and myrtenal | --- | Spain | **13** |
| *Lavandula angustifolia* Mill. | Caryophyllene, β-phellandrene and eucalyptol. | Antimicrobial | Romania | **14** |
| | Linalyl acetate, linalool, lavandulol, 1, 8-cineole, lavandulyl acetate and camphor. | --- | Canada | **15** |
| | Linalool, camphor, linalyl acetate, (Z)-B-ocimine, 1, 8-cineole and (E)-β-ocimene. | Antioxidant | Iraq | **16** |
| | Linalool, linalyl acetate, thujene and α-pinene. | --- | Iran | **17** |
| | 1, 8-cineole, camphor and linalool. | --- | Italy | **18** |
| | Linalyl acetate, linalool, lavandulol, 1, 8-cineole, lavandulyl acetate and camphor. | Cytotoxic | India | **19** |
| | Linalyl acetate, linalool, lavandulol, 1, 8-cineol, lavandulyl acetate and camphor. | Antimicrobial | USA | **20** |

Free radicals (reactive oxygen and nitrogen species, ROS/RNS) are produced in normal and pathological cell metabolism and proved to be aggravated in cases of oxidative stress; they are controlled by endogenous enzymes as superoxide dismutase, glutathione peroxidase, catalase or chemical compounds as α-tocopherol, ascorbic acid, phenol compounds and glutathione (**33**).

Phenolic compounds are a class of secondary metabolites existing in most plants protecting them against ultraviolet radiation, pathogens and herbivores; concerning phenolic compounds in the investigated species, previous studies reported the existence of flavonoids as genkwanin, luteolin, apigenin, apigenin-7-O-glucoside , apigenin-7-O-glucuronide in both species whereas, 6-hydroxyluteolin-7-O- glycoside, scutellarein-7-O-glycoside, vitexin and luteolin 7-O-gulcour-inonide, luteolin 7,4`-O-di-O-glucuronide, luteolin-7-O-glucoside and chrysoeriol-7-O-glycoside existed in *L. dentata* and *L. angustifolia* respectively (**34** and **35**).

Phenolic compounds exhibit a wide range of pharmacological activities depending on their ability to inhibit certain enzymes meanwhile induction of other detoxifying and antioxidant enzymes, so they revealed to be useful treatment and/or prophylaxis of certain diseases as different inflammations, Alzheimer's disease (**36** and **38**), cancers (**38 – 40**), bacterial, protozoal and viral infections (**39, 40, 42** and **43**), heart diseases, stroke, arteriosclerosis, diabetes (**14, 15** and **44**).

In the last years, there is an increasing interest in antioxidants and the preference of natural rather than synthetic sources (**39-41**) hence, the interest in the antioxidant potential of plant extracts, essential oils and/or isolated compounds, so the present study was conducted to lay a phytochemical foundation for assessment of the volatile oils, phenol and flavonoid contents of two Egyptian *Lavandula* species monitored with investigation of their *in vivo* antioxidant potentials.

## MATERIAL AND METHODS:

### Plant material:

Shrubs of *Lavandula dentata* L. were purchased from an Ornamental Plants Cultivation Station, Kerdasa Road, Giza while those of *Lavandula angustifolia* Mill. was purchased from The Medicinal Plants Cultivation Station, Faculty of Pharmacy, Cairo University, on April 2013, their identities were established by Professor Dr. Mohamed El-Sayed Tantawy, Professor of Botany, Faculty of Science, Ain Shams University. Voucher specimens are kept in a herbarium in Pharmacognosy Department, Faculty of Pharmacy Al-Azhar University, Cairo, Egypt; plant material were air-dried, powdered and



 

kept in tightly closed amber coloured glass containers protected from light at low temperature.

**Chemicals:**

Folin-Ciocalteu's reagent, 1,1-Diphenyl-2-picryl-hydrazil (DPPH) and Streptozotocin (Sigma Chemical Co., Saint Louis, MO, USA); gallic acid, quercetin, aluminum chloride and Silica gel 60 $F_{254}$ (E. Merck, Darmstadt, Germany), Glibenclamide (Daonil®, Hochest Company for Pharmaceuticals, Cairo, A.R.E) , solvents of analytical grade and pre-made kits suitable for automatic bio-chemical analyzers for evaluation of blood glucose level (BGL), superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT) in $RBC_S$ and evaluation of serum ascorbic acid (Randox Laboratories, Crumlin, England).

**Determination of physicochemical parameters (45);** ash values; total ash, water-soluble ash and acid-insoluble ash values, moisture content and solvent extractives′ values were determined for each of the powdered plants.

**Investigation of volatile oils (45):**

- ***Preparation of the volatile oils;*** fresh entire aerial parts were comminuted and separately hydro-distilled in Clevenger′s system, the resulted volatile oils were dried over anhydrous sodium sulfate and kept in a refrigerator until analysis.

- ***Determination of percent yields, specific gravities and refractive indices;*** the percent yields, specific gravities and refractive indices were determined for both isolated oils.

- ***GC/Ms analysis of volatile oils;*** Thermo Scientific capillary gas chromatography (model Trace GC ULTRA) directly coupled to ISQ Single Quadruple MS, TG-5MS non-polar 5% Phenyl Methyl poly-siloxane capillary column (30m x 0.25mm ID x 0.25um) system was used where programmed analysis was first carried out; the oven temperature programmed from 40°C (3 min) to 280°C at5°C/min, then isothermal at 280 ºC for 5 min; carrier gas Helium, flow rate 1mL/min; the volume of injected sample was 1µl of sample in diethyl ether; splitless injection technique; ionization energy 70eV, in electronic ionization (EI) mode.

**Preparation of extracts;** for each plant, 50 g each finely-powdered sample were extracted separately in a soxhlet with 250 ml of 80% (v/v) aqueous methanol and distilled water for one hour to yield the methanol and aqueous extracts respectively, the extracts were filtered under vacuum through Whatmann No.1 filter paper, the residue was re-extracted following the same procedure two more times, extracts collected were vacuum dried at 40°C.

**Determination of total phenol content (46);** the total phenol content in aqueous and methanol extracts of each plant was determined spectrophotometrically using Folin-Ciocalteu's reagent where standard curve was done using different concentrations of gallic acid in methanol. The concentrated extracts of tested plants were dissolved each in least methanol volume then completed to 10ml, 100µl of these extracts were separately diluted with 8ml distilled water, to each sample 0.5ml of 50% Folin-Ciocalteu's reagent was added and left 8 min, and then 1.5 ml of 5% sodium carbonate were added, mixed and allowed to stand for 60 min. protected from light. The absorbance was measured using Genesys Spectrophotometer (Milton Roy, INC., Rochester, NY) at 725 nm where methanol was used as blank and concentration of the total phenolic content was calculated as mg gallic acid equivalents per gram dry weight (mg GAE $g^{-1}$).

**Determination of total flavonoid content (47);** the total flavonoid content in in aqueous and methanol extracts was done colourimetrically using aluminum chloride solution where standard curve was done using different concentrations of quercetin in methanol, 100µl were added to a 96 Micro-well plate, 100µl of 2% aluminum chloride solution in methanol were added, after 10 min, their absorbance was measured using Genesys Spectro-photometer (Milton Roy, INC., Rochester, NY) at 415 nm where methanol was used as blank and the concentration of total flavonoids was calculated as mg quercetin equivalent per gram dry weight (mg QE $g^{-1}$).

**Preliminary screening of antioxidant potential (48);** the tested extracts and volatile oils were subjected to qualitative TLC investigation according to the stable DPPH radical technique where 20 µl aliquot of each extract was spotted on silica gel plates and developed using *n*-butanol: acetic acid: water (4:1:5) as a mobile phase, after development, the dried TLC plates were sprayed with 0.2% DPPH solution in methanol and examined after 30 min. where active antioxidants appeared as yellow spots against purple background.

**Biological Studies:**

**Acute toxicity (49);** adult albino mice of either sex were fasted overnight, divided into six groups-six animals each (1:1 for males: females) - were fed with increasing doses of isolated volatile oils and methanol extracts 50, 100, 200, 500, 1000 and 2000 mg/ kg b.wt. where animals were observed for any toxic symptoms and mortalities during the first 24-72 hours post treatment.

**Evaluation of antioxidant potential;** adult albino rats of either sex weighing 150-200 g purchased from The Animal House Laboratory, National Research Center, Cairo, Egypt were used for this study, they were housed in an environmentally control room, maintained at uniform light and temperature conditions of and provided with food and water *ad libitum*.

**Induction of diabetes;** rats were rendered diabetic by a single intraperitonial dose of freshly prepared Streptozo-tocin 45 mg $kg^{-1}$ b.wt. dissolved in saline where diabetes was identified in rats by moderate polydipsia and marked polyuria, 48 h., later fasting blood glucose levels were estimated, rats with blood glucose levels ranging between 200-350 mg $dl^{-1}$ were considered diabetic and included in the experiment.



 

**Experimental design;** one hundred diabetic rats were divided into ten groups - ten animals each (1:1 for males: females) - as follows; **group-1;** received standard saline solution (10 ml $kg^{-1}$b.wt.),**group-2;** received the reference standard Glibenclamide (0.025 g $kg^{-1}$ b.wt.), **group-3;** received *L. dentata* volatile oil (50 mg $kg^{-1}$ b.wt.),**group-4;** received *L. dentata* volatile oil (100 mg $kg^{-1}$b.wt.), **group-5;** received *L. angustifolia* volatile oil (50 mg $kg^{-1}$b.wt.), **group-6;** received *L. angustifolia* volatile oil (100 mg $kg^{-1}$ b.wt.); **group-7;** received *L. dentata* methanol extract (50 mg $kg^{-1}$b.wt.), **group-8;** received *L. dentata* methanol extract (100 mg $kg^{-1}$ b.wt.), **group-9;** received *L. angustifolia* methanol extract (50 mg $kg^{-1}$b.wt.), **group -10;** received *L. angusti-folia* methanol extract (100 mg $kg^{-1}$b.wt.), as well as **group-11** which was formed of normal untreated animals received standard saline solution (10 ml $kg^{-1}$ b.wt.).

All the investigated volatile oils and extracts were given as single daily oral doses; they were sacrificed on 30$^{th}$ day by cervical dislocation and the gained liver and kidney tissues′ homogenates were used for estimation of enzymatic antioxidants such as superoxide dismutase (**50**) and catalase (**51**) and non-enzymatic antioxidants such as vitamin C (**52**) and glutathione (**53**).

## RESULTS AND DISCUSSION:

The physiochemical parameters determined for the powdered aerial parts of *L. dentata* L. and *L. angustifolia* Mill. are compiled in table (2).

**Table 2: Some physiochemical constants determined for the powdered aerial parts of *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae), cultivated in Egypt:**

| Physiochemical parameter | Mean (%W/W) | | |
|---|---|---|---|
| | Description | *L. dentata* L. | *L. angustifolia* Mill. |
| ***Moisture content*** | Loss on drying at 110 $^{o}$C | 15.28±2.30 | 13.40±2.25 |
| ***Ash values*** | Total ash | 10.48±2.33 | 8.55±1.96 |
| | Acid insoluble ash | 1.90±0.85 | 1.70±0.80 |
| | Water soluble ash | 2.70±1.49 | 2.69±1.53 |
| | Water insoluble ash | 5.80±1.36 | 4.10±1.16 |
| ***Extractives by cold maceration*** | Petroleum ether | 6.22±1.75 | 5.90±1.68 |
| | Ethyl acetate | 5.90±1.48 | 5.50±1.51 |
| | Ethanol | 9.06±1.90 | 8.90±1.88 |
| | Aqueous | 10.65±2.15 | 10.05±2.04 |

**Results tabulated representing the means of three successive readings ± standard error.**

The percent yields of the hydro-distilled volatile oils from aerial parts of *L. dentata* L. and *L. angustifolia* Mill. Were 3.47 and 2.95 respectively, physical examination revealed that they share in having pale yellow colour and aromatic characteristic odour but differs in their specific gravities and refractive indices, table (3).

GC/MS analysis of the prepared volatile oils exhibited qualitative and quantitative variations tables, 4, 5 where twenty-six and thirty-one compounds were identified representing 96.43 % and 98.56 % of the total individual composition of *L. dentata* L. and *L. angustifolia* Mill. volatile oils respectively (**54** and **55**).

The percentage of oxygenated compounds of both oils were much higher (83.56% and 96.83%) than those of hydro-carbon compounds (10.09 % and 1.73 %) where the percentage of oxygenated monoterpene compounds were (50.04 % and 84.52 %) represented by ten and twenty one compounds, mainly menthe-1,5-dien-8-ol <*Para*> (26.80%) , 1,8-cineole (4.98%), geraniol (3.92%) and linalyl acetate (18.99%), citronellol <*Alpha*> (17.36%), menthe-1,5-dien-8-ol <*Para*> (16.21%), meanwhile, the percentage of oxygenated sesquiterpene compounds were (33.52% and 12.31%) represented by three and two compounds, mainly caryophyllene oxide (16.40%), guiaol <*Alpha*> (15.36%) and farnsol (12.22%) in the volatile oils of *L. dentata* L. and *L. angustifolia* Mill. respectively (**54** and **55**).

**Table 3: Percent yields, physical characters, specific gravities and refractive indices of the volatile oils of *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae), cultivated in Egypt:**

| Item | *L. dentata* L. | *L. angustifolia* Mill. |
|---|---|---|
| **Percent yield** | 3.47 | 2.95 |
| **Colour** | Pale yellow | Pale yellow |
| **Odour** | Aromatic | Aromatic |
| **Specific gravity** | 0.88942 | 0.89320 |
| **Refractive index** | 1.51840 | 1.52135 |



 

**Table 4: Percent identified chemical constituents of volatile oils of *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae), cultivated in Egypt:**

| Item | *L. dentata* L. | *L. angustifolia* Mill. |
|---|---|---|
| **Total hydrocarbons** | **10.09** | **1.73** |
| - Monoterpenes | 1.53 | 1.11 |
| - Sesquiterpenes | 8.56 | 0.62 |
| **Total oxygenated compounds** | **83.56** | **96.83** |
| - Oxygenated monoterpenes | 50.04 | 84.52 |
| - Oxygenated sesquiterpenes | 33.52 | 12.31 |
| **Others** | 2.78 | 0 |
| **Total identified compounds** | **96.43** | **98.56** |

The percentage of monoterpene hydrocarbon compounds were (1.53 % and 1.11 %) represented by two and three compounds, mainly tricyclene (1.03%), pinene <*Alpha*> (0.50%) and cymene <*Para*> (0.82%), pinene <*Alpha*> (0.17%) while the percentage of sesquiterpene hydrocarbon compounds were (8.56 % and 0.62%) represented by seven and five compounds, mainly aromandrene <*Allo*> (2.07%), humulene <*Alpha*>(1.94%), germacrene-D (1.48%) and bisaboline <*Beta*> (0.40%), cadenine <*Gamma*> (0.15%) in the volatile oils of *L. dentata* L. and *L. angustifolia* Mill. respectively (**54** and **55**).

In conclusion, the volatile oil of *L. angustifolia* Mill. was richer in its content of total oxygenated compounds (96.83%) compared to *L. dentata* L. volatile oil (83.56%) whereas its main constituents were of monoterpene group while those of *L. dentata* L. were of sesquiterpene group, fortunately they are sharing in containing high percent of the monoterpene menthe-1,5-dien-8-ol<*Para*> at levels of 26.80% and 16.21% respectively, meanwhile, the total hydrocarbon compounds in the volatile oil of *L. dentata* L. was comparatively richer in its content (10.09%) compared to the volatile oil of *L. angustifolia* Mill. (1.73%) where its main constituents were of monoterpene group (1.11%) and those of *L. dentata* L. were of sesquiterpene group (8.56%).

The overall gained results exhibited significant qualitative and quantitative diversity in the chemical compositions of the volatile oils of *L. dentata* L. and *L. angustifolia* Mill. which are matched with the reported results in much previous research works (**4** and **9-20**).

Quantitative estimation of the total phenol content of the aqueous and methanol extracts of the tested species using Folin-Ciocalteu's reagent table 6, showed that the aqueous and methanol extracts of *L. angustifolia* Mill. contained higher percent of phenolic compounds (194.95±2.55 and 152.50±2.21) compared to those for *L. dentata* L. (188.50 ±2.07 and 167.10±2.30) mg GAE $g^{-1}$ respectively, the total phenols were measured by in terms of gallic acid equivalent (the standard curve equation is y=0.05X± 0.0545, $r^2$= 0.9873); while quantitative estimation of total flavonoids of tested species using aluminum chloride reagent revealed that the aqueous and methanol extracts of *L. angustifolia* Mill. contains higher percent (98.83±2.68 and 119.95±1.87) compared to those of *L. dentata* L. (98.83± 2.68 and 119.95±1.87) mg QE $g^{-1}$ respectively, the total flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation is y = 0.0067X ± 0.0132, $r^2$ = 0.999).

Oxidative stress in diabetes mellitus revealed the reduction in the antioxidant status and glycation of proteins, inactivation of enzymes, and alteration in structural functions of collagen basement membrane (**56**), in addition, tissue damage resulting from oxidative stress has been implicated in the pathology of a number of disorder diseases such as cancer, inflammatory joint disease, cardiovascular diseases, cataract and could play a role in neurodegenerative diseases and ageing processes (**57**) whereas antioxidants are substances or nutrients which can prevent or slow the oxidative damage (**58**).

Acute toxicity studies revealed that no toxicity symptoms or death in the given doses so the $LD_{50}$ of the tested oils and extracts are greater than 2000 mg $kg^{-1}$b.wt. which made them safe drugs, hence 50 and 100 mg $kg^{-1}$b.wt.were selected as therapeutic doses.

Oxidative stress is a condition of reduction in antioxidant enzymes like SOD and CAT; these enzymes play an important role in maintaining physiological levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to Streptozotocin which play an important role in reducing cellular stress (**59**). The activities of SOD and CAT were significantly decreased in liver and kidney tissues of diabetic control rats due to inadequacy of the antioxidant defenses in combating ROS mediated damage while treatment with different extracts and volatile oils increased the activity of these enzymes and help to control free radicals when compared to diabetic rats compared to the standard drug Glibenclamide, tables (7 and 8).



 

**Table 5: Chemical composition of the volatile oils isolated from the aerial parts of *L. dentata* L.and *L. angustifolia* Mill. (Lamiaceae), cultivated in Egypt:**

| Peak No. | Compound Name | $R_t$* (min.) | Percent | |
|---|---|---|---|---|
| | | | *L. dentata* L. | *L. angustifolia* Mill. |
| ***Monoterpene hydrocarbons*** | | | | |
| **1** | Tricyclene | 8.40 | 1.03 | -- |
| **2** | Pinene <*Alpha*> | 10.24 | 0.50 | 0.17 |
| **3** | Camphene | 10.44 | -- | 0.12 |
| **4** | Cymene <*Para* > | 10.47 | -- | 0.82 |
| ***Oxygenated monoterpenes*** | | | | |
| **5** | 1,8-cineol | 12.96 | 4.98 | -- |
| **6** | Sabinene hydrate <*Cis*> | 13.40 | -- | 1.14 |
| **7** | Linalol oxide <*Cis*> | 13.56 | -- | 2.41 |
| **8** | Terpinolene | 13.70 | -- | 1.04 |
| **9** | Linalool | 13.94 | -- | 4.43 |
| **10** | 2-menthenol <*Cis*> | 14.20 | -- | 0.11 |
| **11** | Camphor <*Dextro*> | 14.46 | -- | 1.65 |
| **12** | Isoborneol | 14.65 | -- | 0.11 |
| **13** | Borneol | 14.86 | -- | 0.43 |
| **14** | Terpinen-4-ol <*Levo*> | 14.94 | -- | 0.89 |
| **15** | Terpinene<*Gamma* > | 15.02 | 0.93 | 0.90 |
| **16** | Linalyl acetate | 15.24 | -- | 18.99 |
| **17** | Geraniol | 15.65 | 3.92 | -- |
| **18** | Nonanoic acid | 16.88 | 3.72 | -- |
| **19** | Isopulegol | 17.40 | 0.75 | -- |
| **20** | Bornyl acetate | 17.52 | -- | 0.37 |
| **21** | Isobornyl acetate | 17.73 | -- | 0.86 |
| **22** | Cuminyl alcohol | 17.85 | 0.64 | 0.90 |
| **23** | Lavendulyl acetate | 17.93 | -- | 3.75 |
| **24** | Decanoic acid | 18.00 | 2.35 | -- |
| **25** | Neryl acetate | 18.17 | -- | 0.72 |
| **26** | Geranyl acetate | 18.44 | -- | 3.04 |
| **27** | Cedrene<*Alpha* > | 18.54 | -- | 7.40 |
| **28** | Citronellol<*Alpha* > | 18.75 | 2.96 | 17.36 |
| **29** | Mentha1, 5 dien-8-ol <*Para* > | 19.30 | 26.80 | 16.21 |
| **30** | Dihydrocarvyl acetate | 20.67 | -- | 1.83 |
| **31** | Carvone | 21.93 | 2.99 | -- |
| ***Sesquiterpene hydrocarbons*** | | | | |
| **32** | Caryophyllene <*Beta* > | 22.79 | 1.11 | 0.07 |
| **33** | Humulene <*Alpha* > | 23.58 | 1.94 | -- |
| **34** | Aromandrene <*Allo*> | 24.60 | 2.07 | -- |
| **35** | Germacrene-D | 25.95 | 1.48 | 0.06 |
| **36** | Bicyclogermacrene | 27.38 | 0.06 | 0.02 |
| **37** | Bisaboline <*Beta* > | 28.09 | 1.05 | 0.40 |
| **38** | Cadenine <*Gamma* > | 28.41 | 0.85 | 0.15 |
| ***Oxygenated sesquiterpenes*** | | | | |
| **39** | Caryophyllene oxide | 29.18 | 16.40 | 0.09 |
| **40** | Guaiol <*Alpha* > | 30.48 | 15.63 | -- |
| **41** | Farnsol | 31.95 | 1.76 | 12.22 |
| ***Others*** | | | | |
| **42** | Octadecatrenoic acid methyl ester | 30.21 | 0.49 | -- |
| **43** | 6-dodecanone | 33.72 | 0.68 | -- |
| **44** | Docosane | 44.22 | 0.58 | -- |
| **45** | 17-octadecynoic acid | 46.86 | 1.03 | -- |

*** $R_t$: retention time in minutes**



 

**Table 6: Total phenol and flavonoid contents of aqueous and methanol extracts prepared from *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae), cultivated in Egypt:**

| Item | *L. dentata* L. | | *L. angustifolia* Mill. | |
|---|---|---|---|---|
| | Aqueous | Methanol | Aqueous | Methanol |
| Total phenol (mg GAE $g^{-1}$) | 188.50 ±2.07 | 167.10±2.30 | 194.95±2.55 | 152.50±2.21 |
| Total flavonoid (mg QE $g^{-1}$) | 90.40±1.57 | 116.15±1.91 | 98.83±2.68 | 119.95±1.87 |

**The tabulated results representing means ± standard errors.**

Vitamin C plays a central role in the antioxidant protective system, protecting lipids oxidation and diminishing the number of apoptotic cells, regenerating the oxidized vitamin E and acts as a non-enzymatic antioxidant (**60**). Diabetes result in significant decrease in vitamin C levels compared to control rats where its level was significantly restored in liver and kidney tissues of treated groups compared to the standard drug Glibenclamide, tables (7 and 8).

GSH has a multifaceted role in antioxidant defense; it is a direct free radical scavenger; act as co-substrate for peroxide detoxification. Diabetic oxidative stress decrease GSH level in liver and kidney tissues compared to control where significant elevation of GSH levels were observed in treated groups compared to the standard drug Glibenclamide, tables (7 and 8).

**CONCLUSION:**

In conclusion, the gained results proved quantitative variation in the estimated physiochemical parameters, qualitative and quantitative diversity of volatile oils′ physical characters, constants and chemical composition, in addition to quantitative variation in total phenol and flavonoid contents of *L. dentata* L. and *L. angustifolia* Mill. (Lamiaceae) cultivated in Egypt meanwhile concerning antioxidant studies; both plants proved to be of promising therapeutic value in preventing diabetic oxidative stress which can be attributed to their significant quenching impact on the extent of lipid peroxidation along with, enhancement of antioxidant defense systems in liver and kidney tissues.

**Table 7: Results of enzymatic and non-enzymatic antioxidant potentials of the volatile oils and methanol extracts of *L. dentata* and *L. angustifolia* at two dose levels (50 and 100 mg/kg b.wt.) and Glibenclamide (0.025 mg/kg b. wt.) on liver tissues of adult albino rats:**

| Antioxidant Parameters | | Groups | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal control | Negative control | GLB | Volatile oils | | | | Methanol extracts | | | |
| | | | | | *L. dentata* | | *L. angustifolia* | | *L. dentata* | | *L. angustifolia* | |
| | | | | | 50 mg | 100 mg | 50 mg | 100 mg | 50 mg | 100 mg | 50 mg | 100 mg |
| Enzymatic | SOD U/g tissue | 7.119 ± 0.089 | 4.074 ± 0.152 | 6.310 ± 0.152 | 5.735 ± 0.068 | 5.984 ± 0.088 | 6.112 ± 0.050 | 6.346 ± 0.098 | 6.877 ± 0.100 | 6.949 ± 0.145 | 6.982 ± 0.040 | 7.502 ± 0.074 |
| | CAT µ mole $H_2o_2$ utilized/min/ mg protein | 58.466 ± 0.273 | 43.140 ± 0.121 | 53.871 ± 0.199 | 46.323 ± 0.134 | 47.365 ± 0.193 | 50.023 ± 0.084 | 51.295 ± 0.125 | 55.577 ± 0.358 | 56.699 ± 0.210 | 57.288 ± 0.181 | 58.701 ± 0.223 |
| Non-enzymatic | Vit. C mg/g fresh tissue | 1.794 ± 0.013 | 0.765 ± 0.020 | 1.441 ± 0.016 | 0.986 ± 0.016 | 1.157 ± 0.061 | 1.398 ± 0.041 | 1.535 ± 0.035 | 1.758 ± 0.022 | 1.876 ± 0.025 | 1.898 ± 0.035 | 1.876 ± 0.027 |
| | GSH µg/mg protein | 47.115 ± 0.141 | 29.543 ± 0.181 | 43.416 ± 0.178 | 24.333 ± 0.128 | 30.242 ± 0.160 | 36.664 ± 0.553 | 39.544 ± 0.212 | 43.965 ± 0.158 | 46.076 ± 0.195 | 45.946 ± 0.085 | 47.993 ± 0.171 |

**The tabulated results representing the means ± standard error, n = 10**



 

**Table 8: Results of enzymatic and non-enzymatic antioxidant potentials of the volatile oils and methanol extracts of *L. dentata* and *L. angustifolia* at two dose levels (50 and 100 mg/kg b.wt.) and Glibenclamide (0.025 mg/kg b. wt.) kidney tissues of adult albino rats:**

| Antioxidant Parameters | | Groups | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Normal control | Negative control | GLB | Volatile oils | | | | Methanol extracts | | | |
| | | | | | *L. dentata* | | *L. angustifolia* | | *L. dentata* | | *L. angustifolia* | |
| | | | | | 50 mg | 100 mg | 50 mg | 100 mg | 50 mg | 100 mg | 50 mg | 100 mg |
| Enzymatic | SOD U/g tissue | 2.321 ± 0.080 | 0.890 ± 0.030 | 1.942 ± 0.037 | 1.138 ± 0.047 | 1.215 ± 0.019 | 1.319 ± 0.029 | 1.464 ± 0.104 | 1.868 ± 0.052 | 1.983 ± 0.042 | 2.129 ± 0.035 | 2.416 ± 0.125 |
| | CAT M mole $H_2o_2$ utilized/min/ mg protein | 29.276 ± 0.095 | 15.679 ± 0.185 | 27.908 ± 0.268 | 18.710 ± 0.159 | 20.783 ± 0.171 | 21.142 ± 0.234 | 22.579 ± 0.162 | 24.938 ± 0.076 | 25.942 ± 0.076 | 28.518 ± 0.231 | 30.799 ± 0.201 |
| Non-enzymatic | Vit. C mg/g fresh tissue | 1.270 ± 0.024 | 0.495 ± 0.024 | 1.676 ± 0.025 | 0.832 ± 0.016 | 0.889 ± 0.017 | 0.887 ± 0.020 | 1.064 ± 0.038 | 1.210 ± 0.010 | 1.211 ± 0.038 | 1.292 ± 0.016 | 1.431 ± 0.017 |
| | GSH µg/mg protein | 48.638 ± 0.297 | 23.453 ± 0.197 | 44.786 ± 0.155 | 32.870 ± 0.116 | 34.895 ± 0.162 | 32.848 ± 0.335 | 35.493 ± 0.219 | 40.835 ± 0.152 | 43.589 ± 0.205 | 42.735 ± 0.185 | 46.683 ± 0.139 |

**The tabulated results representing the means ± standard error, n = 10**

**REFERENCES:**


1. M. Upson and S. Andrews, "*The Genus Lavandula*, A Botanical Magazine Monograph published Royal Botanic Gardens, Kew © University Botanic Garden 1 Brookside Cambridge", (2004), CB2 1JE 01223 336265.
2. H.M. Cavanagh and J.M. Wilkinson, "Biological Activities of Lavender", *Essential Oil Phytother Res*, (2002), 16:301-308.
3. L. Ouahmane; R. Duponnois and M. Hafidi, "Some Mediterranean Plant Species (*Lavandula* spp. and *Thymus satureioides*) Act as Potential 'Plant Nurses' for the Early Growth of *Cupressus atlantica*", *Plant Ecology* ,(2006), 185: 123-134.
4. B. Imelouane; A. Elbachiri; M. Ankit; H. Benzeid and K. Khedid,"Physicochemical Compositions and Antimicrobial Activity of Essential Oil of Eastern Moroccan *Lavandula dentata"*, *Int. J. Agric. Biol*. (2009), 11: 113-118.
5. V.D. Zheljazkov; T. Astatkie and A.N. Hristov, "Lavender and Hyssop Productivity, Oil Content, and Bioactivity as a Function of Harvest Time and Drying", *Ind. Crop. Prod*, (2012), 36: 222-228.
6. M. Saadatian; M. Aghaei; M. Farahpour and Z. Balouchi, "Chemical Composition of Lavender (*Lavandula Officinalis* L.) Extraction Extracted by two Solvent Concentrations", *Global Journal of Medicinal Plant Research*, (2013), 1(2): 214-217.
7. N.G. Bissett, "Herbal Drugs and Phyto-pharmaceuticals", Stuttgart: MedPharm CRC Press, (1994), 566.
8. M. Gamez; J. Jimenez ; C. Navarro and A. Zarzuelo, "Study of the Essential Oil of *Lavandula dentata* L.", *Pharmazie*, (1990), 45:69-70.
9. R.A. Mothana; M.S. Alsaid; S.S. Hasoon; N.A. Al-Mosaiyb; A.J. Al-Rehaily and M.A. Al-Yahya, "Antimicrobial and Antioxidant Activities and Gas Chromatography Mass Spectrometry (GC/MS) Analysis of the Essential Oils of *Ajuga bracteosa* Wall. Ex Benth.and *Lavandula dentata* L. Growing Wild in Yemen", , *Journal of Medicinal Plants Research*, (2012), 6(15), 3066-3071.
10. B. Imelouane; A. Elbachiri; J.P. Wathelet; J. Dubois and H. Amhamdi, "Chemical Composition, Cytotoxic




 

and Antioxidant Activity of the Essential Oil of *Lavandula dentata*", *World Journal of Chemistry*, (2010), 5 (2): 103-110.

**11.** L. Bousmaha; J.B. Boti; F.A. Bekkara; V. Castola and J. Casanova, "Infraspecific Chemical Variability of the Essential Oil of *Lavandula dentata* L. from Algeria", *FlavourFragr.J*, (2006) 21: 368-372.

**12.** T. Dob; D. Dahmane; B. Tayeb and C. Chelghoum, "Chemical Composition of the Essential Oil of *Lavandula dentata* L. From Algeria", *The Int. J. Aromath*, (2005), 15: 110-4.

**13.** M.I. Garcia-Vallejo; M.C. Garcia-Vallejo and A. Valasco-Negueruela,"Composicion Quimica de Aceites Esenciales de *Lavandula dentata* L. En Espana; Quimotipos", Proceedings of the 7th Congreso Nacional de Quimica, Sevilla, Espana, EFCE Publication, *Quim. Agric. Aliment*., (1987), 68, 1: 415-422.

**14.** C. Jianu; G. Pop; A.T. Gruia and F.G. Horhat, "Chemical Composition and Antimicrobial Activity of Essential Oils of Lavender (*Lavandula angustifolia*) and Lavandin (*Lavandula Intermedia*) Grown in Western Romania", *Int. J. Agric. Biol*, (2013), 15: 772-776.

**15.** G. Woronuk; Z. Demissie; M. Rheault and S. Mahmoud, "Biosynthesis and Therapeutic Properties of *Lavandula* Essential Oil Constituents," *Planta Medica*, (2011) 77: (1), 7–15.

**16.** K.J. Hamad; S.J. Al-Ahaheen; R.A. Kaskoos; J. Ahmad; M. Jameel and S.R. Mir, "Essential Oil Component and Antioxidant Activity of *Lavandua angustifolia* From Iraq", *Int. Res. Pharm. J*., (2013), 4(4),

**17.** M.M. Barazandeh, "Essential Oil Composition of *Lavandula latifolia* Medik from Iran", *J. Essent. Oil Res.*, (2002) 14: 103-104.

**18.** R. Naef and A.F. Morris, "Lavender - Lavandin; A comparison. Rivista Ital", *EPPOS*,(1992), (Numero Speciale): 364-377.

**19.** A. Prashar; I. C. Locke and C. S. Evans, "Cytotoxicity of Lavender Oil and its Major Components to Human Skin Cells," *Cell Proliferation*, (2004), 37(3), 221-229.

**20.** M. Lis-Balchin and S. Hart, "Studies on the Mode of Action of the Essential Oil of Lavender (*Lavandula angustifolia* P. Miller)", *Phytother. Res*., (1999), 13: 540-542.

**21.** K. Adam; A. Sivropoulou; S. Kokkini; T. Lanaras and M. Arsenakis, "Antifungal Activities of *Origanum vulgare* Subsp. Hirtum, *Mentha spicata*, *Lavandula angustifolia*, and *Salvia fruticosa* Essential Oils against Human Pathogenic Fungi", *Journal of Agricultural & Food Chemistry*, (1998), 46:1739-1745.

**22.** E. Yarnell, "Essential Oils against Lice", *Quarterly Review of Natural Medicine*, (1998), 3: 177-184.

**23.** M.T. Lis-Balchin, "Lavender. The genus *Lavandula*. In: Book Series: Medicinal and Aromatic Plants-Industrial Profiles", (2002), Vol. 29. Hardman, R. (ed.). Taylor and Francis, New York.

**24.** A.M. Schuwald; M. No¨ldner; T. Wilmes; N. Klugbauer and K. Leuner, "Lavender Oil-Potent Anxiolytic Properties via Modulating Voltage Dependent Calcium Channels", *PLOS ONE*, (2013), 8(4): e59998. doi:10.1371/journal.pone.0059998.

**25.** Y. Sugawara; C. Hara and K. Tamura, "Sedative Effect on Humans of Inhalation of Essential Oil of Linalool: Sensory Evaluation and Physiological Measurements Using Optically Active Linalools", *Analytica Chimica Acta*, (1998), 365:293-299.

**26.** C. Ghelardini; N. Galeotti; G. Salvatore and G. Mazzanti, "Local Anaesthetic Activity of the Essential Oil of *Lavandula angustifolia*", *Planta Med*, (1999), 65:700-3.

**27.** L. Stanojević; M. Stanković; M. Cakić; V. Nikolić; L. Nikolić; D. Ilić and N. Radulović, "The Effect of Hydrodistillation Techniques on Yield, Kinetics, Composition and Antimicrobial Activity of Essential Oils From Flowers of *Lavandula officinalis* L.", *Hem. Ind*., (2011) 65: 455–463

**28.** M. Soković; P.D. Marin; D. Brkić and L.J.V. Griensven, "Chemical Composition and Antibacterial Activity of Essential Oils of Ten Aromatic Plants against Human Pathogenic Bacteria", *Food*, (2007), 1: 220–226.

**29.** S. Cosentino; C.I. Tuberoso and B. Pisano, "*In -vitro* Antimicrobial Activity and Chemical Composition of Sardinian Thymus Essential Oils", *Lett Appl Microbiol*, (1999), 29:130-5.

**30.** B. G. Stanic; S. Pepeljnjak and S.V. Knežević, "*In-vitro* Antibacterial and Antifungal Activity of *Lavandula X intermedia* Emeric ex Loisel. 'Budrovka'", *Molecules*, (2011), 16: 424-4253.

**31.** E. S. Şerban; M. Ionescu; D. Matinca; C.S. Maier and M.T. Bojiţă, "Screening of the Antibacterial and Antifungal Activity of Eight Volatile Essential Oils", *Farmacia*, (2011), 59: 440-446.

**32.** F.D. Dauria; M. Tecca; V. Strippoli; G. Salvatore; L. Battineli and G. Mazzanati, "Antifungal activity of *Lavandula angustifolia* essential oil against *Candida albicans* yeast and mycelial form", *Med Mycol*, (2005), 43(5):391-396.

**33.** L. Amjad and M. Shafighi, "Evaluation of Antioxidant Activity, Phenolic and Flavonoid Content in *Punica granatum* var. Isfahan Malas Flowers", *International Journal of Agriculture and Crop Sciences*, (2013), Available online at www.ijagcs.com JACS/2013/5-10/1133-1139



 

**34.** F. Ferreres; F.A. Barberan and F. Tomas, "Flavonoids from *Lavandula dentata*", *Fitoterapia*, (1986), 57, 199 200.

**35.** T. M. Upson; R. J. Grayer; J. R. Greenham; C. A. Williams; F. Al-Ghamdi and F. H. Chen, "Leaf Flavonoids as Systematic Characters in the Genera *Lavandula* and *Sabaudia"*, *Biochemical Systematics and Ecology*, (2000), 28, 991 1007.

**36.** J. E. Middleton; C. Kandaswami and T. Theoharides, "Effects of Plant Flavonoids on Mammalian Cells: Implications for Inflammation, Heart Disease& Cancer", *Pharmacol. Reviews*, (2000), 52:673-839.

**37.** G. Ferretti; T. Bacchetti; A. Belleggia and D. Neri, "Cherry antioxidants: from farm to table", *Molecules*, (2010), 15: 6993-7005.

**38.** T. Bacchetti; S. Masciangelo; A. Micheletti and G. Ferretti, "Carotenoids, Phenolic Compounds and Antioxidant Capacity of Five Local Italian Corn (*Zea Mays* L.) Kernels", *J Nutr Food Sci*, (2013), 3:6.

**39.** N.M. Abdel-Hady; G.T.M. Dawoud; A.A. El-Hela and T.A. Morsy, "Interrelation of Antioxidant, Anticancer and Antilieshmania Effects of some Selected Egyptian Plants and their Phenolic Constituents ", *J. Egypt .Soc. Parasitol*., (2011), 41(3):785-800.

**40.** A.A. El-Hela; N.M. Abdel-Hady; G.T. Dawoud; A.M. Hamed and T.A. Morsy, "Phenolic Content, Antioxidant Potential and *Aedes aegyptii* Ecological Friend Larvicidal Activity of Some Selected Egyptian Plants", *J. Egypt. Soc. Parasitol*, (2013), 43(1):215-234.

**41.** N.M. Abdel-Hady,"Quantitative Diversity of Phenolic Content in Peels of Some Selected Egyptian Pomegranate Cultivars Correlated to Antioxidant and Anticancer Effects", *Journal of Applied Sciences Research*, (2013), 9(8): 4823-4830.

**42.** N.M. Abdel-Hady, "Efficient Extraction and *in-vitro* Control of *Trichomonas vaginalis* of Peels′ Total Phenols and Flavonoids for Selected Egyptian Pomegranate Cultivars", *Journal of Applied Sciences Research*, (2013), 9(8): 5003-5012.

**43.** M.J. Alves; I.C. Ferreira; H.J.C. Froufe; R.M. Abreu; A. Martins and M. Pintado, "Antimicrobial Activity of Phenolic Compounds Identified in Wild Mushrooms, SAR Analysis and Docking Studies", *Journal of Applied Microbiology*,(2013), ISSN 1364-5072.

**44.** T.F. Eissa; E. González-Burgos; M. Emilia-Carretero and M. Gómez-Serranillos,"Phenolic Composition and Evaluation of Antioxidant and Cytoprotective Activity of *Chiliadenus montanus"*,*Rec. Nat. Prod*, (2013), 7:3 184-191.

**45.** Egyptian Pharmacopeia, English Text, 3rd Edition, University Press, Cairo, (1984).

**46.** K. Zhou and L. Yu, "Total phenolic contents and antioxidant properties of commonly consumed vegetables in Colorado", *Lebensmittel-Wissenschaf and Technologie*, (2006) 39 (10), 1155-1162.

**47.** A. Djeridane; Y. M. Nadjemi; D. Boutassouna; P. Stocker and N. Vidal, "Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds", *Food Chem*, (2006), 97(4):654-660.

**48.** A. Cavin; K. Hostettmann; W.Y. Dyatm and O. Potterat, "Antioxidant and lipohilic constituents of *Tino spora crispa*", *Planta Med*, (1998), 64:393-6.

**49.** S. Nirmala and T. Karthiyayini, "Wound Healing Activity on the Leaves of *Achillea Millefolium* L. by Excision, Incision and Dead Space Model on Adult Wister albino rats", *International Research Journal Of Pharmacy*, (2011), 2(3), 240-245.

**50.** J.A. Williams; G. Wiener; P.H. Anderson and C.H. Mc-Murray, "Variation in the Activities of Glutathione Peroxidase and Superoxide Dismutase and in the Concentration of Copper in the Blood in Various Breed Crosses of Sheep", *Res Vet Sci*, (1983), 34: 253-256.

**51.** G.J. Cohen; D. Dembiec and J. Marcus, "Measurement of catalase activity in tissue extracts", *Anal. Biochem*, (1970), 34, 30-38.

**52.** H.S. Loh and C.W.M. Wilson, "Relationship between Leucocytes and Plasma Ascorbic Acid Concentrations", *British Medical journal*,(1971), 3:733.

**53.** G. Ellman, "Tissue Sulfhydryl Groups", *Archives of Biochemistry and Biophysics*,(1959), 32, 70-77.

**54.** R.P. Adams, "Identification of Essential Oils by Ion Trap Mass Spectrometry", (1989), Academic Press, Inc., San Diego, New York, Boston, London, Sydney, Tokyo, Toronto.

**55.** R.P. Adams, "Identification of Essential Oils by Gas Chromatography/Mass Spectrometry", (1995), Allured Publishing Corporation, Illinois, U.S.A.

**56.** E. Ganesh; A. Chowdhury; T. Malarvani and N. Vardhan, "Microscopical Observation of Kidney for Ameliorative Effects of Vitamin- E&C in Streptozotocin-Induced Diabetic Rats", *International Journal of Life Science And Pharma Research*, (2012), 2: 3.

**57.** O.A. Khalil; K.S. Ramadan; E.N Danial; H.S Alnahdi and N.O. Ayaz, "Antidiabetic Activity of *Rosmarinus officinalis* and its Relationship with the Antioxidant Property", *African Journal of Pharmacy and Pharmacology*, (2012), 6:1031-1036.

**58.** V. Ragini; K.V. Prasad and K. Bharathi, "Antidiabetic and Antioxidant Activity of *Shoreatum buggaia* Rox.", *International Journal of Innovative Pharmaceutical Research*, (2011), 113-121.

**59.** V. N. Shilpa; N. Rajasekaran; V. K. Gopalakrishnan and K. Devaki, "*In-vivo* Antioxidant Activity of *Premna Corymbosa* (Rottl.) against Streptozotocin Induced Oxidative Stress in Wistar Albino Rats", *Journal of Applied Pharmaceutical Science*, (2012), 2 (10), 60-65.




 

 