# Comparison of the Effects of Some Essential Oils as Anti-inflammatory Agents on the activities of Lysosomal Acid-Hydrolases in Rat Liver in-vitro

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**Abstract:** Study aimed to evaluate the effect of three different volatile oils such as: (*Cinnamomum Zeylanicum N.*)(CIN); (*Eucalyptus globules L.*) (EUC); (*Mentha Piperita L.*)(PEP) oils and their combination by two concentrations (100 and 300 µg/ml) on the four marker lysosomal enzymatic activities (ACP, Acid phosphatase; β-GAL, β- galactosidase, β-NAG, β-N-acetyl glucosaminidase, and β-GLU, β-Glucuronidase) in rat liver *in-vitro*. In addition to, the activities of these enzymes in Carbontetrachloride($CCl_4$)-induced hepatotoxicity (*in-vivo*) for each volatile oil by the two concentrations were investigated. It was observed that at the *in-vitro* experiment, the activity of each lysosomal enzyme appeared to be decreased by different values depending on the concentration of the volatile oil. The highest percentage values of the inhibitory effect were observed at the high concentration of the oil. The enzyme activity was altered according to the enzyme type and the oil. On the other hand, at the experiment of $CCl_4$-induced hepatotoxicity (*in-vivo*), the enzyme activity behavior was changed according to sole dose of treatment. At the sole treatments of each volatile oil by the two concentrations, the enzyme activity was decreased by different percentage values of inhibition. It was concluded that (*Cinnamomum Zeylanicum N.*); (*Eucalyptus globules L.*); (*Mentha Piperita L.*) oils by the two concentrations and also their mixtures exerted an inhibitory effect by different percentage of inhibition due to the effect of their antioxidant components of oils on the membrane permeability of the lysosomes.

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## INTRODUCTION

Lysosome contains many acid hydrolases within their membranes **(Nakagawa *et al.*, 2000).** The flavonoids protected lysosomal membranes more effectively against free radicals generated in the aqueous phase, the antioxidant activity of the antioxidant compound, suggesting that factors other than lipid peroxidation may be involved in the suppressive effect of flavonoids. **Decharneux *et al.* (1992)** proposed a more direct action of flavonoids on lysosomal membranes. Additionally, the localization of flavonoids within the membranes may modify membrane fluidity and lipid peroxidation **(saija *et al.*, 1995 and Arora *et al.*, 2000).** A decrease in lysosomal stability is generally paralleled by increased lysosomal enzyme activity in extracellular fluid **(Weissman, 1967).** Lysosomal enzymes are important mediators of acute chronic inflammatory diseases and involved in damage to connective tissue **(Rarichandran *et al.*, 1990).** Autophagy, a lysosomal process involved in the maintenance of cellular homeostasis, is responsible for the cell death **(Meijer and Codogno, 2009),** the lysosomal membrane permeability with a resulting leakage of hydrolases into the cytosol, which are then directly involved in cell death **(Jahreiss *et al.*, 2009).**

**Abdel-Hamid *et al.* (2011)** investigated that lysosomal enzymes disorders contribute to several human diseases, either due to genetic defects in its enzyme expression or the escape of lysosomal enzymes (lysosomes) into extralysosomal medium **(Murray *et al.*, 1999).** It was noticed that some herbs could ameliorate anticancer-induced lysosomal abnormalities, conserving lysosomal integrity.

Functional status of vital organs in aged patients usually have degeneration and deteriorating function of many vital organs diseases as hypertension, diabetes, chronic liver disease, impaired kidney function. Some drugs and medicinal plants that stabilize the lysosomal membrane and inhibit lysosomal enzymes were used in the treatment of some inflammatory diseases **(Wu *et al.*, 2007).** In many pathological conditions, changes in the state of lysosomes take place. The loss of the stability of lysosomal membrane has been observed in the leakage of enzymes from lysosomes **(Stvolinskaya *et al.*, 1992).**

Flavonoids may be effective as antioxidants in spaces between the aqueous phase and the lipid phase owing to their localization near the surface in lipid bilayers. Thus, not only evident antioxidative activity, but also the subcellular localization of flavonoids may contribute to their potencies as antioxidant in biological

system **(Nakagawa *et al.*, 2000)**. Flavonoids are more nutritive dietary components that are widely distributed in plants. It was found that *Rutin* has antioxidant effect due to the polyphenolic flavonoid **(Kamalakannan and Prince, 2006).**

Rutin exhibits multiple pharmacological activities including: Antitumor; anti-inflammatory; anti-ulcer; anti-mutagenic and hepatoprotective activities **(Janbaz *et al*., 2002 and Kim *et al.*, 2005)**. Rutin which have been attributed to antimutagenic, antioxidant, antiviral, bactericide, cancer-preventive, hepatoprotective., and anti-inflammatory activity.

Natural oils are extensively used in cosmetic as well as in folk medicine for the treatment of growing number of more or less specific pathologies. Recently the clinical use of essential oils has expanded worldwide also including therapy against various kinds of inflammatory diseases, such as: Allergy, rheumatism and arthritis. These activities have mainly been recognized through clinical experience, but there have been relatively little scientific studies on biological actions of these natural extracts **(Serafino *et al.*, 2008).** Essential oils can interact with microbial cell membranes and inhibit the growth of some bacteria **(Calsamiglia *et al.*, 2007).**

The marker constituents of the natural antioxidant food-additive Eucalyptus extract were investigated by **(Amakura *et al*., 2009).** Two major compounds: Gallic acid, and allagic acid were identified by HPLC which have antioxidant activity by their radical scavenging ability **(Amakura *et al*., 2009).**

Eucalyptus globules L. oil contains ursolic acid, exhibited a protection against hepatotoxicity, also, euglobals have anti-tumor properties **(Shukla *et al*., 1992 and Whitman and Ghazizadeh, 1994).** Monoterpenoid components of aromatic constituents of Eucalyptus oil are traditionally used as analgesic, anti-inflammatory, and antipyretic remedies.

Phytochemical analysis has shown that the profile of the monoterpenoids changes among the Eucalyptus spp. with potential variations in therapeutic properties **(Serafino *et al.*, 2008).**

It was found that cineole is the main constituent of eucalyptus oil mainly used in inflammatory air way diseases as a mucolytic agent **(Worth *et al.*, 2009).** Eucalyptus spp. contains the essential oil in the leaves which is commonly used for medicinal purpose on average between 70 and 95% of the oil. This compound has: anesthetic; antibrochitis, antiseptic, fungicide, herbicide, pesticide and sedative. Other major components in the oil are: aromadendrene; camphene; cryptone; ρ- cymene; d-limonene; α-pinene; β-pinene; spathulenol; γ-terpinene and α-thujene **(Lis *et al.*, 1998).**

Other important compounds in the leaves, buds, branches and bark include: Antioxidant eridictyol, naringenin, quercetin, rhammazin, rhamnetin, taxifolin and Englobals which are believed to have anti-tumor promoting activity.

Evidence based research regarding the bioactivity of this herb is reviewed **(Mc Kay and Blumberg, 2006).** The phenolic constituents of the leaves include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin and hisperidin. The main volatile components of the essential oil are menthol and menthone **(Gobel *et al*., 1994).**

*In-vitro,* peppermint has significant anti-microbial and anti-viral activities, strong antioxidant and antitumor actions and some anti-allergenic potential **(Mc Kay and Blumberg, 2006)** Mentha piperita herb; plant extract and oil of peppermint health benefits. Animal studies show Mentha piperita have analgesic and anesthetic effects in central and peripheral nervous system immune system influencing actions and anticancer potential **(Yang *et al*., 2010).** Mentha piperita *L.* is one of the most widely consumed single ingredient herbal teas, or tisanes. Peppermint tea, brewed from the plant leaves and the essential oil of peppermint are used in traditional medicines.

The antioxidant activities and the determined major components of six popular and commercially available herb essential oils including lavender, peppermint; rosemary; lemon; grape fruit, and frankincense were compared **(Yang *et al*., 2010).** The major components of the essential oils of these herbs were linalyl acetate; menthol; 1,8-cineole; limonene, and p-menth-2-en-ol, respectively. Radical scavenging activity against ABTS radical was highest in peppermint essential oil.

The chemical composition of the essential oil from peppermint (Mentha piperita L.) was analyzed by GC/FID and GC/MS, the main constituents were menthol (40.7%) and menthone (23.4%). Further components were (+/-)-menthyl acetate, 1,8-cineol, limonene, β-pinene and β-caryophyllene. Mentha Piperita L. oil possessed antiradical activity with respect to DPPH and hydroxyl (OH)· radicals, exercising stronger antioxidant impact on the OH· radical.

Peppermint essential oil demonstrated antioxidant activity in a model linoleic acid emulsion system in terms of inhibiting conjugated dienes formations by 52.4% and linoleic acid secondary oxidized products generation by (76.95) at 0.1% concentration **(Schmidt *et al*., 2009).**

GS/MS study revealed cinnamaldehyde to be the major constituent of cinnamon oil, and it was the predominant active compound found in cinnamon oil **(Simic *et al*., 2004).** Cinnamon aldehyde is a natural antioxidant and the animal studies suggest that an extract of cinnamon bark taken orally may help prevent stomach ulcer **(Matan *et al.*, 2006).** The GC/MS chromatogram of Peppermint oil revealed the presence

of the following compounds: Menthol represented the highest percentage (33.7%); Menthone (20%); 1.8cineole (Eucalyptol) while myrcene was the lowest one (0.15%). The GC/MS chromatogram of Eucalyptus oil revealed the presence of the following compounds: 1.8cineole (Eucalyptol) represented the highest percentage (85%); α-pinene (2.9%) while γ-terpinene was the lowest one (0.7%). The GC/MS chromatogram of Cinnamon oil revealed the presence of the following compounds: Cinnamic-aldehyde represented the highest percentage (75.1%); Linalool (5.2%) while camphene was the lowest one (0.30%) **(Nermien and Gouda, 2011).**

It was investigated by **Svoboda and Hampson (1999)** that the bioactivity of volatile oils of selected temperate aromatic plants: anti-bacterial; antioxidant; anti-inflammatory and other related pharmacological activities. Plant volatile oils as antioxidants were investigated for their protective role for highly unsaturated lipid in animal tissues **(Deans *et al.*, 1993).**

The oils have shown their action as those of hepatoprotective agents in aging mammals. The reason that antioxidants are important to human physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive form such as superoxide, hydrogen peroxide and hydroxyl radicals, collectively known as active oxygen (**Scalzo *et al.*, 2005).** These molecules are formed in living cells by various metabolic pathways. Specific molecules, pollution from tobacco smoke and burning of fossil fuels together with UV radiation and pollutions such as ozone, nitrogen oxide, and sulphur dioxide add to the formation of free radicals, superoxide is converted by an enzyme, superoxide dismutase into $H_2O_2$, it is able to cross all biological membranes **(Siddhuraju and Becker, 2007).**

The aim of this study was to compare the anti-inflammatory activity of some volatile oils: Cinnamomum Zeylanicum N., Eucalyptus globules L. and Mentha Piperita L. oils on specific marker lysosomal enzymes "Acid phosphatase (ACP), β-Galactosidase(β-GAL), β-N-acetyl glucosaminidase (β-NAG), and β-Glucuronidase (β-GLU)" isolated from rat liver either *in-vivo or in-vi*tro.

## MATERIALS and METHODS

### Chemical Agents

Volatile oils under investigation were supplied in standard and purified materials from Dept. of phytochemistry, Medicinal plant Research center, NODCAR, Giza Egypt. All chemicals, solvents and reagents used were of analytical and pure grade. Rutin as antioxidant standard was purchased from Sigma chemical Co., St. Lewis, USA.

### Enzyme Substrates

1- p-nitrophenyl phosphate (sodium salt) was used for acid phosphatase [EC.3.1.3.2].
2- p-nitrophenyl-ß-D-galactopyranoside was used for ß-galactosidase [EC.3.2.1.23].
3- p-nitrophenyl-2-acetamido-2-deoxy- ß-D-glucopyranoside was used for N-Acetyl- ß-glucosaminidase [EC.3.2.1.30].
4- Phenolphthalein glucuronic acid (sodium salt) was used for ß- glucuronidase [EC.3.2.1.31]

*All these substrates were purchased from Sigma Chemical Co. U.S.A.*

### Preparation and Isolation of lysosomal fraction for *in-vitro* study

The lysosomal fraction was separated according to the method of **Tanaka and Iizuka (1968)** male albino rats (Sprague-Dawley) weighing 170-200g, were used.

### Preparation and Isolation of lysosomal fraction for *in-vivo* study

Male albino rats weighing 170-200g were injected with (100µl/100g rat) and after 24hrs rats were decapitated and prepared to separate the lysosomal fractions according to the method of **Tanaka and Iizuka (1968)**.

### Incubation of lysosomes with oils

In a culture tube a mixture of 1.0 ml of lysosomal fraction and 1.0 ml of oil solution at concentration of (100 and 300 µg/ml), the total volume was completed to 3.0ml by the addition of the sucrose buffer solution. In order to avoid lysosomal membrane rupture by sudden elevation of temperature, ice-cold lysosomal preparation was warmed to room temperature 2 minutes before incubation at 37°C. The culture tubes were incubated in a shaking water bath at 37°C at 30minutes; cultures of each concentration were removed and centrifuged at 19.000 r.p.m. for 15 minutes. The resulting supernatant was subjected to enzyme assay to determine the activity of the released enzymes **(Robin and William, 1978).**

### Methods of Enzyme Assay

The activity of four lysosomal acid hydrolases has been measured according to the method described by **Van Hoof and Hers (1968)** with slight modifications by **Younan and Rosleff (1974).** The principle of this method is identical for the first three lysosomal enzymes (Acid phosphatase, N-Acetyl-ß-glucos-aminidase and ß-galactosidase) depending on the reaction of each of the three lysosomal acid hydrolases with the appropriate substrate liberating p-nitrophenol (yellow color) which can be measured colourimetrically at 420 nm, together with the corresponding standard.

While the method used for measuring ß-glucuronidase depends on the reaction of this enzyme with the substrate phenolphthalein glucuronide liberating phenolphthalein (pink color) that can be measured colourimetrically at 542 nm together with the corresponding standard.

**Statistical Analysis of the Results**

The results are expressed as Mean ± SD. The collected data were statistically analyzed by the least significant differences (LSD) at the level 5% of the probability procedure according to **Snedecor and Cochran (1980).**

## RESULTS

**Lysosomal Enzymatic Activity *in-vitro***

The effect of three volatile oils: Cinnamomum Zeylanicum N., Eucalyptus globules L.and Mentha Piperita L. oils at sole and in combination together by two concentrations against standard antioxidant compound "Rutin". The activities of four lysosomal enzymes (ACP; β-GAL; β-NAG, and β-GLU) were studied.

Table (1) indicated the activities of the four lysosomal acid hydrolases in rat liver *in-vitro* under the three volatile oils sole and in combination. A significant decrease ($p<0.05$) in the activities of ACP; β-GAl; β-NAG, and β-GLU were observed for the three volatile oils by two concentrations in comparison to control group. Also, at the combined groups of oils, the lysosomal enzymatic activities appeared to be significant ($p<0.05$) decreased as compared to control group. The percentage values were altered.

**Lysosomal Enzymatic Activity *in-vivo***

Table (2) revealed the effect of the three volatile oils on the four lysosomal enzymatic activities in $CCl_4$-induced hepatotoxicity groups as compared to positive control group. This study was performed to investigate the effect of the volatile oils such as Cinnamomum Zeylanicum N., Eucalyptus globules L.and Mentha Piperita L. oils by two concentrations on the four lysosomal enzymatic activities ACP; β-GAL; β-NAG, and β-GLU in rat liver. An significant decrease ($p<0.05$) values in the activities of ACP; β-GAL; β-NAG, and β-GLU were observed. These percentage values were dependent on the concentration and each enzyme.

## DISCUSSION

It has been suggested that oxygen free radicals generated during cell toxicity or damaging effect and may also be responsible for the damage through the release of lysosomal enzymes **(Kalra and Prasad, 1994).** The increased release of acid hydrolases from lysosomes alters the metabolism of glycoproteins and glycosaminoglycans which are involved in lysosomal structure, these enzymes are involved in the destruction of structural macromolecules in connective tissue due to the enzymatic destruction of proteoglycans **(Reddy and Dhar, 1988).** These release of enzymes also stimulate the inflammatory mediators like oxygen radicals, prostaglandins etc. which stimulate tissue disruption **(Kalra and Prasad, 1994).** The increase in lysosomal enzymes produces a reduction in membrane integrity and the leakage of the enzymes from the enclosed sacs lead to intracellular dysfunction and disruption of potential substrates and organelles such as mitochondria, sacarolemmaeto **(Mayanskaya *et al.*, 2000 and Subashini *et al.*, 2007).** This behavior may be due to the hydroxyl radical is very reactive as it combines with almost all molecules found in living cells. Proteins; lipids; carbohydrates, and DNA in living cells represent oxidizable substrates. The secondary events include changes in membrane structure permeability and fluidity, lysosomal destabilization and stimulation of apoptosis. Lipid peroxidation finally leads to loss of membrane function and integrity leading to cell necrosis and death **(Siddhuraju and Becker, 2007).** Hydroxyl radicals can also react with bases in the DNA and cause mutations. Oxygen free radicals appear to be an important factor in chronic inflammatory joint disease such as rheumatoid arthritis. There are several known reactions of oxygen centered free radicals which are relevant to tissue injury in inflamed joints. Superoxide and hydrogen peroxide can stimulate growth in a variety of malignant cell types. They may have an important role as extracellular messengers for cell growth and viability.

Phenolics, vitamin E and some drugs such as non-steroidal anti-inflammatory can stabilize the membranes by decreasing their permeability and they also have an ability to bind free fatty acids. It has been suggested that volatile oils could act as such agents. It has been found that certain volatile oils and their components are cytostatic to tumor cells lines and can offer potential as novel antiproliferative agents screened for their antioxidative effect using a TBA assay**(Dorman *et al.*, 1995)**. Antioxidant properties are also found in the volatile oil fraction. However, it is very important to realize that in certain cases, antioxidants can be pro-oxidant and can stimulate free radical reactions. Pre-treatment with medicinal plant extract which have antioxidant components such as: Flavonoids, polyphenolic compounds are able to decrease the release of lysosomal enzymes which could due to the membrane stabilizing effect of the medicinal plant on the lysosomal membrane **(Rice-Evancs *et al.*, 1996).**

It was investigated that the antioxidant activity of Eucalyptus oil has an active ingredients which

revealed by the phytochemical analysis as indicated that the profile of the monoterpenoids changes among the Eucalyptus species, with potential variations in therapeutic properties. It was found that, in Eucalyptus globules, the major monoterpenoid component is eucalyptol (1,8-cineole) by 60-90%, that has been reported to inhibit the production and synthesis of tumor necrosis factor-α-TNF, inter leukin-1B (1L-1B),leukotriene B4, and thromboxane B2 in human blood monocytes, anti-inflammatory activity of eucalyptol in patients was observed by **Serafino *et al.* (2008)**.

It was investigated that the antioxidant activity of Rutin appeared to be highest values in the order of Cinnamon, this can be attributed to the higher phenolic content **(Nermien and Gouda, 2011).** The close correlation between the antioxidant activity and the phenolic contents obtained from various natural sources **(Liu *et al.*, 2007; Verzelloni *et al.*, 2007 and Erkan *et al.*, 2008).** It was clearly revealed that the number of phenolic-OH groups in the structure of an antioxidant molecule isn't always the only factor determining its antioxidant activity. Also, position of phenolic-OH groups, double bonds and their conjugation to (-OH) groups and ketonic group; also play important roles in antioxidant activities **(Rice-Evans *et al.*, 1996).** Recently, the polarity and activities and hydrophobicity of antioxidants, besides the above factors play important roles in their activity, especially biomembrane systems **(Wu *et al.*, 2007).**

Table (1): Effect of Cinnamon (CIN); Eucalyptus (EUC), and Peppermint (PEP) oils sole and in combination by two doses (Low dose;100 and High dose ;300 µg/ml) and Rutin (RU) as standard antioxidant in comparable against normal control (NC) on the four marker lysosomal enzymatic activities (ACP, Acid phosphatase; β-GAL, β- galactosidase, β-NAG, β-N-acetyl glucosaminidase and β-GLU, β-Glucuronidase) in rat liver lysosomes after 30 minutes of incubation *in-vitro* (n=8).

| Treatments | The lysosomal enzymatic activities expressed as mean ± S.D by nmole/ml/hr | | | |
|---|---|---|---|---|
| | "ACP" | "β-GAL" | " β-NAG" | " β-GLU" |
| **NC** | 4816.12±0.008 | 1033.92±0.043 | 1459.65±0.032 | 432.75±0.002 |
| ***Relative %*** | 100% | 100% | 100% | 100% |
| **RU 100** | 2463.26±0.024 | 920.99±0.184 | 1015.21±0.047 | 210.53±0.007 |
| | 51%* | 89%* | 41%* | 49%* |
| **RU 300** | 3285.58±0.042 | 935.67±0.019 | 1345.03±0.093 | 350.88±0.014 |
| | 68%* | 91%† | 54%* | 81%* |
| **CIN 100** | 1588.84±0.023 | 273.68±0.065 | 676.02±0.044 | 136.38±0.023 |
| | 33%* | 26% * | 27%* | 32%* |
| **CIN 100** | 2560.00±0.047 | 499.42±0.089 | 877.19±0.031 | 185.84±0.040 |
| | 53%* | 48%* | 35%* | 43%* |
| **EUC 100** | 1789.77±0.036 | 570.76±0.036 | 957.90±0.022 | 181.74±0.022 |
| | 37%* | 55%* | 38%* | 42%* |
| **EUC 300** | 3006.51±0.075 | 809.36±0.028 | 836.26±0.033 | 194.29±0.027 |
| | 62%* | 78%* | 34%* | 45%* |
| **PEP 100** | 1626.05±0.028 | 519.30±0.028 | 486.55±0.018 | 108.77±0.040 |
| | 34%* | 50% * | 20%* | 25%* |
| **PEP 300** | 2887.44±0.05 | 800.00±0.032 | 801.17±0.036 | 149.71±0.058 |
| | 60%* | 77%* | 32%* | 35%* |
| **CIN+ EUC 100** | 1138.60±0.036 | 384.80±0.030 | 970.76±0.082 | 307.60±0.011 |
| | 24%* | 37%* | 39%* | 71%* |
| **CIN+EUC 300** | 3717.21±0.068 | 451.46±0.036 | 1314.62±0.086 | 370.36±0.038 |
| | 77%* | 44%* | 53%* | 86%* |
| **CIN+ PEP 100** | 2150.70±0.046 | 684.21±0.049 | 581.29±0.021 | 257.31±0.006 |
| | 45%* | 66%* | 23%* | 59%* |
| **CIN+PEP 300** | 3226.05±0.091 | 906.43±0.059 | 1014.04±0.046 | 300.58±0.011 |
| | 67%* | 88%* | 41%* | 69%* |

Data are expressed as mean± S.D of eight rats.
Significant different from Negative Control group at *P < 0.05, †: Insignificant at P> 0.05.

Table (2): Effect of Cinnamon (CIN); Eucalyptus (EUC), and Peppermint (PEP) oils by two doses (Low dose;100 and High dose ;300 µg/ml) and Rutin (RU) as standard antioxidant after injection of $CCl_4$ (100µl/100g rat) in comparable against positive control (PC) on the four marker lysosomal enzymatic activities (ACP, Acid phosphatase; β-GAL, β- galactosidase, β-NAG, β-N-acetyl glucosaminidase and β-GLU, β-Glucuronidase) in rat liver lysosomes after 30 minutes of incubation *in-vitro* (n=8).

| Treatments | The lysosomal enzymatic activities expressed as mean ± S.D by nmole/ml/hr | | | |
|---|---|---|---|---|
| | "ACP" | "β-GAL" | " β-NAG" | " β-GLU" |
| **PC** | 3034.42±0.011 | 2902.92±0.053 | 1555.56±0.026 | 1326.32±0.013 |
| *Relative %* | 100% | 100% | 100% | 100% |
| **Ru 100+ PC** | 2195.35±0.032 | 2238.60±0.010 | 792.98±0.031 | 535.67±0.031 |
| | 72%* | 77%* | 51%* | 40%* |
| **Ru 300+ PC** | 2805.58±0.004 | 2500.58±0.087 | 804.68±0.030 | 479.53±0.026 |
| | 92%† | 86%* | 52%* | 36%* |
| **CIN 100+PC** | 1856.74±0.131 | 2350.88±0.020 | 697.08±0.040 | 340.35±0.023 |
| | 61%* | 81%* | 45%* | 26% * |
| **CIN 300+PC** | 2671.63±0.041 | 2608.19±0.017 | 1146.20±0.068 | 442.11±0.029 |
| | 88%* | 90%* | 74%* | 33%* |
| **EUC 100+PC** | 2790±0.041 | 2759.06±0.011 | 674.85±0.059 | 557.89±0.006 |
| | 92%† | 95%† | 43%* | 42%* |
| **EUC 100+PC** | 3003.49±0.012 | 2685.38±0.049 | 1163.74±0.092 | 553.22±0.031 |
| | 99%† | 93%† | 75%* | 42%* |
| **PEP 100+PC** | 1905.12±0.032 | 2122.81±0.009 | 646.78±0.027 | 409.36±0.026 |
| | 63%* | 73%* | 42%* | 31%* |
| **PEP 300+ PC** | 2880.00±0.017 | 2401.17±0.025 | 1250.29±0.035 | 733.33±0.047 |
| | 95%† | 83%* | 80%* | 55%* |

Data are expressed as mean± S.D of eight rats.
Significant different from Positive Control group at *P < 0.05, †: Insignificant at P> 0.05.

It was obviously that the scavenging effect of Rutin and other volatile oils on the DPPH radical decreased in the order of Rutin> Cinnamon> Peppermint at the concentration of 0.2 mg/ml **(Nermien and Gouda, 2011).** The percentage inhibition of the volatile oils was decreased in the order of Cinnamon> Eucalyptus> Peppermint as compared to the Rutin which have a highest inhibitory effect. The OH scavenging activity of Rutin and other similar to those reported by **(Caillet *et al*., 2007).**
The total phenolic contents of the volatile oils under investigation Rutin, Cinnamon, Peppermint, and Eucalyptus exerted a different total phenolic contents. The main phenolic compounds and flavonoids groups are quercetin, kaempferol, myricetin, apigenin and leuteolin **(Siddhuraju and Becker, 2007).**
Total antioxidant capacity and total phenolic contents showed different correlations depending on species, but it might not always correlate with the amount of phenolics **(Scalzo *et al.*, 2005).**

It was found by **Buchbauer and Jirovetz (1994)** that volatile oils either inhaled or applied to the skin act by means of their lipophenolic fraction reacting with the lipid parts of the cell membranes and as a result modify the activity of the calcium ion channels. At certain levels of dosage, the volatile oils saturate the membranes and show effects similar to those of local anesthetics, they can interact with the cell membranes by means of their physiochemical properties and molecular shapes, and can influence their enzymes, carriers, ion channels and receptors.

## CONCLUSION

It was concluded that Cinnamomum Zeylanicum N., Eucalyptus globules L.and Mentha Piperita L. oils by the two concentrations and their mixtures exerted an inhibitory effect by different

percentage values of inhibition due to their antioxidant activity of these oils on the membrane permeability of the lysosomes. In addition to, the inflammatory effects of these oils on the lysosomal enzymes, ACP; β-GAL; β-NAG, and β-GLU were appeared by different degrees of release either *in-vitro* or *in-vivo* studies. Such stabilization of the lysosomes may have a beneficial effect in various hepatic disorders involving abnormal fragility of the lysosomes. The observed effects of these volatile oils under investigation were due to their stabilization and the antioxidant effects on the lysosomal membrane permeability.

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