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RESEARCH ARTICLE

Antioxidant activity of purified Eugenol compound in some Dairy products

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Abstract

A volatile oil of flower buds cloves was extracted through steam distillation using a Clevenger, the qualitative active groups in cloves volatile oil detection was carried out, then the results showed a presence of all active groups except glycosides and Coumarins. Thin layer chromatography technique (TLC) of clove volatile oil was carried out with standard sample compression, the Rate flow was (Rf) of clove volatile oil reached to 0.59, this result is equal to the Rf of standard compound. quantitative and qualitative detection of volatile oil compounds of clove buds leafs was carried out by using high performance liquid chromatography technique (HPLC), the result was showed presence of Eugenol compound in retention time 11.517 minutes while the standard Eugenol was recorded 11.373 minutes which indicates the purity of compound, in addition analysis with Ultra Violet (UV) - Visible to insure the Eugenol compound purity at a wavelength 300 nm. The antioxidant activity of purified eugenol compound in concentrations (0.1, 0.2, 0.4) were tested in three yogurt product Butter, Local cream and Local yogurt (Irbil yogurt) through tree indicators detection due peroxide number, free fatty acid and Total Nitrogen (TN) values for purified compound those compared with negative control (without treatment) and positive control (treatment with Sorbic acid), The concentration 0.4% of the Eugenol compound gave a highly antioxidant activity more than other concentrations through significant decreasing at ($P \leq 0.05$) in peroxide number and the percent of free fatty acid and the total Nitrogen quantity (TN) in the samples that treated with eugenol compound was reached (6.2, 3.6, 3.2) milli-equivalent of O_2/Kg, (4.03, 1.79, 1.42) % and (3.03, 1.44, 1.33) milligram nitrogen/100g respectively comparison with negative control samples (untreated) which reached (19.55 ،28.43 ،17.22) milli-equivalent of O_2/Kg, (18.22, 25.33, 17.56) % and (14.66, 14.27, 10.54) milligram nitrogen/100g, while didn't reached significant with positive control (samples was treatment with sorbic acid) reached (6.9, 3.9, 3.01) milli-equivalent of O_2/Kg, (5.43,1.91,1.15) %, (3.11,1.04,0.93) milligram nitrogen/100g respectively. we conclude the antioxidant activity may be go to the high rang to the purity of eugenol compound and its content to number of aliphatic and aromatic molecules and alcohols and phenols higher than other concentration, and these play important role in antioxidant activity, we can using the eugenol compound instead to chemical compounds that's have highly healthy effect.

INTRODUCTION

Carnation *Syzygium aromaticum* is a plant belong to the family *Myrtaceae*. Culturing in many countries like

India, Madagascar, Sri Lanka, Indonesia and south of China, at present, Carnation *S. aromaticum* culturing in Bangladesh in limited zone (FAO, 1989). *S. aromaticum* contain many compounds such as (Eugenol) which considered as one of essential compound for *S. aromaticum* oil, which produced from flowers budding of *S. aromaticum*, whereas contain Eugenol compound and Eugenol acetate 4- allyl-2-methoxyphnol acetate β-Caryophyllene; trans – (1R, 9S) -8- Methylene – 4, 11, 11- , bicycloundec -4- ene trimethylbicycloundec-4- ene, and 60 – 90 % of another secondary compounds (Kamel et al., 2007).

Many studies proved that *S. aromaticum* has an anti oxidants activity (Singh and Goel, 2012). In addition to containment of many active compounds some of them are Kaempferol and Vanilic acid (Bhowmik et al., 2012). Also a containment of active group some of them are Tannins, Saponins, Alkaloids and Phenols, also considered as important aromatic spices which contains a necessary essential and responsible compounds of antimicrobial activity (Kumar et al., 2012), also has antimicrobial activity against bacteria and fungi (Bhowmik et al., 2012; Pandey and Singh, 2011). Also *S. aromaticum* oil can be used as anti cancer because of his properties as anti oxidant. *S. aromaticum* oil give many medicinal effects including anti infection of trachea, emitting, analgesic, antispasm, degassing, probiotic of renal function and disinfectant, also it can use wildly as sweets flavors, sauce and special spices. Due to medicinal properties of *S. aromaticum*, it's used in drugs especially in preparing of gums, teeth, dyes and juices (Atal and Kapur, 1982). The oil of *S. aromaticum* has inhibition activity for the germs, fungi and Insect repellent (Liu et al., 1997). As well as its antioxidant properties, and used as flavor of certain antimicrobials at food, also used as analgesic and a natural antiseptic used in dentistry in the first place, in addition to that as a treatment to reduce dental pain, also it can be used for medical drugs as flavor that address the Inflammatory bronchial, Common cold, Fiver, Sore throat, also as an antispasmodic, Anti neural pain, gas expellant, anti-infection, stimulator and Uterus activator (Huang *et al.*, 2002; Velluti *et al.*, 2003).

Eugenol compound considered as Phenyl propane compound, which is belong to a phenyl groups, this group has a great importance in the prevalence of aromatic odor of plant could isolates from volatile and constant oils of plant tissues and isolating volatile Terpenes which they salsikih fats (soluble in fats), which are Natural phenolic compounds have an aromatic ring, this aromatic ring has a three carbon atoms linked to the side chain is derived from the Biosynthesis of amino acid like Cyclic Propane Phenylalanine, contains one or more of C6-C3 residues, which they are widespread as Hydroxycinnamic acids, these acids are very important to bullied lignin, in addition to a relation with a growth regulators, and in cases of disease resistance, this Phenolic compound can spoil proteins and interacts with phospholipids (Briozzo, 1989; Deans and Ritchie, 1987).

Materials and methods

Reagents and solutions are prepared according to methods that described by (Stahl, 169) and (Atlas et al., 1995), buds of *S. aromaticum* obtained from national center of herbs, buds classified by Faculty of science in University of Baghdad by the document No. 67 in 21/02/2013. Flowers are grinded for getting flowers powder of *S. aromaticum* and kept in dark bottles till be used, the method of Clevenger is used for extraction of cloves oil from *S. aromaticum* plant, 150 grams of cloves plant buds puts in a round bottom flask volume two litters, then 1850 ml of acidified distilled water added to the flask and joined with Clevenger equipment, the equipment is controlled for getting directed aqueous distilling after put it under heating, during aqueous distilling, oil will separate from water by condensing water vapor, then distilled water collect by a funnel linked with glass continuer, distillation process continues until disappearance of color, after that, oil is separated from the water layer by separating funnel where the neglect of water class to get (3.5 ml) of oily layer brown, methods of Shihata 1951, is followed to detect the major active groups, they are (Tannins, Flavonoids, Phenols, Terpenes, Alkaloids, Coumarins , Glycosides, Resins, Steroids and Sapindales). For qualitative detection about Eugenol compound, the method of Harborn 1988 is used, where the stationary phase is silica gel plate and solution of Normal Hexan- Chlolroform in volumetric ratio (2:3) respectively, to give colored spots when exposed to ultraviolet (UV) ray with a wavelength 264 nm, the migration of colored molecules are measured (Rate fraction –Rf), while the technique of high performance liquid chromatography (HPLC) is used for purification and quantification of phenolic compounds in volatile oil of cloves buds plant, the mobile phase solution is consists of (Acetonitrile: Deionized bidistilled water) in volumetric ratio (40:60) respectively, then mixed by Vortex to remove air bubbles to become ready for use. Separation is done by the column C18 with flow rate 1.4 ml / min and wavelength 210 nm, 10 µl of volatile oil of cloves buds plant, retention time of materials in the extract compared with retention time of standard compound. Optical density was measured by a spectrophotometer – Ultra Violet (UV) type (Shimadzu, made in Japan) and compared with standard Eugenol by using (DMSO) as a control. Purified Eugenol is mixed with samples of Better, local Cream, Yogurt (local mark named as Laban Arbil) with percentage (0.1, 0.2 and 0.4) % for each sample separately, results is compared with control samples (un treated) and better samples, local cream and Laban Arbil treated with Sorbic acid in concentration 1 gm / kgm (positive control) each sample separately, and kept under temperature of 25 °C for one

hour to select optimum concentration for anti-oxidation activity, activity of optimum Eugenol examined with samples of better and local cream and laban Arbil for a period of store (1, 24, 48, 72 and 96) hours at 25 °C separately. The number of peroxide and the percent of total nitrogen and free fatty acid as Oleic acid are estimated in samples according to (Pearson, 1976). Induction period activity, which is the number of days needed to reach the peroxide value of the model to 20 ml equivalent to O_2 / kg of Oil, in Indication that period is obtained Protection factor-(PF) which is Identifying the change in the detection of oil period after addition of compound according to following equation:

$$\text{Protection factor-(PF)} = \frac{\text{Detection period of treated sample in extract IPA}}{\text{IPB Detection period of control sample}}$$

If the range among (1-1.5), protection factor is so weak, but if it will be among (2-1.5) protection factor is intermediate, and if reached to (3-2.5) protection factor will be high, in other hand protection factor will be so high when reached more than 3 according to (Yingming et al., 2004). A program of SPSS in statics for getting t-test in significant limits ($P \leq 0.05$).

Results and Discussion

The volatile oil obtained from buds of *S. aromaticum* plant as a quantity, 3.5 ml per 150 gm from *S. aromaticum* powder, were results of quality detection of active groups for volatile due to detection of Alkaloids, Sapindales, Phenols, Glycosides, Phenols, Tannins, Flavonoids and Terpenes, these results are similar to what provide by (Sunil et al., 2013).

Table (2): Chemical active compounds in *Syzygium aromaticum*

Plant	Active group							
Q.infectoria	**Alkaloids**	**Terpenes and Steroids**	**Coumarins**	**Flavonoids**	**Tannins**	**Sapindales**	**Resins**	**Phenols**
	+	-	-	+	+	+	+	+

Plates of silica gel (thin layer chromatography) (P.64) are used for purification and qualified detection of volatile oil compounds from buds of *S. aromaticum* plant, Where the rate of Retention factor (Rf) was 0.59 Eugenol in extracted samples with dark brown color near separating area compared with Eugenol standard sample which reached to (Rf) (0.59) and equal flow rates in proving get more pure compound of Eugenol as shown in figure (1). This result become similar to (Bhowmik et al., 2012), when he said that *S. aromaticum* contain Eugenol. This area was taken by abrasion, and tested by chemical tests by using the technique of High Performance Liquid Chromatography (HPLC), UV/Visible Spectrophotometer, retention time of Eugenol are fixed by HPLC for purified *S. aromaticum* which reached (11. 517 minute) compared with standard sample which reached (11.373 minute), this similar to (Rahami, 2012), where he provide that *S. aromaticum* contain a high proportion of Eugenol figures (2 and 3).

Table (3): Standard Retention Time and Concentration of standard compounds

Compound	Retention time (minute)	Area	Concentration (ppm)	Percent %
Eugenol	**11.517**	**98729.248**	**1599.4**	**15.99**

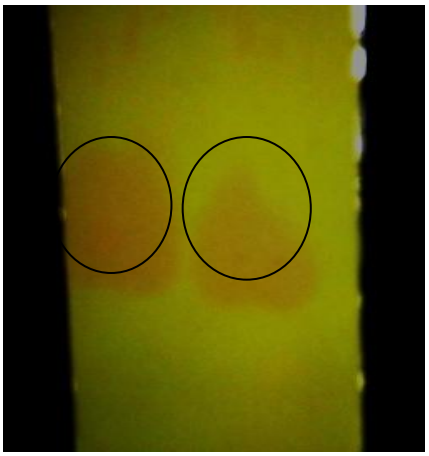

Figure (1): Thin layer chromatography, to detect extract Eugenol from buds of *S. aromaticum* compared with standard Eugenol

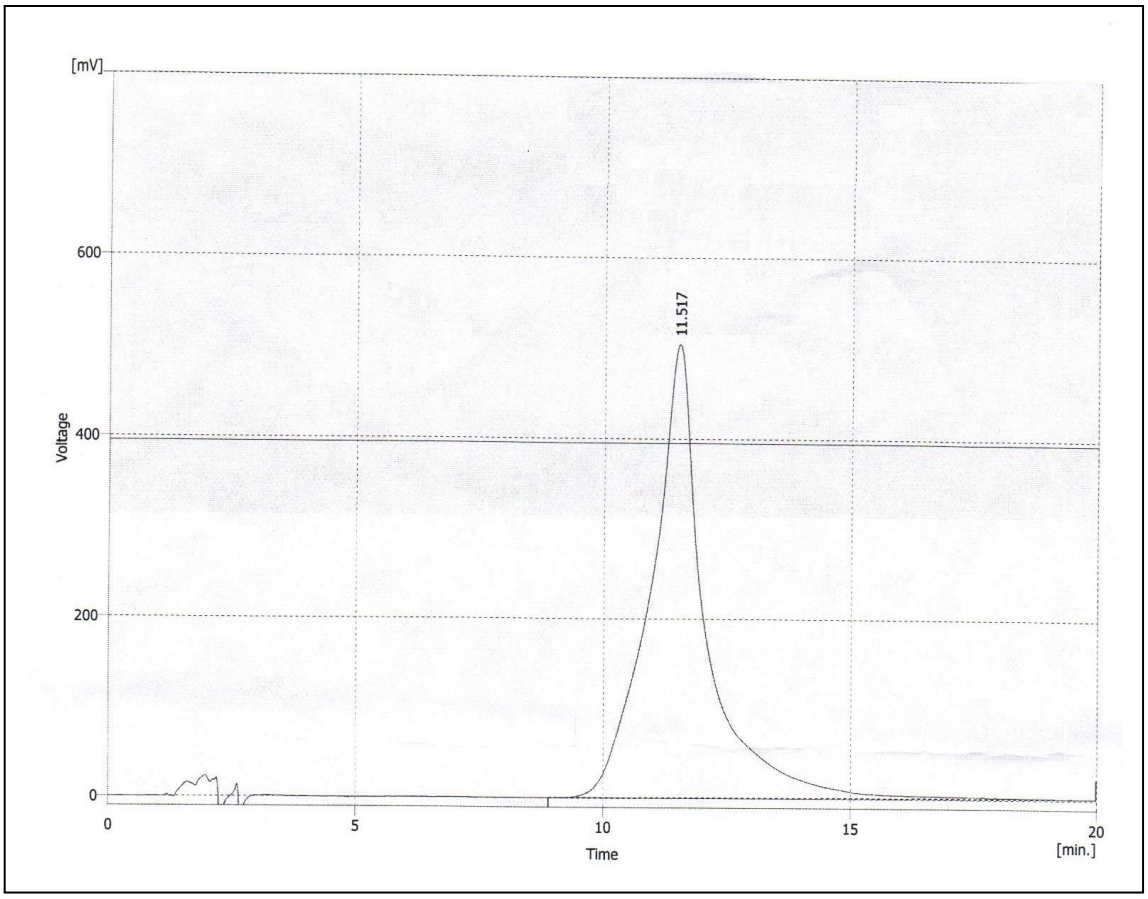

Figure (2): HPLC diagram, to detect Eugenol Compound in extracted Oil from *S. aromaticum* buds at Wavelength 210 nm.

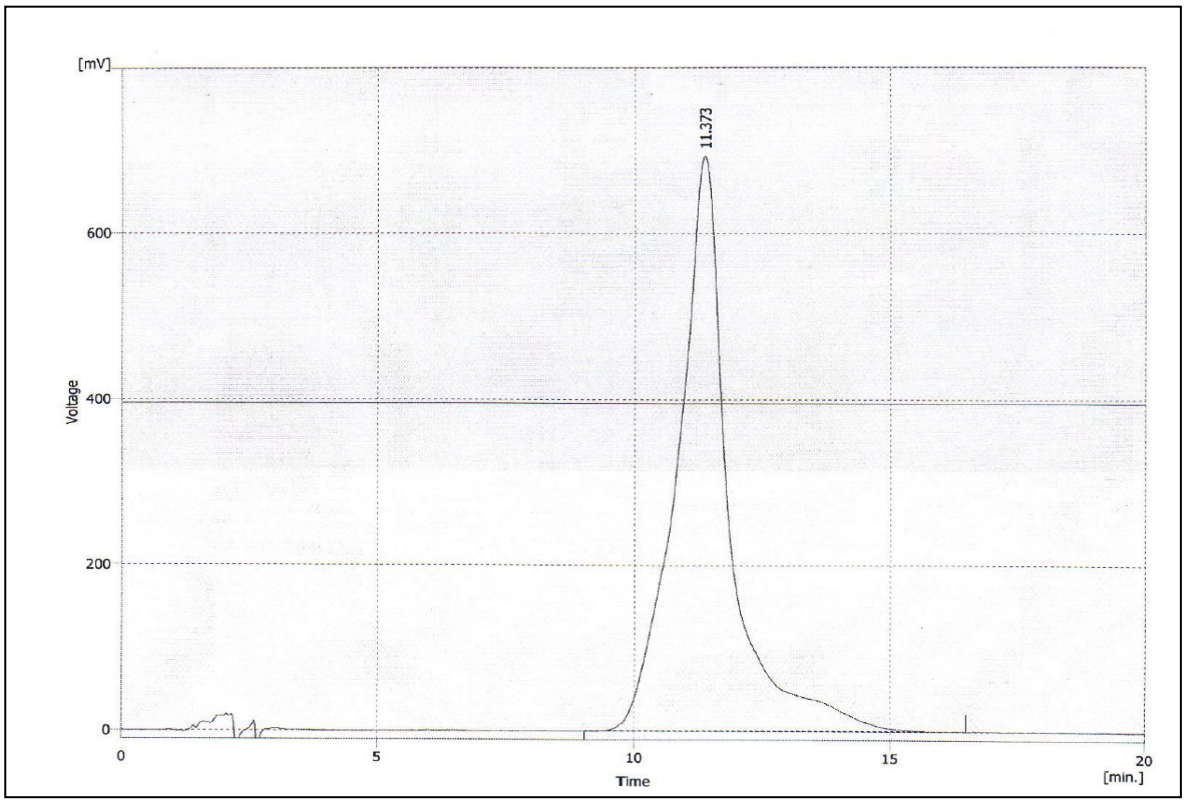

Figure (3): HPLC diagram, to detect Eugenol Compound in standard compound for Oil from *S. aromaticum* buds at Wavelength 210 nm

For determination of extracted sample by using technique of visible Spectrophotometer and Ultra Violet radiation (UV) at wave length 300 nm, many concentration of standard Eugenol are tested and they were 4, 2, 1, 0.5 and 0.25 ppm respectively, the optical density are recorded they were 3.913, 3.411, 3.011, 2.814 and 2.791 nm respectively, the concentration of pure Eugenol was 3.85 ppm according to use obtained equation from the chart of different concentration which applied them optical density of sample to get its concentration, this result is similar to results of (Rahimi, 2012), which proved a compound Eugenol optical density at 3 nm, the chart of standard Sample depending on obtained results, then slop is calculated (Figure 4).

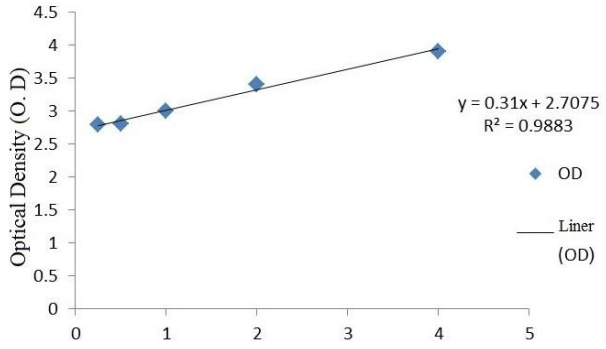

Figure (4): Chart of different concentrations of Standard Eugenol

Eugenol is one of Phenyl Propane like Anethole, Estragol and Cinnamic aldehyde which Existing in metabolic pathway sequences in fixed oils of *S. aromaticum* plant, Eugenol is weak able to soluble in water and have a

significant benefit more than other compounds which used in many things, on the other qualities is a clear pale yellow oil, clear and has a pungent taste, it can be used with the rest of the components of the spice in many biomedical applications in the human body, the purity of the purified sample Indicate to the efficiency of extraction and purification processes, the evaluation of antioxidant for purified Eugenol for the concentration (0.1, 0.2 and 0.4) % for three criterions which they are quantification of peroxide number, proportion of free fats and quantity of Total Nitrogen (TN) for treated with different concentration of Eugenol compared with negative control (untreated sample) and positive control (treated sample with Sorbic acid), we noticed indeed, that purified Eugenol at the concentration [0.4]% give activity as antioxidant better than other concentrations at significant decreasing when ($p \leq 0.05$) of peroxide number, free fats percent and quantity of (TN) for those samples which treated with purified Eugenol at the concentration [0.4] %, the significant decreasing is done when ($p \leq 0.05$) and reached (3.2, 6.3 and 6.2) milli-equivalent of O_2 / kg, (1.42, 1.79 and 4.03) % and (1.33, 1.34 and 3.03) mg N / 100 gm for samples of better cream and yogurt respectively compared with negative control sample (un treated) which reached to (17.22, 28.43 and 19.55) milli-equivalent of O_2/kg, (17.56, 25.33 and 30.22) % and (10.54, 14.27 and 18.66) mg N /100 gm and other concentration of purified Eugenol, the lesser activity of antioxidant was after treatment of samples with concentration of 1% from Eugenol, results was 11.33, 17.67 and 23.67 milli-equivalent O_2/kg, (12.33, 19.63 and 24.5) % and (8.12, 11.67 and 11.67) mg N/100gm respectively, but the differences between concentration of pure Eugenol at 0.4 % and comparison sample (positive control sample), didn't inform the limits of significant at ($p \leq 0.05$), where the effectiveness of anti-oxidants activity to control positive (treated sample with benzoic acid $C_7H_6O_2$) reaching to (3.01, 3.9 and 6.9) milli-equivalent of O_2 / kg, (1.15,, 1.91 and 5.43) % and (0.93, 1.14and 3.11) mg N/100 gm for better sample, local cream and yogurt (Laban Arbil) respectively. There a variation of a containment of pure Eugenol compound for a proportions of active chemical groups some of them are aliphatic and aromatic molecules in addition of alcohols and phenols more than other groups, these groups play an important roles in activity of antioxidants, where (Hodnick et al., 1988) shows that mono phenol activity as antioxidant depend on additional additives, the number and arrangements of phenolic alternatives also its molecular weight, and corresponded to the what was said by (Anderson et al., 2004), the quantity of plant extracts as effective groups represented by phenolic contains which in turn is a natural anti-oxidant.

Table (3): Activity of antioxidant for pure Eugenol compound in butter samples

Type of extract / Treatments	Number of Peroxide (milli-equivalent O_2/kg)	Total Nitrogen (TN) (mg N/100 gm)	Free fatty acids proportion (calculated as oleic acid) expressed as a percentage
	Mean ± standard error		
Negative control (Un treated)	19.55 ^{a} ± 1.00	16.58 ^{a} ± 0.53	30.25 ^{a} ± 2.78
Positive control (Treated with Sorbic acid 100 mg /kg)	6.76 ^{c} ± 0.77	5.43^{bc} ± 2.47	3.11^{b} ± 0.52
Purified Eugenol compound in concentration [0.4] %	6.20 ^{cd} ± 0.56	4.03 ^{c} ± 1.02	3.03 ^{c} ± 1.39
Purified Eugenol compound in concentration [0.2] %	11.60 ^{d} ± 0.45	7.03^{d} ± 0.38	7.03 ^{d} ± 0.26
Purified Eugenol compound in concentration [0.1] %	13.5 ^{b} ± 0.95	18.67 ^{b} ± 0.83	16.67 ^{b} ± 0.15

- Different letters in mono column refer to a presence of significant differences.
- Probability when ($P \leq 0.05$)
- Results are the average of three replications

Table (4): Activity of antioxidant for pure Eugenol compound in local cream

Type of extract / Treatments	Number of Peroxide (milli-equivalent O_2/kg)	Total Nitrogen (TN) (mg N/100 gm)	Free fatty acids proportion (calculated as oleic acid) expressed as a percentage
	Mean ± standard error		
Negative control (Un treated)	$18.22^{a} \pm 1.00$	$16.58^{a} \pm 0.53$	$30.25^{a} \pm 2.78$
Positive control (Treated with Sorbic acid 100 mg /kg)	$3.9^{c} \pm 0.07$	$1.91^{bc} \pm 0.04$	$1.14^{b} \pm 0.05$
Purified Eugenol compound in concentration [0.4] %	$6.3^{cd} \pm 0.56$	$1.79^{c} \pm 1.02$	1.34 c ± 1.39
Purified Eugenol compound in concentration [0.2] %	$11.60^{d} \pm 0.45$	$7.03^{d} \pm 0.38$	$12.03^{d} \pm 0.26$
Purified Eugenol compound in concentration [0.1] %	$18.67^{b} \pm 0.83$	$7.63^{b} \pm 0.19$	$7.67^{b} \pm 0.15$

- Different letters in mono column refer to a presence of significant differences.
- Probability when ($P \leq 0.05$)
- Results are the average of three replications

Table (5): Activity of antioxidant for pure Eugenol compound in local yogurt (Laban Arbil)

Type of extract / Treatments	Number of Peroxide (milli-equivalent O_2/kg)	Total Nitrogen (TN) (mg N/100 gm)	Free fatty acids proportion (calculated as oleic acid) expressed as a percentage
	Mean ± standard error		
Negative control (Un treated)	$14.66^{a} \pm 1.00$	$16.58^{a} \pm 0.53$	$30.25^{a} \pm 2.78$
Positive control (Treated with Sorbic acid 100 mg /kg)	$3.01^{c} \pm 0.77$	$1.15^{bc} \pm 0.047$	$0.93^{b} \pm 0.002$
Purified Eugenol compound in concentration [0.4] %	$3.2^{cd} \pm 0.56$	$1.42^{c} \pm 1.02$	$1.33^{c} \pm 1.39$
Purified Eugenol compound in concentration [0.2] %	$11.60^{d} \pm 0.45$	$7.03^{d} \pm 0.38$	$12.03^{d} \pm 0.26$
Purified Eugenol compound in concentration [0.1] %	$12.33^{b} \pm 0.83$	$12.33^{b} \pm 0.12$	$0.03^{b} \pm 8.12$

- Different letters in mono column refer to a presence of significant differences.
- Probability when ($P \leq 0.05$)
- Results are the average of three replications

For detection about Periods of Storage in antioxidant activity for purified Eugenol compound at [0.4] % in treated butter samples by purified Eugenol compound due to its Inhibition activity for microorganisms results from previous researches for researchers, another experiment was designed and compare with control samples (un treated) and positive control (treated butter with Sorbic acid at concentration of 1gm /kg) for period of storage (1, 24, 72 and 96) hours after treatment at 25 °C, Showed varying results of peroxide number, free fatty proportions and total N of treated samples with Eugenol for different storage periods as shown in figures (5, 6 and 7), as it is noticed a decreasing in peroxide number which reached after comparing with control sample. Eugenol compound at concentration 0.4 % and reached (7.23, 13.57, 16.33, 19.67 and 24.14 ml equivalent / kg, and acquired a significant difference ($P \leq 0.05$) with negative control samples which reached (19.55, 37.67, 41.33, 45.78 and 50.65) milli-equivalent O_2/ kg, while significant did not show comparing with treated samples by Sorbic acid at concentration 1gm/kg which reached to (8.88, 14.23, 19.67, 23.50 and 29.33) milli-equivalent O_2 / kg.

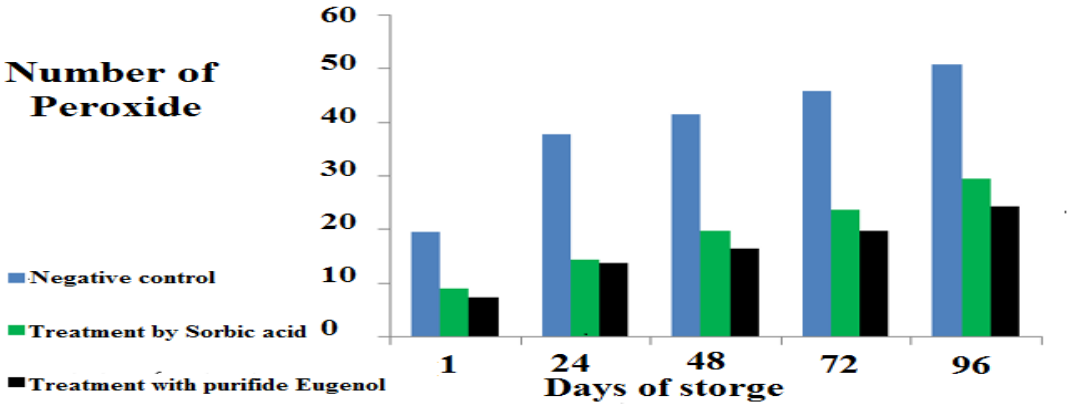

Figure (5): Activity of decreasing peroxide number of Butter samples for different treatments at storage periods (1, 24, 48, 72 and 96) hours

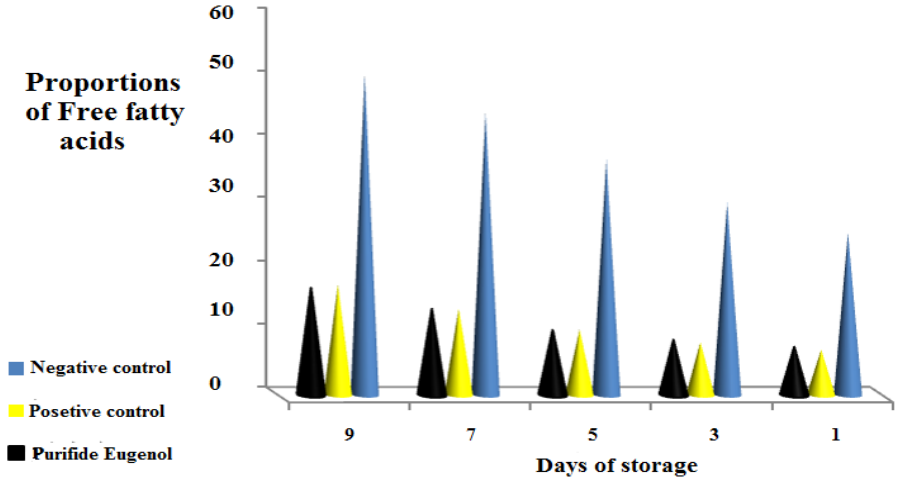

Figure (6): Activity of decreasing proportions for free fatty acids of Butter samples for different treatments at storage periods (1, 24, 48, 72 and 96) hours

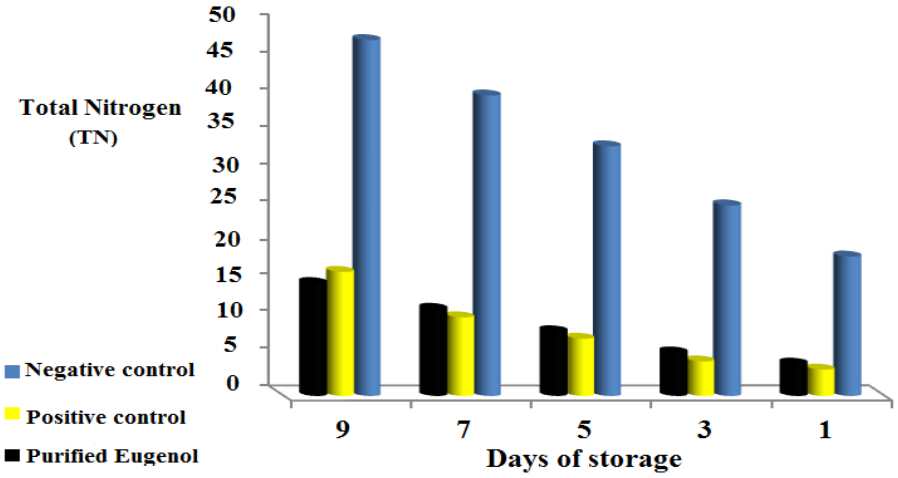

Figure (7): Activity of decreasing Total Nitrogen (TN) of Butter samples for different treatments at storage periods (1, 24, 48, 72 and 96) hours

Table (4): showed the time period to keep peroxide number less than 20) milli-equivalent O_2/kg fat which if more than this number that means for rancidity of fat (Induction period-IP) was changed between control sample and positive control sample (treated with Sorbic acid) and those treated with Eugenol compound, which results showed, treatments with purified Eugenol compound at concentration [0.4] % was better in treatment along three days compared with positive control which was two days, in addition to difference in the period of detection between treatment with pure Eugenol with 0.4% and those treated with Sorbic acid, detection period showed Protection factor (Pf) for samples treated with Eugenol which showed activity of anti-oxidant better than other treatments, as showen in table 4.

Table (4): detection period and protection factor of purified Eugenol compound with concentration [0.4%]

Criterion	Control (untreated butter)	Positive control (treated butter with Sorbic acid	Purified Eugenol at concentration 0.4%
Detection period	1	2	3
Protection factor	1	2	3

- Results are mean of three replicates

From the results of anti-oxidants for different periods of storage, concludes that differences in the effective of containment and nature of the chemical compounds and contain hydroxyl groups (OH^-), their number and degree of polymerization for compounds (Hodnick *et al.,* 1988), as well as contains a lot of amounts from these anti-oxidants which they are non-polar and high phenolic compounds well as being a product of normal medical and therapeutic plants has a significant impact, the purified compound Eugenol advantage in effectiveness of anti-oxidants and protect food products from rancidity operations also damage food a substitute for chemical preservatives, which can have an adverse impact health

References:

Anderson, **R. A**., Broadhurst, C. L., Polansky, M. M., Schmidt, W. F., Khan, A., Flamgan, V. P., Schoene, N. W. and Graves, G. L. (2004). Isolation and characterization of polyphenol type A polymers from cinnamon with insulin-like biological activity. J. Agric. Food. Chem., 52: 65-70.

Atal, C. K. and Kapur, B. M. (1982). Cultivation and Utilization of Aromatic plants. CSIR, Jammu-Tawi, India, pp. 136-741.

Atlas, R. M., Brown, A. E. & Parks, L. C. (1995). Laboratory Manual of Experimental Microbiology-Mosby Company-Yearbook, Inc.

Bhowmik, D., Kumer, K. P., Yaday, A., Srivastava, S. and Paswan, S. (2012) Recent Trends in Indian Traditional Herbs Syzgium aromaticum and its Health Benefits. Journal of Pharmacognosy and Phytochemistry,1(2):6-17.

Briozzo, J. (1989). Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J. Applied Bacteriol. 66: 69-75.

Deans, S. G. and Ritchie, G. (1987). Antibacterial properties of plant essential oils. Int. J. Food Microbiology, 5: 165-180.

Harborne, J. B. (1988). Phytochemical Methods, Aguide to modern techniques of plant analysis. Champman and Hall, London, New York.

Hodnick, W. F., Milosavljeric, E. B., Nelson, J. H. and Pardini, R. S., (1988). Electrochemistry of flavonoids. Biochem. Pharmacol, 37: 2607-2611.

Kamel, C., Hafedh, H., Tarek, Z., Amel, B. K. N., Mahmoud, R., Kacem, M. and Amina, B. (2007). The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae): A short review. Phytother. Res. 21(6): 501-506.

Kumar, K.P., Yadav, A., Srivastava, S., Paswan, S. and Dutt, A. S. (2012) Recent trends in Indian traditional herbs *Syzygium aromaticum a*nd health benefits. Journal of Pharmacongnocy and Phytochemistry, 1 (1): 6-17.

Lee, K. G. and Shibamoto, T. (2001). Antioxidant property of aroma extract isolated from clove buds [*Syzygium aromaticum* (L.) Merr. Et Perry]. Food Chem., 74: 443-448.

Liu, H. Mao, P. and Hong, S. (1997). Study on the virus function of extracorporeal restraining HCMV by Chinese traditional medicine *Eugenia caryophyllata* Thunb. Med. J. Chin. People's Liberation Army, 1: 73-75.

Pandey, A. and Singh, P. (2011) Antibacterial activity of *Syzygium aromaticum* (clove) with metal ion effect against food borne pathogens. Asian Journal of Plant Science and Research, 1(2):69-80.

Rahimi, A. A., Ashnagar, A. and Nikoei, H. (2012) Isolation and characterization of 4-allyl-2-methoxyphenol (eugenol) from clove buds marketed in Tehran city of Iran. International Journal of Chem. Tech. Research, 4(1):105-108.

Shihata, I. M. (1951). A pharmacological study of Anagallis arvensis. MSc. Thesis, Faculty of Vet. Med. Cairo Univ. Egypt.

Singh, J., Anupama, B. and Goel, S. P. (2012). *Eugenia caryophyllata* Thunberg (Family Myrtaceae): A Review. International Journal of Research in Pharmaceutical and Biomedical Sciences, 3 (4):1469-1475.

Stahl, R. (1969). Thin layer chromatography. A laboratory handbook, 2nd ed., Translated by Ashworth M. R., Springer, Verlag, Berlin.

Sunil, K. G., Saikishore, M. B. and Shashikant, P. (2012) Evaluation of flower buds of *Syzygium aromaticum f*or antimicrobial and wound healing activity in Rats. International Journal Pharmaceutical Science, 4 (1): 1746-1750.

Velluti, A., Sanchis, V., Ramos, A. J. and Marı'n, S. (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. Int. J. Food Microbiology, 89: 145-154.

Yingming, P., Ping, L., Hengshan, W. and Min, L. (2004). Antioxidant activities of several Chinese medicinal herbs. Food Chemistry, 88: 347-350.