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# ISOLATION AND IDENTIFICATION OF NATURAL ANTIOXIDANTS AND ANTIMICROBIAL AGENTS FROM ORANGE PEEL AND THEIR APPLICATIONS IN BUTTER AND GHEE INDUSTRY

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## ABSTRACT

The antioxidative activity and antimicrobial agents of methanolic, chloroformic, acetonic and hexanic oil extracts of orange peel was tested in buffaloe's ghee and buffaloe's butter. The crude extract was separated on TLC using two developing systems. The active seven compounds namely, Eugnol (Phenolic) M/Z 281; Phenol, 2, 4-bis (1, 1-dimethylethyl) (Phenolic) M/Z 206; Nootkatone 2(3H)-naphthalenone, 4, 4a, 5, 6, 7, 8-hex (Bicyclomonoterpen) M/Z 218; 1, 2-Benzenedicarboxylic acid butyl 2-methylpropyl (Aromatic ester) M/Z 278; Hexadecanoic acid, ethyl ester (Saturated fatty acid) M/Z 284; Octadecanoic acid, ethyl ester (Fatty acid) M/Z 312 and 1, 2-Benzenedicarboxylic acid, diisooctyl ester (Aromatic ester) M/Z 326 and unknown trace compounds of oil extract were identified using GC-MS. The crude oil extract was added to ghee at three levels (0.05, 0.1, and 0.15%) as antioxidant compared with Antrancine (19) as synthetic antioxident, and also it was added to butter at three levels (0.1, 0.2 and 0.3%) as antimicrobial agent to pathogenic and non-pathogenic microorganisms. Results showed that chloroformic extract was the most active antioxidant in ghee than other oil extracts. The inhibition activity of oil extract was high on *Staphylococcus aureus, Pseudomonas fluorescence, Listeria monocytogenes* and *salmonella* sp., while inhibition activity was lower on *Aspergillus flavus*, *Escherichia coli* and non-pathogenic microorganisms. Generally, oil extract was best at 0.10 and 0.15% as antioxidant in ghee, while it was best at 0.2 and 0.3% in butter as antimicrobial agent against bacterial food poisoning. This extract could, therefore, be recommended for practical applications.

## INTRODUCTION

The parts used from orange are leaves, flowers and peel. Only 55-60% of the fruit are used for juice and the reminder must be utilized or it will become a nuisance (Luh and Woodroof, 1977). According to the FAO (1994), the world production of orange fruits is 58 731 000 tons per year, while the Egyptian production is 1 300 000 tons per year (Ghazi, 1999). So, it can be strongly recommended to produce β-carotene from orange peel. Peel of citrus fruit is considered one of the by-products to which attention should be paid to use commercially. One of its more important contents is the volatile oil.

Marshall *et al.* (1985) reported that if butter has been contaminated in the manufacturing process, and if conditions such as poor dispersion of water and high temperature favor microbial growth, spoilage may occur. They suggested that psychrotrophic bacteria are prominent in this type of deterioration. Moreover, Jay (1978) and Kaul *et al.* (1979) have reported that spoilage of butter is generally due to mold growth. From the fact that several

outbreaks of food poisoning associated with consumption of butter have occurred (National Center for Disease Control, 1970 and Marth, 1985).

Recently, natural volatile oils play an important role in the food aspects, medicine and cosmetics (Shams El-Dean, 1977). Abd El-Galeel *et al.* (1998) found that oil content (%) in peel of unripe and ripe orange was 0.70, 72.0 and 0.26, 0.23 with extraction by distillation and cold pressing, respectively. They, also, reported that citrus peels are considered good source of volatile oils, where, decanal the major aldhyde, linalool the major alcohol and linalyl acetate is considered the major ester among all of the investigated extractable volatile oils.

Therefore, the purpose of this research is to use orange peel (an industrial by-product) as a source of oil extract, as natural antioxidant and antimicrobial agent in buffaloe's ghee and butter. The search also deals with separation and identification of active compounds using TLC and GC-MS spectrum using standard parent peak.

## MATERIALS AND METHODS

Baladi orange fruit (*Citrus sinensis*) was obtained from local market in Zagazig city, Egypt, during the season of 1999. Upon receiving the fruits, peels were removed manually using knifes, cutting to slices, directly extracted with different solvents (methanol, acetone, chloroform and hexane) using ratio 50:10 (v/w) solvent : sample for 12, 24 and 48 hr.

**Oil extract:**

Peel oil extract obtained by solvent extraction for different periods at 25°C was filtered, then the solvent was evaporated using rotary evaporator under vacuum at 50°C to obtain crude oil extract as end product.

**Thin layer chromatography (TLC):**

The obtained oil extract was spotted on TLC silica gel G plates and two solvent systems were used. The first solvent was chloroform : ethyl acetate : formic acid (50:40:10, v/v/v). The second solvent was isopropanol: ammonia : water (80:10:10, v/v/v) according to Eisa (1999). The examination under UV lamp (365 nm) was carried out and the components were marked for $R_f$ value.

**Gas chromatography-mass spectrometry (GC-MS):**

The obtained peel oil extract was identified by GC-MS, as mentioned by El-Shawaf (2000). The analysis was operated in Central Laboratory, Food Industries Department, Fac. of Agric., Cairo Univ., Egypt, using GC-HP Model 6890.

**Antioxidative activity:**

The obtained oil extract of citrus peel at levels of 0.05, 0.10 and 0.15% was added directly at melting point of ghee and stirred to ensure complete dissolution. Antrancine (19), as a known antioxident, was added to the same sample for comparison. Oxidative stability of sample was determined in oven at 60 $\pm$ 1°C for 15, 30, 45 and 60 days according to Alaiz *et al.* (1995).

**Chemical analysis:**

The antioxidant activity of all samples were evaluated by determination of peroxide value as milliequivalent-$O_2$/Kg sample, according to A.O.A.C (1990), and thiobarbituric acid (TBA), as malonaldhyde/Kg sample, according to Pearson (1970). Antioxidant effectiveness (AE %) was calculated from the following equation according to Adegoke and Krishna (1998).

$$AE\ \% = \frac{(PV)\ of\ control - (PV)\ of\ test\ sample}{(PV)\ of\ control} \times 100.$$

Where:

PV = Peroxide value (meq-$O_2$/Kg).

AE = Antioxidant effectiveness.

**Microorganisms:**

*Staphylococcus aureus*, *Salmonella* sp., *Escherichia coli* and *Listeria monocytogenes* were obtained from Dairy Dept., Fac. of Agric., Mansoura Univ., Egypt.

*Pseudomonas fluorescence*, *Aspergillus flavus* and *Penicillium requforti* were obtained from Dept. of Microbiol., Fac. of Agric., Mansoura Univ., Egypt.

**Microbiological analysis:**

***Listeria monocytogenes*:**

*L. monocytogenes* was counted on Mc Brid's *Listeria* agar (Lovett *et al*., 1985).

***Escherichia coli*:**

The coliform group was counted on violet red bile agar (VRBA) according to APHA (1972).

***Staphylococcus aureus*:**

*Staphylococcus aureus* (cfu/ml) was counted by plating on Baird-Parker medium (Oxoid). The plates were incubated at 37°C for 48 hr, then counted according to Otero *et al.* (1988).

***Salmonella* sp.:**

*Salmonella* sp. was counted on the high selective *Salmonella* and *Shigella* agar (SS agar), Difco, 1984). The plates were incubated at 37°C for 24 hr.

**Yoghurt culture:**

DRI-VAC yoghurt lactic culture CH2 no. 2559 was used. The suitable medium was lactic acid agar proposed by Elliker (1956) for culture activity.

**O-Culture:**

DRI-VAC lactic culture (O-culture no. CH54 production no. 021506) was used.

***Streptococcus* and *Lactobacillus*:**

*Streptococcus salivarius* subsp. *thermophilus* CH1 no. 01606 and *Lactobacillus deluberckii* subsp. *bulgaricus* CH-14 no. 010785 were kindly obtained from DRIVAC lactic culture CH14 Hansen's Laboratories, Copenhagen, Denmark.

The strains were subcultured weekly in slopes of specific media broth and incubated at 37°C for 24 hrs. Stock cultures were stored at 4°C between transfers. Before use, stock cultures were activated by two successive transfers at 24 hrs intervals. A second transfer of the cultures were made to reconstituted skim milk powder (11% w/v) solids, which were then incubated at 37°C for 24 hrs. Inocula were prepared from the second above culture activity.

**Fungi medium for enumeration:**

Potato dextrose agar (PDA) was used according to Adekunle and Ayeni (1974).

**Inhibition (%)**

Inhibition was measured using the following equation according to Gonzalez *et al.* (1993).

Inhibition (%) =

$$\frac{(\text{CFU/ml in initial control}) - (\text{CFU/ml in associativ e culture})}{(\text{CFU/ml in initial control})} \times 100$$

**Sensitivity of organisms:**

After incubation at the suitable temperature for microbe, each culture was tested for its inhibitory activity of extract against microorganisms using the diffusion disc assay method according to Hassan *et al.* (1994) as follows: two petri dishes were filled with 15 ml of nutrient agar medium and inoculated with 0.1 ml of the test organisms. After the agar had solidified, two sterilized filter paper Whatman No. 3 (diameter 6 mm as disks) were immersed in each extract for three seconds, then were placed on the agar surface. A third petri dish was only inoculated with the pathogenic and non-pathogenic organisms as a control. The same steps were repeated with the all other microorganisms. Then the petri dishs were kept in the refrigerator for 2 hr. for diffusion then incubated at 30°C for 24 hrs for bacteria and for 5 days for fungi before examination for zones of inhibition.. The sensitivity of each microbe for the different extract concentrate were recorded as follows:

1- Zones diameter more than 15 mm was considered to be highly sensitive (+++).
2- Zones diameter ranging from 5-15 mm was considered to be moderate sensitive (++).
3- Zones diameter less than 5 mm was considered to be slightly sensitive (+).
4- No zones, were considered to be insensitive (-) according to El-Alfy (1992)

**Antimicrobial agent in butter:**

Chloroformic extract was added at 0.1, 0.2 and 0.3% to determine its effect on the count of pathogenic and non-pathogenic microorganisms during their growth in butter at 30°C for 48 hrs for bacteria and 5 days for fungi.

## RESULTS AND DISCUSSION

Table (1) shows the effect of different solvents and time on the amount of oil yield as percent of citrus peel. Data showed that methanol extract had higher oil than acetone extract, chloroform extract and hexane extract, respectively. This might be due to the polarity of solvent, where methanol had higher polarity than acetone, chloroform and hexane, respectively. These results are in agreement with those reported by Eisa (1999) and El-Shawaf *et al.* (2000).

Data in table (1) revealed also that the suitable time for extraction was 24-48 hr. Increasing extraction time led to increase in extracted oil from citrus peel. These results are in agreement with those obtained by El-Shawaf *et al.* (2000). Data in the same table (1) showed that the low amount of oil extract was 0.6% in hexane solvent. The high amount of oil extract was 3.0% in methanol solvent. These results are in agreement with those obtained by Heath *et al.* (1981), who reported that the colored peel filled with a highly aromatic essential oil which yielded 0.40-0.50% from peel containing 2-6% of citral and limonene (90%).

**Table (1): Effect of different solvents on the amount of oil yield (%) extracted from citrus peel at different time.**

| Solvent to dry sample ratio (v/w) | Extraction periods (hr) at room temperature | Type of solvent | | | |
|---|---|---|---|---|---|
| | | Acetone (mg %) | Chloroform (mg %) | Hexane (mg %) | Methanol (mg %) |
| 50/10 | 12 hr | 0.80 | 0.70 | 0.60 | 2.50 |
| 50/10 | 24 hr | 0.98 | 0.92 | 0.90 | 2.90 |
| 50/10 | 48 hr | 1.02 | 0.97 | 0.95 | 3.00 |

Table (2) shows the major compounds of citrus peel oil extracted with different solvents and separated on TLC using the developing system. Data revealed that methanol extract had many compounds separated on TLC. Five compounds were separated with the first solvent (chloroform : ethylacetate and formic acid, 50 : 40 : 10 v/v/v), while four compounds were separated with the second solvent system (isopropanol : amonia : water, 80 : 10 : 10, v/v/v). Data showed that the first solvent was preferable than the second solvent for high separation and high resolution, depending on the polarity of the solvent and its purity. Also, data revealed that all oil of citrus peel extract take the same trend with the two solvent systems. Table (2) showed that the $R_f$ of Antrancine (19) as synthetic antioxidant are 0.563 and 0.445 for the first and second solvent systems, respectively. And it appears as one zone only on TLC, meaning that its purity and one compound. The difference in $R_f$ for any compound depended on the polarity and the concentration of solvent.

**Table (2): Major compounds of peel citrus extracted with different solvents separated on TLC by two developing system as high resolution.**

| Developing system | Fraction compounds of peel extract under UV lamp (363 nm) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Methanolic extract | | Acetonic extract | | Chloroformic extract | | Hexanic extract | | Standard* | |
| | $R_f$ | Colour | $R_f$ | Colour | $R_f$ | Colour | $R_f$ | Colour | $R_f$ | Colour |
| Chloroform : Ethyl acetate : formic acid 50 : 40 : 10 (v/v/v) | 0.094 | Blue | 0.094 | Blue | 0.094 | Blue | 0.750 | Green | 0.563 | Violet |
| | 0.156 | Slight blue | 0.156 | Slight blue | 0.156 | Slight blue | 0.844 | Slight blue | | |
| | 0.250 | Violet | 0.375 | Violet | | | | | | |
| | 0.750 | Slight green | 0.750 | Slight green | 0.750 | Slight green | | | | |
| | 0.844 | Slight blue | 0.844 | Slight blue | 0.844 | Slight blue | | | | |
| Isopropanol : Amonia : Water 80 : 10 : 10 (v/v/v) | 0.031 | Green | 0.031 | Green | 0.031 | Green | 0.781 | Slight green | 0.445 | Violet |
| | 0.125 | Blue | 0.063 | Blue | 0.125 | Blue | | | | |
| | 0.781 | Slight green | 0.781 | Slight green | 0.781 | Slight green | | | | |
| | 0.813 | Violet | 0.813 | Violet | 0.813 | Violet | 0.813 | Violet | | |

*** Antrancine (19).**

Table (3) shows the effect of citrus peel oil extract at different concentrations on peroxide value of ghee during storage at 63 ± 1°C for two months. Data indicated that chloroformic extract was better than other oil extracts for protection ghee from oxidative rancidity. Also, data showed that methanolic extract was the second for protection ghee from oxidative rancidity than both acetonic extract and hexanic extract for all concentrates of oil extract. During storage, data showed that peroxide value was affected with addition of antioxidant of oil extract, depending on the number of active compounds and its concentration which determine by GC-MS. The use of oil extract as antioxidative in ghee prevented oxidation and prolonged shelf life as natural preservative compared with control.

**Table (3): Effect of peel extract as antioxidant on peroxide value of ghee at 63 ± 1°C during storage periods.**

| Treatments | Conc. (%) | Peroxide value* during storage at 63 ± 1°C | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 15 (days) | 30 (days) | 45 (days) | 60 (days) |
| Control | | 1.60 | 3.20 | 4.60 | 6.00 | 11.20 |
| Antrancine (19) | 0.075% | 1.60 | 1.64 | 2.00 | 3.20 | 7.20 |
| Ghee + Acetone extract | 0.05 | 1.60 | 2.70 | 3.60 | 3.85 | 6.50 |
| | 0.10 | 1.60 | 2.40 | 3.20 | 3.60 | 6.00 |
| | 0.15 | 1.60 | 1.65 | 2.40 | 3.20 | 5.20 |
| Ghee + Chloroform extract | 0.05 | 1.60 | 2.60 | 2.85 | 3.70 | 4.92 |
| | 0.10 | 1.60 | 2.00 | 2.40 | 3.20 | 4.80 |
| | 0.15 | 1.60 | 1.68 | 2.00 | 2.80 | 3.60 |
| Ghee + Hexane extract | 0.05 | 1.60 | 2.68 | 3.68 | 4.10 | 7.85 |
| | 0.10 | 1.60 | 2.08 | 3.60 | 3.80 | 7.20 |
| | 0.15 | 1.60 | 2.00 | 3.20 | 3.64 | 4.36 |
| Ghee + Methanol extract | 0.05 | 1.60 | 2.70 | 3.20 | 3.82 | 5.10 |
| | 0.10 | 1.60 | 1.98 | 2.40 | 3.20 | 4.20 |
| | 0.15 | 1.60 | 1.80 | 2.00 | 2.84 | 3.60 |

***(PV) for each sample was analysed twice.**

The results presented in Table (4) showed that the antioxidant effectiveness (AE%) of citrus peel oil in ghee at 63 ± 1°C during storage periods was lower in chloroformic extract commpared with synthetic antioxidant. Methanolic extract had lower AE% compared with synthetic antioxidant than both hexanic extract and acetonic extract. This means that chlorformic extract was best than other solvent extract as antioxidative agents.

**Table (4): Antioxidative effectiveness (AE%) of peel extract of ghee at 63 ± 1°C during storage periods.**

| Treatments | Conc (%) | Antioxidative effectiveness % (AE%) during storage | | | |
|---|---|---|---|---|---|
| | | 15 (days) | 30 (days) | 45 (days) | 60 (days) |
| Antrancine (19) | 0.075% | 48.75 | 38.88 | 47.33 | 53.09 |
| Ghee + Acetone extract | 0.05 | 81.18 | 73.66 | 58.17 | 46.84 |
| | 0.10 | 71.80 | 64.97 | 54.00 | 42.37 |
| | 0.15 | 48.36 | 47.57 | 47.33 | 35.23 |
| Ghee + Chloroform extract | 0.05 | 78.05 | 57.36 | 55.67 | 32.73 |
| | 0.10 | 59.30 | 47.57 | 47.33 | 31.66 |
| | 0.15 | 49.30 | 38.88 | 40.67 | 20.94 |
| Ghee + Hexane extract | 0.05 | 80.55 | 75.40 | 62.33 | 58.89 |
| | 0.10 | 61.80 | 73.66 | 57.33 | 53.09 |
| | 0.15 | 59.30 | 64.97 | 54.67 | 27.73 |
| Ghee + Methanol extract | 0.05 | 81.18 | 64.97 | 57.67 | 34.34 |
| | 0.10 | 58.68 | 47.57 | 47.33 | 26.30 |
| | 0.15 | 53.05 | 38.88 | 41.33 | 20.94 |

The results given in table (5) shows that thiobarbituric acid (TBA) was decreased with increase the concentrations of oil extract for all treatments commpared with control during storage periods of ghee. Data revealed that chloroformic extract reduction (TBA) of ghee was more than other extract of oil compared with control sample and synthetic antioxidant until 0.15 concentration of oil. Also, data showed that slightly increase in TBA during storage periods at 63 ± 1°C than other solvent extract compared with control samples. This might be due to the protection of ghee against oxidation or retardation of spoilage with the active component presented in oil extract as shown in Figs (1 to 7) of GC-MS. Other unknown components in oil extract play an important role as antioxidative agents auch as carotenoids (Palozza *et al.* , 1994 and Ismail, 1998)..

Data in table (6) show that the diameter of inhibition zones for pathogenic microorganisms was 11.0, 7.0, 15.0 and 10.0 mm for *Staphylococcus aureus*, *Listeria monocytogenes*, *Pseudomonas fluorescens* and *Aspergillus flavus* for methanolic extract, respectively. While, the diameter of inhibition zone for non pathogenic microorganisms was 7.0, 7.0, 7.0 and 8.0 mm for *Lactobacillus deluberckii* subsp. *bulgaricus*, *Streptococcus salivarius* subsp. *thermophilus*, yoghurt culture and *Penicillium requforti*, respectively. On the other hand, chloroformic extract had high effect on microorganisms, where inhibition zone were 22.0, 21.0, 15.0 and 5.0 mm for *Staphylococcus aureus*, *Listeria monocytogenes*, *E. coli, Salmonella* sp., and *Aspergillus flavus*, respectively. While the same above

extract had no effect on non-pathogenic microorganisms except, *Penicillium requforti*, where the inhibition zone was 4.50 mm. This means that the chloroformic extract had lower effect on non-pathogenic microorganisms. On the other hand, acetonic extract and hexanic extract have effect on *Staphylococcus aureus* only, where the inhibition zone for each were 8.0 and 7.0 mm, respectively. Generally, methanolic extract had antimicrobial component for both pathogenic and non-pathogenic microorganisms. While, chloroformic extract had antimicrobial agent for pathogenic microorganisms only, and no effect on non-pathogenic microorganisms. Acetonic extract and hexanic extract had antimicrobial agent for *Staphylococcus aureus* and less effect on other microorganisms.

**Table (5): Effect of peel extract on thiobarbituric acid (TBA) of ghee at 63 ± 1°C during storage periods.**

| Treatments | Conc. (%) | TBA (malonaldhyde/Kg sample) during storage | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 15 (days) | 30 (days) | 45 (days) | 60 (days) |
| Control | | 0.049 | 0.913 | 1.716 | 2.184 | 2.465 |
| Antrancine (19) | 0.075% | 0.049 | 0.070 | 0.819 | 1.014 | 1.529 |
| Ghee + Acetone extract | 0.05 | 0.049 | 0.080 | 0.840 | 1.570 | 1.650 |
| | 0.10 | 0.049 | 0.055 | 0.757 | 1.466 | 1.646 |
| | 0.15 | 0.049 | 0.042 | 0.234 | 1.225 | 1.544 |
| Ghee + Chloroform extract | 0.05 | 0.049 | 0.102 | 0.229 | 1.460 | 1.740 |
| | 0.10 | 0.049 | 0.117 | 0.226 | 1.435 | 1.716 |
| | 0.15 | 0.049 | 0.070 | 0.187 | 1.248 | 1.404 |
| Ghee + Hexane extract | 0.05 | 0.049 | 0.069 | 0.078 | 1.072 | 1.670 |
| | 0.10 | 0.049 | 0.062 | 0.070 | 1.053 | 1.630 |
| | 0.15 | 0.049 | 0.005 | 0.062 | 0.920 | 1.544 |
| Ghee + Methanol extract | 0.05 | 0.049 | 0.172 | 0.195 | 1.198 | 1.620 |
| | 0.10 | 0.049 | 0.164 | 0.187 | 1.170 | 1.513 |
| | 0.15 | 0.049 | 0.156 | 0.164 | 1.092 | 1.482 |

**(TPA) for each sample was analysed twice.**

**Table (6): Effect of peel extract on microorganisms and sensitivity as inhibition zone (mm).**

| Microorganisms | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Methanol extract | Acetone extract | Chloroform extract | Hexane extract |
| Pathogenic microorganisms: | | | | |
| *Staphylococcus aureus* | 11.0 | 8.0 | 22.0 | 7.0 |
| *Listeria monocytogenes* | 7.0 | Nil | 21.0 | Nil |
| *Escherichia coli* | Nil | Nil | 15.0 | Nil |
| *Salmonella* sp. | Nil | Nil | 15.0 | Nil |
| *Pseudomonas fluorescence* | 15.0 | Nil | Nil | Nil |
| *Aspergillus flavus* | 10.0 | Nil | 5.0 | Nil |
| Non-pathogenic microorganisms: | | | | |
| *Lactobacillus deluberkii* subsp. *Bulgaricus* | 7.0 | Nil | Nil | Nil |
| *Streptococcus salivarius* subsp. *Thermophilus* | 7.0 | Nil | Nil | Nil |
| Yoghurt culture | 7.0 | Nil | Nil | Nil |
| O. culture | Nil | Nil | Nil | Nil |
| *Penicilliumm requforti* | 8.0 | Nil | 4.50 | Nil |

**15-20, very highly sensitive**
**10-15, highly sensitive**
**5-10, moderate sensitive**
**1-5, slightly sensitive**
**Nil, insensitive.**

Table (7) shows the effect of chloroformic extract of citrus peel on some pathogenic and non pathogenic microorganisms in butter at different concentrations. Data revealed that chloroformic extract had high effect at 0.1% on *Staphylococcus aureus* as pathogenic bacteria and on *Lactobacillus deluberrckii* subsp. *bulgaricus* as non pathogenic bacteria. Also, data showed that chloroformic extract take the same trend at 0.2% and 0.3% for non pathogenic bacteria. While, chloroformic extract had higher inhibition activity at 0.2% on *Pseudomonas fluorescence* (90.0%) than inhibition activity of *Staphylococcus aureus* (88.0%). On the other hand, chloroformic extract at 0.3% had high effect on some pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas fluorescence* and *Listeria monocytogenes*, where, inhibition activity were 99.0%, 96.0% and 94.0%, respectively. These results are in agreement with those reported by Yousef *et al.* (1991). They reported that synthetic antioxidant, *e.g.*, BHA (100-300 ppm), BHT (300-700 ppm) and TBHQ (10-30 ppm) inhibited *Listeria monocytogenes* in tryptose broth. Payne *et al.* (1989) indicated that minimum inhibitory concentrations of phenolic compounds including some currently used antioxidants food additives, against *L. monocytogenes* on an agar medium were recently reported. Chang and Branen (1975) found that 400, 400 and 150 ppm of BHA effectively inhibited groth of *E. coli*, Salmonella typhimurium and *Staphylococcus aureus*. Erickson and Tompkin (1977) reported that TBHQ at 30 ppm completely inhibited the growth of *Staphylococcus aureus*, but 300 ppm of the additive only delayed growth of *Pseudomonas fluorescens*.

The effect of chloroformic extract on food-poisoning bacteria or type of deterioration and/or non-pathogenic due to the active component present the oil extract which determined by GC-MS such as Eugnol, Phenol, 2, 4 bis (1, 1-dimethylethyl), Nootkatone 2 (3H)-Naphthalenone, 4, 4a, 5, 6, 7, 8-hex, 1, 2-Benzendicarboxylic acid, butyl 2-methylpropyl, Hexadecanoic acid, ethyl ester, Octadecanoic acid, ethyl ester and 1, 2 Benzendicarboxylic acid, diisooctyl ester, as shown in Table (8) and Figs. (1 to 7), respectively, compared with the parent peak. Generally, oil extract of citrus peel able added to butter to eliminate of contaminated butter by different microorganisms especially when hygienic measures are inadequate. These results are in agreement with those reported by Murshall *et al.* (1985).

**Table (8): GC-MS for separation of chloroformic extract of peel as antioxidant and antimicrobial agent.**

| Compound | R.T. | Area % | Ions (M/Z) | Matching% | Structure |
|---|---|---|---|---|---|
| Eugenol | 19.79 | 4.73 | 281 – 164 – 149 – 131 – 121 – 103 – 91 – 77 – 65 - 55. | 99.0 | Fig. (1) |
| Phenol | 21.57 | 1.56 | 206 – 191 – 175 - 163 – 147 – 141 – 133 – 123 – 115 – 107 – 97 – 91 –77 – 65 – 57 - 51. | 93.0 | Fig. (2) |
| Nootkatone.2(3H)-Naphthalenone, 4, 4a, 5, 6, 7, 8-hex. | 25.17 | 2.14 | 218 – 203 – 190 – 175 – 161 – 147 – 133 – 124 – 115 – 107 – 97 – 91 – 85 – 79 – 67 - 55. | 93.0 | Fig. (3) |
| 1, 2-benzendicarboxylic acid, butyl 2-methylpropyl | 26.52 | 1.09 | 278 - 223 – 205 - 181 - 160 – 149 - 135 - 121 – 104 - 93 - 76 - 65 – 55. | 91.0 | Fig. (4) |
| Hexadecanoic acid, ethyl ester | 26.70 | 0.18 | 284 - 255 – 241 - 213 - 199 – 187 - 157 - 143 – 131 - 115 - 101 – 88 - 77 - 69 - 55. | 98.0 | Fig. (5) |
| Octadecanoic acid, ethyl ester | 28.55 | 0.75 | 312 – 283 – 269 – 255 – 241 – 227 – 213 – 199 – 185 – 171 – 157 – 143 – 129 – 115 – 101 – 88 – 79 – 69 - 55. | 97.0 | Fig. (6) |
| 1,2-benzendicarboxylic acid, diisooctyl ester | 32.71 | 82.33 | 326 – 279 – 261 – 249 – 231 – 221 – 207 – 191 – 180 – 167 – 149 – 132 – 121 – 104 – 93 – 83 – 71 - 57. | 91.0 | Fig. (7) |

**R.T.: Retention time.**

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## فصل وتعريف مضادات أكسدة ومضادات ميكروبية طبيعية من قشر البرتقال وتطبيقاتها فى الزبد والسمن

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تهدف الدراسة إلى إستخدام قشور البرتقال فى إنتاج مركبات ذات صفات مضادة للأكسدة ومضادات للميكروبات الممرضة وغير الممرضة بإستخدام زيت قشور البرتقال المستخلص بالميثانول والأسيتون والكلوروفورم والهكسان . وتم فصل الزيت وإستخدامه كمضاد أكسدة طبيعى فى صناعة السمن الجاموسى بنسب 0.05 ، 0.1 ، 0.15% ومضاد للميكروبات فى الزبد الجاموسى بنسب 0.1 ، 0.2 ، 0.3% . وتم التعرف على المركبات النشطة بإستخدام كروماتوجرافيا الطبقة الرقيقة (TLC) مستخدماً اللون والـ $R_f$ تحت الأشعة فوق البنفسجية ( 365 نانومتر ) . ثم إستخدم جهاز GC-MS للتعرف على المركبات النشطة ووزنها الجزيئ وهى : Eugnol (Phenolic) ووزنه الجزيئ 281 ، Phenol, 2, 4-bis (1, 1-dimethylethyl) (Phenolic) ووزنه الجزيئ 206 ، Nootkatone 2(3H)-naphthalenone, 4, 4a, 5, 6, 7, 8-hex (Bicyclomonoterpen) ووزنه الجزيئى 218 ، 1, 2-Benzenedicarboxylic acid butyl 2-methylpropyl (Aromatic ester) ووزنه الجزيئى 278 ، Hexadecanoic acid, ethyl ester (Saturatted fatty acid) ووزنه الجزيئى 284 ، Octadecanoic acid, ethyl ester (Fatty acid) ووزنه الجزيئى 312 ، 1, 2-Benzenedicarboxylic acid, diisooctyl ester (Aromatic ester) ووزنه الجزيئى 279 .

، وأوضحت النتائج أن النشاط التثبيطى لمستخلص الكلوروفورم كان عالى بالنسبة للبكتيريا العنقودية وبكتيريا السيدوموناس فلورسنس والليستريا مونوسيتوجينس وكذا بكتيريا السلامونيلا ؛ بينما كان النشاط التثبيطى منخفض للمستخلص على فطر الأسبرجلس فلافس وبكتيريا القولون وكل البكتيريا الغير ممرضة

، وأوضحت النتائج أن مستخلص الكلوروفورم أعطى أفضل النتائج كمضاد أكسدة طبيعى عند تركيز (0.1 ، 0.15% ) مقارنة بمضاد الأكسدة الصناعى ( أنترانسين 19 ) خلال فترة التخزين . كذلك أعطى نفس مستخلص الكلوفورم تأثير تثبيطى عالى على الميكروبات المحدثة للتسمم الغذائى عند تركيز ( 0.2 ، 0.3 % ) ، وبذلك يمكن التوصية بإستخدامها فى النواحى التطبيقية .

**Table (7): Effect of chloroformic extract of peel on some pathogenic and non-pathogenic microorganisms mixed in butter.**

| Microorganisms | Concentration of oil peel extract in butter sample | | | | | | | | | | | Control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.0 | | 0.10 % | | | 0.20 % | | | 0.30 % | | | |
| | Microbial count x $10^3$ | Log of Microbial count | Microbial count x $10^3$ | Log of survivors | Inhibition activity (%) | Microbial count x $10^3$ | Log of survivors | Inhibition activity (%) | Microbial count x $10^3$ | Log of survivors | Inhibition activity (%) | Microbial count X $10^6$ |
| ***Pathogens:*** | | | | | | | | | | | | |
| *Staphylococcus aureus* | 20.0 | 4.301 | 3.90 | 3.591 | 80.50 | 2.10 | 3.380 | 88.00 | 0.20 | 2.301 | 99.00 | 24.0 |
| *Listeria monocytogens* | 10.0 | 4.000 | 8.40 | 3.924 | 16.00 | 4.00 | 3.602 | 60.00 | 0.60 | 2.778 | 94.00 | 18.0 |
| *Escherchia coli* | 40.0 | 4.602 | 36.0 | 4.556 | 10.00 | 26.5 | 4.423 | 33.75 | 17.0 | 4.230 | 57.50 | 43.0 |
| *Salmonella sp.* | 25.0 | 4.398 | 22.0 | 4.342 | 12.00 | 11.0 | 4.041 | 56.00 | 2.00 | 3.301 | 92.00 | 31.0 |
| *Pseudomonas fluorescence* | 10.0 | 4.000 | 5.30 | 3.724 | 47.00 | 1.00 | 3.000 | 90.00 | 0.25 | 2.398 | 96.50 | 14.0 |
| *Aspergillus flavus* | 28.0 | 4.447 | 20.0 | 4.301 | 28.57 | 13.2 | 4.121 | 52.86 | 13.0 | 4.114 | 53.57 | 33.0 |
| **Non Pathogens:** | | | | | | | | | | | | |
| *Lactobacillus deluberckii* subsp. *bulgaricus* | 18.0 | 4.255 | 12.0 | 4.079 | 33.33 | 9.0 | 3.954 | 50.00 | 8.00 | 3.903 | 55.56 | 24.0 |
| *Streptococcus salivarius* subsp. *thermophilus* | 40.0 | 4.602 | 35.0 | 4.544 | 12.50 | 31.0 | 4.491 | 22.50 | 30.0 | 4.477 | 25.00 | 43.0 |
| Yoghurt culture | 45.0 | 4.653 | 40.0 | 4.602 | 11.11 | 38.0 | 4.580 | 15.56 | 30.0 | 4.477 | 33.33 | 49.0 |
| O. culture | 49.0 | 4.690 | 45.0 | 4.653 | 8.16 | 43.0 | 4.633 | 12.24 | 46.0 | 4.663 | 6.12 | 52.0 |
| *Penicillium requforti* | 15.0 | 4.176 | 13.1 | 3.491 | 12.67 | 13.0 | 4.114 | 13.33 | 12.8 | 4.107 | 14.67 | 18.0 |