Sys Rev Pharm 2020; 11(4): 561 – 569
A multifaceted review journal in the field of pharmacy
E-ISSN 0976-2779 P-ISSN 0975-8453

Inhibit of the Virus Infections by using Aromatic Oils, *Saccharomyces cerevisiae, Boswellia Carterii* and Zinc

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Article History: *Submitted: 23.01.2020* *Revised: 26.03.2020* *Accepted: 30.04.2020*

ABSTRACT

Anti-virus treatments are a type of anti-microbiology, a class that includes antibiotics and anti-microbiological drugs, including fungi. These treatments were relatively harmless to the host, so they can be used to treat patients. Therefore, it must distinguish between the anti-viral drug and its killer, which is not considered a drug category, but rather destroys the virus body outside the living cell. Most antiviral drugs currently available help in the treatment of immune deficiency disease in patients.

The results showed significant differences between influenza vaccinated animals with diet therapy with inhaling essential oils, and non-vaccinated with absent of diet therapy and inhaling essential oils for the percentages from differential number of white blood cells in rats. The results showed a clear rise in the differential number of leukocytes in the blood samples of the experiment animals that were fed on yeast (*Saccharomyces cerevisiae*) enriched with lemon oil, and zinc, in addition to chewing gum. The white blood cell count was calculated during fixed periods 2, 8 and 10 days from vaccinated with influenza virus for all experiment animals, the results were (53, 96, 9, 7) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 2 days, (46, 92, 8, 5) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 8 days, and (42, 85, 7, 4) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 10 days, of the test group fed on yeast enriched with lemon oil, zinc, and chewing gum, and then it was vaccinated, compare between control group.

There were no clear symptoms for experimental animals with influenza vaccine (effective and not weakened) that was fed to the food above and inhaled eucalyptus oil with a clear increase in the number of white blood cells compared to the control group and the negative group for feeding. While the symptoms were clear on the negative control group (not to use diet therapy) and vaccinated with effective influenza vaccine.

The diet therapy was prepared from a group of effective essential oils, which were used in two ways, the first was through direct inhalation, that consist of (25 ml of oil eucalyptus, 25 ml of oil lemon and 100% oxygen gas) by heat, which third frequency of use in the one day for 10 days. The second components used by mouth that consist of (25 ml/kg oil lemon, 5µg/kg zinc, 8 mg/kg *Saccharomyces cerevisiae*, and 5 mg/kg chewing gum (*Boswellia Carterii*), which third frequency of use in the one day for 10 days. In this way, we increase immunity and prevent the virus from binding to the cell by inhibiting an enzyme angiotensin-converting enzyme 2.

Key words: infection virus, aromatic oils, *Saccharomyces cerevisiae, Boswellia Carterii* and Zinc.

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DOI: 10.31838/srp.2020.4.83

INTRODUCTION

Influenza virus is a group of viruses that infect humans and animals and transmit from one to another (1). A virus is biological agent that which reproduces inside the host cell of living organism. When the infected by the virus, host cell is produce thousands of copies of original virus at extraordinary rate (2).It affects the respiratory system, and some are mild as colds or as severe as pneumonia. The SARS which spread between 2002-2003, is an example of the Coronavirus, it has been transmitted from animals to humans (3). It spread in the Middle East in 2012, and there is another new strain of corona virus called respiratory syndrome of the Middle East, which has moved from camel to humans, according to the researchers. The means of spreading the virus among people is through sneezing, coughing, and a healthy person touching another infected person, or touching the surfaces carrying the virus through the hands, then touching the nose, mouth, or eyes. When infected with coronavirus, the affected person has a set of symptoms, including high temperatures, shortness of breath, coughing, and runny nose (4). Most of the infections with the coronavirus appear as moderate colds, while there are strains that lead to more severe acute pneumonia. Coronaviruses cause inflammation acute and chronic respiratory system in animals and human (5). Human coronavirus, previous to the emergence of SARSCoV, there are two type human coronaviruses (OC43, 229E), both etiologic agents .There had been speculation that about of association human coronaviruses with serious human diseases such as multiple sclerosis (6).

MATERIAL AND METHODS

Essential oils are easy-to-volatile vegetable oil extracts that can be obtained from plant parts, as they are distinguished by their aromatic scents, such as eucalyptus oil (7). *Eucalyptus camadulensis* trees were forest trees, it found in Iraq from the north to south and its original homeland was in Australia. Eucalyptus trees contain different proportions of essential oils used for medicinal purposes. It differs from fatty oils by completely evaporating and leaving no trace behind. Essential oils consist of many different ingredients (8). They are soluble in fats and do not contain any fatty components. The solubility of essential oils in water is weak, and they are in the form of liquid droplets above the surface of the water being less dense than water. Essential oils were responsible for the characteristic aroma of the plant, and they evaporate and volatilize at normal temperatures (9). Essential oils can be easily separated from the vegetable parts carrying them using distillation and extraction methods. This extraction method was an alternative to the steam distillation method for extracting the oils and the resulting oils are characterized as being a low-impurity oil, and that mono turbines and other compounds were extracted with ether and petroleum ether or acetone (10). Where extraction was carried out at low temperatures, and the evaporation and distillation of solvents was carried out under vacuum pressure in order to keep the oil components

from crashing. The benefits of this method were use in the extraction of volatile polar compounds and the extraction of the Soxhlet extractor system among the best. Eucalyptus oil was extracted from the distillation of fresh leaves. Eucalyptus oil contains Cineole, and it must not be less than 75% of the percentage of essential oils in order to be suitable for medical purposes (11). Eucalyptus oil was a colorless or pale yellow liquid. Oil is used to relieve the symptoms of respiratory infections, coughing and congestion. Eucalyptus oil also contains Rutin, terpenoids, flavonoids and phenolic acid. Essential oils were extracted from the samples by the Soxhlet apparatus by used two types of organic solvents (12).

Experimental animals

Eighty of laboratory animals (rat) were 3 weeks of age. It divided in four group, each group consists of twenty rats, the animals were fed on a diet consisting of 8mg /kg yeast, 25 ml/kg lemon oil, 5mg/kg chewing gum in addition to 5µg/kg zinc. Where the feed was given for 10 days before the start of vaccinated thereat. In addition to exposing it three times a day to essential oils, which are eucalyptus and lemon oil through heating.

Blood samples

Blood samples were collected from the vein during (two, eighth and ten days) to a count of white blood cells (13).

Calculation of the percentage differential number of white blood cells

By preparing a blood smear on slides, the differential number of white blood cells was calculated (14).

Assay of RT PCR

The method used of RT-PCR kit for detection the infection with influenza virus by following the kit instructions . The forward of N gene primer was 5" CATCCAG CAA ATA CACCATCCA 3" and the revers primer was 5" GCATCTCTGAGTA- TTTTTATGG 3" ,The solutions of template RNA were, primer ,5x QIAGEN Ones-tep RT-PCR Buffer, dNTP Mix,5x Q solution and RNAase free water were thawed and placed on ice .The prepared mix reaction was according table 1 in kit instructions. all the components in reaction mix contains except RNA template . the prepared was negative control without template RNA (15).

Table 1: Reaction components of one-step RT-PCR by using Q Solution.

Component	Volume/reaction	Final concentration
Reaction Mix One Step RT-PCR Buffer,5x	10 µl	1×;2.5 mM Mg+2
dNTP Mix (10 mM)	2 µl	400 µM of each dNTP
Primer A	1 µl	0.6 µM
Primer B	1 µl	0.6 µM
RNase free water	19 µl	-
One Step RT-PC Enzyme Mix	2 µl	-
5x Q Solution	10 µl	-
Template RNA	5µl	-

Added 2 µg/reaction of template RNA to PCR tubes, PCR tubes was put on ice. Wait the thermal cycler until reached 50°C. Then the tubes of PCR placed in the thermal cycler. program outlined was programmed in the thermal cycler according the table 2. Bromophenol blue as loading dye was added for each tubes. The samples loaded on agarose gel and put of the gel to electric 5 to 8 V/cm. ethidium bromide was used to staining the gel, which fluoresces under UV.

Table 2: The conditions of thermal cycler

Step	Temperature	Time
Reverse transcription	50°C	30 min.
Initial PCR activation	95°C	15 min.
3 step cycle		
Denaturation	0.5 - 1min.	94 °C
Annealing	0.5 - 1min.	50 °C
Extension	1min.	72 °C
Number of cycles	35	
Final extension	10 min.	72 °C

Preparation of the stock solution from extract

The extract mater was dissolved in methanol by rate 1mg/ml.1µl from extract was injected in GC-MS to analysis (16).

Identification by GC-MS

Spectrum of GC-MS was Identification of conducted using the Standard database of National Institute and Technology. The component stored of spectrum was known for more compounds in the library NIST. Ascertained was molecular weight, structure and name of the component material (17).

GC-MS assay

GC-MS analysis were by used a perkinelmer GC clauses 500 system with gas chromatography. Where interfaced the mass spectrometer equipped from through elite-1, the column of fused silica capillary to GC-MS detection. Ionizing energy of 70 eV in electron ionization system was used. Flow rate 1ml / min with used Helium gas as the carrier and injection 1µl in injector was employed of temperature 250 c, ion source 280 °c. the programmed of oven temperature which increased 10 °c \ 1 min , where it was started from 110 °c to reach 280 °c, and it was ended in 9 min(18).

RESULT AND DISCUSSION

The result showed was positive of diagnosed influenza virus with a RT PCR technique for identification it and was positive (figure1).Which isolated from infected animals obtained from the central virus Laboratory.

Figure 1: Agarose gel analysis of RT PCR of virus. Agarose gel 0.8 gm/ml in 5 V/cm at1 hour under TAE buffer, UV transilliuminator was exposed of gel.

GC-MS assay

In present study of eucalyptus leaf chemical composition by GC-MS, it was contain methyl estermandoctadacanoic acid and hexadacanoic acid as a major component , as shown in a table (3).

Table 3: Chemical constituent the presence in eucalypts leaf

No.	Compound	Zone percent	Retention time
1	methyl ester + Sulfuric acid	12.867	3.445
2	Eucalyptol	1.567	5.566
3	Levoglucosenone	1.467	6.545
4	Butylatedhydroxytoluene	8.775	11.899
5	2-naphtheline methanol	1.067	13.456
6	Methyl tetradecanoate	2.818	14.311
7	methyl ester + Hexadecanoic acid	35.899	16.265
8	9-dimethoxybicyclo (3,3,1)nona-2,4-dione	2.11 910	16.655
9	Octadecanoic acid, methyl ester	19.6112	18.273

The diet therapy was prepared from a group of effective essential oils, which were used in two ways, the first was through direct inhalation, that consist of (25 ml of oil eucalyptus, 25 ml of oil lemon and 100% oxygen gas) by heat, which third frequency of used in the one day for 10 days .The second components used by mouth that consist of (25 ml/kg oil lemon, 5µg/kg zinc, 8 mg/kg *Saccharomyces cerevisiae*, and 5 mg/kg chewing gum (*Boswellia carterii*), which third frequency of used in the one day for 10 days. In this way, we increase immunity and prevent the virus from binding to the cell by inhibiting an enzyme angiotensin-converting enzyme 2.

Table 4: The concentrations of essential oils used by inhalation and number of times it were repeated.

No.	The Vapor	Concentration ml/kg	Frequency of use in the one day	The period in days
1	Eucalyptus oil	25	3	10
2	lemon oil	25	3	10
3	Oxygen gas	100 %	3	10

Table 5: The concentrations of diet therapy (oil lemon, *Saccharomyces cerevisiae and* chewing gum) which used by mouth and number of times it were repeated.

No.	The material	Concentration	Frequency of use in the one day	The period in days
1	oil lemon	25	3	10

		ml/kg with 5 µg/kg zinc		
2	*Saccharomyces cerevisiae*	8 mg/kg	3	10
3	chewing gum (*Boswellia Carterii*)	5 mg/kg	3	10

The results showed significant differences ($p<0.05$) between the influenza vaccinated animals with taken diet therapy with inhaling essential oils, and non- vaccinated with absent of diet therapy and inhaling essential oils for the percentages from differential number of white blood cells in rats, as shown in a table (6-9). The results showed a clear rise in the differential number of leukocytes in the blood samples of the experiment animals that were fed on yeast (*Saccharomyces cerevisiae*) enriched with lemon oil, and zinc, in addition to chewing gum .The white blood cell count was calculated during fixed periods 2, 8 and 10 days from vaccinated with influenza virus for all experiment animals, the results were (53, 96, 9, 7) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 2 days, (46, 92, 8, 5) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 8 days, and (42, 85, 7, 4) for (Neutrophil, Lymphocyte, Monocyte and Eosinophil) sequentially, after 10 days, of the test group fed on yeast enriched with lemon oil, zinc, and chewing gum, and then it was vaccinated, compare between control group table (6). There were no clear symptoms for experimental animals with influenza vaccine (effective and not weakened) that was fed to the food above and inhaled Eucalyptus oil with a clear increase in the number of white blood cells compared to the control group and the negative group for feeding. While the symptoms were clear on the negative control group (not to use diet therapy) and vaccinated with effective influenza vaccine.

Table 6: Average percentages of differential number of white blood cells (control group).

no	Mouse group / WBC		Negative Control group	variance standard	
1	After 2 day	N	29	time	type
		L	69	*	*
		M	2	*	*
		E	1	*	*
2	After 8 day	N	29	*	*
		L	69	*	*
		M	2	*	*
		E	1	*	*
3	After 10 day	N	30	*	*
		L	69	*	*
		M	2	*	*
		E	1	*	*

(N : Neutrophil , L : Lymphocyte , M : Monocyte , E : Eosinophil).
L.S.D.=0.9 , * : There were significant differences.

Figure 2: Average percentages of differential number of white blood cells (control group).

Table 7: Average percentages of differential number of white blood cells (Positive Control group).

no	Mouse group / WBC		Positive Control group	variance standard	
1	After 2 day	N	35	time	type
		L	78	*	*
		M	4	*	*
		E	2	*	*
2	After 8 day	N	34	*	*
		L	77	*	*
		M	4	*	*
		E	2	*	*
3	After 10 day	N	36	*	*
		L	77	*	*
		M	3	*	*
		E	2	*	*

N : Neutrophil , L : Lymphocyte , M : Monocyte , E : Eosinophil
L.S.D.=1.2,* : There were significant differences.

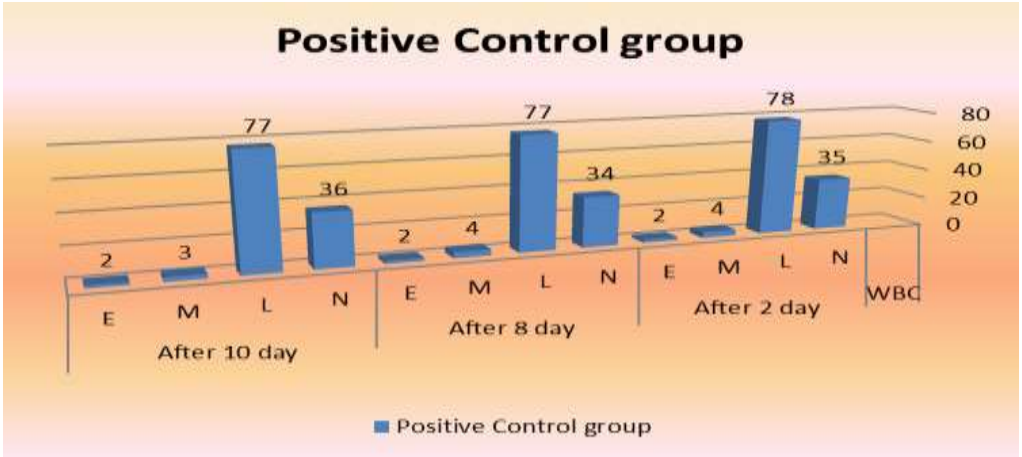

Figure 3: Average percentages of differential number of white blood cells (Positive Control group).

Table 8: Average percentages of differential number of white blood cells (Negative Test group).

no	Mouse group / WBC		Negative Test group	variance standard	
1	After 2 day	N	45	time	type
		L	90	*	*
		M	7	*	*
		E	5	*	*
2	After 8 day	N	43	*	*
		L	88	*	*
		M	6	*	*
		E	4	*	*
3	After 10 day	N	39	*	*
		L	80	*	*
		M	5	*	*
		E	3	*	*

N : Neutrophil , L : Lymphocyte , M : Monocyte , E : Eosinophil
L.S.D.=1.4,* : There were significant differences.

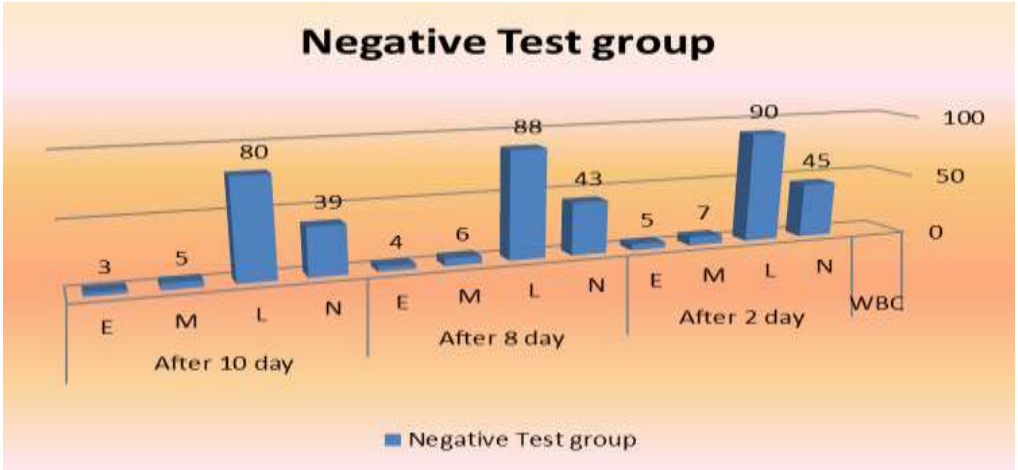

Figure 4: Average percentages of differential number of white blood cells (Negative Test group).

Table 9: Average percentages of differential number of white blood cells (Positive Test group).

no	Mouse group / WBC		Positive Test group	variance standard	
1	After 2 day	N	53	time	type
		L	96	**	**
		M	9	**	**
		E	7	**	**
2	After 8 day	N	46	**	**
		L	92	**	**
		M	8	**	**
		E	5	**	**
3	After 10 day	N	42	**	**
		L	85	**	**
		M	7	**	**
		E	4	**	**

N : Neutrophil , L : Lymphocyte , M : Monocyte , E : Eosinophil
L.S.D.=1.8,** : There were high significant differences.

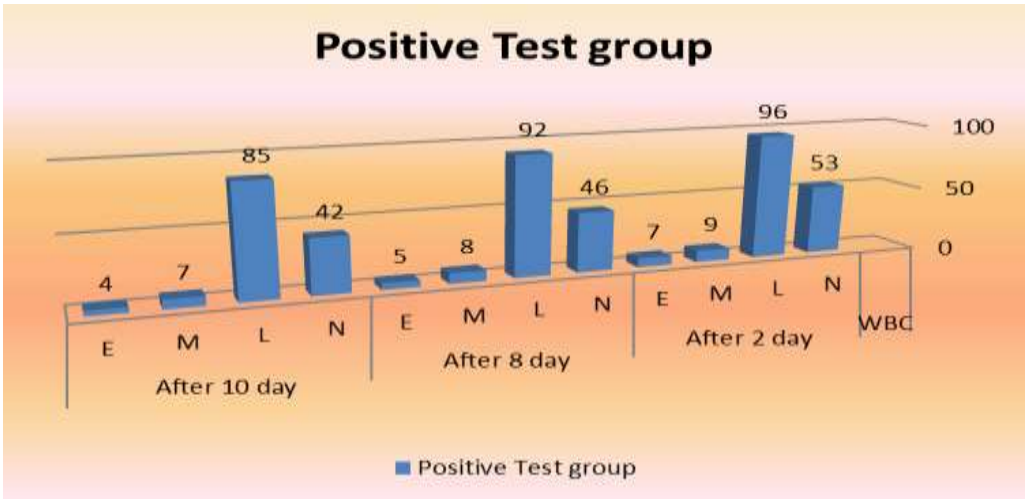

Table 5: Average percentages of differential number of white blood cells (Positive Test group).

Through the results, it was shown the effective role of lemon oil, yeast dough and zinc, in addition to bitter chewing gum in stimulating immunity and raising the pH of blood , and the role of eucalyptus oil in sterilizing the respiratory system through the high affinity for binding to receptors on cell surfaces, in addition to the large role of it in destroying the virus through heat during it vaporization, and the other factor has a familiarity with the glycoprotein of the viruses that prevent their association with receptors on cells, for animals experience compared to control group (19).

concluded of the results that the using of yeast (*Saccharomyces cerevisiae*) enriched with lemon oil, and zinc, in addition to bitter chewing gum lead to improve immunity of animal and raised of pH in the blood , this improvement was done by synergism effective of yeast enriched with lemon oil, and zinc, in addition to bitter chewing gum as organic form. Anti-virus treatments was a type of anti-microbiology, a class that includes antibiotics and anti-microbiological drugs (20). These treatments were relatively harmless to the host, so they can be used to treat patients. Therefore, it must distinguish between the anti-viral drug and it was killer, which was not considered a drug category, but rather destroys the virus body outside the living cell (22). Most antiviral drugs currently available help in the treatment of immune deficiency disease in patients. Finding antiviral drugs that are safe to use and at the same time with an effective effect was extremely difficult, because viruses enter the host's cells to multiply and as a result it was difficult to target the virus without harming the cells of the organism. Antivirals emerged as a result of the great cognitive expansion in molecular research, which helped researchers to learn about the molecular structure of viruses in addition to knowing their vital functions. Through this, great progress was made by creating new antivirals (23).

Eucalyptus oil was distilled oil from eucalyptus leaves, this plant belongs to family myrtaceae, which was grown all over the world, and eucalyptus oil has a wide application in history, such as industrial uses, antiseptics and medicines (24). Eucalyptus leaves were distilled with steam to extract eucalyptus oil. Eucalyptus oil contains a number of highly effective chemical and organic compounds that are effective therapeutic compounds, the most important of which is the cienol compound that gives a sharp and pungent scent of oil with antimicrobial and antioxidant effectiveness (25). One of the most important benefits of Eucalyptus essential oil was its ability to treat some of the problems that afflict the respiratory system. It was capable of removing symptoms of the common cold, such as difficulty breathing and nasal congestion, by adding several drops of eucalyptus oil to boiling water and inhaling it was ascending fumes that prevent shrinking the muscles surrounding the airways, moisturize the airways, reduce irritation and help get rid of the accumulated secretions (26). It was an effective treatment for bronchitis. In addition, studies have indicated that it may represent a treatment with effective outcomes for asthma and sinusitis (27).Lemon oil was composed of a group of important nutrients of high interest, as it helps to increase the strength of the immune system in the body through the production of white blood cells and the resistance to disease, including viral infections, in addition to its role in reducing body temperature (28). Essential oil of eucalyptus and oil lemon vapor and the isolated compounds citronellol and sienol were very active against virus with exposures for 15 minutes (29). In gas phase, inhibitory activity at 15-25 µL/mL concentration. Under that conditions the vapors were showed no measurable adverse effect on epithelial cell monolayers. the suggests for these oils in vapor phases could be potentially which that use to therapy for viruses (30). The result showed agreed with evaluated effects on proteins of influenza virus by used of oil vapors in inhibitor of virus infection by cutting of attachment between recieptor of the cell and anti-gene (virus), some oil vapors have characteristic antivral therapeutic benefits for influenza patients, and other membranes which containing respiratory viruses (31). Eucalyptus oil is used in severe respiratory conditions, including asthma and sinusitis, in addition to bronchitis and viral infections through inhalation of oil vapor by means of an evaporator or vapor maker by diluting the oil with hot water and then inhaling vapor (32). Antivirals are a class of antivirals that are specifically used to treat a viral infection. It was mechanism of similar action to the antibiotics used to kill bacteria and fungi, there was a specialization in the work of antivirals according to their type, but its work differs from that of other antibiotics in that it does not destroy the cause of the disease but rather prevents its growth from the beginning (33). Yeast was a type of single-celled fungi, and contains a large amount of proteins and minerals, including iron, zinc, chromium and fiber, yeast was rich in minerals and proteins, when consumed it was an important factor to improve the body's immunity, as well as it was rich in vitamin B and anti-oxidants that prevent the formation of free radicals in the body that allow oxidation and union of substances, and the yeast also enhances the effectiveness of white blood cells, which is the first defense line against any infection.(34).Where yeast was added to *Boswellia Carterii* (chewing gum) because of it was benefits, as eating and contributes to prevent of respiratory diseases, including viral infections. The results of previous studies indicate that eating yeast contributes to reducing the risk of influenza, especially in healthy people who have recently received the flu vaccine (35). *Boswellia Carterii* contains inflammatory corticosteroids. It was noteworthy that the cortisone in chewing gum has a much better effectiveness than the artificial cortisone. In addition to that, the cortisone in chewing gum has no side effects, such as an industrial that causes side effects. There were several antagonists manufactured after separating and extracting cortisone from chewing gum. *Boswellia Carterii* contains volatile oils included in manufacture of perfumes and treatment of bronchitis (36). It was considered a natural antibiotic and was used as a marinade for respiratory infections, including viral, through its effective role in strengthening the immune system, as well as a barrier in preventing the link between the virus and the cell in addition to it was effective role in preventing the pH of blood from decreasing, and this was an important factor in preventing viral infection (37). Zinc plays a vital role in the presence of proteins that help regulate the immune system and produce immune cells. Zinc was found in the strongest muscles of the body, especially in white and red blood cells, pancreas, kidneys, retina, liver, skin, bones, prostate gland and semen in men that contain large amounts of zinc (38). The body uses zinc to increase the body's immunity by activating the lymphocyte T cells, which control the regulation of the immune response, and prevent infection. The American Dietetic Association also indicated that zinc deficiency increases the possibility of several diseases. The zinc contributes to a 50% reduction in the period of virus infection and its severity, within 24 hours of the onset of

symptoms. About 1.1 billion people in the world suffer from zinc deficiency, due to several reasons, including the aging of people and people of this category who are vulnerable to deficiency in this element due to their taking of medicines that reduce the absorption of this element and get rid of it through edification, or not eating foods rich in zinc (39).

CONCLUSION

1-The role of essential oil of Eucalyptus its ability to treat of problems that afflict the respiratory system. In addition to its effective role in making the pH of respiratory system alkaline, in addition to its effective role in inhibiting the enzyme Angiotensin-converting enzyme 2 present in receptors on the sites where the link between the virus and the cell occurs.

2-In addition to the role of the active groups in the essential oil, which have high affinity for binding to the glycoproteins of the virus, which is an effective inhibitor.

3-Lemon oil was composed of a group of important nutrients of high interest, as it helps to increase the strength of the immune system in the body through the production of white blood cells , and its role in reducing body temperature. Yeast was a type of fungi, which contains a large amount of proteins and minerals, including iron, zinc, chromium and fiber. yeast was rich in minerals and proteins, it was an important factor to improve the body's immunity, as well as it was rich in vitamin B and anti-oxidants that prevent the formation of free radicals in the body that allow oxidation and union of substances.

4-*BoswelliaCarterii* contains inflammatory corticosteroids. It was noteworthy that the cortisone in chewing gum has a much better effectiveness, it has no side effects, such as an industrial that causes side effects. *Boswellia Carterii* contains volatile oil which used for treatment of bronchitis. It was considered a natural antibiotic for respiratory infections, including viral, through its effective role in strengthening the immune system, and it was effective role in making the pH of blood alkaline, and this was an important factor in preventing viral infection.

5-Zinc plays a vital role in the presence of proteins that help regulate the immune system and produce immune cells.

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