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Rosemary Plantarum Kariesh Cheese: Phytochemical, Micriobiological, Virology Activities and Sensory Evaluation

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Abstract

Fortified foods with some plant extracts and probiotic bacteria in food area are getting a lot of attention nowadays to bring new functional foods. Studying the synergistic effects of adding probiotics and plant extracts together is the aim of this study that having an increasing global research interest. Rosemary (Rosmarinus officinalis), as an additive plant extract, was tested for some phytochemical compounds, as it has been found to contain 7 components of them. Additionally, antimicrobial activity of Rosemary plant extracts was tested, via gel agar diffusion method for different pathogenic bacteria and fungi. Meanwhile, antiviral activity of Rosemary revealed that the all tested Rosemary extracts have antiviral activities against rotavirus infections on MA 104 cell lines where aqueous extract, alcoholic extract, and oil extract reduced the virus titers by -2, -1.75, and -3 log10 TCID50/ml, respectively. Generally, ethanol – petroleum ether extract showed the higher antimicrobial activity rather than other rosemary extracts. Furthermore, synergetic study on probiotic Kariesh cheese fortified with rosemary extracts and probiotic bacteria, Lactobacillus plantarum and Lactococcus lactis was carried out in different treatments in combination with Staphylococcus aureus. Rosemary plantarum Kariesh cheese with different treatments were examined microbiologically and sensory attributes were only performed for cheeses subject to safety control. conclusion: Rosemary have antimicrobial against different pathogenic bacteria and fungi. antiviral activity, synergetic study on Kareish cheese examined microbiologically and for Sensory attributes. Sensory attributes showed superior quality rather than the plane Kariesh cheese.

Keywords: Antimicrobial – Rosemary - Kariesh cheese – Antiviral - phytochemical.

Introduction

The common delicious white soft cheese consumed is Kariesh cheese in Egypt and many other countries. That produced by using lactic acid *(Lactococcus lactis)* acidification coagulants, containing about 10% fat and 70% moisture, [1]. Several dairy products, have been developed by adding plant additives and probiotic bacteria in order to get new functional dairy products to Fulfills the desires and needs of consumers [2, 3, 4]. Moreover, there have been various attempts recently to produce types of Kariesh cheese with nutritional and biological additives to improve their qualities [5, 6]. The most lactic acid bacteria (LAB) are Lactobacillus, Lactococcus and Bifidobacterium that have been beneficial for the human health and having probiotic properties [7, 8]. Several studies reported that *L. plantarum* induces the innate immune system (cytokine) responses and postbiotics that provide a protective benefit against COVID-19 and SARS- COV-2 [9, 10]. Furthermore, *L. plantarum* is associated with various fermented milk products as some cheeses and fermented plant foods such as pickles and kimchi [11]. Rosmarinus officinalis L. (Rosemary) is an aromatic evergreen shrubby herb highly distributed in the Mediterranean region, due to the presence of pigments, bioactive phytochemicals, flavors, Rosemary used as food flavorings, in cosmetics, in industry and in medicine [12, 13]. It is a well-known and greatly valued medicinal herb that is widely used for their useful compounds and properties in different area as in pharmaceutical uses and folk medicine as it contains tritepenes, ursolic acid, oleanolic acid, and micromeric acid [14, 15]. A large number of phenolic diterpenes such as carnosic acid, and carnosol have been identified in Rosmarinus, the presence of polyphenolic compounds with antioxidant activity has been reported [16]. In addition, several flavonoids and antioxidant activity, have been identified from rosemary [17, 18]. Moreover, rosemary extracts contain important active constituents and flavonoids derived from flavones [19, 20]. Additionally, various authors have reported that bioactive extracts from rosemary herb had anti-inflammatory, antidiabetic, antiviral and antimicrobial activities, several recent studies have related them with the rosemary antioxidant capacity [19, 4]. Furthermore, studied the Effect of Rosemary supplementation on Probiotic Yoghurt, as functional probiotic milk product [3]. The aim of study: This study aimed to improve nutritional, hygienic and sensory attributes of Kariesh cheese to obtain new functional dairy product by adding

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Rosemary extracts, *Lactobacillus planetarium and Lactococcus lactis* in the cheese. Microbiological-viral studies on phytochemical, antioxidant, and sensory attributes of the cheese were concern.

Materials and methods:

Rosemary extraction and phytochemical screening tests:

Preparation of Rosemary Aqueous Extracts:

Methanol Extraction:

Rosemary herb plants, was obtained from a medicinal herb garden. Rosemary aqueous extract was prepared by the method according to [21]. The Rosemary methanolic extract fractionated with chloroform, n-hexane, n-butanol, ethyl acetate, and water, respectively, were concentrated till dryness and then stored at -20ºC until used**.**

Oil extraction of Rosemary and Distillation method:

The dried plant material was ground prior to the hydro distillation and then was submitted to hydro distillation using Clevenger type apparatus, and heated for three hours according to the [22]**,** then essential oil was collected and stored at -20ºC until used

Preparation of Rosemary Aqueous Extract*:*

Rosemary aqueous extract was prepared by the method followed by [21]. Twenty five (25 g) of Rosemary sample was washed with tap water twice, and stored at room temperature, then blended with an electric blender (Vitamix, Black A3300 USA). The sample was put into a conical flask and extracted with distilled water (100 mL) for 24 h on a vibrator water bath at different Temperatures of 20 Cº, 40Cº, and 60 Cº. the samples was centrifuged at 3000 rpm for 10 min, then filtered using Whatman filter paper. Finally, the extract was filtered using a Millipore filter (0.22 m micro-filters, Merck & Co., Inc., Germany) into 10 mL tubes and kept at –20 C until further usage.

Phytochemical screening tests for Rosemary extracts and essential oils:

Phytochemical screening tests for methanol Rosemary extracts were carried according to [23]**.** The screening tests were for sterols and triterpenoids[24]**,** carbohydrates and/or glycosides/ Molish test [25], reducing sugar [26], tannins [27]**,** phenolic , flavonoids compounds , saponins, Fixed oils and Fats, alkaloids , amino acids & proteins, and glycosides "Borntrager's test" [28] coumarone, quinines and cardiac glycosides[29] and volatile oils [30]**.** Essential oils of, Rosemary, was prepared and extracts of its fractions of volatile oils were kept at -20ºC until use for antibacterial antivirus activities investigation.

Microbiological activity of Rosemary:

Reference strains:

Pathogenic microorganisms were obtained from Microbiology laboratory of dairy department, National research centre. The microorganisms used in this study were: - *B. cereus* (ATCC 33018), *S. aureus* (ATCC 2023), *E. coli 0157:H7* (ATCC 6933), Salmonella typhimurium (ATCC 14028), *Yersinia enterocolitica subsp. enterocolitica* (ATCC9610), *Listeria monocytogens*V7, *Pseudomonas aeruginosa* (ATCC 9027) and *Aspergillus flavus* (ATCC 3357). Lactic acid bacterial (LAB) strains used in this study were as follows: *Lactococcus lactis sub spp. lactis* and *Lactobacillus plantarum* obtained (Dsaz 0174) from Chr. Hansen's Lab., A/S Copenhagen- Denmark. All these strains were supplied in Clean Cultures from the Microbiology lab, Dairy department of the food industry and nutrition Institute, National research centre, Egypt. LAB strains were cultured and enumerated onto MRS culture media.

Antibacterial activity of Rosemary extract against foodborne pathogenic bacteria:

Agar well diffusion method, with slight modifications, by [31, 32] was considered, where each Brain heart infusion (BHI) agar plate was spread with one test indicator strain, separately using sterile swab sticks. After absorption, the sterile 6-mm corkborer was used to make wells on the agar film with equal depth. The wells were then filled up with approximately 30 µL of Rosemary extracts. The plates were incubated at 37 °C for 24 h. In the case of *Aspergillus flavus*, a spore suspension (10^6 spores /ml) was prepared and 100µl of it was spread on YPD agar dishes and incubated for 5 days at 25 °C. The diameters of inhibition zones were measured in mm around the wells to measure the degree of indicators sensitivity. Control negative plates were prepared as the same described above but with sterilized distilled water. The experiments were carried out in triplicates. Rosemary extracts dissolved in dimethyl sulfoxide (DMSO) and the wells containing DMSO but no spice extracts were used as negative control**.**

Antivirus activity:

Cell culture and virus:

MA104 monkey kidney cell lines were used to propagate simian rotavirus SA-11 (RV SA-11) stock. MA104 were cultivated in DMEM (Dulbecco's Modified Eagle Medium) with 10% of heat inactivated FBS (fetal bovine serum), 100µg/ml streptomycin, and 100 units/ml penicillin, in 5% CO2 incubator. After pre-inactivation of RV SA-11 stock with trypsin 10 mg/ml trypsin at 37 ºC for 30 min, ten-fold was prepared and replicated in the cell lines then the cytopathic effect was investigated after 3 days of incubation. The TCID50/100 µl (50% tissue culture infectious doses/100 µl) was calculated as reported previously by karber method [33]

Cytotoxicity assay:

We evaluated the cytotoxicity by MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, as documented previously by [34]**.** In brief, cells were grown in 96- well plates (5 X 10^3 cells / well). After 24 h incubation, the growth medium was discarded then the MA 104 cells were incubated with different dilutions (1:10, 1:100, 1:1000, 1:10000,

1:100000, 1:1000000) of Rosemary essential oil. Untreated cells were included as control. After incubation period of 48 hours, the culture medium was removed and replaced by 100 µL of fresh medium containing MTT reagent in each well. After incubation period for 4 h at 37°C, culture medium containing MTT reagent was removed then 100 µL of dimethylsulfoxide (DMSO) was added to each well. Viable cells were then measured using a Spectrophotometer reader (Asys Expert Plus microplate reader, UK) at 570 nm. The cell survival rate was calculated as the average OD value of bacteria treated cells/ average OD value of cell control. The 50% cytotoxic concentration (CC50) is defined as the bacterial concentration that can reduce 50% of cell viability to compare with cell control. A non-cytotoxic concentration of each essential oil used for antiviral assays**.**

Study of antiviral activity of rosemary extracts against RV SA11 by $TCID_{50}$ assay.

MA 104 cell lines were seeded in 96-well microtiter plate as described above then $TCID_{50}$ assay was performed as described by [35]. In brief, 10-fold dilutions of pre activated RV (about 10^6 $\log_{10}$ $TCID_{50}$/ml) were prepared. One hundred microliter of each dilution was incubated with 100 µl of a non- toxic dilution of each test compound for 1 h at 37°C in CO2 incubator. After that, one hundred microliter of the above mixture was incubated with MA 104 cells in 96-well plates for at 37°C in CO2 incubator. After discarding, the mixed solution the cell lines were rinsed by phosphate buffer saline then incubated with two hundred microliters of test medium that contain 2 µl of trypsin. Virus control wells (infected untreated-cells) and cell control wells (cells with only medium) were involved in each experiment. All microtiter plates were incubated at 37°C under 5% CO_2 for 3 days. Ten wells were used for each dilution of the virus either with or without oil. We estimated virus titer reductions by the difference between the titers of virus in presence and in absence of the rosemary extract.

Rosemary-plantarum-Kariesh cheese making:

Rosemary-plantarum-Kariesh cheese was made from manufactured according to [36, 37] and modified with addition of rosemary extract and starter culture *Lactococcus lactis sub spp. lactis* and the probiotic culture *Lactobacillus plantarum* (1:1) and spiked with *Staphylococcus aureus* in different 6 trials as shown in table (1). Rosemary and bacterial cultures were added to warm skim milk before cheese making. Activated cultures of lactic acid bacteria (2%) of each of *Lactobacillus plantarum* and *Lactococcus lactis* were mixed by 1:1 ratio and used in making Kariesh cheese. The warmed (30°C) inoculated milk kept overnight until curdling, followed by ladling into plastic frames lined with muslin cloth. Salt 1% was dispersed on the curd, and it was then pressed by using proper weights. The resultant cheeses were stored at 4°C for 14 days.

Table 1: different trials and treatments of Kariesh Cheese under study:

Abbreviations	Treatments
C1	2% starter culture *Lac. lactis* only
C2	2% starter culture and 0.40 µ/ml Rosemary
C3	2% starter culture and *S. aureus* at ~ 6.0 log cfu /ml
T1	2% starter culture and *S. aureus* at ~ 6.0 log cfu /ml with 0.40 µ/ml Rosemary
T2	2% starter culture and 2 % *L. plantarum* with 0.40 µ/ml Rosemary
T3	2% starter culture and *S. aureus* at ~ 6.0 log cfu /ml and 2 % *L. plantarum* with 0.40 µ/ml Rosemary

Sensory attributes of Rosemary plantarum Kariesh cheese:

Cheese samples were sensory evaluated freshly and after 15 days of storage by ten panellists of staff members of Dairy Department at Food Industries and Nutrition Division, National Research Centre, using the score sheet according to [38]**.** The scores of judging were 60 for flavor, 30 for body & texture and 10 for appearance.

Results and discussion

Phytochemical analysis of Rosmarinus officinalis

Alcoholic (methanolic) extract of Rosmarinus officinalis reveal that the Percentage of Rosmarinus officinalis methanol extract was 13.56 % as shown in Table (2). Meanwhile, phytochemical screening of Rosemary, Rosmarinus officinalis L. leaves revealed the present wide variety of biologically active compounds as essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, glycosides, steroids, quinones and terpenoids as the major phytochemical groups as shown in Table (3).

Therefore, Rosemary is used for food flavorings, in cosmetics, in traditional medicine and in industry Due to the presence of rich components, bioactive components [12, 13, 39]**.** Moreover, rosemary plant contains important constituents as diterpenes, phenolic acids, and flavonoids derived from flavones, apigenin and luteolin [20, 19]. Hence, several studies have related these compounds to the antioxidant capacity, antidiabetic, anti-inflammatory, antimicrobial, and antiviral activities [40,19]**.** Particularly, carnosic acid, and carnosol as polyphenolic compounds in Rosmarinus officinalis have been resulted in the antioxidant activity [16]. Additionally, several flavonoids, such as genkwanin, hispidulin, glucoside, luteolin, -glucuronide and others, phenolic compounds, and diterpenoid quinones also have been identified from rosemary [41, 42, 43]. There are several studies on supplementing Kariesh with some herbal extracts and probiotic bacteria in order to get higher nutritional, healthy and sensory properties as functional dairy product [72]. Also, similar studies have been carried on Rosemary Extracts for Their Chemical Composition, Antimicrobial, Antiviral, and Antioxidant activities [44].

Antimicrobial effect of rosemary extracts against foodborne pathogenic microorganisms

Results in Table (4) indicate that Rosemary extract show higher antibacterial activities against Gram positive bacteria than Gram negative bacteria. The more resistant of Gram negative (G-ve) bacteria could be attributed to its cell wall lipopolyssacaride which prevent that active compounds reach the cytoplasmic membrane of G-ve bacteria [45, 46]. In the same trend, Rosemary extract showed inhibitory effect against *Aspergillus flavus.* On the other hand, *S. aureus and B. cereus* showed more sensitivity than other microorganism with the three types of Rosemary extracts. The results obtained in the current study for the antimicrobial activity of rosemary against food borne pathogenic bacteria and *Aspergillus* was consistent with [44, 4]. Likewise, recent studies have shown that this antimicrobial activity was mainly due to the presence of high contents of 1,8-cineole [47]. However, different mechanisms and hypothesis of essential oils, from leaves of R. officinallis, had been given assume its antimicrobial actions [48, 49, 17, 50, 51]. However, mechanisms of antibacterial action of many spices and derivatives is not yet clear [52]. Likewise, recent studies have shown that this antimicrobial activity was mainly due to the presence of high contents of 1,8-cineole [47] and camphor [53]. The antibacterial activity of essential oils, from leaves of R. officinallis, is primarily determined by content of α-pinene [54]. Although some hypothesis had been given, assume different mechanisms. These hypothesis attributed the antimicrobial actions due to hydrophobic and hydrogen bonding of phenolic compounds to membrane proteins, followed by partition in the lipid bilayer [48], perturbation of membrane permeability [49, 55]. Membrane disruption [17]. Destruction of electrons transport systems [49, 51] and Cell wall perturbation [50].

Cytotoxicity and antiviral activity of Rosemary extracts on MA 104 cell lines

Our Results demonstrated that the higher concentrations of Rosemary extracts result in the higher the cytotoxicity effect on MA 104 cell lines (Table 2). Similar studies have been carried by [44] for Rosemary Extract and Essential Oil, for Antiviral activity and their results were not far from the current results. Also, as shown in (Table 2 and Figure 1). Our findings demonstrated that the three extracts, particularly oil extract, have antiviral activities against RV infections when they were incubated with virus prior to cell infections. The antiviral activities of rosemary agree with previous study documented by [56] who indicated that rosemary extract exhibited antioxidant effects and antiviral activity. These antiviral activities of rosemary due to saponins, polyphenols and polysaccharides that were isolated from rosemary and inhibit the herpes virus's replication [57], these findings demonstrated that these compounds have the ability to bind to viral capsids preventing them to attach to cell receptors and thereby prevented their penetrations and entry into host cells.

Microbiological analysis of Rosemary Plantarum Kariesh Cheese during storage:

Behavior of *S. aureus* in Rosemary Plantarum Kariesh Cheese during storage*:*

Due to the previous results of the antibacterial activity in table (3), *S. aureus* showed the most sensitive microorganisms to Rosemary extracts. And then, we prepared Kariesh cheese fortified with Rosemary and *L. plantarum* contaminated with *S. aureus* to study its behavior and the effect of these factors on the growth of this pathogenic bacteria strain in the manufactured Kariesh cheese during storage at 4 C° for 14 days. These factors (in table 1) include: starter culture (C3, T1 and T3), the Rosemary (T1) and the Rosemary with incorporated probiotic strains (T3). Results in Table (6) reveal that the count of *S. aureus* in Kariesh cheese (C3) decreased only about 0.5 log cfu/g at the end of the storage period (14 days) at 4°C indicating low effect of the starter culture factor on the growth of pathogen. Regarding the second factor which is the effect of Rosmary with starter culture in Kariesh cheese (T1) on the growth of *S. aureus*, results reveal high effect of Rosemary on *S. aureus* that is manifested in 3.0 log cfu/g reduction in its growth count, which illustrates the potent inhibitory activity of the Rosemary. The current results were in line with [58] how indicated Rosemary significantly reduces the growth of pathogenic bacteria and slowing down the growth of *S. aureus*. Extract components of Rosemary has synergic effect and interact with the bacterial cell membrane with different mechanisms so, the membrane structure and functionality are loss [59, 60]. Also, our results were in accordance with [61] who reported the antibacterial effect of rosemary in cheese stored at 10 °C for several days. Results for the effect of *L. plantarum* and starter culture in Kariesh cheese (T3) on the growth of *S. aureus*, reveal that *L plantarum* and rosemary showed high effect of inhibition on *S. aureus* as *S. aureus* count reduction was 4.0 log cfu/g Table 6. Moreover, in this concern results obtained were in agreement with the results by [61] about the single and combined action of the acidifying power and the production of bacteriocins of *L. plantarum* against pathogenic bacteria in Laboratory Cheese Model. Generally, from the literature, several studies reported that LAB capable of producing several different substances with antimicrobial power, as organic acids, hydrogen peroxide, ethanol and bacteriocins [62]. Furthermore, rosemary is used as a natural antioxidant to improve food shelf life [63].

Behavior of L. plantarum in Rosemary Plantarum Kariesh Cheese during storage:

Functional fermented dairy products like Kariesh cheese play important roles in ensuring healthy and well-being and prevention of many diseases [64]. Microorganisms like probiotic bacteria can play a significant role to this regard [65]. Therefore, it was important to follow up the growth and behavior of *L. plantarum* strain that was imbedded into the manufacture Kariesh cheese in T2 and T3 trials, treatments, during the storage period. Results in Table (6) show that the growth and behavior *of* L. plantarum were almost the same trend in all trials. Generarly, it can be noticed that the growth of *L. plantarum* in the two treatment (T2 and T3) increased within the first week of storage, then the count declined till reached 7.5 -7.8 log cfu/g for T2 and T3 at the end of storage period, respectively.

The obtained results agree with [66] who indicated that *L. plantarum* was not inhibited by any concentrations of rosemary tested. [61] Indicated that, in cheese samples all strains of *L. plantarum* grew well, reaching counts around 10^8 and 10^9 cfu/g after 7 days. It is important to note that at the end of storage period of the probiotic strain, the count did not fall below the count 10^6 cfu/g which is the minimum bacterial count for the dairy product to be defined as probiotic [67]

Starter culture, L. lactis sub spp. lactis*:*

Results in Table 6, reveal that the count of stature culture, *L. lactis sub spp. lactis*, fluctuated between counts of 6.4 to 7.8 log cfu/g, during cold storage in all controls and treatments. Hence, the obtained results agree with [6] who reported that survival of stature culture *L. lactis sub spp. lactis* in Kariesh cheese during storage and confirmed the survival of the microorganisms is the important criterion for the quality and health characteristics.

Total bacterial count in Kariesh cheese during the cold storage period:

Results shown in Table 6 indicates that at the end of storage period (14 days) all of control and treatments of Kariesh cheese showed total bacteria counts range from 8.1-6.52 log cfu/g for T3 and T 2, respectively, which could be acceptable figures. Furthermore, addition of rosemary extracts did not show a significant microbial effect on the total bacterial count of Kariesh cheese with different treatments during the storage period of cheese. Also, the results obtained were in agreement with [6], for Total bacterial counts in Kariesh cheese during the cold storage period.), finally, molds and yeasts were not detected in all treatments of karish cheese untie the 14 days of storage

Sensory evaluation of the rosemary plantarum Kariesh cheese, during cold storage:

The sensory evaluation results of plain cottage cheese and cottage cheese with added rosemary and Plantarum bacteria, as shown in Table 7, reveal no significant differences in terms of appearance, texture, taste and composition. The sensory evaluation results of plain cottage cheese and cottage cheese with rosemary and Plantarum bacteria added to it, as shown in Table (7), showed no significant differences in terms of appearance, texture and composition at the end of manufacturing and during the cold storage period up to fourteen days. While, the taste improved slightly in cheese with added rosemary and plantarum bacteria compared to cheese without additives, this was during the storage period. From the above, it is clear that the individual sensory evaluation elements were reflected in the overall sensory evaluation, with the treated cheese being superior to the plain cheese. The results also showed that the best sensory properties of the cheese, whether with rosemary and plantarum added or without additives, were after seven days of cold storage. All the treatments of the kareish cheese were appreciated by the 10 panelists for sensory evaluation (total score), as for appearance (in range 6.0 – 7.3), body & texture (in range 27.1-28.9), flavor (in range 52.2 -55.1) and total scores (84.5, 89.9 and 81.1) control 2, over the control 1 (80.0, 84.1 and 79.3) , after 0,7 and 14 days of cold storage, respectively. Obviously, the highest acceptability was for control 2 (contained *Lac. lactis* and, rosemary aqueous extracts), when fresh and during the storage period. It was also noticed an increase in scores of all sensory evaluation factors by increasing the storage period up to 7 days then decreased up to end, after 14 days. This improvement in sensory properties may be due to the increased production of volatile fatty acids that are produced by LAB bacteria during storage [68, 69]. Also, the results obtained agreed with [6] particularly during the first 7 days of Kareish cheese storage. Additionally, there are several recommendations to use these additives to cheese and foods to improve its healthy nutritional, sensory quality and as natural preservatives [68, 70, 71]

: Table (2): Percentage of Rosmarinus officinalis methanol extract %

Extract	%
Alcoholic (methanolic) extract of Rosmarinus officinalis	13.56

Table (3): Phytochemical screening tests of Rosmarinus officinals extract

Test	For Rosmarinus officinalis
Sterol and/or terpenes	(+)
Carbohydrates and/or glycosides	(+)
Reducing sugar	(-)
Alkaloids	(-)
Saponins	(-)
Phenolic	(+)
Tannins	(-)
Flavonoides	(+)
Coumarins	(-)
Amino acid and Protein	(-)
Anthraquinone Glycosides	(-)
Hydroxyanthraquinone	(-)
Quinones	(+)
Cardiac glycosides	(-)
Fixed oils	(+)
Volatile oils	(+)

(+) and (-) refer to present and absent of certain compounds, respectively.

Table (4): Antibacterial activity of Rosemary extract (Concentration 100mg/ml).

Foodborne microorganisms	Inhibition zoon of Rose Mary (diameter, mm)			
	Aqueous Extract	Alcoholic extract	Petroleum ether extract	Control
Salmonella spp.	3.5 ±0.11^{Db}	5.0 ±0.012^{Ba}	5.0 ±0.09^{Ca}	0.0±0.0^{1Ac}
E. coli O157:H7	2.1±0.014^{Ea}	2.5±0.092^{Ca}	2.5±0.221^{Da}	0.0±0.01^{Ab}
Yersinia enterocolitica	3.8±0.09Cc	5.0±0.112^{Bb}	10.0±0.21^{Ba}	0.0±0.01^{Ad}
Staph. aureus	20 ±0.121^{Ab}	20±0.118^{Ab}	23.0±0.211^{Aa}	0.0±0.01^{Ac}
B. cereus	18.0±0.016^{Bc}	20.0±0.214Ab	21.0±0.321^{Aa}	0.0±0.01^{Ad}
Listeria monocytogenes	2.5±0.019^{Ea}	2.5±0.076^{Ca}	2.5±0.111^{Da}	0.0±0.01^{Aa}
Pseudomonas aeruginosa	0.0±0.018^{Fa}	0.0±0.00^{Da}	0.0±0.01^{Da}	0.0±0.01^{Aa}
Streptococcus mutants	0.0±0.099^{Fa}	0.0±0.0.0^{Da}	0.0±0.00^{Da}	0.0±0.01^{Aa}
Aspergillus flavus	3.9±0.081^{Cc}	5.0±0.119^{Bb}	7.5±0.018^{Ca}	0.0±0.01^{Ad}

0.0: No inhibition zone, means with the different capital superscript letters (A,B,C) within the same column indicate significant ($p<0.05$) differences, means with the different small superscript letters (a,b,c,...) within the same row are significantly ($p<0.05$) different

Table (5) Results of antiviral activity of compounds on RV by $TCID_{50}$ / /50µl measurement

Extract	Non-toxic dilution of	Virus titers without extract	Virus titers with extract	Reduction value of virus titers*	% Inhibition
Aqueous extract	1:10000	10^6	10^4	10^2	33
Alcoholic extract	1:100000	$10^{6.25}$	$10^{4.5}$	$10^{1.75}$	28
Oil extract	1:1000	10^6	$10^{3.25}$	10^3	50

*Reduction of virus titer was estimated as " virus titer without extract - virus titer with extract ".

Table (6): Microbiological analysis of Kariesh cheese fortified with Rosemary and *L. plantarum* during storage time.

Storage time	Treatments	T.C	*S. aureus*	*L. plantarum*	*Lac. lactis*	*M&Y*
Zero time	Control 1	7.23			7.20	0.0
	Control 2	7.22			7.25	0.0
	Control 3	7.81	6.19		7.21	0.0
	T1	7.84	6.10		7.10	0.0
	T2	7.73		8.19	7.13	0.0
	T3	7.95	6.2	8.26	7.19	0.0
12 hr.	Control 1	7.44			7.5	0.0
	Control 2	7.36			7.35	0.0
	Control 3	8.11	6.06		7.25	0.0
	T1	7.97	5.81		7.43	0.0
	T2	8.32		8.32	7.34	0.0
	T3	8.02	5.11	8.33	7.61	0.0
24 hr.	Control 1	7.55			7.9	0.0
	Control 2	7.61			7.52	0.0
	Control 3	7.95	6.0		7.31	0.0
	T1	6.81	4.1		7.32	0.0
	T2	7.68		8.35	7.40	0.0
	T3	7.73	3.74	8.41	7.74	0.0
7 days	Control 1	7.42			7.1	0.0
	Control 2	7.53			7.21	0.0
	Control 3	7.71	5.90		7.11	0.0
	T1	6.68	3.35		7.6	0.0
	T2	7.42		8.1	7.49	0.0
	T3	7.28	3.25	8.12	7.51	0.0
14days	Control 1	7.19			6.61	2.0
	Control 2	7.34			6.81	1.9
	Control 3	7.19	5.72		7.1	0.0
	T1	6.52	3.1		6.41	0.0
	T2	7.12		7.5	7.10	0.0
	T3	7.11	2.20	7.8	6.91	0.0

Control 1 starter only and *L. plantarum,* Control 2 = starter culture and Rosemary, T1 *S. aureus* with Rosemary, T2 Rosemary with *L. plantarum,* T3 *S. aureus* with Rosemary and *L. plantarum, Mold and yeast Not be detected except control1 and 2 at 14 days*

Table (7): sensory attributes of Rosemary Plantarum Kariesh Cheese during Storage

	Appearance (10 points)			Body & texture (30 points)			Flavor (60 points)			Total scores (100 point)		
	Storage period (days)											
	0	7	14	0	7	14	0	7	14	0	7	14
Control 1	6.0^{Db} ± 0.18	7.2^{Ba}± 0.24	7.31^{Da} ± 0.091	27.1^{Ec}± 0.16	28.5^{Cb}± 0.09	26.6^{Ga}± 0.12	52.2^{Db}± 0.21	53.5^{Gab} ± 0.11	52.1^{Ha}± 0.34	80.0 ^{Dc} ± 0.1	84.1 ^{Eb} ± 0.15	79.3 ^{Ga} ± 0.12
Control 2	6.1^{Db}±0.09	7.1^{Ba}± 0.11	7.1^{Da}± 0.21	27.5^{Ec}± 0.41	28.9^{Cb}± 0.22	27.3^{Fa}±0.032	55.1^{Da}± 0.19	54.1^{Fb} ±0.1	53.8^{Ga}± 0.05	84.5^{Dc}± 0.09	89.9^{Eb}± 0.21	81.1^{Fa}±0.087

Control1 = free of pathogen and rosemary contain only starter, Control 2= Rosemary and L. plantarum with starter culture. (mean± SD or SE) as: means with the different capital superscript letters (A,B,C) within the same column indicate significant ($p<0.05$) differences, means with the different small superscript letters (a,b,c,...) within the same row are significantly ($p<0.05$) different.

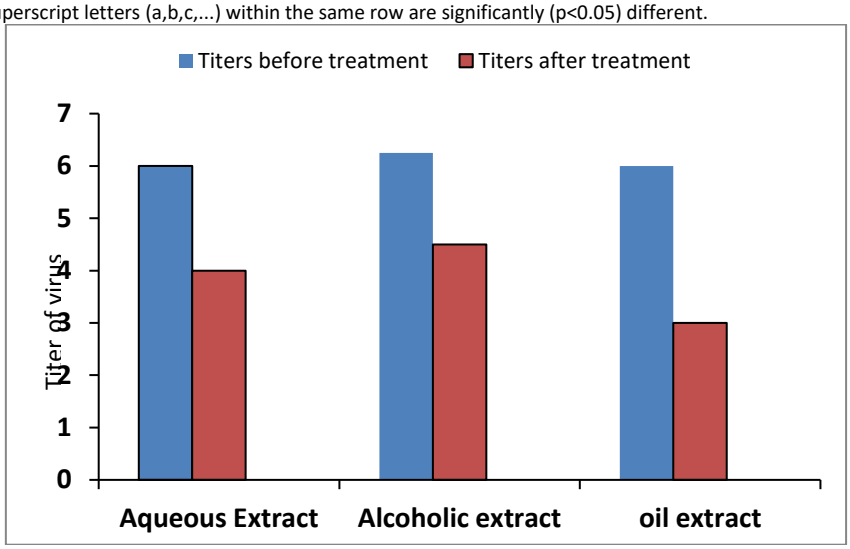

Fig 1. Antiviral activities of Rosemary extracts against RV infection on MA 104 cell lines

Conclusions

Rosemary *(*Rosmarinus officinalis L), using different extracts, was found to contain 7 phytochemical components, where the most dominant components were terpenoids, flavonoids and phenolic acids antimicrobial activity of Rosemary plant extracts was positive, via gel agar diffusion method, against different pathogenic bacteria and fungi. Additionally, Rosemary extract showed also antiviral activity applying Cytotoxicity assay by MTT method and using MA104 cell lines. Synergetic study on Kariesh cheese fortified with rosemary aqueous extract and *Lactobacillus plantarum* and the starter culture *Lactococcus lactis* were examined microbiologically and for Sensory attributes. Sensory attributes of rosemary plantarum Kariesh cheese showed superior quality rather than the plane Kariesh cheese.

Conflicts of interest

"There are no conflicts to declare"

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