Eur Food Res Technol (2008) 226:583–590
DOI 10.1007/s00217-007-0592-1

ORIGINAL PAPER

Reevaluation of bioactivity and antioxidant activity of *Myrtus communis* extract before and after encapsulation in liposomes

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Received: 16 January 2007 / Revised: 1 February 2007 / Accepted: 5 February 2007 / Published online: 24 February 2007

Abstract The antioxidant and antimicrobial activity of *Myrtus communis* extract was determined before and after its encapsulation in liposomes of different composition. Evaluation of the sunflower oil oxidation by the Rancimat stability test and malondialdehyde formation by HPLC were used to measure the antioxidant action in comparison with common commercial antioxidants, such as butylated hydroxytoluene and α-tocopherol. The thermal-oxidative decomposition of the samples, the modification of the main transition temperature for the lipid mixture and the splitting of the calorimetric peak in the presence of the antioxidants were studied by differential scanning calorimetry. The size and the surface charge of liposomes were also studied. The extract showed antioxidant and antimicrobial activity. At concentrations up to 160 ppm, the extract showed superior activity than α-tocopherol. When the extract was encapsulated in liposomes, its antioxidant as well as its antimicrobial activity proved to be superior from that of itself in pure form.

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Keywords *Myrtus communis* · Liposomes · Encapsulation · Rancimat · DSC · MDA

Introduction

Myrtus communis (Myriaceae) is an evergreen sclerophyll shrub or small tree [18]. The leaves emit an aromatic and refreshing smell somewhat reminiscent of myrrh or eucalyptus; the taste is very intensive, quite unpleasant and strongly bitter. The plant grows abundantly in the North Western to Eastern Mediterranean. Myrtle is a spice finding no wide application because of its bitterness, despite the pleasant odor. Its culinary importance is limited to the region of origin. Foods flavored with the smoke of myrtle are common in rural areas of Italy or Sardinia. The most important constituents of myrtle oil are myrtenol, myrtenol acetate, limonene, linalool, α-pinene, 1,8-cineole, β-caryophyllenein addition to *p*-cymene, geraniol, nerol and the phenylpropanoid, methyleugenol [8, 21]. However, there is considerable variability in the composition of oil from different locations. In folk medicine, the fruit of this plant is used in the treatment of many types of infectious disease, including diarrhea and bloody diarrhea, and the leaves are used as antiseptic and anti-inflammatory agent, and as a mouthwash, for the treatment of candidiasis. Many plants from the Myriaceae family are reported to have antibacterial or antifungal activities [17, 25].

Antioxidants have been widely used as food additives to avoid the degradation of foods. They also have an important role in preventing a variety of lifestyle-related diseases and ageing because these are closely related to the active oxygen and lipid peroxidation

[11]. As indicated by Lalas and Tsaknis [14] doubts about the safety and use of synthetic antioxidants first arose in the early 1960s. Consequently, there has been much interest in the antioxidant activity of naturally occurring substances. The fats and oils industry increasingly seeks natural sources for antioxidants taking into account the growing consumer preference for "natural" products [14]. The plant kingdom offers a large range of compounds with desired activity. Extracts of many plants (such as spices, herbs, etc.) have shown to have various degrees of antioxidant activity [5] and antibacterial and antifungal properties [20]. However, as indicated by Chorianopoulos et al. [4] the commercial application of plant extracts and essential oils in food preservation could be difficult due to their limited antibacterial activity. More attention and effort is now focusing on product formulation and efficient delivery systems to enhance product and ingredient stability and assist or enhance absorption [12]. This can be accomplished by the use of liposomes.

Liposomes are spherical particles with one or multiple concentric membranes in which a fraction of the solvent can be encapsulated. Liposomes are constructed of polar lipids. Typical compositions include phosphatidylcholines and phosphatidylethanolamines, often containing negatively charged lipids, such as phosphatidylserine and phosphatidylinositol. In addition ceramides (such as sphingomyelin), and sterols (cholesterol, ergosterol, sitosterol, etc.) are also included. As indicated by Barratt [2], liposomes were first proposed as carriers of biologically active substances in 1971. They resemble to the lipid membrane part of cells. Only, liposomes can encapsulate both hydrophilic and lipophilic materials (plant extracts). It is also important that liposomes encapsulating extracts possess new physicochemical characteristics and bioactivity [27] which can enhance extract's original activity. The modified activity of plant extracts encapsulated in liposomes has been reported previously [10].

In this work, we have evaluated the modified activity (antioxidant and antimicrobial) of the methanol extract of *M. communis* (indigenous in Greece) before and after encapsulation in liposomes and its activity was compared with that of known synthetic [butylated hydroxytoluene (BHT)] or natural (α-tocopherol) antioxidants and antimicrobial agents (netilmicin and intraconazole). Liposomes were applied because, due to their unique properties, they are able to enhance the performance of products by increasing ingredient-solubility (easier incorporation of water-soluble compounds into oil-based products), improving the bioavailability and the in vivo and in vitro stability [10].

Materials and methods

Leaves of *M. communis* were collected in the area of Attiki (Athens, Greece) and deposited at the Herbarium of the Division of Pharmacognosy-Chemistry of Natural Products (Athens, Greece).

The dried aerial parts were powdered and extracted with methanol at room temperature for 24 h and the extract was concentrated under vacuum (using a Büchi Rotavapor R-215 and B-160 Vacobox pump—BÜCHI Labortechnik AG, Flawil, Switzerland).

The extraction of a smaller quantity of the same plant material was also performed using the same procedure but with absolute ethanol as solvent. This extract was co-chromatographed in comparison with the methanol extract using thin layer chromatography on Silica gel F254 TLC plates (Merck Ltd, Darmstand, Germany) in various systems of different polarities (Cyclohexane:dichloromethane:methanol from 100:0:0 to 0:70:30). The spots were detected before and after spraying with vanillin (Sigma Chemicals Company Ltd, St Louis, USA) and Neu's reagent (Sigma Chemicals Company Ltd) as described by Lalas and Tsaknis [14], showing a completely similar chromatographic profile for both solvents. In further experiments only methanol extract was used.

Evaluation of the antioxidant action by the Rancimat method

The method used was adapted from Lalas and Tsaknis [14]. The antioxidants (pure extract in concentrations 80, 120, 160 and 240 ppm or BHT at 200 ppm and α-tocopherol at 200 ppm—both from Sigma Chemicals Company Ltd, St Louis, USA) were accurately weighed into sunflower oil (Elais S.A., Athens, Greece) and their action were determined using a Rancimat 679 (Metrohm Ltd, Herisau, CH 9101, Switzerland) along with another sample of sunflower oil without antioxidant (control). One milliliter of the appropriate solvent (methanol) was added and mixed well in order to dissolve the antioxidant. The conditions were set at 90 °C and 15 l/h. The comparison of the activity of pure extract (160 ppm) and the extract encapsulated in liposomes (prepared using 160 ppm of extract), against the oxidation of sunflower oil using the same experimental conditions (90 °C and 15 l/h), was also determined. During this experiment a sample of sunflower oil with empty liposomes was used as control.

Protection factor

The protection factor (P.F.) for Rancimat method was calculated as P.F. = (induction period with antioxidant)/

(induction period without antioxidant). A protection factor greater than 1 indicates inhibition of the lipid oxidation. The higher the value, the better the antioxidant activity [13].

Determination of malondialdehyde by HPLC

The method used was adapted from Gortzi et al. [10]. The determination of malondialdehyde (MDA) was carried out after accelerated oxidation of sunflower oil under UV light. Sunflower oil and the added antioxidant (pure extract, BHT, α-tocopherol) in concentrations of 100 and 150 ppm, were accurately weighed into a glass petri dish (87 mm internal diameter and 15 mm in height) and mixed well. The dishes were placed (half immersed) in a water bath (50 °C) and directly exposed to UV light produced by a lamp situated 50 cm above (General Electric 260 nm UV Germicidal lamp G25T8, 25 Watt, 45 cm in length and 3 cm in diameter) for 12 h. Then, the oils were collected and stored in dark glass-bottles with nitrogen and stored at −16 °C until MDA analysis by HPLC the same day. The method used for the determination of MDA was adapted from Tsaknis et al. [29]. HPLC was performed using a Waters System consisted of a Waters 600E HPLC pump equipped with a Waters μ-bondapack C18 (300 × 3.9 mm i.d.) column. The mobile phase was 1% acetic acid:acetonitrile (85:15, v/v) and the flow rate was set at 2.5 ml/min. The retention time of MDA was 1.44 min. Chromatograms were monitored at 254 nm using a Waters 486 Tunable Absorbance Detector (Millipore Corporation, Waters Chromatography Division, Massachusetts, MA 01757, USA).

Preparation of liposomes

The liposomes were prepared as described by Gortzi et al. [10], using common lipid compositions [1] since as indicated by El Jastimi et al. [6] phosphatidylcholine/cholesterol liposomes show little leakage of contents. Liposomes contained egg L-α-phosphatidylcholine (PC) (10 mg/ml) and cholesterol (C) (2 mg/ml) for determination of antioxidant and antimicrobial activity or PC (10 mg/ml), C (2 mg/ml) and phosphatidylglycerol (PG) (1 mg/ml) for determination of antimicrobial activity were prepared by the mechanical shaking technique (thin film method). PC, C and PG were obtained from Sigma Chemicals Company Ltd (St Louis, USA). The 99% purity of the lipids was verified via thin layer chromatography on silicic acid-coated plates (Merck Ltd, Darmstadt, Germany) as described previously [19]. The mixture of lipids was dissolved in chloroform:methanol (3:1) in a 50-ml round-bottom flask and the organic solvent was removed by a rotary evaporator until a thin film was formed to the walls. When *M. communis* extract was used (in a quantity of 1.5 mg/ml) as antioxidant or antimicrobial agent, it was dissolved in methanol and then mixed in a round-bottom flask with PC and C (antioxidant and antimicrobial activity) or PC, C and PG (antimicrobial activity), respectively. The organic solvents were evaporated under a stream of nitrogen at 35 °C (above the lipid transition temperature). The lipid film was suspended in 2 ml of a phosphate buffer saline solution (PBS) (pH 7.4) (Sigma Chemicals Company Ltd) and vigorously vortexed for 15 min. Sonication of the preparation (in order to reduce the size and homogenize liposomes) was carried out in a Branson bath-type sonicator. This suspension was allowed to hydrate for 2 h in the dark at room temperature (in order to anneal any structural defects) [1] and then centrifuged at 6,500 rpm at 4 °C using a Sorvall General-Purpose RC-3 Automatic Refrigerated Centrifuge (Ivan Sorvall INC., Newtown Connecticut, USA) in order to dispose non-incorporated molecules of extracts or lipids from the liposome suspension [26]. The supernatant of the centrifuged suspension contained the prepared multilamellar vesicles (MLV). When it was not possible to use the prepared liposomes the same day, they were freeze-dried with a Virtis model, Sentry 5L (Virtis Company INC., Gardiner, New York, USA) in order to prevent storage problems [22].

Liposome sizing measurements

A total of 50 μl of the liposome dispersion were diluted with 20 ml of filtered buffer (0.22 μm pore size, polycarbonate filters, Millipore, UK) and sized immediately by photon correlation spectroscopy using a Model 4700C (Malvern Instruments Ltd, Malvern, Worcestershire, UK) (which enables the mass distribution of particle size to be obtained, according to manufacturers). Measurements were made at 25 °C with a fixed angle of 90° and sizes quoted are the *z*-average mean (dz) for the liposomal hydrodynamic diameter. The size of liposomes was also confirmed by image analysis of electron micrographs using a LEICA Q500MC image analysis system (Leica Cambridge Ltd, Cambridge, UK).

Liposomal surface charge

Liposome dispersions were diluted with PBS pH 7.4 and their electrophoretic mobility was measured at 25 °C by photon correlation spectroscopy using a Zetasizer 5000 (Malvern Instruments, UK). Zeta potentials of the dispersions were calculated by the instrument according to the Smolowkovski equation.

Scanning electron microscopy

Electron microscopy analysis of liposome was carried out according to Gortzi et al. [10] on a JEOL JSM-6360 Scanning electron microscope (JEOL Ltd, Tokyo, Japan). The liposome dispersion placed earlier on aluminum stubs (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was dried and then sputtered with gold.

Vesicle dispersions were kept at 5 ± 1 °C and at fixed time intervals (once a week for the first 2 months) liposomes size distribution was determined by scanning electron microscopy (SEM).

Differential scanning calorimetry

The method used was adapted from Gortzi et al. [10]. The extract was encapsulated in liposomes (PC:C 10:2) and its antioxidant action was estimated using differential scanning calorimetry (DSC). A Perkin Elmer DSC-6 calorimeter (Perkin Elmer Corp., Norwalk, CT, USA) was employed to study the oxidation stability of the samples.

Samples of 4 mg were placed in DSC aluminum crucibles closed with lids perforated with a hole (internal diameter—1 mm) in the center in order to allow the sample to be in contact with the oxygen stream. The purge gas foaming the reaction atmosphere was oxygen. An empty crucible, hermetically sealed, was used as reference.

The starting temperature of oxidation was determined as the onset temperature of the oxidation peak. The temperature program was: Heat from 30 to 180 °C (100 °C/min), hold for 1 min at 180 °C and finally heat from 180 to 370 °C (10 °C/min).

Antimicrobial bioassay

The antibacterial activity of the extract was determined, using the diffusion technique of Bauer-Kirby (disc method) [3] according to Gortzi et al. [10], by measuring the zone of inhibition against four Gram positive bacteria: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus mutans* (ATCC 31989) and *Staphylococcus viridans* (ATCC 19952), and four Gram negative: *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047) and *Klebsiella pneumoniae* (ATCC 13883), as well as against three human pathogenic fungi *Candida albicans* (ATCC 10231), *Candida tropicalis* (ATCC 13801) and *Candida glabrata* (ATCC 28838) and against the food-pathogen bacteria of *Listeria monocytogenes*. Standard antibiotics netilmicin and intraconazole (both from Sanofi, Diagnostics Pasteur, Paris, France) were used in order to control the sensitivity of the tested bacteria and fungi, respectively. The tested compounds were dissolved in methanol. For each experiment, a control disc with pure solvent and two discs with empty liposomes (each with different lipid composition: PC:C:PG or PC:C) was used as blind control. All the paper discs had a diameter of 6 mm and were deposited on the surface of the seeded tryptic case soy agar (Scharlau Chemi S.A., Barcelona, Spain) petri dishes. Six microliters of a solution of 1 mg/ml of the extracts have been put on the discs. The plates were inoculated with the tested organisms to give a final cell concentration of 10^7 CFU/ml and incubated for 48 h at 37 °C. The fungi were grown on Sabouraud's agar (Pronadisa, Conda Lab. Madrid, Spain) at 25 °C for 48 h. The results are expressed as mm of zone of inhibition.

Statistical analysis

Results, were expressed as the means and standard deviation (SD in parenthesis), of at least three simultaneous assays carried out in all methods. Statistical significance of the differences between the mean values was assessed by ANOVA test.

Results

The antioxidant action of *M. communis* extract was determined using three methods: namely Rancimat stability test, MDA formation and oxidative stability by DSC. Rancimat and DSC methods assess different aspects of the oxidative process and are based on generation of volatiles and thermal release, respectively, indicating the onset of advanced oxidation (termination), while the MDA method assesses the antioxidant action at a much lower temperature and determines a secondary product (MDA) at a different stage of oxidation.

The extract (Figs. 1, 2) in all concentrations showed antioxidant action. Its action was also compared with that of common commercial antioxidants BHT and α-tocopherol. At concentrations up to 160 ppm, it showed superior action than α-tocopherol (significant at $P < 0.05$). As it appears in Fig. 1 the increase of antioxidant activity was not proportional to concentration. As observed by Schuler [24] and Gortzi et al. [10], the activity of certain antioxidants does not increase linearly with the increase of their concentration. In high levels of addition can even act pro-oxidantly.

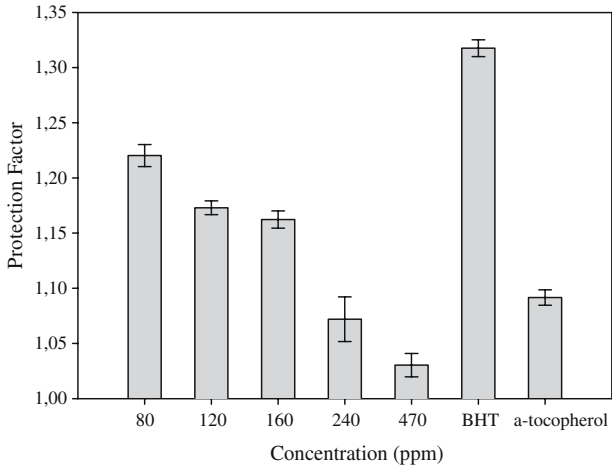

Fig. 1 Protection factor (Rancimat method) of *Myrtus communis* pure extract (80, 120, 160 and 240 ppm), BHT (200 ppm) and α-tocopherol (200 ppm) in sunflower oil

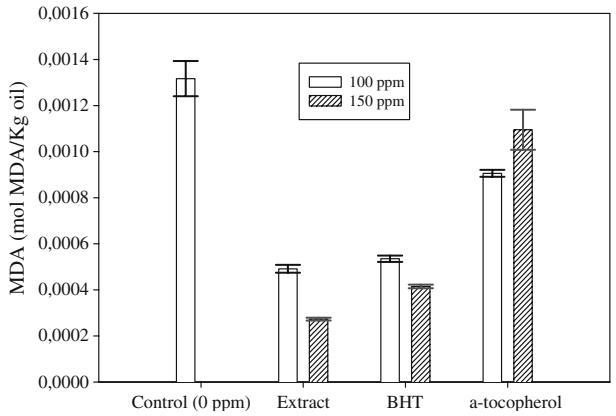

Fig. 2 Malondialdehyde concentration in sunflower oil with *Myrtus communis* pure extract, BHT and α-tocopherol (all at 100 and 150 ppm)

The encapsulation of the extract in liposomes (PC:C 10:2) produced a more intense (significant at $P < 0.05$) antioxidant action than the same extract in pure form (25% higher antioxidant activity) (Table 1). It should be stated that 160 ppm (used for the direct comparison of the activity of encapsulated or non-encapsulated extract—Table 1) was the initial amount of extract used for encapsulation. The exact percentage of encapsulation was difficult to be determined and it was not our purpose to do so during this study, since our interest was focused on the modification of the bioactivity.

Auto oxidation of fats, fatty acids and lipids is a well-established exothermic process and the methods of thermal analysis are valuable for study thermostability and thermo-oxidation [16]. DSC is a good technique to determine the kinetic parameters of the non-inhibited and inhibited fatty acid oxidation. The extrapolated temperature of the start of the oxidation process

Table 1 Induction period (h) and protection factor on sunflower oil after addition of liposomes (pure or encapsulating extract)

Sample description	Induction period[a]	Protection factor
Sunflower oil + liposomes (PC:C 10:2 mg/ml) (no extract)	19.6 (0.8)	–
Sunflower oil + 160 ppm *M. communis* (MeOH extract)	23.5 (0.9)	1.2
Sunflower oil + liposomes (PC:C 10:2 mg/ml) with 160 ppm *M. communis* (MeOH extract)	29.5 (0.6)	1.5

[a] Values are means of triplicate determinations. Standard deviation is given in parenthesis

is useful to evaluate antioxidant activity [16] based on the measurements of the incubation period. The effectiveness of an antioxidant represents the possibility of blocking the chain radical process by interaction with the peroxyl radicals, which are responsible for the duration of the incubation period [9]. In Fig. 3 the DSC curves of *M. communis* extract and liposomes (PC:C 10:2) suspensions in the absence and in the presence of extract are given. The analysis was carried out with PC:C liposomes. This preparation was selected since the system PC:C + *M. communis* extract presented the highest antimicrobial activity (Table 3). Thermal-oxidative decomposition of pure extracts and liposome preparations was studied. In comparison to the Rancimat stability test, DSC analysis is concluded to be useful as well as faster method, which can be applied for the evaluation of oxidative stability of samples containing volatile antioxidants and other lipid systems containing water [10]. The method used in DSC analysis was based on experiments in which the temperature of the extrapolated onset of the thermo-oxidation process and the temperature of maximum heat flow were determined from the resulting measurement curves for exothermic reaction [16]. An exothermic peak is observed in the range of 180 to 360 °C related to auto-oxidation process of the samples. Using the curves, the onset temperature at which the auto-oxidation process begins is determined [16] as 218 °C (empty liposomes-control), 237 °C (*M. communis* extract) and 272 °C (liposomes + *M. communis* extract). As it appears in Fig. 3 the addition of extract encapsulated in liposomes had more intense antioxidant action than itself in pure form. The shape of DSC plots of liposome preparations are similar to those of pure extract oxidation curves. However, at the same heating rate, the temperature of the start of oxidation reaction is significantly ($P < 0.05$) higher. The modified antioxidant action of extract during its encapsulation was expected since the complex (liposome membrane-fraction) possesses new

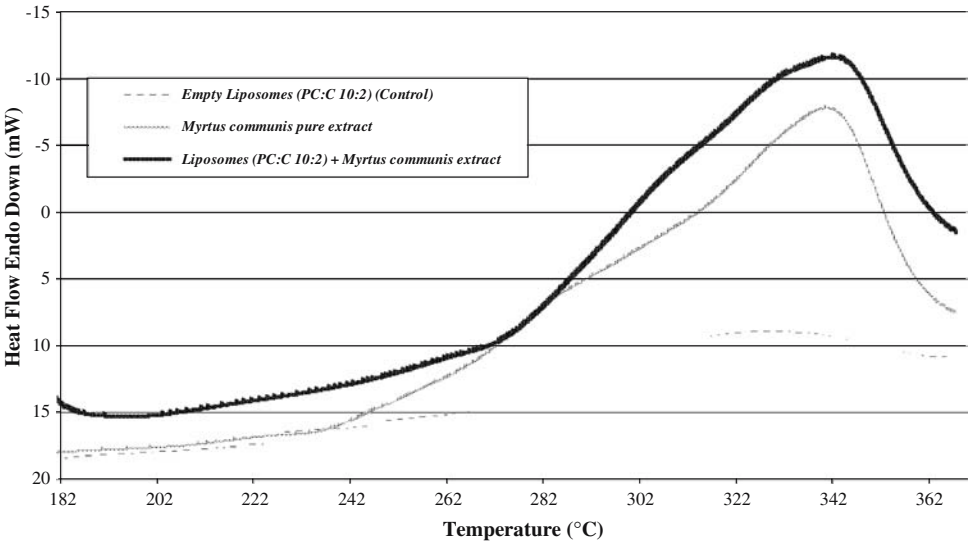

Fig. 3 DSC oxidation (DSC-heat flow/temperature) of *Myrtus communis* pure extract and extract encapsulated in liposomes. Illustration 1 Scanning electron micrographs of MLV liposomes (before and after encapsulation of *M. communis* extract) obtained by thin film method (pH 7.4, room temperature)

physicochemical characteristics and bioactivity depended on structure, size and *z*-potential of the preparation [10, 27]. Also, the antioxidant action of pure liposomes (no extract added) of the same lipid composition (PC:C 10:2) appeared much lower (significant at $P < 0.05$) than that of extract, implying that the encapsulation of the components of the extract in the aqueous part of liposomes and the possible link of the lipophilic in lipid bilayers stabilized the liposome membranes.

Morphological studies using optical and SEM were performed in order to verify vesicle formation and to provide knowledge on their shape, structure, and size. SEM images have a characteristic three-dimensional appearance (due to the manner in which the image is created) and are useful for judging the surface structure of the sample. Scanning electron micrographs (Illustration 1) showed that the liposome vesicle, obtained by the thin film method, were spherical in shape. The tendency of the liposomes to aggregate was also observed. Physical stability of vesicular (self aggregation) dispersions was also investigated by SEM visualization. Vesicle dispersions were kept at 5 ± 1 °C and at fixed time intervals (once a week for the first 2 months) liposomes size distribution was determined by SEM again, while vesicle size had not significant ($P < 0.05$) increased.

Liposome (PC:C) size and surface charge were studied for extract-incorporating liposomes and empty liposomes for comparison (Table 2). The mean diameter of the vesicles ranged from 230 to 270 nm and the surface charge of all the vesicles was negative, ranging from −8.9 to −11.9 mV. The incorporation of extract caused a significant ($P < 0.05$) change in the liposome surface structure and affected liposome size and *z*-potential. Indeed, the extract-incorporating liposomes

Table 2 Size (expressed as mean diameter) (nm) and *z*-potential (mV) of the PC:C (10:2) and PC:C:PG (10:2:1) liposomes before and after *M. communis* extract encapsulation

Sample composition	Size[a] (nm)	*z*-potential[a] (mV)
PC:C (no extract)	250 (20)	−10.4 (1.5)
PC:C + *M. communis* extract	285 (15)	−9.0 (0.7)

[a] Values are means of five subsequent determinations from three different samples. Standard deviation is given in parenthesis

were slightly larger (270–300 nm) (a phenomenon also observed by other authors [15] and had a lower negative surface charge (−8.3–−9.7 mV), which increases with the amount of substances incorporated in the lipid membrane. The extract composition seems to play a significant role in the way it can be packed in the lipid bilayer.

The methanolic extract of *M. communis* has also been studied for its antimicrobial activity, before and after encapsulation in liposomes (Table 3). The extract appeared active against most of the studied microorganisms (10–14 mm zone of inhibition). After its encapsulation, the antimicrobial activity appeared stronger (12–18 mm zone of inhibition). In general, the Gram positive bacteria appeared as the least resistant ones while *L. monocytogenes* and *C. albicans* showed the higher resistance. After encapsulation, the exhibited antimicrobial activities appeared stronger (significant at $P < 0.05$), mostly against the Gram positive bacteria. When using liposomes as carriers for the targeting of bioactive compounds [7], the physicochemical properties of the liposomal formulations are undoubtedly the main determinants of their final targeting efficiency. It is well known and documented that liposome size, surface properties and stability have a profound effect on their biodistribution and thereby will

Table 3 Antimicrobial activity (mm zone of inhibition) of *M. communis* methanol extract before and after encapsulation in liposomes

Tested compound	Liposome preparation (mg/ml)	*S. aureus*	*S. epidermidis*	*S. mutans*	*S. viridans*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*C. glabrata*	*L. monocytogenes*
Myrtus extract	–	14 (0.3)	13 (0.2)	14 (0.3)	14 (0.1)	12 (0.2)	12 (0.1)	12 (0.2)	12 (0.1)	10 (0.1)	12 (0.2)	12 (0.1)	10 (0.2)
Myrtus extract PC:C:PG (10:2:1)		15 (0.2)	16 (0.2)	16 (0.2)	17 (0.3)	14 (0.1)	14 (0.1)	13 (0.2)	14 (0.1)	14 (0.2)	14 (0.2)	15 (0.2)	13 (0.1)
Myrtus extract PC:C (10:2)		16 (0.1)	16 (0.3)	18 (0.3)	18 (0.2)	14 (0.1)	14 (0.2)	13 (0.1)	13 (0.1)	13 (0.1)	15 (0.1)	16 (0.3)	12 (0.1)
Netilmicin	–	21 (0.4)	25 (0.3)	24 (0.2)	25 (0.3)	20 (0.3)	23 (0.5)	22 (0.3)	24 (0.2)	–	–	–	22 (0.1)
Intraconazole	–	–	–	–	–	–	–	–	–	20 (0.1)	22 (0.2)	23 (0.2)	–

Values are means of triplicate determinations. Standard deviation is given in parenthesis

highly influence the possibility of targeting any encapsulated bioactive compound to specific tissues [23]. The antimicrobial properties of plant extracts have been of great interest in both academia and the food industry, because of their possible use as natural additives. Thus, a growing tendency to replace synthetic antimicrobial with natural ones has emerged [10]. Due to their strong antimicrobial activity, the extract from the herbal parts of *M. communis* used in our study could be considered a natural source that can be freely used in the food industry as a culinary herb. Especially, the dramatically increased antimicrobial activity after the encapsulation in liposomes can promote the use of the above mentioned extract as potent preservative and conservation agent not only in food industry but also in cosmetics and medical preparations.

The present study demonstrated the potential antioxidant and antimicrobial-food preservative ability of *M. communis* (indigenous in Greece). The encapsulation in liposomes modified the activity of the extract. Both, antimicrobial and antioxidant activity appeared improved and the commercial application in food preservation could be considered. However, further investigation should be carried out on the modified solubility of extract, as well as the rate of release of its antioxidant–antimicrobial components from liposomes. The *M. communis* is a self-growing plant widely distributed. The production of extract and its exploitation as potential natural antioxidant and food preservative could be of economic benefit.

Acknowledgments This study has been co-funded by 75% from European Union and 25% from the Greek Government under the framework of the Education and Initial Vocational Training Program—EPEAEK I Archimedes.

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