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Effect of liposomal complexes of quercetin-rich flavonoids from French Marigold (*Tagetes patula* L.) on Jurkat cell viability

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ABSTRACT

Background: Natural polyphenols are naturally used in traditional medicine to treat various diseases. Despite their healthful properties, ingesting phenolic compounds in food form does not provide a sufficient concentration for systemic therapeutic effects due to their low solubility in water, poor absorption, and fast metabolism. This problem has been solved by creating various composite pharmaceuticals from phenolic compounds using different methods to stabilize polyphenols.

Objective: The purpose of this study was to assess the effects of the DPPA (1,2-palmitoyl phosphatidic acid) and DPPC (dipalmitoyl phosphatidylcholine) liposomes on the protective effects of a quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L*.) on the viability of Jurkat cells. The study will examine both intact cells and cells that have been incubated under oxidative stress conditions.

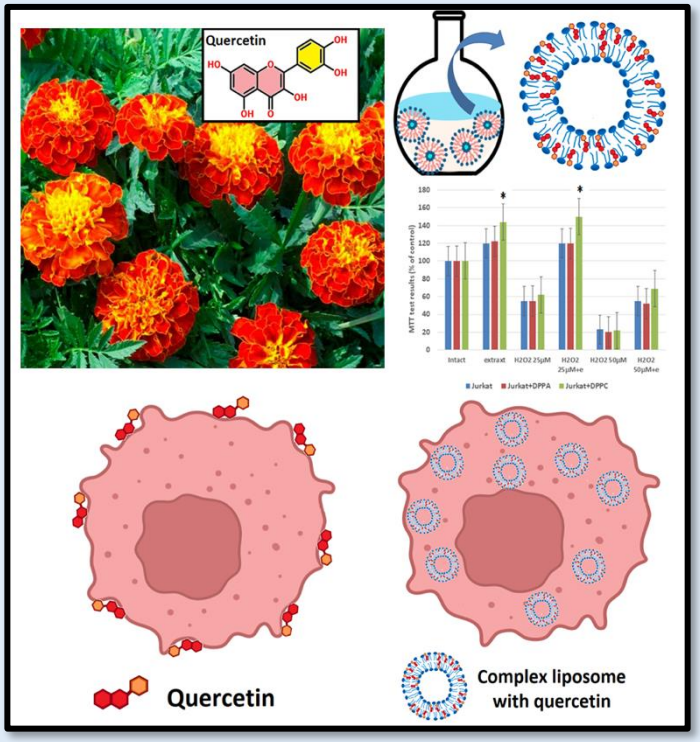
Materials and Methods: Quercetin-rich flavonoid fraction was extracted from a French Marigold (*Tagetes patula* L.) by thin-layer chromatography (TLC), High-pressure liquid chromatography (HPLC), and Liquid Chromatography-Mass Spectrometry (LC-MS) methods. Extract alone and in complex with dipalmitoyl phosphatidylcholine (DPPC) and 1,2-dipalmitoyl phosphatidic acid (DPPA) liposomes were added to the Jurkat cells culture at a rate of 2 mg/mL−1.The 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) test assayed Cell viability by evaluating cellular dehydrogenase activity.

Results: Flavonoids were separated and identified in the marigold extracts by TLC, HPLC, and LC-MS methods. The spectrophotometric absorption spectrum of the quercetin-rich flavonoid fraction extracted from French Marigold

(*Tagetes patula* L.) shows two peaks corresponding to benzoyl (254nm) and cinnamyl (375nm) aromatic rings. In the complex of quercetin-rich flavonoid fraction with DPPC and DPPA liposomes, the spectrophotometric absorption peak at 254nm was not detected, while the absorption intensity of the peak at 375nm was sharply reduced. The quercetin-rich flavonoid fraction alone and in combination with DPPC liposome increased intact and incubated under low- and high-intensity oxidative stress conditions Jurkat cells' viability but did not reveal effect in combination with DPPA liposome.

Conclusions: The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) forms stable complexes with DPPC and DPPA liposomes that allow the storage of high content of phenolic compounds in lipid nanocapsules. The use of the liposomal system in the pharmaceutical and food industry allows for carried and controlled bioactive-compound release, which is considered one of the main strategies to improve and enhance the quality of food, providing preventative healthcare for the population and decreasing the risk of disease.

Keywords: bioactive-compound, polyphenols, Quercetin, Liposomes, Jurkat cells, therapeutic effects, pharmaceuticals



INTRODUCTION

Flavonoids are a significant group of polyphenols found in plants and are used in traditional medicine to treat various diseases [1- 5]. In recent decades, scientists have turned their attention to natural ingredients [6-8] to identify compounds with effective pharmacological properties and investigate their molecular mechanisms of action. Despite their healthful properties, ingesting phenolic compounds in orally does not provide a sufficient concentration for systemic therapeutic effects due to their low solubility in water, poor absorption, and fast metabolism [9].

This problem has been solved by creating various composite pharmaceuticals from phenolic compounds

using different methods (cyclodextrin coatings, simple emulsions, lipid nanoparticles, or liposomes [10-13]) to ensure the stabilization of polyphenols. The genus Tagetes (Asteraceae) is originally from the Americas, but some of its species, such as *Tagetes erecta* and *Tagetes patula,* were introduced to European countries from India and North Africa in the 16th century and became naturalized there [14]. *Tagetes patula* was brought to Georgia from the Indian Subcontinent where its petals were ground and made into a popular *Tagetes patula* [15, 16]. Tageta Patula (yellow flower) is used in Georgian cuisine as the main spice when making cold dishes prepared with fatty, cholesterol-rich chicken meat (''sacivi'). The traditional use of the rich antioxidant flavonoids of the yellow flower (*Tagetes patula*) in these dishes supports their easy digestion. Tageta Patula is known to have health-beneficial properties and has been used in folk medicine for centuries to treat rheumatism, gastro-interstitial, kidney, hepatic, and respiration system disorders [17]. Studies have confirmed the efficacy of oral administration of *Tagetes patula* extracts against acute and chronic inflammation in animal and cellular models [18,19]. Both the hydrophilic and lipophilic fractions from *Tagetes patula* petals showed high antiradical and immunomodulatory capacities [20]. Thus, it can be assumed that the combination of hydrophilic and lipophilic antioxidants in marigold flowers contributes to their health benefits. Additionally, the major flavonoid of Tagetes, quercetin, has anti-cancer activity [21]. Therefore, rich in antioxidant flavonoids yellow flowers (*Tagetes patula* traditionally are used as a health optimization/prevention from chronic diseases and the management of symptoms associated with those diseases and can be attributed to Functional Food Species (FFS) (22-24).

In the present study, we investigated the effects of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) alone and incorporated into DPPA (1,2-palmitoyl phosphatidic acid) and DPPC (dipalmitoyl phosphatidylcholine) liposomes on the viability of the intact and incubated under oxidative stress conditions Jurkat cells.

MATERIALS AND METHODS

Plant Material: The flowers were obtained from local Georgian *T. patula* plants, which were grown at an experimental plot near Tbilisi from commercially supplied seeds. A certified herbalist from our laboratory proved the authentication of our sample. The flowers were air-dried in a dark dry place at a temperature of 25–30°C and then stored in a closed glass container in a cool, dry place. The moisture content of the dried flower samples did not exceed 5%. For the authentication of the chemical constituents, we used an authentic sample of quercetin (Extrasynthese, Genay, France).

Extraction and Purification of Marigold Constituents: The isolation process of *Tagetes patula* flowers involved sequential solvent extraction. After 48 hours of extraction on a Soxhlet apparatus using 1,2-dichloroethane, a 600g sample of dried, pulverized plant material was extracted until the disappearance of color. After dichloroethane extraction, the plant matter residue was re-extracted using ethanol to isolate compounds with higher polarity (solvent/plant matter ratio 1:5). The solvents were evaporated at 40°C under a vacuum. To separate the individual compounds from the dichloroethane extract, column chromatography was performed using a chloroform-hexane solvent system on a silica gel column. The fractions were separated from the column by gradually increasing the chloroform content in the system while using hexane. In the ethanolic extract, various substances were separated using a silica gel column. This was done by eluting the column with a mixture of dichloroethane and methanol, and then characterizing the obtained fractions using thin-layer chromatography (TLC). The elution process started with dichloroethane and was followed by a gradual increase in the methanol content in the system. Different fractions

were obtained by eluting with 2, 3, 5, 7, and 10% methanol in dichloroethane. Finally, rechromatography of fractions Sephadex LH-20 and silica gel column, and further purification on a polyamide column using elution with aqueous ethanol were used to separate different compounds [18].

***Thin layer chromatography (TLC)*:** The TLC separation method was used with Merck (Germany) silica gel plates. The solvent systems of dichloroethane-methanol (9:1) and chloroform-methanol (9:1) were used to separate lipophilic compounds. On the other hand, the samples from ethanolic extracts of more polar compounds were isolated using a solvent system consisting of chloroform, methanol, and water in a ratio of 26:14:3. The resulting chromatograms were examined under UV light at both 254 and 360 nm wavelengths. The flavonoids were identified as yellow spots that became visible after heating the plates sprayed with a 1% ethanolic solution of aluminum chloride, both before and after the application of staining reagents. Upon spraying the plates with a 20% sulfuric acid solution, other compounds were detected. When the sprayed plates were heated to 100°C, the compounds were revealed as spots with blue to green shades, depending on the specific compounds. [18].

High-pressure liquid chromatography (HPLC): Separation was carried out on a Waters chromatograph (USA) using a Radia-Park C18 (8x10) chromatographic column, column temperature 35^0 C, flow rate 1.8ml per minute. Sample quantity 20µl solvent system A-0.1% trifluoroacetic acid, B-60% acetonitrile-water (by volume). To 0.2 grams of dry spice powder add 40-degree alcohol, the ratio of material to solvent is 1/15, that is, 3 ml. For good extraction, the mixture was stirred, treated with ultrasound, and then left for 24 hours at room temperature. A portion of the obtained extracts was taken for analysis and filtered through a 0.22 millipore filter before HPLC analysis [18].

Liquid Chromatography-Mass Spectrometry (LC-MS): Yellow crystals of quercetin are soluble in ethyl acetate, ethanol, and methanol, but are not dissolved in benzene and chloroform. The procedure of identification of quercetin involved dissolving the samples in high-performance liquid chromatography-grade methanol, followed by filtration through a Millex-HV Durapore (PVDF) membrane with a pore size of 0.2µm. The filtered samples were then injected into the Ultra-performance LC-Quadruple Time of Flight (UPLC-QTOF) device (Waters Premier QTOF, Milford MA, USA) for mass spectral analyses. The Ultra-Performance Liquid Chromatography (UPLC) column was linked to a Photodiode Array (PDA) detector through an online connection and then to a Mass Spectrometry (MS) detector that had an electrospray ion (ESI) source and was operated in ESI-positive mode.

A UPLC BEH C18 column (Waters Acuity) (2.1 × 50 mm i.e., 1.7µm) was used for separation. The chromatographic and MS parameters involved using a mobile phase of 0.1% formic acid in water (phase A) and 0.1% formic acid in acetonitrile (phase B). The program for linear gradient was executed in the following manner: A change from 100% to 95% over 0.1 minutes, followed by a transition from 95% to 5% over 9.7 minutes. The solution was then held at 5% for 3.2 minutes and finally brought back to the initial conditions (95% A) in 4.2 minutes. The flow rate was maintained at 0.3 ml/min and the column temperature was kept at 35°C. The QTOF Premier MS instrument was used to detect the masses of the eluted compounds. The UPLC-MS runs were performed with the following settings: capillary voltage of 2.8 kV, cone voltage of 30 eV, and collision energy with argon as the collision gas set to 5 eV. The range of the runs was 70 to 1,000 D. Sodium formats was used for calibrating the MS system, while Leu-enkephalin was used as the lock mass. To ensure accurate masses, MassLynx software version 4.1 (Waters) was used for instrument control and calculation [18].

The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) was identified was identified with the help of chemical formulae calculated based on information of accurate mass, isotopic pattern, and UV/visible spectra, in comparison with data of authentic standards of quercetin (Extrasynthese, Genay, France) (Melting point 307-310^{0}C, M^+ 302, UV spectrum 374 and 256 nm (bands 1 and 2)). The decomposition of the first band of the spectrum by the addition of sodium methylate and acetate confirms the presence of a hydroxyl at position 7. The bathochromic shift of aluminum chloride and hydrochloric acid addition at 52 nm in the first band proves the simultaneous presence of hydroxyl at C-3 and C-5 atoms [18].

Cell Culture and Experimental Design: The Jurkat cells, which are a type of human T-cell leukemia lymphoblastoid, were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). These cells were grown in RPMI 1640 bioactive medium (Gibco, Grand Island, NY, USA) as suspension culture and kept at 37°C under 5% humidified CO_2. The medium was supplemented with inactivated embryonic bovine serum (Sigma, St Louis MO, USA), L-glutamine (4mM), penicillin (100 U/ml), and streptomycin (100 U/mL). The cell experiments were conducted at cell densities ranging from 0.3 to 0.6 x 10^6 cells/ml [18].

To replicate oxidative stress conditions, H_2O_2 (Sigma) was added to the Jurkat culture to achieve concentrations of 25 µM and 50µM, which correspond to low and intermediate stress severity levels, respectively [25]. In the control treatment, water was added instead of H_2O_2. The Jurkat cells were treated with a quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) at a rate of 2mg/mL, both in complex and without liposomes.

Cell Viability: The 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide (MTT) test was used to measure cell viability by assessing mitochondrial dehydrogenase activity [26, 27]. To conduct the test, cell suspensions with a concentration of 2×10^6 cells mL^{-1} were incubated with H_2O_2 and marigold extract, as described above. After the incubation period, the cells were collected and washed via centrifugation at 1500 g for 5 minutes. The cells were re-suspended in a fresh medium and to re-suspended cells a 30µl of MTT was added with a concentration of 8 mg mL−1 to 100µl of cell suspension.

The mixture was then incubated for 4 hours at 37°C in an atmosphere containing 5% CO2. After incubation, the resulting colored formazan crystals were dissolved in 100µl of dimethyl sulfoxide (DMSO). The cellular dehydrogenase activity was measured by the absorption values of the solutions at 570 nm wavelength. The experiments were conducted six times for accuracy. To evaluate the effects of different treatments on cell activity and viability, their absorbance values were expressed as a percentage of thousands of untreated cells (control: $I_{Abs\ treated}$/ $I_{Abs\ control}$) [18].

Preparation of dipalmitoyl phosphatidylcholine (DPPC) and 1,2- dipalmitoyl phosphatidic acid (DPPA) liposomes: For the preparation of complex liposomes containing quercetin, a ratio of 3:1 of quercetin and lipids was selected. Since quercetin is a molecule of hydrophobic nature, to the mixture of lipids molecules and quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) we first added a small amount of organic solvent, 1µl of methanol, and then added 3 ml of distilled water heated to 60^0 C.

A mixture of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) (3 mg) and lipids (1 mg) (DPPC and DPPA from Lipoid, Newark, New Jersey) was dissolved in 3ml distilled water at 50 °C followed by intensive shaking until a homogeneous liposomal suspension without aggregates of quercetin molecules was obtained. Since liposomes

encapsulate about 50% of GTCs, we added 0.2µg of GTC–liposome complexes to 100 ml of the incubated cell suspension. Quercetin is highly soluble in lipids and alcohol, but it is not very soluble in water [28], the quercetin molecule can bind to hydrophobic parts of the liposomes and can also be incorporated into the internal volume (lumen) of the liposomes. Quercetin-rich flavonoid fraction extracted from a French Marigold (*Tagetes patula L.*) was placed inside liposomes in complexes prepared by the method described by Mdzinarashvili et al., (2016) [29]. To equalize the liposome diameters (200 nm), the suspension of complex liposomes was finally extruded through a nanoporous membrane [29].

Statistics: To evaluate the statistical credibility of the influence of different effects on cell viability, and the differences between the treated and control groups, a pairwise comparison using Student's t-test was performed. The values of ≤0.001, ≤0.01, and ≤0.05 were designated as highly significant, significant, and marginally significant, respectively. (df = 10 (Degree of Freedom), if 6-6 samples were measured in the control and experimental groups) The SPSS Software was used to analyze the results.

RESULTS AND DISCUSSION

Composition of *T. patula* Extracts: The group of flavonols quercetin, quercetagetin, patuletin, and their glycosylated and di-glycosylated derivatives), belonging to the flavonoids were separated and identified in the marigold extracts by TLC, HPLC, and LC-MS methods. Such composition of flavonoids is typical of *T. patula* (Figures 1, 2, 3) [18, 30, 31]. The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) was used for further trials with Jurkat cells (Figure 4).

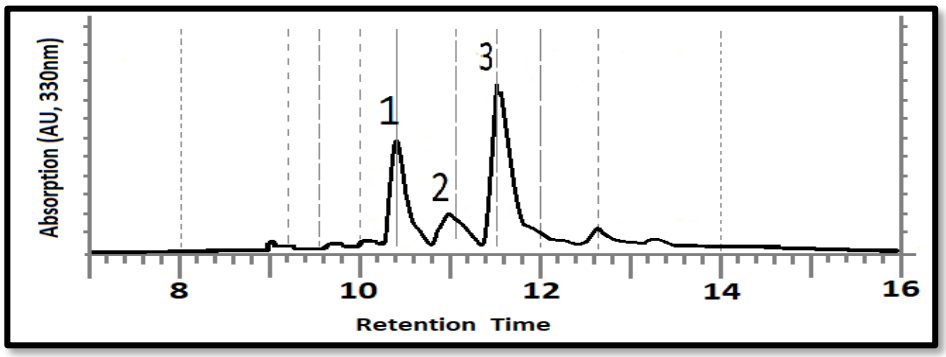


Figure 1. HPLC chromatograms of three main fractions (1 – quercetagetin diglycosides, 2 – quercetin diglycosides, 3 – quercetin and quercetin glycosides) extracted from French Marigold (*Tagetes patula* L.)

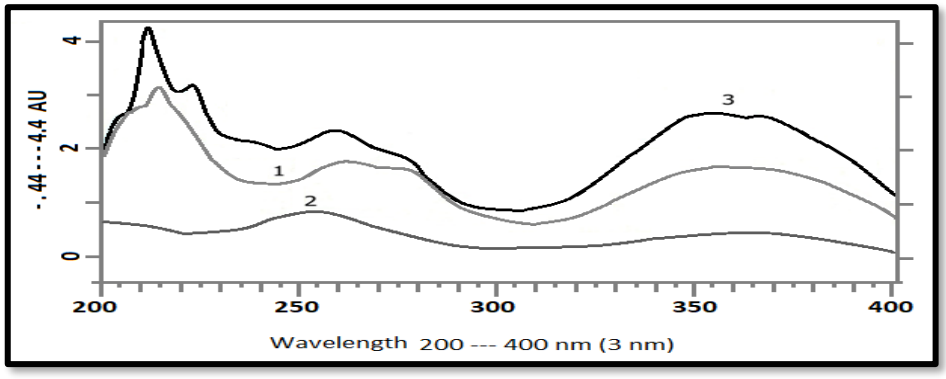


Figure 2. The ultraviolet (UV)-spectrum of fractions (1 – quercetagetin di-glycosides, 2 – quercetin di-glycosides, 3 – quercetin and quercetin glycosides) extracted from French Marigold (*Tagetes patula* L.) (quercetin-rich flavonoid fraction is shown in blue)

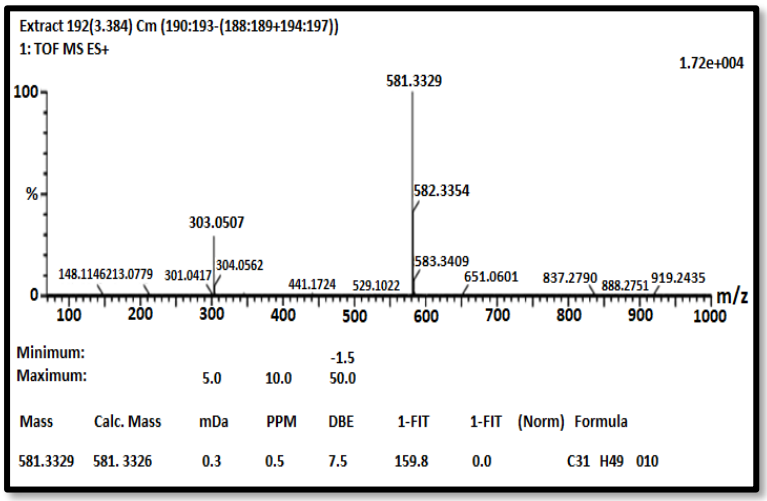


Figure 3. The of mass-spectrum quercetin and its glycoside fractions

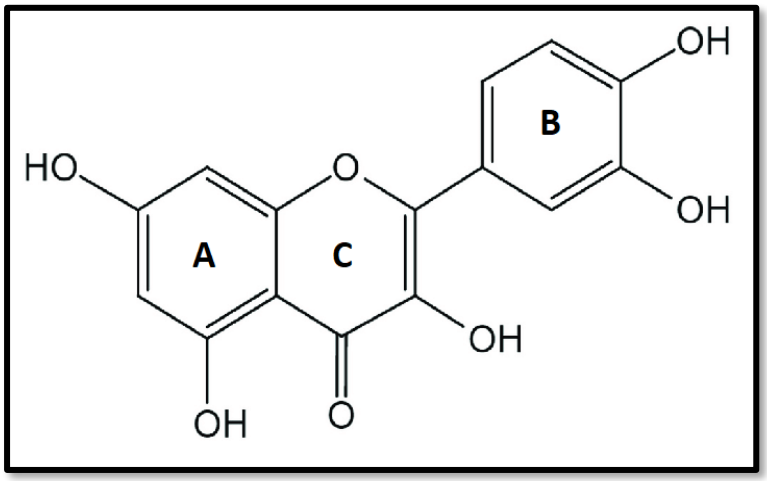


Figure 4. The quercetin molecule (A-benzoyl, B– cinnamyl)

Spectrophotometric absorption spectrum of pure quercetin in MDPPA and MDPPC liposomes: The absorption spectrum of the quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) shows two peaks at 254 nm and 375 nm, which belong to different conjugated aromatic rings: the first absorption peak at 254 nm corresponds to benzoyl. The second absorption peak at 375 nm corresponds to cinnamyl [32] (Figure 5).

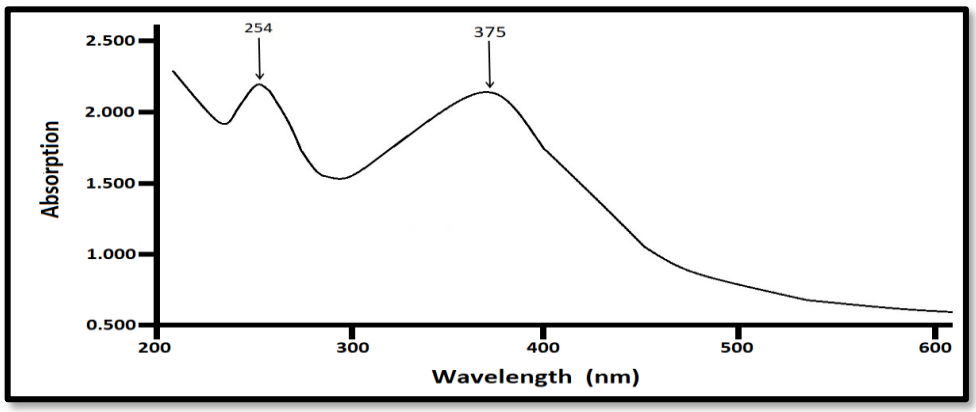


Figure 5. The spectrophotometric absorption spectrum of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.)

In the complex of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) with DPPC and DPPA liposomes, the spectrophotometric absorption peak at 254 nm wavelength, characteristic for the benzoyl group of quercetin, was not detected, while the absorption intensity of the peak at 375 nm wavelength, characteristic of the cinnamyl group, was sharply reduced (Figure 6, 7).

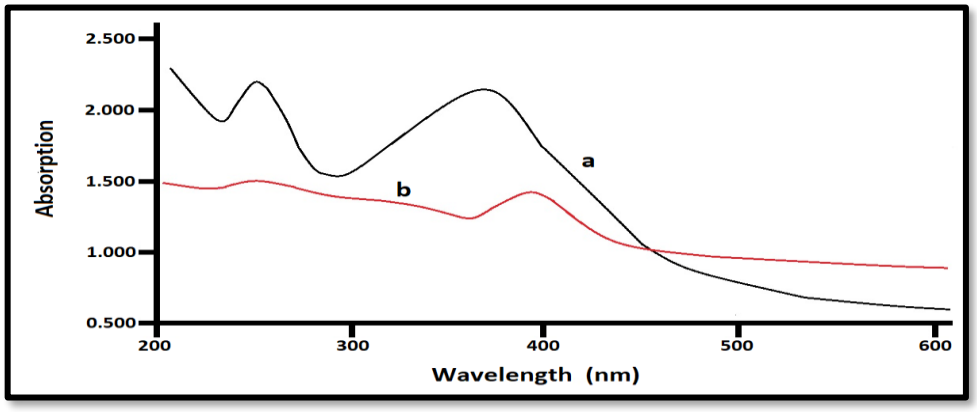


Figure 6. The spectrophotometric absorption spectrum of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) (a) and DPPC liposome-encapsulated quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) (b)

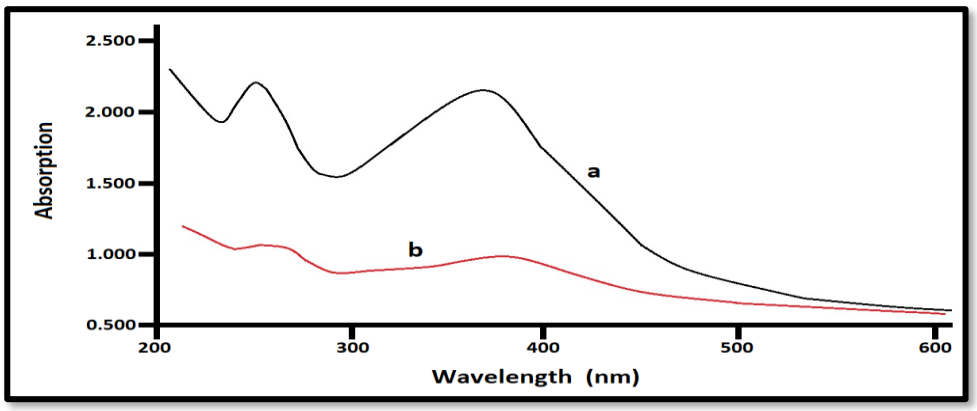


Figure 7. The spectrophotometric absorption spectrum of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) (a) and DPPA liposome-encapsulated quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) (b)

Effects of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula* L.) on Jurkat Cells Viability: When a quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) was added to an intact Jurkat culture, there was an increase in cell viability by 20% compared to the control level (Figure 8). This increase may be attributed to an enhancement in mitochondrial dehydrogenase activity and cell proliferation level.

Oxidative stress induced by exogenous H_2O_2 in a dose-dependent manner reduced the viability of Jurkat cells (Figure 8). The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) had enough high protective effects against H_2O_2-induced damage of the Jurkat cells (at low and intensive oxidative stress conditions). Zhang et al [33] showed that quercetin is superior to other phenolic compounds in protecting Jurkat cells from H2O2-induced death.

Fig. 5 shows that DPPC and DPPA liposomes alone did not affect intact and incubated under oxidative stress Jurkat cells viability.

The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) in combination with DPPC liposome increased the viability of intact Jurkat cells by 20% and did not change in combination with DPPA liposome.

The viability of Jurkat cells incubated at both low- and high-intensity oxidative stress conditions under the influence of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) in combination with DPPC liposome increased by 25%, and 18%, respectively, but did not change when incubated with DPPA liposome-encapsulated quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) (Figure 8).

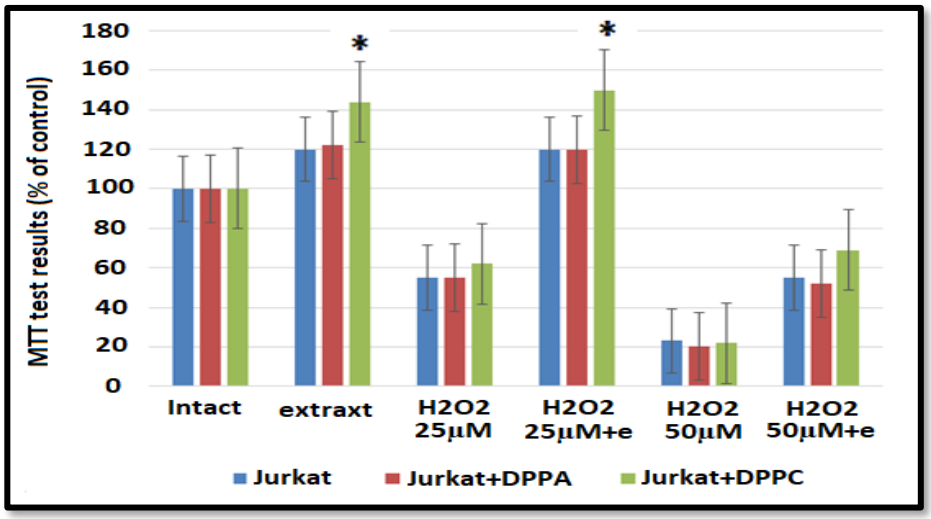


Figure 8. Effects of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) on the viability of the Jurkat cells. The viability of Jurkat cells was calculated as a percentage of the untreated control. Error bars indicate standard deviations from six replicates. Values with asterisks (*) were significantly different from control ($p \leq 0.05$).

DISCUSSION

Based on the analysis of spectrophotometric data, we obtained information on the structure of the complex of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) with DPPC/DPPA liposomal nanoparticles.

According to research, the extract of marigold fraction flavonoids rich in quercetin has two peaks in its absorption spectrum at 254 nm and 375 nm wavelengths. These peaks belong to the conjugated aromatic rings of benzoyl (254 nm) and cinnamyl (375 nm) in the quercetin molecule [32] (Figure 5). DPPC and DPPA liposomes have similar structural organizations at a basic level - both lipid molecules create standard bilayer liposomes; their lipid tails are stabilized by a "hydrophobic" interaction, while the hydrophilic lipid heads are held together by electrostatic interaction.

The absorption spectrum of the complex formed by the quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) with DPPC and DPPA liposomes was analyzed using spectrophotometry. In the spectrophotometric absorption spectrum of the quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.*) encapsulated into the DPPC and DPPA liposomes, the peak characteristic for the benzoyl group (254 nm) was not detected (Figures 3, 4), which

should be related to the connection of the hydrophobic benzoyl group to the hydrophobic tails of the liposomes. It was observed that the intensity of the peak at 375 nm, which is characteristic of the cinnamyl group, decreased significantly (Figures 6, 7). The cinnamyl group is hydrophilic, causing it to be located on the surface of the lipid bilayer of DPPC and DPPA liposomes. It partially sinks into the layer of the lipid heads, leading to a decrease in the absorption intensity detected by the peak at 375 nm. This result confirms the formation of stable complexes of the quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L*.) with DPPC and DPPA liposomes. There are significant differences between the DPPC and DPPA liposomes. The DPPA liposomes with negative (–P-COO–) surface charge in a neutral aqueous environment will tend to be repelled by negatively charged (ζ-potential) cell membranes, whereas polar phosphatidylcholine head of DPPC liposomes contains both a negative (–P-COO–) and a positive (N+) charged groups [34, 35]; consequently, the DPPC liposomes are attracted to the negatively charged (ζ-potential) cell membranes.

The study results showed that liposomes composed of pure DPPC and DPPA did not change the viability of Jurkat cells incubated under varying degrees of oxidative stress. The combination of the quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L*.) with DPPC liposomes increased the viability of intact Jurkat cells by 20%. Under low- and high-intensity oxidative stress conditions, the incubation of Jurkat cells with the combination of the quercetin-rich flavonoid fraction extract with DPPC liposomes increased cell viability by 25% and 18%, respectively. The effectiveness of the quercetin-rich flavonoid fraction extract in combination with DPPC liposomes may be attributed to two factors: firstly, the high storage capacity of phenolic compounds in the lipid nanocapsules, and secondly, the interaction of the liposomes with the cell membrane structures, which can lead to the release of the quercetin-rich flavonoid fraction within the cell cytoplasm and therefore improving the delivery process's efficiency. Consequently, DPPC liposomes increased the antioxidant effect of the quercetin flavonoid fraction on intact Jurkat cells and those, incubated under oxidative stress conditions, therefore DPPC liposomes modulated quercetin's bioactivity and therapeutic effects.

Quercetin has anti-inflammatory and anti-cancer properties, but its effectiveness is limited due to low stability, poor solubility, and insufficient bioavailability. However, the use of liposomes enhances its efficacy. Both literature data and our study results demonstrate that the use of liposomes as drug carriers for quercetin can lead to improved therapeutic outcomes. Quercetin liposomes have the potential to be a significant tool for addressing current complications in inflammatory processes and cancer treatment. The liposomal nano system used to deliver quercetin in vivo experimental model of hepatic ischemia and reperfusion injury revealed the ability to downregulation of pro-inflammatory markers and enhanced recovery, the in vitro model demonstrated its anti-inflammatory, proliferative, pro-apoptotic, and anticancer potencies [36-39].

In recent times, there has been a rising trend among consumers to adopt a healthy lifestyle, which includes the consumption of natural and healthy food. This has led to the development of novel functional food products. Liposomes are a promising technology in the food industry, as they are natural, non-toxic, biodegradable, and versatile systems that can be used for both hydrophilic and hydrophilic components. They are particularly useful for improving the stability of such components.

Liposomes are tiny spheres that can contain various components such as additives, preservatives, and antimicrobials. Encapsulation in liposomes can improve bioavailability and enhance flavors and beneficial properties. Liposomes have unique features such as targeted delivery, controlled release, drug protection, superior therapeutic effects, and clearance prevention compared to traditional drug delivery systems. The use of liposomal technology has proven to be beneficial in promoting the effectiveness of functional food and nutraceutical applications. In the food industry, adopting a liposomal system has been recognized as one of the most effective ways to enhance nutrient stability, customize absorption, and improve overall food quality. Liposome-based functional products offer opportunities to determine the impact of each component in the product formulation and its stability. To increase the effectiveness of liposomes it is important to consider the relationship between bioactive substances, phospholipid types, cholesterol content, and various wall materials, as well as the stability of liposomal vesicles. The pharmaceutical and food industries have been using the liposomal system to carry and control the release of bioactive compounds. Liposomes are one of the main strategies to improve nutrient stability, increase individualized absorptivity, and enhance food quality. Liposomes are considered an effective way to improve preventative healthcare for the population and reduce the risk of diseases by providing enhanced nutrient delivery.

CONCLUSION

Tageta Patula (yellow flower) is widely used in Georgian cuisine as the main spice when making cold dishes prepared with fatty, cholesterol-rich chicken meat (''sacivi'). The traditional use of the rich antioxidant flavonoids of yellow flowers (*Tagetes patula*) in food products supports their easy digestion and also gives them the ability to manifest many positive healing effects. Therefore, antioxidant flavonoids of the yellow flower (*Tagetes patula*) are traditionally used as a health optimization/prevention of chronic diseases remedy and for the management of symptoms associated with them and can be attributed to FFS (22-24).

The quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L.)* forms stable complexes with DPPC and DPPA liposomes. This complex gives the possibility to the storage of high content of phenolic compounds in lipid nanocapsules.

The negative (–P-COO–) surface charge of the DPPA liposomes tends them repel negatively charged cellular membranes, whereas the polar phosphatidylcholine head of DPPC liposomes which contains negative (–P-COO–) also the positive (N^+) charged groups is attracted to the cellular membrane, leads the release of quercetin-rich flavonoid fraction extracted from French Marigold (*Tagetes patula L*.) within the cell cytoplasm and ensures an enhancement efficiency of the delivery process.

The nanotechnologies used in our research allowed us to increase the bioavailability of the quercetin-rich fraction of yellow flowers and, accordingly, to enhance their action. The use of the liposomal system in the pharmaceutical and food industry allows for carried and controlled bioactive-compound release, which is considered one of the main strategies to improve and enhance the quality of food, providing preventative healthcare for the population and decreasing the risk of disease.

List of abbreviations: DPPA - 1,2-palmitoyl phosphatidic acid; DPPC - dipalmitoyl phosphatidylcholine; TLC - thin layer chromatography; HPLC - High-pressure liquid chromatography; LC-MS - Liquid Chromatography-

Mass Spectrometry; MTT - 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide; DMSO - dimethyl sulfoxide; FFS – Functional Food Species

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3. I. Chkhikvishvili - experimental studies
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7. D. Chkhikvishvili – manuscript draft
7. S. Kalmakhelidze data analysis
8. A. Sharashenidze – Manuscript editing
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