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HIBISCUS SABDARIFFAE FLOS AND *CALENDULAE FLOS* EXTRACTS AS POTENTIAL ANTOXIDANTS FOR PRESERVATION OF PHARMACEUTICAL EMULSIONS

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Abstract: Aqueos extract from *Hibiscus sabdariffae flos* and ethanolic (50°) extract from *Calendulae flos* were obtained, analysed and incorporated in oil/water emulsions in order to determine the antioxidant activity which makes them possible preservatives. The constituents of interest (anthocyanins, flavonoids, polyphenolcarboxylic acids, carotenoids) have been identified and dosed successfully using spectrophotometric methods, ensuring about the quality of extracts. Simple emulsion with 10% sunflower oil was chosen to evaluate the antioxidant activity in emulsions, comparing them with references in concentrations between 0.25% and 5% (ascorbic acid, ascorbyl palmitate, tocopherol and rosemary extract). Both assays applied (ferric reducing power and organoleptic examination) have revealed the ability of the proposed extracts to improve emulsions preservation.

Keywords: hibiscus extract, marigold extract, antioxidant activity, emulsions, preservation

INTRODUCTION:

Plant matrix contains a complex of substances with antioxidant activity (acids, flavonoids, anthocyanins, carotenoids and others) being a guiding side for new sources of preservatives. The research in this field came from food industry.

Toxicity studies on synthetic antioxidants have led to worrying results, especially for butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA). *In vitro*, toxic activity was found to be higher for BHT versus BHA (60-65% hemolysis after 12 minutes - BHT and 50% after 20 minutes - BHA) (Jayalakshmi et al., 1986). In vivo, administration of BHT at mice, in diet, revealed pleural, peritoneal, testicular and pancreatic haemorrhages. Modification of mice thyroid, enzyme induction and stimulation of carcinogenic DNA synthesis (Chen et al., 2014) were also observed.

The research on natural sources for preserving meat products have led to favorable results. Spice extracts (anise, pepper, coriander, rosemary, garlic, onion) (Embuscado, 2015; Sebranek et al., 2005), pomegranate juice and pomegranate extract (Naveena et al., 2008) have proven their antioxidant potential by maintaining the organoleptic properties of chicken and pork products. Extracts form cloves (*Eugenia caryophyllata*) and grape seeds have been investigated on silver carp fillets. Addition of extracts increased the duration of fillets preservation (organoleptic, 4°C, 18 days) with three days compared to blank (without antioxidant) (Shi et al., 2014).

Extracts rich in carotenoid were employed to ensure food emulsion stability by lowering their rancidity. The antioxidant activity of the following carotenoid-rich extracts: tomato extract, paprika and annato (significant content of bixin = polar carotenoid) was demonstrated by testing in O/W emulsion (10% sunflower oil, emulsifier: tween 20). On peroxidation and formation of conjugated dienes (first oxidation stages products) the effect is insignificant. In analyzes for volatile aldehydes (secondary oxidation stages products) such as pentanal, hexanal, 2-heptenal, demonstrated the antioxidant effect by lowering the content of these aldehydes in the emulsions. The antioxidant effect is considered to be variable depending on the oxidation stage (Kiokias et al., 2012).

Rosemary methanolic extract (RE) was analysed compared to BHA and BHT in terms of antioxidant and antimicrobial activity. The following conclusions have been reached: the antioxidant effect of RE is based mainly on rosmarinic acid and carnosic acid that were found in its composition; associated with BHT and BHA a synergism of antioxidant action was observed; the extract provided superior antimicrobial capacity compared to BHT and benzoic acid, BHA had similar activity (minimal inhibitory concentration on *S. aureus*: BHA <RE <benzoic acid <BHT and *E. coli*: BHA <RE <benzoic acid = BHT) (Romano et al., 2009). Rosemary extract and semisynthetic derivatives of rosmarinic acid (rosmarinates) have been researched for the preservation of polyolefins (polymers), lubricants, oils and cosmetics, with favorable results (Doudin et al., 2016).

Hibiscus seed extract (HE) and hibiscus seed oil (HO) were mixed with sunflower oil checking antioxidant activity under accelerated conditions. Stabilization of sunflower oil meet the sequence: HE> HO> tocopherol> sunflower oil without antioxidant (Nyam et al., 2012).

These results, together with many others gathered over a significant period of research have encouraged the development of producers who made natural excipents. Here are some examples found on online-market: Herbalox Rosemary Extract (based on rosemary extract; available in three forms: oil dispersible, water dispersible and water soluble), Duralox Oxidation Management System (includes spice extracts, ascorbic acid, tocopherols), Fortium (rosemary extract, green tea extract and tocopherol

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mixtures) (Fernandez-Lopez et al. 2005, Sebranek et al. 2015, Schilling et al., 2018).

Starting from the above, the objectives of this research are the following: obtaining, content analysis and evaluating the antioxidant activity in emulsions (10% sunflower oil) for extracts coming from hibiscus and marigold, in the perspective of using them as preservatives.

MATERIALS AND METHODS:

Vegetal material, reagents, equipment

Hibiscus and marigold flowers were purchased from stores specialized in vegetal products sales, from Bucharest in 2017. All the reagents and solvents were purchased from Karl Roth (Germany), unless stated otherwise. The standards (ascorbic acid, ascorbyl palmitate, tocopherol) meet analytical requirements being acquired from Sigma-Aldrich (Germany). The emulsion components (*Acacia gum*, *Sunflower oil)* and *rosemary extract* were purchased from an online store specialized in natural ingredients (Elemental srl). The equipment employed was: rotary evaporator (Buchi R-215), lyophilizer (Christ Alpha apparatus). All spectrophotometric determinations were performed using a Jasco V-530 spectrophotometer (Jasco, Japan).

Extraction

Hibiscus extract was obtained by water-extraction with 1500 ml of distilled water for 15 minutes at reflux. The vegetal product (75 g) was brought in a round bottom flask and added distilled water. The heating was done by electric mantles keeping it at reflux. After cooling, the content was filtered through cotton-plug and the filtrate was concentrated with the rotary evaporator (Buchi R-215) and lyophilized (Christ Alpha apparatus). Marigold extract was obtained in the same manner using instead of water, 50% ethanol, (drug to solvent ratio 1:20). Until the analysis, these extracts were stored in a desiccator.

Extract analysis

Phytochemical analysis

Qualitative examination of the extracts consists of chemical reactions for main classes of active compounds, according to pharmacognostical protocol (Gîrd et al., 2010).

Preparation of the working solutions used for phytochemical screening, quantitative analysis (except for carotenoids determination) and antioxidant activity: 0,5 g of hibiscus extract and marigold extract, respectively, were disolved in 25 mL of water (for hibiscus extract) or 50% ethanol (for marigold extract). The obtained solutions were codified H (for hibiscus extract) and M (for marigold extract). The concentrations of H and M solutions were 1%.

Quantitative analysis

The quantitative determination has targeted the evaluation of the compounds with known antioxidant activity: anthocyanins, flavonoids, polyphenolcarboxilic acids, total phenolic compounds and carotenoid, by using spectrophotometric methods.

Anthocyanidins (expressed as cyanidin chloride equivalents) were determined according to European Pharmacopoeia 7th Edition, 2011, (*Myrtilli fructus* recens monography). Flavonoid content (expressed as rutin equivalents) was evaluated based on their chelating reaction with aluminium chloride (*Cynarae folium*, *Romanian Pharmacopoeia* Xth Edition, 1993). Polyphenolcarboxilic acids were assessed based on the formation of oxymes in the presence of sodium nitrite/hydrochloric acid and sodium hydroxide (*Ash leaf*, *European Pharmacopoeia* 7th Edition, 2011). Total phenolic compounds content (expressed as tannic acid equivalents) were established using Folin-Ciocâlteu reagent according to Singleton (1965), modified by Nencu et al. 2013. The use of tannic acid as standard was supported by other authors (Garg et al., 2012, Mandade et al., 2011). Carotenoids content (expressed as β-carotene equivalents) was established based on the carotenoid absorbance at λ = 460 nm (Ciulei et al, 1995). Briefly, 1.25 g marigold extract was extracted with absolute ethanol (extract-to-solvent ratio 1:10). The resulted filtrate was subdued to saponification with 10% potassium hydroxide solution in methanol (to release carotenoids from the ester combinations). Next, the mixture was extracted twice with 10 mL ethanol and made up to 25 mL with the same solvent. The absorbances were determined spectrophotometrically at λ=460 nm (maximum absorbance wavelength of β-carotene) against blank (absolute ethanol). The measurement was repeated three times. The results were acquired by absorbance interpolation to a linear β-carotene calibration curve.

The results of the spectrophotometric determinations were obtained using calibration curves pre-obtained under the same conditions: cyanidine chloride (y=0.0172 + 3.0369x, where y= absorbance, x = concentration of sample, R2=0,9993); rutin (y=0.0002 + 0,3150x, where y= absorbance, x = concentration of sample, R2=0,9997), caffeic acid (y=0.0123x + 18,189, where y= absorbance, x = concentration of sample, R2=0,9998), tannic acid ((y=0.0533 + 0,0605x, where y= absorbance, x = concentration of sample, R2=0,9998), β-carotene (y=0.0355 + 0,0224x, where y= absorbance, x = concentration of sample, R2=0,9996).

All spectrophotometric determinations were performed using a Jasco V-530 spectrophotometer (Jasco, Japan). All determinations (except for carotenoids) were performed on 1% H and M solutions.

Emulsions preparation

In current research, the oil emulsion was prepared according to *Romanian Pharmacopoeia* Xth Edition, *Emulsiones monograph* (*Emulsio oleosa)* (*Romanian Pharmacopoeia* Xth Edition, 1993).

The formulations of simple emulsion and the extracts and references emulsions, respectively, are found in table 1.

The technique preparation is given below.

Tab. 1.

The emulsions formulations

EMULSIONS								
Ingredients	ES	1	2	3	4	5	6	7
Vegetal extracts (hibiscus extract/marigold extract)/ Reference (ascorbic acid/ ascorbyl palmitate/ rosemary extract/ tocopherol)	-	0.25	1	2	2.5	3	4	5
Sunflower oil	10	10	10	10	10	10	10	10
Acacia gum	5	5	5	5	5	5	5	5
Water	85	84.75	84	83	82,5	82	81	80
ES = simple emulsion; 1- formula for emulsion of 0.25% of hibiscus extract, marigold extract, ascorbic acid, tocopherol, ascorbyl palmitate, rosemary extract; 2- formula for emulsion of 1% of hibiscus extract, marigold extract, ascorbic acid, tocopherol, ascorbyl palmitate, rosemary extract, 3- formula emulsion of 2 % of hibiscus extract, marigold extract, ascorbic acid, tocopherol, ascorbyl palmitate, rosemary extract; 4- formula for emulsion of 2.5% of hibiscus extract, marigold extract, ascorbic acid, tocopherol, ascorbyl palmitate, rosemary extract, 5- formula for emulsion of 3% of hibiscus extract, marigold extract, ascorbic acid, ascorbyl palmitate, rosemary extract, 6- formula for emulsion of 4% of hibiscus extract, marigold extract, ascorbic acid, ascorbyl palmitate, rosemary extract, 7- formula for emulsion of 5% of hibiscus extract, marigold extract, ascorbic acid, ascorbyl palmitate;								

Preparation of the simple oil emulsion. Acacia gum is accurately weight with a weighing machine (Precisa 220A) and transferred into ceramic mortar. 10 g of sunflower oil (internal phase) are added to acacia gum and triturated homogenously with pestle. When the mixture gets homogenous, 7.5 g of water (external phase) is added and mixed well to form the primary emulsion. After the primary emulsion is formed (cracking sound is produced), water is added in small quantities, and mixed well to form the final emulsion (100g).

Preparation of the emulsions with the vegetal extracts/ascorbic acid

First, the primary emulsion was obtained. Different quantities of extracts/ascorbic acid were dissolved by sonication in 50 g of water and then, the mixture obtained was added to the primary emulsion gradually. Water was added till 100g of the final emulsions were produced. The final concentration of the extracts and the ascorbic acid in the final emulsions were: 0.25, 1%, 2%, 2.5%, 3%, 4%, 5%.

Preparation of the emulsions with tocopherol, rosemary extract and ascorbyl palmitate

For the emulsions with ascorbyl palmitate, the same quantities as for ascorbic acid were dissolved by sonication in 10g of sunflower oil. Then the steps were analogously to the simple oil emulsion. The final concentrations of the references in the final emulsions were: 0.25, 1%, 2%, 2.5%, 3%, 4%, 5%.

Because some technical difficulties occurred, for the rosemary extract the emulsion with 5% concentration could not be obtained. For tocopherol emulsions only the preparations with 0.25%, 1%, 2% and 2.5% concentration were obtained. The storage conditions respected the indications found in *Romanian Pharmacopoeia* Xth Edition: tightly closed containers, 8-15°C.

Emulsions analysis

Organoleptic examination. The emulsions were analysed following the rancid smell, phase separation and microbiological contamination over a period of two weeks. The rancid smell and the phase separation were established only by organoleptic means. The microbiological contamination was established by the appearance of a brown layer and by microscopic examination of the mold using a stereo-microscop (Carl Zeiss, Stemi 305, ob.4x) and microscopic (Carl Zeiss, ob.100x.).

The microscopic preparation consisted in taking a small portion of a culture on a sterile clean slide (after ethanol decontamination of the slide). The slides were covered with a cover slip and viewed under the light of the microscope. The microscopic identification of microbiological contamination was established by comparing mold features with data from fungal atlas (Leboffe et al., 2011, Malloch., 1981)

Antioxidant activity. Ferric reducing power assay was the method employed for the evaluation of the antioxidant activity of all the emulsions. The method was applied according to Oyaizu et al.,1986.

Ferric reducing power assay: 2.5 ml solution (H/M) mixed with 2.5 ml of 1% potassium hexacyanoferrate (III) solution and 2.5 ml phosphate buffer (pH = 6.6), were brought in water bath (50°C) for 20 minutes. Then 2.5 ml of 10% trichloroacetic acid were added. 2.5 ml of the resulted solution were treated with 2.5 ml of water and 0.1 ml of 0.1% ferric chloride solution, left at rest for 10 minutes. Absorbances (λ = 725nm) were measured against blank (without analysis solution). Antioxidant capacity was estimated using a gallic acid standard curve characterized by the following equation: $y = 0.046 x + 0.152$ (y=sample absorbance; x=concentration in gallic acid microequivalents.

Statistical analysis. Results for spectrophotometric and antioxidant determinations represent the average ± standard deviation (SD) of three replicates, from three independent determinations and were calculated using Microsoft Office 2007 (Excel programme).

RESULTS AND DISCUSSIONS:

Composition of extracts

The results of the phytochemical screening and quantitative determinations are found in tables 2 and 3, respectively. Carbohydrates, mucilages, polyphenolcarboxylic acids, flavonoids, saponosides (except for marigold extract) and tannins are found in both extracts. Carotenoids are identified only in marigold. Athocyanidins are found only in hibiscus extract. With a few exceptions, the chemical compositions of the two extracts are similar. The

results of the phytochemical screening prove that phenolic antioxidants and carotenoids were not degraded under the process of obtaining the extracts. For both extracts there were quantified polyphenolcarboxylic acids, flavonoids and total phenolic compounds (hydrophilic antioxidants). Carotenoid (lipophilic antioxidants) content was established only for marigold extract. Anthocyanidins (hydrophilic antioxidants) content was assessed just for hibiscus extract.

Organoleptic examination

The results of the phase separation and emulsions organoleptic degradation (rancid smell) over the two-week surveillance are found in table 4. The microbiological contamination was suggested by the appearance of a brown layer and the microscopic image of the mold (fig. 2). By analyzing scientific data we identified the presence of the hyphae and conidia in all the emulsions as detailed in table 4. Those anatomical characteristics suggested a fungus development (Leboffe et al., 2011). For example, figure 2 shows images of the simple emulsion taken after nine days.

Tab. 2.

Chemical composition of the obtained extracts

Active constituent	Hibiscus	Marigold
carbohydrates	+	+
mucilages	+	+
polyphenolcarboxylic acids	+	+
flavonoids	+	+
anthocyanidins	+	-
carotenoids	-	+
tannins	+	+
saponosides	+	- (degradation under obtaining process)

Note: (+) = present; (-) = absent

Tab. 3.

Quantitative analysis of interest constituents in extracts

	HIBISCUS EXTRACT	**MARIGOLD EXTRACT**
anthocyanins (g% cyanidine chloride equivalents)	1.80 (SD=0.049)	-
flavonoids (g% rutoside equivalents)	-	0.11 (SD=0.007)
polyphenolcarboxylic acids (g% caffeic acid equivalents)	0.57 (SD=0.009)	2.58 (SD=0.022)
carotenoids (g% β-caroten equivalents)	-	0.01 (SD=0.0004)
total polyphenols (g% tanic acid equivalents)	21.38 (SD=0.285)	19.36 (SD=0.318)

Note:(SD – standard deviation from three replicates)

No important results came from the emulsions with 0.25% antioxidants, because of advanced phase separation phenomena and microbiological contamination. The maximum preservation period was 3 days irrespective of the antioxidant used.

For 1% concentrations, emulsions with ascorbic acid, tocopherol and ascorbyl palmitate led to identical results as at 0.25%. For extracts (rosemary, marigold and hibiscus) at 1%, a slight improvement in preservation was noticed.

In comparison with hibiscus extract and reference, the marigold extract protected better against microbiological contamination (the surface of brown layer was smaller than for hibiscus extract). The emulsion with rosemary extract had a poor rancid smell, but both processes were actually significant in altering the quality of the emulsion.

Emulsions with antioxidants between 2% and 5% could be analysed throughout the proposed period (2 weeks), possibly thanks to the high antioxidant concentrations.

The following aspects were observed (table 4, fig.1):

1. Emulsions with extracts looked more pleasant in comparison with emulsions with references: those with rosemary extract were yellowish-green, those with marigold extract were yellowish-brown and those with hibiscus extract were pinkish-red (fig.1).

2. The rancid smell appeared after 5 days in the simple emulsion and in those with lower antioxidant concentrations (2% tocopherol, 2% and 2.5% ascorbic acid, 2% ascorbyl palmitate); and after 7 days in all of them, except for those with rosemary extract at concentrations between 2.5% - 4%.

Also, the hibiscus extract emulsion had a poorer rancid smell than the references, but more intense than the emulsion with rosemary extract. These may be due to the superior antioxidant capacity of both extracts (rosemary and hibiscus extracts) and to the fact that odorant compounds found in both extracts may mask the rancid smell of the emulsions.

3. Phase separation was the most advanced in the case of emulsions with rosemary extract, which were difficult to prepare, especially at concentrations higher than 2.5%. Emulsions with marigold and hibiscus extracts re-dispersed easier when shaken, compared to the others (at the early stages of phase separation).

4. Microbiological contamination started after 7 days in the simple emulsion (fig.2), after 10 days in the other emulsions, except for the marigold extract where it started after 12 days. For emulsions with 2.5% - 4%

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rosemary extract, contamination began on day 10, but it was slower than in the others.

5. Phase separation phenomena, rancid smell and microbiological contamination were very advanced after 14 days and further examination was not possible.

6. At the end of the organoleptic examination, emulsions with rosemary extract had the least rancid smell, followed, at a significant rate, by those with hibiscus extract; those with marigold extract were the least microbiological contaminated.

Thus, from organoleptic point of view, the vegetal extracts behaved as multipotential excipients, noticing the tinctorial power, flavoring potency, antioxidant activity and antimicrobial properties.

Tab. 4.

Organoleptic examination results

EMULSION	ES	E1, E2, C1, P1	C2-C5, P2-P5, R1	R2, R3, R4	M1-M5	H1-H5
onset of rancid smell	after 3 days	after 5 days	after 7 days	after 10 days	after 7 days	after 7 days
onset of phase separation	after 5 days	after 5 days	after 5 days	after 5 days	after 5 days	after 5 days
onset of microbiological contamination	after 7 days	after 10 days	after 10 days	after 10 days	after 12 days	after 10 days

Note:ES=simple emulsion; C1-C5=ascorbic acid emulsions; P1-P5=ascorbyl palmitate emulsions; E1-E2=tocopherol emulsions; R1-R4=rosemary extract emulsions; M1-M5=marigold extract emulsions; H1-H5=hibiscus extract emulsions; 1=2%; 2=2.5%; 3=3%; 4=4%, 5=5%;

Fig. 1 Aspect of emulsions prepared with the obtained extracts.

Fig. 1. Aspect of emulsions prepared with the obtained extracts

Antioxidant activity in emulsions

The results of other studies indicate that non-polar antioxidants are more effective in emulsified oils and polar ones in bulk oils (Yehye et al., 2015). This fact is attributed to the ability of antioxidants to accumulate in the phase where lipid oxidation is predominant. Polar antioxidant concentrates on the oil-air and oil-water interfaces, where oxidation occurs due to increased oxygen and pro-oxidant concentrations. In emulsions, non-polar antioxidants accumulate in the lipid phase and the oil-water interface, where interactions develop between droplet surface peroxides and prooxidants (Yehye et al., 2015).

The activity of antioxidants in emulsions also depends on emulsifier type, which influences the distribution of the antioxidant at the interface. It is assumed that the emulsifier competes with the antioxidant to locate at the interface, self-assembling and capturing the antioxidant and bringing it into the aqueous phase, accentuating oxidation in the lipid phase by depleting the antioxidant (Jayasinghe et al., 2013).

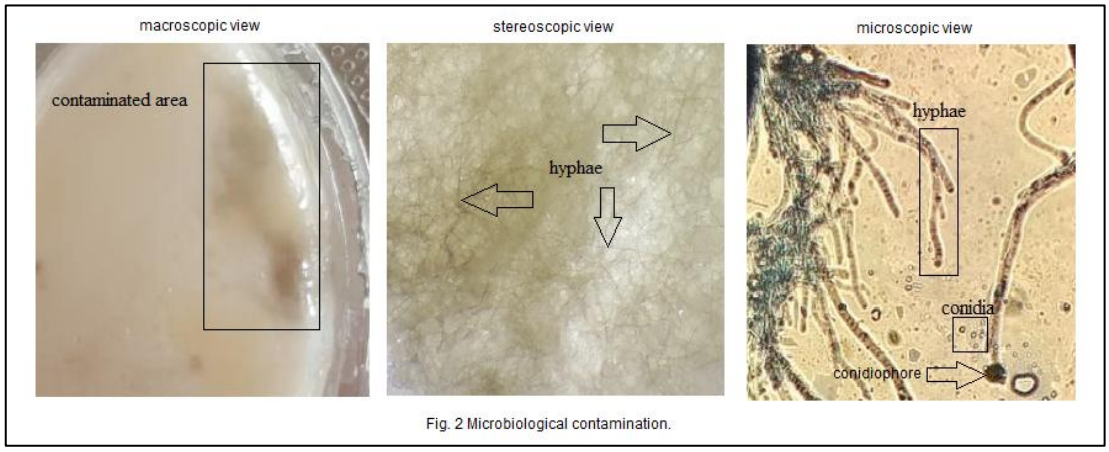

Fig. 2. Microbiological contamination

Emulsions were analysed after 3 days and after 7 days of storage, respectively.

Data after 3 days (fig. 3):

1. Simple oil emulsion also had some antioxidant capacity (reduced compared to other emulsions) possibly because of the pre-existing compounds in sunflower oil used for preparation;

2. The best results were for tocopherol (2.5%), ascorbic acid (3%, 4%, 5%) and ascorbyl palmitate;

3. The extracts showed lower capacity compared to synthetic substances;

4. In the case of ascorbyl palmitate there was an almost constant antioxidant capacity independent of the concentration, possibly due to its solubilization only up to a certain limit in the analysed system;

5. For extracts the highest antioxidant activity, was remarked for hibiscus extract and the lowest, for rosemary extract, marigold extract being intermediate. The antioxidant power increased, directly depending on the concentration of the extract in the emulsion.

Data after 7 days (fig. 3):

1. There were registered negative values expressed in gallic acid microequivalents in the case of simple emulsion and for those with 2% and 2.5% ascorbic acid, possibly by consumption of the antioxidant and pro-oxidant products formation in the system.

In previous research (Jayasinghe et al., 2013), oxidative stability of O/W emulsions (10% fish oil) was investigated using natural extracts (indian gooseberries - *Emblica officinalis*, sweet basil leaves - *Ocimum basilicum*) compared to ascorbic acid and α-tocopherol (35°C, 120h, in comparison with sample without antioxidant). The result of the research stated that the emulsion with ascorbic acid had lower stability, concluding that ascorbic acid activated oxidation. Moreover natural extracts rich in polyphenols and tocopherols were good antioxidants.

2. As far as ascorbic acid emulsions are concerned, it was not possible to make a correlation between the antioxidant activity, antioxidant concentration in the emulsion and the storage period. An explanation is that the method applied might not have been appropriate, so that to generate an accurate analysis of this system.

3. Palmitate emulsions showed a decrease in antioxidant capacity compared with the analysis after 3 days; in addition, after 7 days, the increase in antioxidant capacity with concentration could be observed. It is possible that after the consumption of the antioxidant (by the oxidative process), which was probably at solubility limit, the antioxidant that remained in suspension started to be less soluble.

4. Emulsions with extracts maintained antioxidant activity values similar to those obtained after 3 days, suggesting higher oxidative stability in these systems.

Thus, the analysed extracts proved their efficiency as antioxidants in O/W emulsions (10% sunflower oil). Antioxidant capacity was comparable to that of rosemary extract (already placed on the market as antioxidant for different products) and increased with the concentration of the extract in the medium.

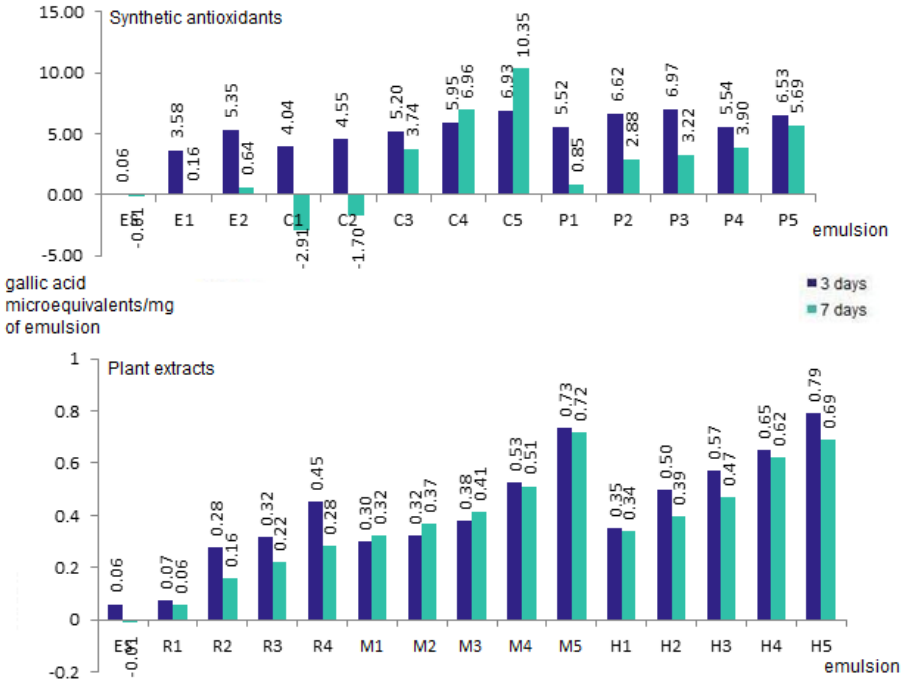

Fig. 3 Antioxidant activity in emulsions.
ES = simple emulsion; C1-C5 = ascorbic acid emulsions; P1-P5 = ascorbyl palmitate emulsions; E1-E2 = tocopherol emulsions; R1-R4 = rosemary extract emulsions; M1-M5 = marigold extract emulsions; H1-H5 = hibiscus extract emulsions; 1=2%; 2=2.5%; 3=3%; 4=4%; 5=5%;

Fig. 3. Antioxidant activity in emulsions.
ES = simple emulsion; C1-C5 = ascorbic acid emulsions; P1-P5 = ascorbyl palmitate emulsions; E1-E2 = tocopherol emulsions; R1-R4 = rosemary extract emulsions; M1-M5 = marigold extract emulsions; H1-H5 = hibiscus extract emulsions; 1 = 2%; 2 = 2.5%; 3 = 3%; 4 = 4%; 5 = 5%.

CONCLUSIONS:

The main purpose of the current study is to establish whether some selected vegetal products (*Hibiscus sabdariffae flos* and *Calendulae flos*) can be natural sources of antioxidant preservatives for O/W emulsions. Dry extracts obtained from hibiscus and marigold were analysed and it was proved that they contain antioxidant compounds (polyphenols and carotenoids) in significant amounts.

Regarding emulsions, it is necessary to take into account the preparation technique, the emulsifier, the concentration in which the antioxidant is used, as well as its nature; this complexity makes research in this field difficult but, exciting at the same time. The extracts obtained proved their antioxidant activity in emulsions (10% sunflower oil). Their antiradical activity increases in a direct proportion with the extract amount. The behaviour of the extracts was established as multipotential excipients (tinctorial power, flavouring potency, antimicrobial effect, emulsion stabilizers, antioxidant activity). Further study could involve other methods of antioxidant evaluation, synergism mechanism between active compounds found in extracts and synthetic antioxidants, different types of emulsions with complex composition. Vegetal sources from which the extracts are derived, as well as the ways of obtaining are variable and represent a direction of continuous research and development for antioxidants.

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