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In vitro Antioxidant Potential and Anti-*Escherichia coli* Effect of Crude Extracts from Common Edible Yellow Flower Petals

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Abstract: Infections with *Escherichia* (*E.*) *coli* is an important problem in clinical practice. Finding natural, non-toxic ways to prevent infections or control the proliferation of these strains is a current topic. The purpose of this study was the antioxidant analysis and the inhibition of *E. coli* multiplication of the extract of four species of common edible flowers (tulip, daffodil, freesia and chrysanthemum), yellow and white chrysanthemum, by in vitro study. The petals were extracted with a mixture of three solvents (ethanol/water/acetic acid, 50/49.50/0.50). After concentration, the antibacterial effect against *E. coli* was determined, by the well diffusion method by measure the diameter of the inhibition zone. At a general evaluation of the results it was observed that the content in bioactive compounds was correlated with the biological effect in vitro. Also, the primary results were influenced by the degree of coloration of the petals. DPPH radical inhibition was approximately 80% for *Tagetes erecta*. These extracts can be successfully used to combat recurrent infections with coliform strains associated with the presence of an inflammatory process, favored by certain physiological imbalances (oxidative stress).
Keywords: *pattern, antiradical, diameter, inhibition*

I. Introduction

Ornamental flowers through their petals are a raw material little used in studies aimed at obtaining pharmaceutical products/supplements. In Romania, they are used only in the form of teas, although the potential of the domain is significant through diversity and the potential of exploitation [1]. Many of the common ornamental flowers (freesia, daffodil, tulips, for example) are consumed, having a role to improve the appearance and taste of the culinary preparations [2].

By diversifying the sources of nutraceuticals, it has been shown that edible petals are rich in bioactive compounds, with characteristics similar to other vegetables (antioxidant, antimicrobial effect, etc.) [3]. The antimicrobial effect is one of the main effects and has been shown to have a direct action on highly pathogenic strains. Also, the effect is harnessed to obtain compounds (through biotransformation) that act in the natural control of antibiotic use [4]. The use of this substrate in the development of pharmacological products comes from the enhancement of knowledge in folk medicine [5].

Thus, the best-known edible flower is the rose, which has uses in different industrial fields. The color of flowers represents an indication of the existence of bioactive compounds, such as flavonoids or carotenoid compounds, which determine antioxidant properties and protect against oxidative stress [6]. Starting from here, the present in vitro study considered the use of petals of edible flowers of yellow color to obtain extracts with antioxidant and antimicrobial effect, against the strains of *E. coli*.

II. Experimental Section

II.1 Chemicals

The chemicals and reagents were purchased, from Sigma-Aldrich GmbH (Sternheim, Germany). All the other unlabeled chemicals and reagents were of analytical grade.

II.2 Substrates and extraction process

Three common edible flower species were purchased: freesia - Refesia freesia - sample noted F, daffodil - Narcissus poeticus - sample noted N, Chrysanthemum indicum (yellow chrysanthemum) - sample noted Cg, white chrysanthemum - sample noted Ca and Tulipa gesneriana (yellow tulip)) - sample noted Lg. Petals were harvested and washed, extracted with a mixture of three solvents: ethanol/water/acetic acid (50/49.50/0.50, v/v/v), in a ratio of 20% [7]. The mixture was kept 24h, under shaking in the dark. Finally, the mixture was filtered through a 0.45 µm Millipore filter and kept in tightly sealed bottles in the dark.

II.3 Bioactive compounds quantification

The determination of the total phenol content was done spectrophotometrically using the Folin-Ciocalteu reagent (diluted 1/10), and the samples were read at 765 nm [8;9].

The determination of the total flavonoid content was performed spectrophotometrically using aluminum chloride as a reagent, and the samples were read at 420 nm [10].

The determination of the total content of carotenoid compounds was performed spectrophotometrically, at 470, 653 and 666 nm, based on previously protocol [8;11].

II.4 *In vitro* antioxidant determination

The antiradical activity (AI%) was determined spectrophotometrically using 1 mM DPPH reagent, at a λ value of 517 nm. In parallel, a control was used and the following formula: AI (%) = [(Am - Ap) / Am] × 100, where: Am - the absorbance of the control, Ap - the absorbance of the samples [12]. Ascorbic acid (AA), 1 mg/mL, was used as a control.

The reduction power was spectrophotometrically determined using 1% potassium ferricyanide reagent, and readings were made at 700 nm. The higher the value, the higher the antioxidant potential [13]. Ascorbic acid (AA), 1 mg/mL, was used as a control.

II.5 *In vitro* antimicrobial determination

It was performed by agar well diffusion assay, by a slightly modified protocol [7, 14]. LB medium, poured into 90 mm sterile Petri dishes, was used. The environment was inoculated with a fresh culture of E. coli with a uropathogenic potential, which is in the culture collection of the Pharmaceutical Biotechnology Laboratory (Faculty of Biotechnologies, USAMV Bucharest). 4 holes of 5 mm are made in the medium and 50 µL of concentrated extract are added. Store at 30°C for 24 hours, then read the inhibition diameter in mm. 5% phenol was used as a control.

II.6 Statistical Analysis

Evaluations of all the parameters investigated were performed in triplicate, with the results expressed as the mean ± standard deviation (SD) values of three observations. The mean and SD values were calculated using the IBM SPSS Statistics 23 software package (IBM Corporation, Armonk, NY, USA). To do the calculations, the significance level was set at: Significant = $p \leq 0.05$; very significant = $p \leq 0.01$; and highly significant = $p \leq 0.001$ using the normal distribution of the variables. The differences were analyzed by ANOVA followed by a Tukey post hoc analysis. The IBM SPSS Statistics software package (IBM Corporation, Armonk, NY, USA) was used to analyze and correlate the experimental data [15].

III. Results and Discussion

III.1 Major bioactive compounds determination

The amount of phenols represents the main group of compounds on which the expression of the biological response depends. Their role is essential in characterizing new functional products (extracts), from which the subsequent exploitation in different products from the cosmetic and/or biopharmaceutical industry has depended [15]. For the total phenolic content, the results are presented in Table 1.

There were no significant statistical changes ($p \leq 0.001$), a variation that was characteristic for all four extracts tested. N and F had similar values of about 1600 µg/mL gallic acid equivalent. These values were about 85% higher compared to chrysanthemum extract. There was a difference of only 15% between the two chrysanthemum extracts, which was correlated with the presence of carotenoid compounds.

These results were supported by the results of the extraction capacity. The values of the total phenol content were directly proportional to the extraction yield, and the increasing order was: Ca <Cg <F <N (data not show). Between the two species of chrysanthemum the difference was about 18%. The value for N exceeded 62%, which corresponded to the increase of the concentration of phenolic compounds.

Table 1. *Total phenolic compounds (TPC), flavonoids compounds (TFC) and carotenoidic compounds (TCC) from crude extracts from edible flowers*

Compounds (µg/mL)	Crude extracts				
	Ca	Cg	F	Lg	N
Total carotenoidic compounds	81.83 ±0.00[a]	249.35 ±10.50[c]	281.36 ±13.66	1195.64 ±67.00[c]	377.31 ±5.60[b]
Total flavonoids compounds	3.86 ±0.13[a]	14.76 ±0.28[b]	16.35 ±1.04[a]	14.68 ±0.12[c]	20.88 ±0.08[a]
Total phenolic compounds	225.98 ±9.78[c]	262.57 ±4.52[c]	1600.96 ±28.27[c]	241.22 ±22.05[b]	1616.64 ±41.49[c]

Different letters mean significant statistical differences (a - P ≤ 0.05; b - P ≤ 0.01; c – P ≤ 0.001), n = 3.

III.2 *In vitro* antibacterial activity

The crude extracts have shown a high capacity to inhibit the *E. coli* bacterium. The main result was that *E. coli* inhibition was not directly correlated with total phenol content. The increasing order of the inhibition spectrum was: Cg = Lg <F <Ca <N (Figure 1). N determined a larger diameter by ≈ 87% compared to control ($p \geq 0.05$). The difference between Ca and Cg was about 50%. The results were not correlated with the phenol content, which did not show high differences (Table 1). The results obtained for N showed a significant variation ($p \geq 0.05$), which was interpreted based on the instability of some compounds present in the extract.

III.3. *In vitro* antioxidant activity.

The *in vitro* evaluation of the crude extract was done by three methods. DPPH inhibition activity demonstrated a high potential for N and F samples, with values of approximately 80% ($p \leq 0.05$). They were four times higher compared to the two extracts of chrysanthemum and 50% higher compared to the ascorbic acid 0.5 mg/mL.

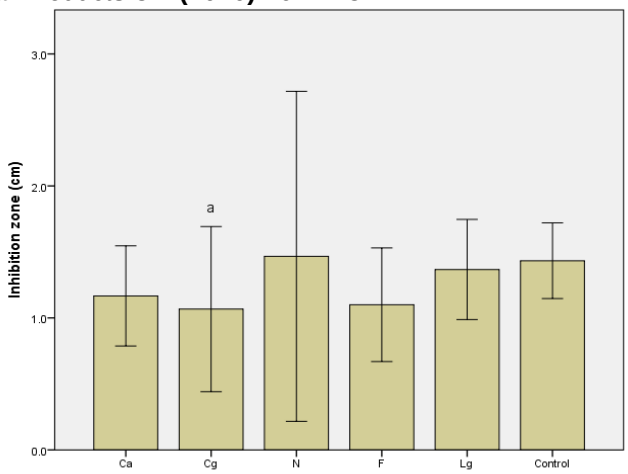

Figure 1. *Diameter (cm) of the E. coli ATCC inhibition zones*
Different letters mean significant statistical differences (a - P ≤ 0.05; b - P ≤ 0.01; c – P ≤ 0.001), n=3.

The extracts showed a significantly lower reduction power (p ≤ 0.001), 50% lower compared to 0.5 mg/mL ascorbic acid. The minimum value was recorded for Cg, below 0.4. These results have shown that extracts from this edible substrate have a reduced amount of reductones [16].

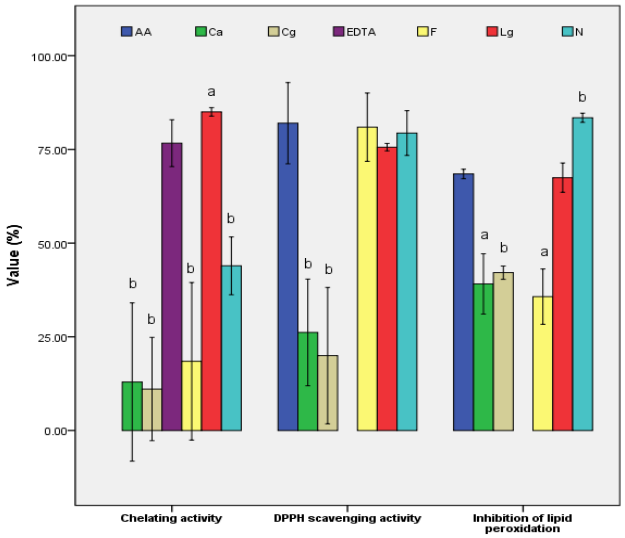

Figure 2. *DPPH scavenging activity, chelating activity and inhibition of lipid peroxidation from common edible flower petals*
Different letters mean significant statistical differences (a - P ≤ 0.05; b - P ≤ 0.01; c – P ≤ 0.001), n=3. AA – ascorbic acid; EDTA – ethylenediaminetetraacetic acid

The chelating activity was reduced and followed the order determined for the rest of the *in vitro* methods. Only Lg exceeded the control value, reaching of up to ≈80% (P ≤ 0.05). The rest of the samples showed values below 50%, with a minimum for Ca and Cg (P ≤ 0.01). These results were valid for the determination of minimum amounts of total flavonoids (Table 1). The same behavior was observed for the inhibition of lipid peroxidation. Lg had a value similar to AA, as control (73.5%). In contrast, N exceeded 75% (P ≤ 0.01), which corresponded to the maximum antiradical response. These results were correlated with the ratio between the amount of carotenoid compounds and phenols.

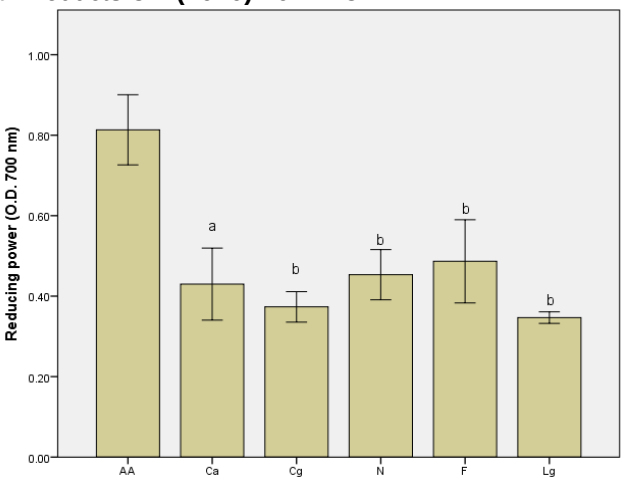

Figure 3. *Reducing power from common edible flower petals*
Different letters mean significant statistical differences (a - P ≤ 0.05; b - P ≤ 0.01; c – P ≤ 0.001), n=3; AA – ascorbic acid

IV. Discussions

The content of bioactive compounds was directly responsible for expressing the biological effect *in vitro*. In the case of this study it was correlated with the degree of coloring of the edible flowers taken into operation. Contrary to studies using another substrate, the *in vitro* effect was inversely colored with flavonoid content (Table 1). This aspect was also determined in a previous study, due to the presence of nonflavonoid phenolic compounds in the extracts [17]. The degree of correlation between the content of flavonoids and phenols was positive, but low. Depended on the level of these compounds, with a maximum of R^2 of 0.45 ± 0.01 for all extracts.

The data obtained correlated with those presented for the antioxidant evaluation of edible flowers extracts of Tagetes erecta [18]. In both studies the level of DPPH radical inhibition was approximately 80%. It was correlated with a high level of total polyphenols and with the total amount of carotenoid compounds (Table 1). Thus, the *in vitro* antioxidant effect was interpreted as a result of the action of the two groups of compounds and not of the presence of flavonoids. These have other activities, which in other studies [17] have been responsible for a hypoglycemic effect, for example.

The antimicrobial effect is another point of interest in the use of edible flower extracts. Inhibition spectra for *E. coli* were obtained from the extracts made with dichloromethane from *Laurus nobilis* leaves [14]. Antimicrobial activity depended on the presence of a mixture of water and ethanol as a solvent. The acetic acid only increased the extractive yield, which resulted in a higher biological value and a higher use of the substrate. A similar antimicrobial effect was also determined for *Pseudomonas aeruginosa*, *Klebsiella peumoniae*, but also for the pathogenic yeast *Candida albicans*, for *Calluna vulgaris* flowers [19]. The biological effect was an important one, demonstrating the value of this substrate in the production of functional supplements (creams or capsules, for example) for the improvement of infections with pathogenic strains that can affect the urinary tract. The results confirmed the conclusions of a previous study [20], which claimed that edible flowers represent a substrate that is under-utilized by nutraceuticals. The limiting factor comes from the possibility of contamination with compounds (chemical additives) that support their growth and storage in a commercial form and which have toxic effect on the human body.

In addition, the instability of the biological effect makes *in vivo* use more difficult, and clinical exploitation is uncertain under certain conditions. It is possible that the solvent will in time determine this instability due to low pH, as well as certain operating conditions in case of high temperatures. This aspect makes the unique use as a source of control of some pathogenic strains to be combined with other variations (e.g., red ones) where stability is higher due to the presence of proanthocyanides. In addition, the presence of these products (extracts) enhances the topical effect of some synthetic drugs. Their inclusion determines the exploitation of new resources that can determine the development of products that reduce the toxicity and which lead to the defense against the effects of oxidative stress. The extent of antimicrobial proliferation has been determined by an anti-inflammatory effect, and combining with antioxidant protection supports a topical action (dermatocosmetic products, for example) [21,22].

IV. Conclusion

The study showed the efficiency of the extracts from edible flower petals against the *E. coli* strain. The results showed that the carotenoid component has a greater influence on the expression of this activity *in vitro*. In contrast, the antioxidant value was more pronounced in the case of Lg, N and F to the detriment of chrysanthemums. So, these extracts can be successfully used to combat recurrent infections with coliform strains associated with the presence of an inflammatory process, favored by certain physiological imbalances (oxidative stress).

Author Contributions: A.U. & E.V. designed the experiments, analyzed the data, and wrote the paper. The authors discussed and made comments on the results.

Conflicts of Interest: The authors declare no conflict of interest.

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