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Antioxidant and Antimicrobial Activity of Edible Flower Extracts Obtained by Different Extraction Methods

Atividade Antioxidante e Antimicrobiana de Extratos de Flores Comestíveis Obtidos por Diferentes Métodos de Extração

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Abstract

There is a great demand in the market for natural products derived from plants. The antioxidant activity and phenolic compounds provide several beneficial effects to human health. In addition, the antimicrobial properties of substances present in plant extracts and essential oils, produced by plants have been recognized empirically for centuries and have been scientifically proven in recent years. The objective of this study was to evaluate the content of phenolic compounds, total flavonoids, and antioxidant and antimicrobial capacity in vitro of flower extracts of edible rose petals (*Rosa x grandiflora* Hort.), sunflower (*Helianthus annuus* L.) and calendula (*Calendula officinalis* L.) obtained by conventional and ultrasound-assisted extraction methods. For the extraction, two methods were used (conventional and ultrasound-assisted) and two extraction temperatures were used, 20 ºC and 60 ºC. Cereal ethyl alcohol was used as a solvent. For the extracts characterization, analyzes of total phenolic compounds, total flavonoid content, antioxidant capacity and antimicrobial activity were performed. Rose extracts differed from the other species for the content of total phenolic compounds. Their extract obtained by the ultrasound-assisted method at 60 ºC presented a higher content of total phenolic compounds (28.99 g EAG mL^{-1} extract), higher flavonoid content (20.26 g EQ mL^{-1} extract) and higher antioxidant activity (IC_{50} of 0.75 mg mL^{-1}). The extracts tested did not present antimicrobial activity. The results of this study demonstrate that flower petal extracts may be a viable alternative as a natural antioxidant in place of synthetic antioxidants.

Keywords: *Calendula Officinalis* L.; *Helianthus Annuus* L.; *Rosa x Grandiflora* Hort.

Resumo

Existe uma grande demanda no mercado por produtos naturais derivados de plantas. A atividade antioxidante e os compostos fenólicos proporcionam diversos efeitos benéficos à saúde humana. Além disso, as propriedades antimicrobianas de substâncias presentes em extratos vegetais e óleos essenciais, produzidos por plantas, são reconhecidas empiricamente há séculos e têm sido comprovadas cientificamente nos últimos anos. O objetivo deste trabalho foi avaliar o conteúdo de compostos fenólicos, flavonóides totais e capacidade antioxidante e antimicrobiana in vitro de extratos de pétalas de flores comestíveis rosa (Rosa x grandiflora Hort.), girassol (Helianthus annuus L.) e calêndula (Calendula officinalis L.), obtidos por métodos de extração convencional e assistido por ultrassom. Para a extração, foram utilizados dois métodos (convencional e assistido por ultrassom) e duas temperaturas de extração, 20 ºC e 60 ºC. Álcool etílico de cereais foi usado como solvente. Para a caracterização dos extratos, foram realizadas análises de compostos fenólicos totais, teor de flavonóides totais, capacidade antioxidante e atividade antimicrobiana. Os extratos de rosa diferiram das outras espécies quanto ao conteúdo de compostos fenólicos totais. Seu extrato obtido pelo método assistido por ultrassom a 60 ºC apresentou maior teor de compostos fenólicos totais (28,99 g EAG mL^{-1} de extrato), maior teor de flavonóides (20,26 g EQ mL^{-1} de extrato) e maior atividade antioxidante (IC_{50} de 0,75 mg mL^{-1}). Os extratos testados não apresentaram atividade antimicrobiana. Os resultados deste estudo demonstram que os extratos de pétalas de flores podem ser uma alternativa viável como um antioxidante natural em substituição aos antioxidantes sintéticos.

Palavras-chave: *Calendula Officinalis L.; Helianthus Annuus L.; Rosa x Grandiflora Hort.*

1 Introduction

There is a great demand in the market for natural products derived from plants, such as green tea, herbs and flowers decoction, and the phytotherapeutic products formulation. These products may include aerial parts of plants, seeds, fruits, roots and flowers, used in various commercial applications, such as teas, gastronomic preparations, extracts and essential oils (FRANZEN *et al.*, 2019; CHEN *et al.*, 2020).

Edible flowers are widely explored in the development of floral teas, food colorings, aromas, beverages, bakery products, or as unprocessed products in retail. They have become increasingly popular, evidenced by the increase of cookbooks, magazine articles and websites on the subject, going beyond the scientific knowledge related to their nutritional potential (FRANZEN *et al.*, 2018; 2019; JI-YU *et al.*, 2020).

Flowers are used in cooking to enhance the sensory attributes of the gastronomic preparations, such as color, aroma and flavor. However, some are incorporated into wines and flavored liqueurs, such as the classic Chartreuse green liqueur, of French origin, which uses clove petals as an ingredient. In addition, large species, such as pumpkin flowers, can still be stuffed and fried (CHEN *et al.*, 2020; LÓPEZ-HORTAS *et al.*,

2020).

Edible flowers have proteins, lipids, carbohydrates, minerals and vitamins A, B, C and E in their constitution, which are important for healthy eating. These are considered sources of polyphenolic compounds, which present high antioxidant activity. The variety of colors reflects the different types of carotenoids and anthocyanins present in the flowers chemical composition. The anthocyanins content is associated with flavonoid levels, and therefore with the antioxidant activity, which makes them a source for nutraceuticals for human consumption (KHAN *et al.*, 2019; JI-YU *et al.*, 2020; LÓPEZ-HORTAS *et al.*, 2020).

The antioxidant activity and the phenolic compounds present in flowers provide several beneficial effects to human health. The importance of ingesting food with antioxidant potential for the chronic diseases prevention, such as cardiovascular, cancer and aging-related degenerative brain diseases has been shown in research. These phytochemicals, in addition to the antioxidant action, have anti-inflammatory, anti-obesity, hypoglycemic and protective properties for the neurological, hepatic and gastrointestinal systems (CHEN *et al.*, 2020; JI-YU *et al.*, 2020).

Plants extracts are used as natural antioxidants to prevent lipid oxidation, which is a major concern in the food industry, because it generates products that are undesirable such as lipid degradation and the production of volatile compounds that can make food sensorially unacceptable, as well as producing potentially toxic substances. The industry has preferably used natural antioxidants, but there is still little knowledge about the edible flowers antioxidant capacity, a good alternative for consumers looking for healthier food (KHAN *et al.*, 2019; LIJUN *et al.*, 2020).

Currently, many classic methods also called conventional can be applied for the bioactive compounds extraction, however, these extraction methods have disadvantages, such as, for example, high energy consumption, low extraction efficiency and long processing time. For economic viability of an industrial process, it is essential to work with more efficient extraction methods, for which factors need to be established such as temperature, time, type of solvent and methodology. With the increase in the application of green chemistry, attention is focused on methods/techniques called ecologically correct. During the ultrasound-assisted extraction process, the acoustic cavitations generated by the ultrasound promote the solvent penetration into the cell walls of the plant matrix, facilitating the intracellular content release, in combination with agitation that increases the contact area between the solvent and the target compounds. (KHAN *et al.*, 2019; LÓPEZ-HORTAS *et al.*, 2020).

The methods of extracting unconventional bioactive compounds, such as the ultrasound-assisted method, have been increasingly studied as an alternative to conventional/ classic methods, as a solution to improve extractions with high concentrations of bioactive compounds. Despite the cost disadvantage with technology applied to unconventional methods, there is a great advantage, which is the increase in the levels of compounds obtained and greater selectivity, when compared to conventional methods (LÓPEZ-HORTAS *et al.*, 2020; SHARMA *et al.*, 2020).

Rose (*Rosa x grandiflora* Hort.) can be consumed in many foods as ingredients or in nature, to provide an exotic touch. Rose tea as an ingredient, normally, an infusion is made first to concentrate the flavor. Because rose petals are very rich in vitamins, it has a regenerative effect on the skin and can also be used to fight colds and flu , as well as for digestive problems, freeing the body from toxins, as they have analgesic, anti-inflammatory, antipyretic, soothing, healing and diuretic properties. Sunflower (*Helianthus annuus* L.) is rich in proteins and vitamins of the B complex, and today, in addition to the oil, flower buds can be used. The main properties of this species are the cholesterol regulation in the blood, improvement in cardiovascular health, fighting degenerative problems, and helping hormone formation for the proper digestive system functioning. The calendula petals (*Calendula officinalis* L.) have been used in decorations for cakes, sweets and savory coverings. The use of this species in food requires the pollen removal since it can cause allergic reactions. Calendula flowers are rich in substances such as carotenoids and essential oils and have a spicy palate (FRANZEN *et al.*, 2018; 2019).

Studies on the antimicrobial activities of plant extracts and essential oils from native plants have been reported in many countries around the world, which have a diverse flora and a rich tradition in the use of medicinal plants for use as antibacterial or antifungal (GISHEN *et al.*, 2020; HAO; XIAO, 2020; MOGLAD *et al.*, 2020; SHARMA *et al.*, 2020).

Innumerable plants are known for their biological, antimicrobiological, food activities and as phytotherapeutic agents in the treatment of various diseases, being intensively researched by the pharmaceutical and food industry. However, little is known about the preliminary phytochemical studies of numerous natural plant species (MENEZES FILHO; CASTRO, 2019a; 2019b).

The phytochemical study becomes necessary in order to be able to know the groups of chemical compounds present in plants and, thus, through quantification and separation tests of each group, to evaluate what can be used in agriculture, in the treatment of diseases that affects humans, animals and plants (MENEZES FILHO; CASTRO, 2019b).

Thus, the objective of this study was to evaluate the content of phenolic compounds, total flavonoids and the in vitro antioxidant and antimicrobial capacity of edible flower petals extracts.

2 Material and Methods

The study was carried out in three stages, with the edible

flowers production, obtaining of extracts and the extracts characterization.

The edible flowers production was carried out in the Floriculture Sector of the Department of Phytotechnics at the Federal University of Santa Maria (UFSM), located in Santa Maria, RS (29º 43' S; 53º 43' W and elevation of 95 m). Calendula flowers (*Calendula officinalis* L.) were grown in open-air beds with dimensions of 10 m in length and 1 m in width, with direct seeding, comprising 30 plants m^{-2}. The sunflower cultivation (*Helianthus annuus* L.) was in a greenhouse, in 5-L plastic containers with commercial H-Decker substrate, 3 plants per $container^{-1}$ and with distribution of 8 containers m^{-2}. Rose flowers (*Rosa x grandiflora* Hort.) were collected from greenhouse grown plants, with two years of cultivation. All the species were irrigated daily and grown without the use of fertilizers and chemical products. The flowers were harvested by hand, in the morning period, and allocated in thermal packaging, then transported to the physicochemical laboratory.

The samples preparation was carried out in the physicochemical laboratory at the Department of Technology and Food Science of UFSM. The petals were manually removed and placed in trays for pre-drying in a forced air circulation oven (Marconi®) at 55 ºC for 72 h. After drying, they were crushed in a domestic blender (Walita Liqfaz®), packed in hermetically sealed plastic containers and subjected to freezing temperature (-2 ºC) until the moment for the extracts preparation.

Two extraction temperatures were used for both extraction methods, 20 ºC and 60 ºC, and the extracts were filtered through a qualitative paper filter (n.1). Subsequently, the obtained filtrate was concentrated in a rotary evaporator (Marconi® MA 120 Rotary Evaporator) to eliminate the alcohol (the solvent – cereal ethyl alcohol 96º GL). The extracts were placed in an amber bottle and stored in a freezer (-12 ºC) until the moment of analysis.

For the conventional extraction method, flower petal extracts were obtained according to the methodology used by Viera *et al.* (2017). The crushed petals were mixed with the solvent (cereal ethyl alcohol 96º GL) in the ratio of 1:20 (w v^{-1}). The mixture was kept stirring in a thermostated bath (Marconi® Ultrathermostated Bath Model MA 184) for 120 minutes.

For the ultrasound-assisted extraction method, the methodology by Viera *et al.* (2017) was used, using a USC-1450 Unique® ultrasonic bath, operating at a constant frequency of 40 KHz and ultrasonic power of 135 W, with the solvent (cereal ethyl alcohol 96º GL) in the ratio of 1:20 (w v^{-1}) with extraction period of 120 minutes.

For the determination of total phenolic compounds in the extracts, the *Folin-Ciocalteau* method was used with standard curve of gallic acid [y = 0.016x+0.1012] with [R^2 = 0.9961], with adaptations in the technique described by Miliauskas *et al.* (2004) to estimate the TPC concentration in the sample.

In properly identified test tubes, 400 µL of extract was added, diluted 1:500 for the rose species and 1:100 for the other species, and 2 mL of Folin-Ciocalteau 2N, diluted 1:10. The solutions were completely homogenized and incubated at room temperature (27 ºC) for 3 minutes, then 1.6 mL of sodium carbonate 7% (Na_2CO_3) was added previously filtered and again they were incubated in a water bath at 50 ºC for 5 minutes. After cooling, samples readings were taken using a UV-Vis spectrophotometer (Biospectro, model: SP-220) at 760 nm. TPC was expressed as g of gallic acid equivalents (GAE) per mL of extract. The analyses were performed in triplicate, for a greater accuracy in the results.

Total flavonoid content was determined using the colorimetric assay developed by Zhishen *et al.* (1999). A known volume (0.5 mL) of the extract was added to a test tube and at time zero, 150 µL of a 5% sodium nitrite ($NaNO_2$) solution was added. After 5 minutes, 150 µl of a 10% aluminum trichloride ($AlCl_3$) solution was added and allowed to stand for 6 minutes further and then 1 ml of 1 M NaOH was added, followed by the addition of 1.2 mL of distilled water. The absorbance at 510 nm was used for UV-Vis spectrum recording using a spectrophotometer (Biospectro SP-220, Brazil). The extract absorbance was compared to a standard quercetin curve [y=0.0028x+0.1036, R^2 = 0.9968] to estimate the flavonoid content concentration in the sample. The flavonoid content was expressed as g of quercetin equivalents (QE) per mL of extract.

The antioxidant capacity was determined by the reduction of the stable radical 2.2-diphenyl-1-picryl-hydrazila (DPPH) through the antioxidants action present in the sample (extracts), according to the methodology of Kim *et al.* (2002). Aliquots of 0.5 mL of methanolic solution containing different concentrations were added to 2.5 mL of DPPH solution. The mixture was stirred gently and allowed to stand at room temperature (27 ºC) in the dark for 30 minutes. Subsequently, the absorbance was read at 517 nm. The elimination activity was measured as the decrease of absorbance in the samples in comparison to the standard DPPH solution (control). The radical standard solution was prepared for use daily. The results were expressed as percentage of radical elimination activity (%) of the DPPH radical, according to Equation 1 [$\%DPPH_{\text{radical eliminated}}=((A_0-A_a)/A_0)*100$]. Where A_0 is control absorbance and A_a is sample absorbance. The effective concentration had 50% of radical inhibition activity (IC_{50}) expressed as mg mL^{-1} of extract, which was determined from the graph of free radical elimination activity (%) against extract concentration.

The flower petals extracts (*Rosa x grandiflora*, *Helianthus annuus* and *Calendula officinalis*) were tested individually against *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Aspergillus niger*. The strains were donated by the Department of Industrial Pharmacy of UFSM. The strains were maintained at -18 ºC

in the appropriate culture media added with 10% glycerol, and streaked every 15 days for slants of tryptase soybean agar (TSA) for the bacteria and culture medium PDA (Potato-Dextrose-Agar) for the fungus, and kept at 4 ºC.

The extracts antibacterial activity detection on the tested microorganisms was performed by the disc diffusion method (CLSI, 2009). For the disc diffusion technique, the microorganism suspensions were prepared in 0.9% NaCl solution and compared to the turbidity of 0.5 on the Mcfarland scale (equivalent to approximately 1.5 x10^8 colony forming units mL^{-1}). Using sterile swabs, the bacterial suspensions were seeded on the Petri dishes surface containing about 15 mL of Mueller-Hinton agar with thickness of approximately 4 mm. Sterilized filter paper discs with a diameter of nine millimeters were impregnated with 25 µL of each extract and deposited on the inoculated plates, incubated at 36 ºC for 24 h. The inhibition controls used were: chloramphenicol (Chloramphenicol®) 0.4% (25 µL $disc^{-1}$) used as positive control, and discs impregnated with distilled water (25 µL $disc^{-1}$) as negative controls. After the incubation time, the halos formation was analyzed. The inhibition zone diameter (halo) was measured in millimeters. The tests were performed in quadruplicate and the results were expressed in millimeters as the arithmetic mean value of the inhibition halos diameter that were formed around the discs.

To evaluate the antifungal activity of flower petal extracts the method adapted by Fiori *et al.* (2000) was used. Pure extracts were incorporated into the PDA (Potato-Dextrose-Agar) culture medium at 4% concentration (1 mL extract for 25 mL PDA/dish). After 1 hour of PDA medium containing petal extracts being verted in the Petri dishes, the spores of the *Aspergillus niger* pathogen were inoculated, then in the sequence the dishes were incubated at room temperature (25 ºC ±1) in the dark for 7 days in an incubator (Biomatic®). The controls were: Ciclopirox olamine 10 mg mL^{-1} (Loprox®) as positive control, only the PDA medium with pathogen spores as negative control and only the PDA medium as medium control. The tests were performed in quadruplicate to confirm the results.

To obtain the extracts, an experimental design completely randomized was used, in a 2x2x3 scheme (extraction method, temperatures and edible flowers), with three replicates. The extraction methods were conventional and ultrasound-assisted, at two temperatures, 20 ºC and 60 ºC, and the edible flowers studied were calendula, sunflower and rose. Data variance analysis (ANOVA) ($p<0.05$) was performed with the support of the SISVAR software (FERREIRA, 2011), the data normality was verified by the Shapiro-Wilks test and the means interaction was compared by the Scott-Knott test ($p<0.05$).

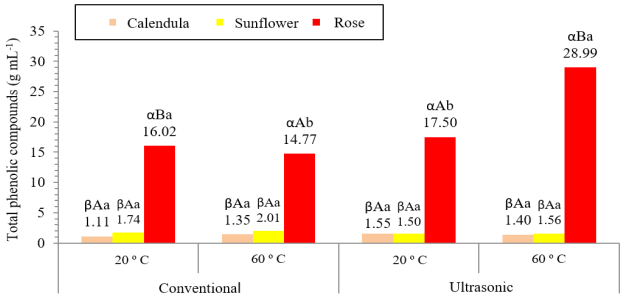
3 Results and Discussion

In the extracts characterization, total phenols were expressed in gram of gallic acid equivalent per mL of extract (g GAE mL^{-1} extract), the higher the value, the higher the content of total phenolic compounds. According to Figure 1, rose extracts presented higher content of total phenolic compounds exhibit statistical difference from the other species ($p<0.05$). Their extract obtained by the ultrasound-assisted method at 60 ºC presented the highest content of total phenolic compounds (28.99 g GAE mL^{-1} extract) while calendula extract obtained by the conventional method at a temperature of 20 ºC presented the lowest phenolic compounds values (1.11 g GAE mL^{-1} extract).

It was observed that the rose species by the ultrasound-assisted method, regardless of temperature, was more efficient in relation to the other species and the conventional method in the extraction of total phenolic compounds. This can be attributed to obtaining different phenolic compounds by the influence of the different extraction methods and temperatures used, presenting a better response. Bandeira *et al.* (2018) state that this difference in compounds may be related to the type of treatment provided to the sample and also to the samples geographical origin. Habermanna *et al.* (2016) in rose extracts, phenolic compounds were found to be more abundant, which may be very interesting because these compounds have been correlated to various biological effects, including antioxidant, antiobesity and antitumor activities.

Phenolic compounds are abundantly produced by plants , this group has active pharmacological actions, in the healing process, in burns, in antioxidant and anti-tumor capacity and in inflammatory processes. Phenolic compounds act as hypoglycemic, astringent, antidiarrheal and antioxidant free radical scavengers of singlet oxygen (MENEZES FILHO; CASTRO, 2019a).

Figure 1 - Total phenolic compounds (g mL^{-1}) present in flower petals subjected to extraction by conventional and ultrasound-assisted methods, and temperature variation of 20 ºC and 60 ºC



Notes: The means followed by Greek letters differ statistically the flower species, Roman capital letters differ statistically the extraction methods, and Roman lowercase letters differ statiscally the extraction temperatures by the Scott-Knott test at 5% level of error significance. Coefficient of variation 10.46%.
Source: Research data.

Kim *et al.* (2013) when analyzing the amount of total phenols in edible plant extracts, obtained a wide range of variation from 3.13 to 72.30 mg GAE/g^{-1} extract, the most efficient extraction being with ethanol, compared to the other solvents used. Wang *et al.* (2003) found values of 62

mg g^{-1} of phenolic compounds in artichoke leaves and 14 mg g^{-1} in artichoke fruits. When studying the phenolic amount of broccoli and asparagus, Sun *et al.* (2007) found similar amounts for methanolic extracts (4.9 mg g^{-1} in dry basis). For the aqueous extracts, the authors found a higher result for asparagus (4.9 mg g^{-1} in dry basis) than for broccoli (4.5 mg g^{-1} in dry basis).

A study by Asolini *et al.* (2006), in which the phenolic concentrations of various plants used as teas were analyzed; salvia and chamomile ethanolic extracts presented phenolic compounds around 25 mg GAE mL^{-1}, an approximate value to that found in the ethanolic extract (25.66 mg GAE mL^{-1} extract) from dehydrated *Pereskia aculeata* leaves analyzed by Rodrigues (2016), however, both were lower than those observed in the roses extract obtained by the ultrasound-assisted method at 60 ºC in the present study.

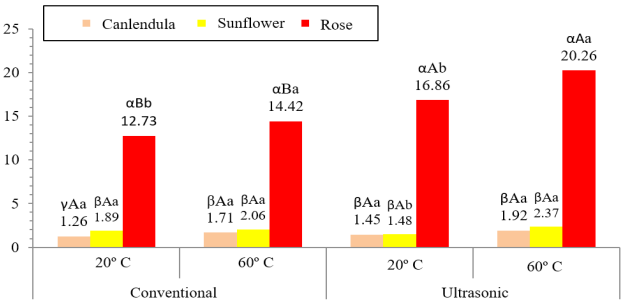
According to Pereira (2009), when performing *in vitro* analysis in plant extracts a direct relationship was observed, the higher the phenolic content, the higher the extracts antioxidant activity. Caetano *et al.* (2009), in their study with acerola agroindustrial residue, found a phenolic content in its hydroethanolic extract (80%) regardless of the temperature used in the extraction process, 1.78 mg in catechin equivalent mL^{-1}.

The phenolic compounds solubility in a given solvent is a peculiar characteristic of the phytochemical, which explains the lack of a universal procedure and points to the need for the careful selection of the extraction method for each natural antioxidant source. Therefore , considering that in plants there are polyphenols with diversified polarity, for the efficient extraction of these constituents, the use of solvents with different polarities is required (CAETANO *et al.*, 2009; PONZILACQUA *et al.*, 2019). The solvents effectiveness will depend on the polyphenols polarity present in the sample, as well as on the polymerization degree and the interaction with other constituents (JI-YU *et al.*, 2020; CHEN *et al.*, 2020).

Kalemba-Drożdż and Cierniak (2019) point out that for the extraction process efficiency, it is necessary to combine at least two extraction cycles, using organic solvents solutions, with different polarities, in order to extract compounds with different chemical structures. In general, the extraction duration and temperature are parameters to be improved in order to reduce time and the extraction process cost (PONZILACQUA *et al.*, 2019; JI-YU *et al.*, 2020). Although the temperature favors the phenolic compounds extraction, it should be emphasized that it may also trigger its degradation with possible damage to the antioxidant action (CAETANO *et al.*, 2009; KALEMBA-DROŻDŻ; CIERNIAK, 2019; CHEN *et al.*, 2020).

Figure 2 shows the mean values of total flavonoid content of flower petal extracts obtained by the conventional and ultrasound-assisted methods at different temperature conditions, cereals ethyl alcohol as solvent and during a period of 120 minutes of extraction. The results showed that there was a significant difference ($p<0.05$) in the extracts between the methods and the species, and presented flavonoid values of 12.73 (conventional 20 ºC), 14.42 (conventional 60 ºC), 16.86 (ultrasound-assisted 20 ºC) and 20.26 (ultrasound-assisted 60 ºC) g QE mL^{-1} rose petal extract, 1.89 (conventional 20 ºC), 2.06 (conventional 60 ºC), 1.48 (ultrasound-assisted 20 ºC) and 2.37 (ultrasound-assisted 60 ºC) g QE mL^{-1} sunflower petal extract and 1.26 (conventional 20 ºC), 1.71 (conventional 60 ºC), 1.45 (ultrasound-assisted 20 ºC) and 1.92 (ultrasound-assisted 60 ºC) g QE mL^{-1} calendula petal extract.

Figure 2 - Flavonoids (mg mL^{-1}) present in flower petals, subjected to extraction by conventional and ultrasound-assisted methods, with temperature variation of 20 ºC and 60 ºC



Notes: The averages followed by Greek letters differ statistically the flower species, Roman capital letters differ statistically the extraction methods, and the Roman lowercase letters differ statistically the extraction temperatures by the Scott-Knott test at 5% level of error significance. Coefficient of variation 5.49%.
Source: Research Data

The roses extract obtained by the ultrasound-assisted method at 60º C presented the highest flavonoid content (20.26 g QE mL^{-1} extract), differing significantly ($p<0.05$) from the conventional method and from the other species studied, while the calendula extract (1.26 g QE mL^{-1} extract) obtained the lowest result. A study carried out by Pereira (2009) found a value of 12.69 mg QE g^{-1} for flavonoids in a sample of *Achyrocline satureioides*, a result that is lower than those found in rose petal extracts of this study.

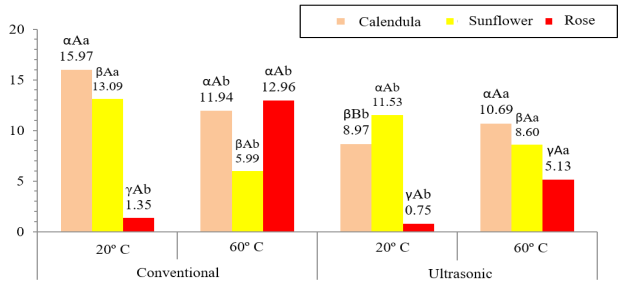
Rodrigues (2016) observed in the extracts of leaves of *Pereskia aculeata* Mill. flavonoid values of 16.31, 4.98 and 7.73 mg QE mL^{-1} extract, values similar to those found in this study. For the total flavonoid content, it was observed that the rose species showed the highest values for the two methods and for the two temperatures, differing statistically from the other species (calendula and sunflower).

Various functions are attributed to flavonoids, as plant cell protection, in plants in full sun, against attack by insects, fungi, viruses, bacteria, as potent antioxidant agents, anti-tumor, anti-inflammatory actions, such as antihistamine and antiviral (MENEZES FILHO; CASTRO, 2019a).

IC_{50} is a parameter used to determine the plants antioxidant potential. It demonstrates the amount of plant needed to reduce DPPH by 50%, simulating how the plant will act on a free radical in the body (ROSA *et al.*, 2013). The results of

potential antioxidants in edible flower petal extracts are shown in Figure 3 through the IC_{50}. Rose petal extracts showed the best results obtained in the DPPH radical elimination assay and were able to promote the free radicals elimination. These results were consistent with those obtained by Barros *et al.* (2011) for *Rosa canina* L. petals. Other research studies revealed that phytochemicals such as anthocyanins, flavonoids, phenolic acids, alkaloids and glycosides in edible flowers exert a high antioxidant activity (CHEN *et al.*, 2020; JI-YU *et al.*, 2020).

Figure 3 - IC_{50} (mg mL^{-1}) present in flower petals, subjected to extraction by conventional and ultrasound-assisted methods, and temperature variation of 20 ºC and 60 ºC.



Notes: The averages followed by Greek letters statistically differ the flower species, Roman capital letters differ statistically from extraction methods and, Roman lowercase letters differ statistically the extraction temperatures, by the Scott-Knott test, at a level of 5% error significance. Coefficient of variation 12.59%.
Source: Research Data.

When analyzing the results, it was verified that the rose petals extracts obtained at 20 ºC in both methods presented a higher antioxidant activity, differing significantly ($p<0.05$) from the other species. There was difference between the species and between the temperatures, regardless of the method used in the extractions. It was verified that the ultrasonic extraction at a temperature of 20 ºC used in the rose species was the most efficient, presenting the IC_{50} value of 0.75 mg mL^{-1} of extract to inhibit 50% of the DPPH radical.

The plant *Ginkgo biloba* (L.) Hoffmanns., considered to have high antioxidant activity, presented an IC_{50} of 0.04 mg mL^{-1} in an experiment conducted by Mensor *et al.* (2001). Silva *et al.* (2012) found lower value for IC_{50} (0.13 mg mL^{-1}) in *Achyrocline satureioides* (Lam.) DC. extracts extracted with 80% ethanol, and Pereira (2009) found a value of 5.26 mg mL^{-1} for the same species, considering that the lower the IC_{50} value, the higher the antioxidant capacity of the analyzed plant material. Based on these results, it may be stated that the rose species showed high antioxidant activity, according to the DPPH method.

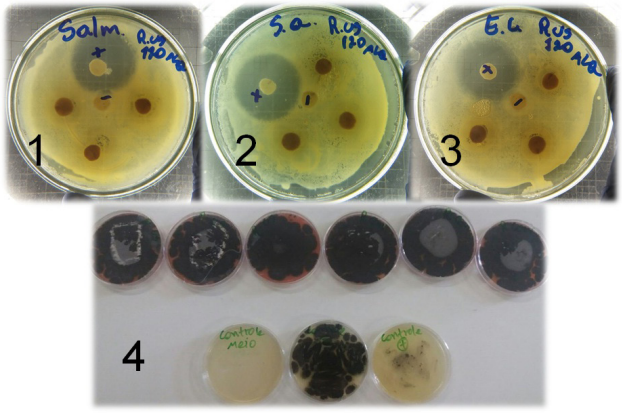
Rodrigues (2016) found in the ethanolic extract of *Pereskia aculeata* Mill. leaves values for IC_{50} of 3.87 mg mL^{-1} of extract, inhibition values higher than those found in both methods of cold extraction for the rose species, that is, a higher amount is needed of *Pereskia aculeata* Mill. plant extract than rose petal extract to inhibit 50% of the DPPH radical. The *Malpighia emarginata* DC. hydroethanolic extract, elaborated by Caetano *et al.* (2009), independent of concentration and temperature of production, showed high sequestration capacity of the DPPH radical. Therefore, it is evident that the temperature for obtaining extracts does not influence their antioxidant action in a striking way.

The calendula species presented a higher extract concentration for IC_{50} (15.97 mg mL^{-1} of extract to inhibit 50% of the DPPH radical) for the conventional method at a temperature of 20 ºC, resulting in a low antioxidant activity when compared to the other species analyzed in this study. It should be noted that different authors have presented IC_{50} values of natural antioxidants with great differences, making it difficult to compare the results. Flavonoids are also considered safe compounds, with low potential to induce organic toxicity (LIJUN *et al.*, 2020). However, they may exhibit pro-oxidant activity, explaining some mutagenic and cytotoxic effects (ZHANG *et al.*, 2019). The flavonoids pro-oxidant and antioxidant properties, such as those derived from quercetin, depend on the environment in which they are inserted into, as well as their structure and chemical concentration (PARK *et al.*, 2019).

In general, the extracts that presented the best antioxidant activities were those that exhibited the highest concentrations of phenolic compounds and flavonoids. The rose species was shown to be more efficient against the DPPH radical inhibition and thus presented greater antioxidant activity. These results support the potential of edible flowers, especially roses, as phenolic compounds sources with bioactive potential, having a high phytochemical interest for the food industry.

The rose, sunflower and calendula petals extracts showed no antimicrobial activity on the microorganisms *Staphylococcus aureus* ATCC 25923, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028, *Escherichia coli* ATCC 25922 and *Aspergillus niger* (Figure 4).

Figure 4 - Result of the antimicrobial activity of flower petal extracts, subjected to extraction by conventional and ultrasound-assisted methods, against the microorganisms *Salmonella enterica* subsp. *enterica* serovar Typhimurium (1), *Staphylococcus aureus* (2), *Escherichia coli* (3) and *Aspergillus niger* (4).



Source: The authors.

Rodrigues (2016) also did not find in his study with *Pereskia aculeata* Mill. extracts any antimicrobial activity on the microorganisms *Staphylococcus aureus*, *Salmonella enterica* subsp. *enterica* serovar Enteritidis, *Salmonella entérica* subsp. *enterica* serovar, Choleraesuis, *Salmonella enterica* subsp. *entérica* serovar Typhimurium, *Escherichia coli*, *Enterococcus faecalis* and *Enterobacter aerogenes*, according to the disc diffusion test.

The rabbiteye blueberry extracts (*Vaccinium ashei* Reade) obtained through different extraction methodologies, elaborated by Piovesan *et al.* (2017), also did not present antimicrobial activity against the microorganisms *Escherichia coli*, *Salmonella cholerestasus*, *Salmonella enteritidis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Viera *et al.* (2017) in their study preparing red onion (*Allium cepa* L.) skin extracts found a negative antimicrobial activity towards the microorganisms tested (*Escherichia coli*, *Salmonella cholerestasus*, *Salmonella enteritidis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*). Although the petal extracts of the studied flowers contain a high content of phenolic compounds and flavonoids, the antimicrobial activity does not seem to be closely related to these pigments. State that the cause of these contrary results may be due to the extraction process, the source of the tested plants and the microorganisms they were tested for.

In the disc diffusion and antifungal tests there was an absence of antimicrobial activity of the substances present in the flower petal extracts, or a small concentration, thus, it did not reach the minimum inhibitory concentration for the microorganisms in the tests.

4 Conclusion

The rose petal extracts obtained by the ultrasound-assisted method with temperatures of 20 ºC and 60 ºC presented higher contents of phenolic compounds and of flavonoids. Thus, in order to obtain rose petals ethanolic extracts with a higher content of phenols and total flavonoids, the ultrasound-assisted extraction is the most indicated. The results indicate that the rose petal extract presented antioxidant capacity. The extract obtained by ultrasound-assisted at a temperature of 20 ºC using cereal ethyl alcohol 96 ºGL as solvent was the one that presented the highest antioxidant activity, verified by the DPPH method. The extracts did not present antimicrobial activity in the disc diffusion and antifungal test. The use of flower petal extracts showed to be a viable alternative as a natural antioxidant replacing synthetic antioxidants, with the possibility of industrial application in food products.

References

ASOLINI, F.C. *et al.* Antioxidant and antibacterial activities of phenolic compounds from extracts of plants used as tea. *Braz. J. Food Techn.,* v.9, n.1, p.209-215, 2006.

BANDEIRA, A.M.P. *et al.* Antioxidant activity and physicochemical characteristics of honeys from the eastern Amazon region, Brazil. *Act. Amaz.,* v.48, n.1, p.158-167. 2018. doi: 10.1590/1809-4392201702721

BARROS, L. *et al.* Use of HPLC–DAD–ESI/MS to profile phenolic compounds in edible wild greens from Portugal. *Food Chem*., v.127, n.2, p.169-173, 2011. doi: 10.1016/j.foodchem.2011.01.009

CAETANO, A.C.S. *et al.* Extração de antioxidantes de resíduos agroindustriais de acerola. *Braz. J. Food Techn.,* v.12, n.1, p.155-160, 2009. doi: 10.4260/BJFT2009800900006

CHEN, D. *et al.* Components identification and nutritional value exploration of tea (*Camellia sinensis* L.) flower extract: Evidence for functional food. *Food Res Int*., v.132, n.2, p.109-110, 2020. doi: 10.1016/j.foodres.2020.109100

CLSI. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard – Tenth Edition*. West Valley Road. 2009.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. *Ciênc. Agrotec.,* Lavras, v.35, n.6, p.1039-1042, dec. 2011. doi: 10.1590/S1413-70542011000600001

FRANZEN, F.L.; LIDÓRIO, H.F.; OLIVEIRA, M.S.R. Edible flower considerations as ingredients in food, medicine and cosmetics. *JAPLR*, v.7, n.1, p.271-273, 2018. doi: 10.15406/japlr.2018.07.00238

FRANZEN, F.L. *et al.* Composición química de pétalos de flores de rosa, girasol y caléndula para su uso en la alimentación humana. *Cien. Tec. Agropec*., v.20, n.1, p.149-158, 2019. doi: 10.21930/rcta.vol20_num1_art:1252

GISHEN, N. Z. *et al.* In vitro antimicrobial activity of six Ethiopian medicinal plants against *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. *Eur. J. of Integ. Med.,* v.36, n.1, p.101-121. 2020. doi: 10.1016/j.eujim.2020.101121

HABERMANNA, E. *et al.* Antioxidant activity and phenol content of extracts of bark, stems, and young and mature leaves from *Blepharocalyx salicifolius* (Kunth) O. Berg. *Braz. J. Biol.,* v.76, n.4, p.898-904, dec. 2016. doi: 10.1590/1519-6984.03815

HAO, D.; XIAO, P. Pharmaceutical resource discovery from traditional medicinal plants: Pharmacophylogeny and pharmacophylogenomics. *Chin. Herb. Med.,* v.12, n.2, p. 104-117, 2020. doi: 10.1016/j.chmed.2020.03.002

JI-YU, N. *et al.* Screening and evaluation of radical scavenging active compounds in the essential oil from *Magnolia biondii* Pamp by electronic nose coupled with chemical methodology. *Ind. Crop. Prod*., v.144, n.2, p.112-160, 2020. doi: 10.1016/j.indcrop.2019.112060

KALEMBA-DROŻDŻ, M.; CIERNIAK, A. Antioxidant and genoprotective properties of extracts from edible flowers. *J. Food Nutr.* Res., v.58, n.1, p.42–50. 2019. doi: 10.13140/RG.2.2.17195.49442/1

KHAN, I. A. *et al.* Inhibitory effect of *Chrysanthemum morifolium* flower extract on the formation of heterocyclic amines in goat meat patties cooked by various cooking methods and temperatures. *Meat Scien*., v.147, n.1, p.70-81, 2019. doi: 10.1016/j.meatsci.2018.08.028

KIM, D.O. *et al.* Vitamin C equivalent antioxidant capacity (VCEAC) of phenolics phytochemicals. *J. Agr. Food Scien. Tech.,* v.37, n.1, p.153-161, 2002. doi: 10.1021/jf020071c

KIM, S.J. *et al.* Evaluation of the antioxidant activities and nutritional properties of ten edible plant extracts and their application to fresh ground beef. *Meat Scie.,* v.93, n.2, p.715-722, 2013. doi: 10.1016/j.meatsci.2012.11.029

LIJUN, Z. *et al.* Studies on the volatile compounds in flower

extracts of *Rosa odorata* and *R. chinensis*. *Ind. Crop. Prod*., v.146, n.2, p.112-143, 2020. doi: 10.1016/j.indcrop.2020.112143

LÓPEZ-HORTAS L. *et al.* Antioxidant capacity of the extracts from flowers of *Erica australis* L.: Comparison between microwave hydrodiffusion and gravity (MHG) and distillation extraction techniques - Formulation of sunscreen creams. *Ind. Crop. Prod*., v.145, n.2, p.112-179, 2020. doi: 10.1016/j.indcrop.2019.112079

MENEZES FILHO, A.C.P.; CASTRO, C.F.S. Análise Fitoquímica Preliminar de Extratos Foliares de Orchidaceas (*Cattleya walkeriana* Gardner), (*Oncidium cebolleta* Sw.), (*Encyclia linearifolioides* Kraenzl.) e (*Polystachya concreta* (Jacq.) Garay & H. R. Sweet). *Ensaios Cienc.*, v.23, n.1, p.16-23, 2019a. doi: 10.17921/1415-6938.2019v23n1p16-23

MENEZES FILHO, A.C.P.; CASTRO, C.F.S. Análise Fitoquímica dos Extratos Etanólicos de *Euphorbia splendens* (Borjer ex. Hooke) e *Hyptis suaveolens* (L.) Poit. *Ensaios Ciênc.*, v.23, n.2, p.98-103, 2019b. doi: 10.17921/1415-6938.2019v23n2p98-103

MENSOR, L.L. *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phyt. Res*., v.15, n.1, p.127-130, 2001. doi: 10.1002/ptr.687

MILIAUSKAS, G.; VENSKUTONIS, P. R.; VAN BEEK, T. A. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, v. 85, p. 231-237, 2004. doi: 10.1016/j.foodchem.2003.05.007

MOGLAD, E.H. *et al.* Antimicrobial and wound healing activities of certain Sudanese medicinal plants. *Saudi J. Biol. Scie.*, v.27, n.7, 2020. doi: 10.1016/j.sjbs.2020.05.017

SILVA, G.P.R. *et al.* Embutido emulsionado com adição de isolado proteico à base de pescado (*Micropogonias furnieri*). *Unoesc Ciênc. ACET*, v. 3, n. 2, p. 179-186, 2012.

PARK, C.H. *et al. In Vitro* Antioxidant and Antimicrobial Properties of Flower, Leaf, and Stem Extracts of Korean Mint. *Antioxidants (Basel, Switzerland)* vol. 8,3 75. 26 mar. 2019. doi: 10.3390/antiox8030075

PEREIRA, M.G. Natural antioxidants application in mechanically deboned poultry meat (MDPM). Santa Maria: Universidade Federal de Santa Maria, Santa Maria, 2009.

PIOVESAN, N. *et al.* Microwave-assisted extraction of bioactive compounds from blueberry (*Vaccinium ashei* Reade) and their antioxidant and antimicrobial capacity. *Food* Res. Int., v.24, n.1, p.2526-2533, 2017.

PONZILACQUA, B. *et al.* Effects of medicinal herb and Brazilian traditional plant extracts on *in vitro* mycotoxin decontamination. *Food Control,* v.100, n.1, p. 24-27, 2019. doi: 10.1016/j.foodcont.2019.01.009

RODRIGUES, A.S. Antioxidant and antimicrobial activity of ora-pro-nobis extracts (*Pereskia aculeata* Mill.) and application in mortadella. 2016. 91 f. Dissertação (Mestrado em Ciência e Tecnologia dos Alimentos) - Universidade Federal de Santa Maria, Santa Maria, 2016.

ROSA, C.S. *et al.* Evaluation of the effect of extract of carob flour (*Ceratonia siliqua* L.) on oxidative stability and color of frozen hamburgers. *Semina: Ciências Agrárias,* v.34, n.5, p.2277-2286, 2013. doi: 10.5433/1679-0359.2013v34n5p2277

SHARMA, K. *et al.* Synergistic antioxidant and antimicrobial activities of essential oils of some selected medicinal plants in combination and with synthetic compounds. *Ind. Crop. Prod*., v.154, n.2, p.1120-1130, 2020. doi: 10.1016/j.indcrop.2020.112569

FIORI, A.C.G. *et al.* Antifungal activity of leaf extracts and essential oils of some medicinal plants against *Didymella bryoniae*. *J. Phytopathol*., v.148, n.7/8, p.483-487, 2000. doi: 10.1046/j.1439-0434.2000.00524.x

SUN, T. *et al.* Evaluation of the antioxidant activity of asparagus, broccoli and their juices. *Food Chem.,* v.105, n.1, p.101-106, 2007. doi: 10.1016/j.foodchem.2007.03.048

VIERA, V. B. *et al.* Extraction of phenolic compounds and evaluation of the antioxidant and antimicrobial capacity of red onion skin (*Allium cepa* L.). *Inter. Food Res. J.,* v.24, n.2, p.990-999, 2017.

WANG, M. *et al.* Analysis of antioxidative phenolic compounds in artichoke (*Cynara scolymus* L.). *J. Agric. Food Chem.,* v.51, n.3, p.601-608, 2003. doi: 10.1021/jf020792b

ZHANG, X. *et al.* Identification and quantitative analysis of phenolic glycosides with antioxidant activity in methanolic extract of *Dendrobium catenatum* flowers and selection of quality control herb-markers. *Food Res. Intern.,* v.123, n.1, p.732-745, 2019. doi: 10.1016/j.foodres.2019.05.040

ZHISHEN, J.; MENGCHENG, T.; JIANMING, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* v.64, n.4, p.555-559, 1999. doi: 10.1016/S0308-8146(98)00102-2.