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# PHYTOCHEMICAL COMPOSITION AND *IN VITRO* BIOLOGICAL PROPERTIES OF SEVERAL *RUDBECKIA HIRTA* AND *TAGETES ERECTA* FLOWER EXTRACTS

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**Abstract**

The present research focuses on the investigation of the phytochemical profile and biological properties of two ornamental species from the *Asteraceae* family. Taking into consideration that certain compounds found in such plants can be used as valuable resource for the pharmaceutical and cosmetic industries, we chose to examine the chemical profile in correlation with the biological activities of *Rudbeckia hirta* and *Tagetes erecta* flower extracts. Three types of extracts were obtained for each plant product, using solvents with different polarities. Secondary metabolites found in the extracts, such as polyphenols and flavonoids (e.g., caffeic, chlorogenic and gentisic acids, patuletin, quercetin, quercetagetin, quercetagitrin) were quantitatively analysed using HPLC-MS methods. The studied compounds were found to be present in most of the analysed samples in variable quantities, with the highest quantities being found in the ethanolic plant extracts. Positive dose-related values revealed that the investigated extracts exhibit good antioxidant activity through the three investigated mechanisms (ferric reducing capacity, superoxide radical scavenging capacity, hydroxyl radical scavenging capacity). Furthermore, all extracts showed promising antifungal and antibacterial activities, with *Tagetes erecta* extracts exhibiting better pronounced antibacterial activity, while the most promising antifungal action was observed for *Rudbeckia hirta* extracts.

**Rezumat**

Studiul a urmărit investigarea profilului fitochimic și a proprietăților biologice a două specii ornamentale din familia *Asteraceae*. Având în vedere că anumiți compuși găsiți în astfel de plante pot fi utilizați ca resursă valoroasă pentru industria farmaceutică și cosmetică, am ales să studiem profilul chimic în corelație cu activitățile biologice ale extractelor obținute din flori de *Rudbeckia hirta* și *Tagetes erecta*. Pentru fiecare produs vegetal s-au obținut trei tipuri de extracte, utilizând solvenți cu polarități diferite. Metaboliții secundari găsiți în extracte, precum polifenolii și flavonoidele (acizii cafeic, clorogenic și gentizic, cvercetol, cvercetagetină, cvercetagitrină, patuletină), au fost analizați cantitativ folosind metode LC-MS. Compușii studiați s-au dovedit a fi prezenți în majoritatea probelor analizate în cantități variabile, cele mai mari concentrații regăsindu-se în extractele etanolice. Valorile obținute în cadrul testării capacității antioxidante au arătat că extractele investigate prezintă o activitate antioxidantă bună prin toate cele trei mecanisme cercetate (capacitatea de reducere a ionului feric, capacitatea de chelatare a radicalului superoxid, capacitatea de chelatare a radicalului hidroxil). În plus, toate extractele au prezentat activități antifungice și antibacteriene promițătoare, extractele de *Tagetes erecta* prezentând o activitate antibacteriană mai bună, în timp ce acțiunea antifungică cea mai eficientă s-a observat pentru extractele de la *Rudbeckia hirta*.

**Keywords**: *Asteraceae*, LC-MS, antioxidant, antifungal activity

## Introduction

The *Asteraceae* (*Compositae*) family is the vastest family of flowering plants and includes around 24.000 recognized species [7, 17]. Plants found in this family have been used for their medicinal, nutritional and ornamental properties for a long time [2, 22]. *Asteraceae* species were found to exhibit different therapeutic properties such as: anti-inflammatory, antimicrobial, antioxidant, hepatoprotective and diuretic [22]. Despite their worldwide distribution and their potential as sources of natural therapeutic agents, a large number of the *Asteraceae* species, especially those commonly

used for ornamental purposes, remain under-evaluated [12].

The *Rudbeckia* genus generally includes mostly perennial and sometimes biennial or annual herbs. Species from this genus have long been used by natives as food and medicine especially in North America, with applications ranging from snakebite treatment to gynaecological disorders aid [25]. *Rudbeckia hirta*, in particular, has been widely included in the traditional medicine, given its properties and great geographic range. It has been used for its analgesic, anthelmintic, antimicrobial and ornamental properties, as well as a snakebite remedy, heart, digestive and kidney medicine [9, 25].

*Tagetes erecta* L. is another well-known plant of the *Asteraceae* family and can be easily found in most parts of South America. The *Tagetes* genus comprises of more than 50 species, with *T. erecta*, *T. tenuifolia* and *T. patula* being the most cultivated worldwide [28]. The most abundant secondary metabolites found in species belonging to the *Tagetes* genus are carotenoids, flavonoids, thiophenes and terpenoids [3, 23]. *Tagetes erecta* is probably the most popular species of the genus and it is used especially as an ornamental plant, but also for its high content in lutein, which, after extraction, is included in dietary supplements for eye health [6].

Consequently, taking into account the large variety of active constituents found in these two ornamental plants and their ethnopharmacological use, we focused on investigating the biological effects of various extracts obtained from flowers, correlating the obtained data with the phytochemical profile.

## Materials and Methods

*Plant material and obtaining of extracts*

The plant material was represented by the dried flowers of two ornamental species from the *Asteraceae* family, *Rudbeckia hirta* and *Tagetes erecta*. The plants were cultivated in ecological conditions in the North-Eastern Romania in 2020. Voucher specimens are deposited at the Department of Drug Analysis, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy, Iași, Romania.

10 g of pulverized plant material were extracted using 200 mL of either water, ethanol, or ethyl acetate, respectively, thus obtaining three types of extracts for each plant. The extracts were prepared using a magnetic stirrer at room temperature for 3 hours, followed by filtration and evaporation of solvent using a rotary evaporator. The dry extracts were kept at 4°C until further use.

*Total phenolic content (TPC)*

The quantity of polyphenols found in the extracts was established using a well-known spectrophotometric method using Folin-Ciocâlteu reagent, with several modifications [2, 24]. The dry extracts were re-dissolved in dimethyl sulfoxide prior to the determination. The absorbance was measured at 765 nm, with gallic acid being used as standard. The experiments were carried out in triplicate and the results were expressed as mg gallic acid equivalents (GAE)/g dry extract.

*LC-MS analysis*

The chemical analysis of polyphenols was carried out using an Agilent Technologies 1100 HPLC Series system coupled with an Agilent Ion Trap 1100 SL mass spectrometer equipped with degasser, binary gradient pump, column thermostat and autosampler. A reverse-phase analytical column was employed for this determination. The chromatographic analysis was performed using a previously described method [18]. The detection of substances was performed using both UV and MS modes. Calibration curves of the following standards were used: caffeic, caftaric, chicoric, chlorogenic, *p*-coumaric, ferulic, gallic and gentisic acids, apigenin, fisetin, hyperoside, isoquercitrin, kaempferol, luteolin, myricetin, patuletin, quercetin, quercitrin and rutin in the 0.5 - 50 µg/mL range with good linearity ($R^2 > 0.999$). Similar conditions were employed for the determination of quercetagetin and quercetagitrin in the samples: a mobile phase consisting of a methanol: 0.1% acetic acid (v/v) mixture and gradient elution starting with 75% of 0.1% acetic acid (v/v) in water and 25% methanol and increasing to 40% methanol at min 4. The chromatographic data was processed using ChemStation and DataAnalysis software from Agilent, Santa Clara, USA.

*The ferric reducing capacity*

This antioxidant method is based on the capacity of reducing agents to reduce potassium ferricyanide to potassium ferrocyanide, that reacts with ferric ions, forming a blue compound with maximum absorbance at 700 nm. The experiments were performed in triplicate and the results were expressed compared to quercetin, which was used as positive control [15].

*Superoxide anion radical scavenging capacity*

The superoxide anion radical generated by the nicotinamide-adenine-dinucleotide (reduced)-phenazine methosulphate reduces tetrazolium nitrate (NBT) to a blue formazan with maximum absorbance at 560 nm. The percentage of superoxide anion radical scavenging activity was calculated using the following formula: 100 × (Ac - Ap)/(Ac), where Ac is the absorbance of the control and Ap is the absorbance in the presence of the sample (extracts, positive control). Experiments were performed in triplicate and quercetin was used as positive control [1, 26].

*Hydroxyl anion radical scavenging capacity*

Hydroxyl radicals formed in the reaction between ferrous ions and hydrogen peroxide react with salicylic acid, forming a pinkish-purple compound with maximum absorbance at 562 nm. The percentage of hydroxyl radical scavenging activity will be calculated using the following formula: 100 × (Ac - Ap)/(Ac),

where Ac is the absorbance of the control and Ap is the absorbance in the presence of extracts or positive control. The experiments were performed in triplicate and the results were expressed comparatively to quercetin [11].

*Antimicrobial activity testing*

The antimicrobial activity was studied using Gram positive bacteria (*Staphylococcus aureus* ATCC 25923), Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and a pathogenic yeast (*Candida albicans* ATCC 10231), through disk diffusion methods [4, 5, 10, 27]. Mueller Hinton agar (Oxoid) and Mueller-Hinton agar, Fungi (Biolab) were inoculated with the suspensions of the tested microorganisms. Sterile stainless-steel cylinders were applied on the agar in Petri plates and a volume of 100 µL solution of the tested extracts (5 mg/mL) was added into the cylinders. The plates were left 10 minutes at room temperature to ensure equal diffusion of the sample in the medium and then incubated at 35°C for 24 hours. Commercially available antimicrobial susceptibility discs containing ciprofloxacin (5 µg/disk), piperacillin/tazobactam (110 µg/disk) and fluconazole (25 µg/disk) were used as reference antimicrobial drugs. After incubation, the diameters of the inhibition zones were measured. All assays were carried out in triplicate.

## Results and Discussion

*Total phenolic content*

Following the analysis of the obtained results, it can be easily observed that the highest amount of polyphenols is found in the ethanolic extract obtained from inflorescences of *Rudbeckia hirta* (118.07 ± 0.067 mg GAE/g dry extract), while the lowest amount was found in the extract obtained from *Tagetes erecta* inflorescences using ethyl acetate (1.90 ± 0.131 mg GAE/g dry extract) (Table I). Overall, the ethanolic extracts of both species presented the highest yield of polyphenolic compounds, while ethyl acetate proved to be the solvent with the least capacity of extracting polyphenols from the plant material.

**Table I**

Polyphenol content of the studied *Rudbeckia hirta* and *Tagetes erecta* extracts

| **Extract** | **Code** | **mg GAE/g dry extract** (mean ± SD) |
|---|---|---|
| *Rudbeckia hirta* aqueous extract | $R_{H2O}$ | 57.963 ± 0.05 |
| *Rudbeckia hirta* ethanolic extract | $R_{EtOH}$ | 118.07 ± 0.067 |
| *Rudbeckia hirta* ethyl acetate extract | $R_{EtOAc}$ | 17.363 ± 0.2 |
| *Tagetes erecta* aqueous extract | $T_{H2O}$ | 63.78 ± 0.098 |
| *Tagetes erecta* ethanolic extract | $T_{EtOH}$ | 88.543 ± 0.067 |
| *Tagetes erecta* ethyl acetate extract | $T_{EtOAc}$ | 1.90 ± 0.131 |

*LC-MS analysis*

Taking into account the standards used for this analysis, the presence of gentisic, caffeic and chlorogenic acids was highlighted for *Tagetes erecta* aqueous extract and the only two compounds that could be quantified from this extract belong to the flavonoids class, namely quercetagetin (3.259 mg/g dry extract) and quercetagitrin (1.827 mg/g dry extract). For *Rudbeckia hirta* aqueous extract, only gentisic acid could be identified. The ethanolic extract obtained using *Tagetes erecta* flowers contained mostly quercetagitrin (47.574 mg/g dry extract), quercetagetin (5.260 mg/g dry extract), patuletin (0.352 mg/g dry extract), chlorogenic acid (0.191 mg/g dry extract) and quercetin (0.050 mg/g dry extract), while luteolin and kaempferol could only be identified in this extract (Table II). On the other hand, the ethanolic extract of *Rudbeckia hirta* contained mainly quercetagitrin, quercitrin, *p*-coumaric acid, patuletin and ferulic acid, while gentisic, caffeic and chlorogenic acids were only identified.

**Table II**

LC-MS quantification of polyphenolic compounds (mg/g dry extract)

| **Compound** | $R_{H2O}$ | $R_{EtOH}$ | $R_{EtOAc}$ | $T_{H2O}$ | $T_{EtOH}$ | $T_{EtOAc}$ |
|---|---|---|---|---|---|---|
| Caffeic acid | - | < LOQ* | < LOQ | < LOQ | - | - |
| Chlorogenic acid | - | < LOQ | - | < LOQ | 0.191 | - |
| *p*-coumaric acid | - | 0.225 | 0.845 | - | - | - |
| Ferulic acid | - | 0.025 | 0.071 | - | - | - |
| Gentisic acid | < LOQ | < LOQ | - | < LOQ | - | - |
| Kaempferol | - | - | - | - | < LOQ | < LOQ |
| Luteolin | - | - | - | - | < LOQ | - |
| Patuletin | - | 0.054 | 0.092 | - | 0.352 | 0.295 |
| Quercetagetin | - | - | - | 3.259 | 5.260 | - |
| Quercetagitrin | - | 4.20 | - | 1.827 | 47.574 | 2.032 |
| Quercetin | - | - | < LOQ | - | 0.050 | 0.034 |
| Quercitrin | - | 0.242 | - | - | - | - |

* < LOQ – below limit of quantification

Flavonoids such as quercetagitrin, patuletin and quercetin could be quantified in the $T_{EtOAc}$ extract, with kaempferol only being identified. On the other hand, the $R_{EtOAc}$ extract contained *p*-coumaric (0.845 mg/g dry extract), patuletin (0.092 mg/g dry extract) and ferulic acid (0.071 mg/g dry extract), as well as caffeic acid and quercetin.

*The ferric reducing capacity*

Iron is involved in the synthesis of free radicals, which contribute to the intensification of oxidation reactions of lipids, proteins and nucleic acids and also determine the synthesis of peroxyl radicals. The ferric reducing capacity of extracts depends on the presence of compounds with functional groups, such as hydroxyl groups, that release protons and electrons involved in the reduction process. Implicitly, it depends on the presence of phenolic compounds in the extracts, as well as on the number of hydroxyl groups found in their structure [8]. This assay is important for highlighting the antioxidant capacity of extracts, given that $Fe^{3+}$ ions can induce oxidation reactions in biological environments [21], reactions that are amplified in case of excess iron [20]. The ethanolic extracts of both species present pronounced activity compared to other types of extracts and were the only ones for which the half maximal effective concentration ($EC_{50}$) could be calculated (Figure 1).

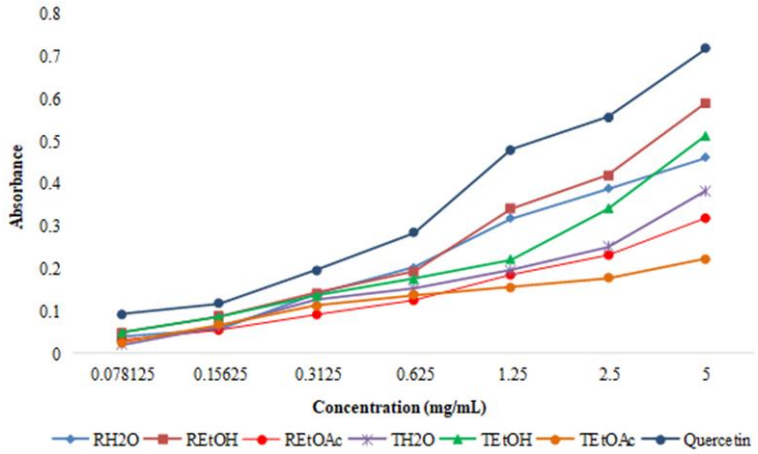


**Figure 1.**
Ferric reducing capacity of the tested extracts and of quercetin

Moreover, the ethanolic extract of *Rudbeckia hirta* presented pronounced activity than the same type of extract obtained from *Tagetes erecta*, with an $EC_{50}$ concentration of 419.02 ± 1.481 µg/mL final solution, compared to 545.39 ± 3.235 µg/mL final solution, respectively.

*Superoxide anion radical scavenging capacity*

The superoxide radical scavenging activity of all tested extracts increased with concentration, as it can be seen in Figure 2.

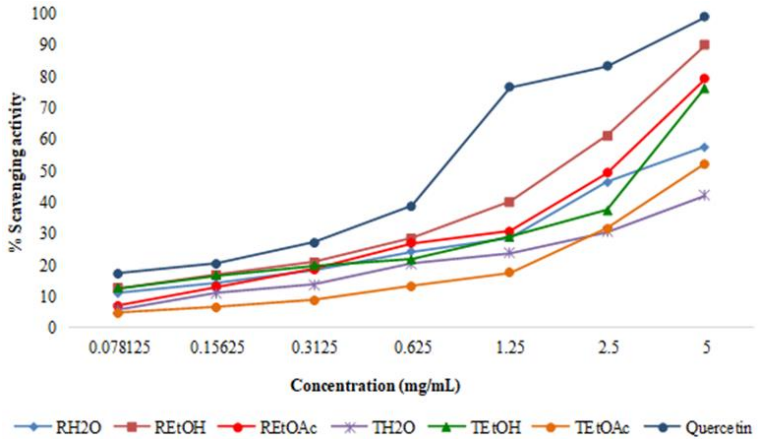


**Figure 2.**
Superoxide radical scavenging activity of the tested extracts and of quercetin

Comparing the obtained $EC_{50}$ values, it can be noticed that the intensity of the antioxidant activity through this mechanism decreases in the following order: $R_{EtOH} > R_{EtOAc} > T_{EtOH} > R_{H2O} > T_{EtOAc}$. The $EC_{50}$

could not be determined for the extract coded $T_{H2O}$. *Rudbeckia hirta* ethanolic extract showed the lowest value (437.89 ± 4.67 µg/mL final solution) out of all the tested extracts. Comparatively, quercetin, which was used as a standard, showed an $EC_{50}$ value of 193.41 ± 2.27 µg/mL final solution. *Tagetes erecta* ethyl acetate extract showed the highest $EC_{50}$ value (1170.7 ± 15.52 µg/mL final solution) and the lowest antioxidant activity can be correlated with the low amount of polyphenols and flavonoids present in this extract.

The superoxide radical anion is less reactive than the hydroxyl anion, but by dismutation through a Fenton-type reaction, it generates the hydroxyl radical, which will cause oxidation especially of lipids found in the cell membrane or in the intra- and extracellular matrix. For example, a *Clerodendrum cyrtophyllum* Turcz ethyl acetate extract presented a higher superoxide radical anion scavenger capacity compared to the corresponding petroleum ether and butanol extracts. However, for the same extract, the scavenger effect of the hydroxyl radical is greater in the case of the butanol extract. Implicitly, these differences are determined by the categories of compounds that are extracted, depending on the used solvent [29].

*Hydroxyl anion radical scavenging capacity*

The results obtained during this antioxidant assay are represented comparatively in Figure 3.

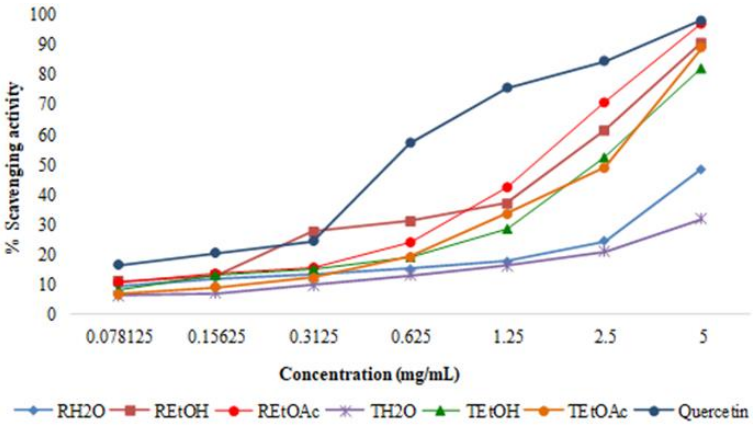


**Figure 3.**
Hydroxyl radical scavenging activity of the tested extracts and of quercetin

Taking into consideration the $EC_{50}$ values, the intensity of the observed activity decreases in the following order: $R_{EtOAc} > R_{EtOH} > T_{EtOH} > T_{EtOAc}$. For $R_{H2O}$ and $T_{H2O}$, whose value could not be determined. The ethyl acetate extract obtained from *Rudbeckia hirta* showed the lowest $EC_{50}$ value (142.15 ± 2.72 µg/mL final solution) and, implicitly, the most promising antioxidant activity, while the used positive control, quercetin, showed a value of 50.60 ± 0.47 µg/mL final solution. It should be noted that the good antioxidant activity of the $R_{EtOAc}$ extract cannot necessarily be correlated with a large amount of polyphenolic acids, given that the total content of polyphenols is relatively low in this extract, but it can most probably be associated with the presence of more non-polar compounds that were not the focus of the present research.

The hydroxyl radical causes the oxidation of lipids, nucleic acids and proteins along with the impairment of their functions, as well as the precipitation of fibrinogen with the risk of pathological phenomena such as atherosclerosis, diabetes or cancer [14]. Polyphenols present in plant extracts have the ability to release hydrogen that will stabilize the hydroxyl radical by blocking its oxidizing effect. Polyphenols must release hydrogen relatively quickly in order to neutralize the hydroxyl radical, as it presents increased reactivity and short half-life [13]. Plant extracts obtained using ethanol or ethanol-water mixtures generally contain higher amounts of polyphenols compared to those obtained with the use of water, which explains their greater ability to neutralize hydroxyl radicals [19].

*Antimicrobial activity testing*

The diameters of the inhibition zones corresponding to the tested samples were measured and expressed in mm, as mean ± SD (Table III).

The extracts showed a good antibacterial activity against the tested Gram-positive bacteria, except for *Rudbeckia hirta* ethanolic extract and *Tagetes erecta* ethyl acetate extract, which did not present any kind of activity. However, these two samples, as well as the other extracts, exhibited promising activity against the Gram-negative tested strains, particularly against *Pseudomonas aeruginosa*, with inhibition zone values close to that of the used positive control, especially for the $T_{EtOAc}$ extract. Overall, the best antibacterial activity was observed in the case of the ethanolic extract of *Tagetes erecta* ($T_{EtOH}$). Considering the antifungal potential, all samples have shown good activity against the tested strains. Nonetheless, *Rudbeckia hirta* ethanolic extract ($R_{EtOH}$) had the best antifungal activity against *C. albicans*, with a similar activity to that of fluconazole.

The antimicrobial spectrum of these samples may be explained by the differences in their qualitative and quantitative chemical composition. It can be noticed, that, generally the ethanolic extracts presented the best antimicrobial activity, implying that their high content in polyphenols contributes to the displayed action.

**Table III**

Antimicrobial activity of the tested extracts and of positive controls

| Samples | Diameter of the inhibition zones (mm – mean ± SD) | | | |
|---|---|---|---|---|
| | ***S. aureus* ATCC 25923** | ***E. coli* ATCC 25922** | ***Pseudomonas aeruginosa* ATCC 27853** | ***Candida albicans* ATCC 10231** |
| $R_{H2O}$ | 13.0 ± 0.00 | 15.0 ± 0.00 | 18.0 ± 0.00 | 23 ± 0.00 |
| $R_{EtOH}$ | 0 | 15.1 ± 0.05 | 20.0 ± 0.00 | 26 ± 0.00 |
| $R_{EtOAc}$ | 12.0 ± 0.00 | 14.0 ± 0.00 | 20.0 ± 0.00 | 20 ± 0.00 |
| $T_{H2O}$ | 14.0 ± 0.00 | 16.0 ± 0.00 | 21.1 ± 0.05 | 20 ± 0.00 |
| $T_{EtOH}$ | 15.1 ± 0.05 | 18.0 ± 0.00 | 21.0 ± 0.00 | 19 ± 0.00 |
| $T_{EtOAc}$ | 0 | 17.0 ± 0.00 | 22.1 ± 0.05 | 19 ± 0.00 |
| Ciprofloxacin (5 µg/disc) | 30.7 ± 0.06 | 30.0 ± 0.00 | *NT | *NT |
| Piperacillin/Tazobactam (110 µg/disc) | *NT | *NT | 28.0 ± 0.00 | *NT |
| Fluconazole (25 µg/disc) | *NT | *NT | *NT | 30.0 ± 0.00 |

*NT – not tested

The results indicate that secondary metabolites found in these species possess good antioxidant, antibacterial and antifungal properties. The ethanolic extracts of both *Rudbeckia hirta* and *Tagetes erecta* species presented the highest content and diversity of polyphenolic compounds. Moreover, these two extracts presented, overall, the best antioxidant activities through the tested mechanisms and the most promising antimicrobial properties.

Our results complete some of the most up-to-date studies on ornamental *Asteraceae* plants, proving that species such as *Rudbeckia hirta and Tagetes erecta* constitute important resources of secondary metabolites with potential therapeutic value. However, supplementary chemical and *in vivo* studies must be carried out, in order to further evaluate such applications, as well as their safety in administration, given that herbal products could also accumulate heavy metals that play an additional risk to their potential toxicity [16].

## Conclusions

The phytochemical profile and biological *in vitro* activities of three types of extracts obtained from the flowers of two under-evaluated species of the *Asteraceae* family, *Rudbeckia hirta* and *Tagetes erecta* were investigated in the present study. The research data suggests that the solvent used for extraction is of extreme importance when searching for active compounds, in this case ethanol being the most indicated solvent for polyphenols' extraction. Even though most of the studied extracts presented biological activity, the intensity and efficacy depend on the extractability of different constituents, as well as on the chemical variability of the plant material. Overall, both ornamental species could be considered significant resources of natural compounds with promising antioxidant and antifungal potential, but further studies regarding their chemical profile and toxicity still need to be done.

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## Conflict of interest

The authors declare no conflict of interest.

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