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THE BIOLOGICAL ACTIVITY *IN VIVO* AND *IN VITRO* OF *CENTAUREA CYANUS, LACTARIUS PIPERATUS* AND *RIBES RUBRUM*

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Abstract

Plants are a valuable source of secondary metabolites, which due to their biological activity, are widely used in various industries, such as cosmetology, pharmaceutical, and food. For this purpose, the plants from the flora of Romania are collected. We can say that both in vivo and in vitro plant culture remains a world ar research topic. The research aims to discover biological activity, discuss the scientifically proven benefits of plants, understand the challenges of in vivo and in vitro research, and evaluate new mechanisms of biological activity of Centaurea cyanus, Lactarius metabolites, and Ribes rubrum extract, discuss the scientifically proven benefits of plants, understand the challenges of in vivo and in vitro research, and evaluate new mechanisms of biological activity. More reports have been written in vitro activity of the C. cyanus lyophilized extract obtained from the flower leaves, demonstrated anti-hemolytic activity. Different species of the genus Lactarius present various biochemicals, which can be useful together with the morphological characteristics, and they can facilitate the taxonomic correlation of the Lactarius species. R. Nigrum leaves have shown the best effect in wound healing.

Key words: *biological activity, in vitro, in vivo, plant extracts.*

INTRODUCTION

Throughout history, herbs have proven to be good medicine in treating various wounds, so detailed clinical studies have begun to be performed on their benefits, leading to the discovery that they have a multitude of effects, such as recovery tissue, hydration, uniformity, cell renewal, etc. (Das et al., 2017).

Numerous studies have been made in recent years related to traditional medicine and its chemical composition and its effect on human health.

Among the medicinal plants used in folk medicine to treat lesions are the species *Ribes*. These are easy to notice, being green shrubs throughout the year with variously colored fruits, either black or red, with a good taste. They were found in temperate climates, especially the northern parts of the world (Heywood et al., 2007). These species are not only used for medical purposes but also in various preparations, such as cake, ice cream, jam, but also in alcoholic and fermented beverages (Weigend, 2007).

Known as cornflower, *Centaurea cynanus's* extract was used in various treatments such as gastric, ocular, nerve, dermatological diseases, stimulant, tonic, and diuretic (Pieroni et al., 2004; Redžić, 2007; Al-Snafi, 2015). The blue coloration of the petals contains protocyanin, and results from a complexion of the quinoidal base of anthocyanidin and the metal ions. The extracts of the *Centaurea cyanus* present *in vitro* functional properties: anti-inflammatory, antioxidant and antibacterial, as well as cytotoxic effect and gastroprotective activity.

The research objectives are: discussing the scientifically proven benefits, discovering the biological activities, and *in vitro* and *in vivo* research of the three plants: *Centaurea cyanus*, *Ribes rubrum* and *Lactarius piperatus*.

MATERIALS AND METHODS

The materials used are represented by scientific publications from around the world.

The research aims to discover biological activity, discuss the scientifically proven benefits of plants, understand the challenges of

in vivo and *in vitro* research, and evaluate new mechanisms of biological activity of *Centaurea cyanus*, *Lactarius* metabolites, and *Ribes rubrum* extract, discuss the scientifically proven benefits of plants, understand the challenges of *in vivo* and *in vitro* research, and evaluate new mechanisms of biological activity.

RESULTS AND DISCUSSIONS

IN VIVO AND *IN VITRO* BIOLOGICAL ACTIVITY

Centaurea cyanus - Cornflower

Escher et al. (2018) have studied the anti-hemolytic activity of the lyophilized extract of *Centaurea cyanus* following the procedures made by Antonini and Brunori (1971). The study object was the evaluation of the chemical composition and the functional properties *in vivo* and the increase of the extraction situations of the aqueous extract obtained from the flower petals of *Centaurea cyanus* flowers are being used as aqueous extras in the traditional medicine. In the specialty literature, no study has been found regarding the statistical optimization of phytochemical extraction conditions in this plant, being necessary to investigate the influence of time and temperature on phytochemical extraction and its properties *in vitro*.

Anti-hemolytic activity *in vitro*

The anti-hemolytic activity of lyophilized *C. cyanus* extract was made according to the procedures described by Antonini and Brunori (1971), and the study used a blood sample. The *in vitro* activity of the *C. cyanus* extract demonstrated a dose-dependent effect, a protective effect against hemolysis with increasing concentration and a beneficial interaction with the erythrocytes. The erythrocytes are susceptible to oxidative damage due to the formation of the cell membrane by polyunsaturated fatty acids; the presence of oxygen and iron in hemoglobin can ensure hemolysis (Çimen, 2008). Hemolysis consists of releasing hemoglobin into the plasma, which can induce cell apoptosis and the interruption of oxygen consumption, leading to body damage (Tracz et al., 2007).

Many studies have demonstrated that the erythrocytespresent benefits. For example, Singh and Rajini (2008) have observed that the potato peel extract protects the human erythrocytes against *in vitro* oxidative damage induced by the iron sulfate and ascorbic acid. Bioactive compounds present in plant extracts protect the erythrocyte membrane against damage caused by reactive oxygen species (Singh and Rajini, 2008). Belwal et al. (2017) observed that the *Berberis asiatica* leaf extract presented anti-hemolytic activity and protective activity against erythrocyte damage. This activity was related to the mixture of compounds, which acts synergistically.

Cell viability

Escher et al. (2018) determined the *in vitro* cell viability of *Centaurea cyanus* aqueous extract by evaluating the cytotoxic effect in the HepG-2, Caco-2 and A549 cell lines. The extract exhibited significant values for all cell lines (IC_{50} and GI_{50}> 9 00 µg/ mL) resulting in antiproliferative action and low cytotoxicity. According to Radan et al. (2017), a high concentration (2000 µg/ ml) of aqueous *Centaurea ragusina* L. extract decreased cell viability by 15% compared to untreated cells, corroborating the low cytotoxicity in different *Centaurea* species. In another study, the extract from whole fruit samples of *Centaurea bruguierana* did not show cytotoxicity in Caco-2 cells (IC_{50}> 1000 µg/ml) (Ostad et al., 2016).

Lactarius piperatus

Biological activities of *Lactarius* metabolites

Lactarius species include various species, from which a multitude of compounds are isolated with defined functions that present biological activities that are not dependent on the evolutionary process. The biological properties of the *Lactarius* species compounds are unknown because they have not been studied at a high level.

Anke et al. (1991) discovered the antifungal, phytotoxic and toxic effect on some cells activities of twenty unsaturated dialdehydes from organisms vegetal and fungous, velleral, isovelleral and isoisovelleral, the shyntetic derivatives of the isovelleral compound, are the most important activity. Isovelleral is considered the most active biologically isolated

secondary metabolite of the *Lactarius* species. It had low results in the range of 0.5-5 µg/ml for Gram-positive and Gram-negative and in the range of 0,1-5 µg/ml for certain species of fungi (Vidari et al., 1995).

After the research Vidari et al. (1995) noticed the change of structure of a natural sesquiterpene aldehyde, found in the *Lactarius* species' composition led to antimicrobial and cytotoxic effects. Also, he observed the reduction of mutagenicity, but the introduction of a hydroxyl group in the aldehyde structure is having an inverse impact on the antimicrobial activity. The results of antimicrobial and mutagenic activity were not simultaneous. Therefore they can be separated by a chemical derivatization process. By reducing one or more aldehyde groups of dialdehydes, the compounds of the *Lactarius* species presented a drastic decrease in the antibacterial and antifungal activities (Vidari et al., 1995). It was also observed that the following compounds: deterol against *Acinetobacter calcoaceticus*, lactoviolin against tubercle bacilli (Vidari et al., 1995) chrysoreic and rubrocinctal A against *Bacillus subtilis* and *Staphylococcus aureus*, phenol against *Candida albicans* had moderate antimicrobial activity.

From this study, phenol was presented as a strong contact allergen. An *in vitro* antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis,* and other bacteria and fungi (Vidari et al., 1995) was observed at the compound associated with the flavonoid A, which has a particular activity.

A variety of isolated compounds from the Lactarius species, besides the presence of various biological activities, present a resistance in the penetration of meat and fungi's latex, as they are suspected in gastrointestinal disorders. The compounds derived from the *Lactarius* species have different tastes, ranging from hot to unpleasant.

Responsible for the hot taste and the biological activities of several terpenoids is the unsaturated dialdehyde, being generally found for many biologically active sesquiterpene isolated from *Lactarius* species (Gamier et al., 1990).

A variety of successful methods have been presented to show a quantitative relationship between the structure of unsaturated dialdehydes and their activity, such as velleral, isivelleral and isoisovelleral.

The PLS (Partial least squares) technique showed a good correlations between the chemical and molecular properties of unsaturated dialdehydes and the mutagenic activity in the Ames *Salmonella* test, membrane toxicity measured as induction of ATP secretion in ELD cells, effect on cell membrane permeability in human neuroblastoma SH-SY5Y cells and inhibitory effect on D1 dopamine receptors (Vidari et al., 1995). It was verified that the most fundamental structural descriptors were the dipol moment, LUMO-HOMO energy difference, lipophilicity, atomic charges of unsaturated dialdehyde funtionality and geometric properties, an example being the distance and dihedral angle between dialdehyde groups.

The appearance of metabolites in *Lactarius* species and their biological significance

Recently, studies have been performed on metabolites isolated from *Lactarius* species, although they are present in many species of this type. For different *Lactarius* species, only certain compounds were present, these being related to other types, while some are specific to certain species. Similar isolation techniques have been used, but some metabolites are irregularly present in the same species.

The swedish authors did not identify Izovelleral, instead, it was found in *Lactarius torminosus* by american authors. It was stated that the isovelleral and velleral were isolated from the *Lactarius piperatus* and *Lactarius rufur*. The subsequent analysisshown that these fungi do not even produce traces of these sesquiterpenes. Neither the isolation of velleraoctone and pyrovellerolactone from *L. vellereus* nor blenin A from *Lactarius torminosus* couldn`t be repeated in further investigations. Irregularities occur due to the existence of particular subspecies and their development in various habitats, which makes it easy to confuse the *Lactarius* species (Vidari et al., 1995).

After several analyses, it was observed that any damage to this fungi species' bodies leads to the significant and rapid change of the content into secondary metabolites. It can be easily perceived as having a change of taste or color.

***Ribes rubrum* - Red currant**

***In vivo* and *in vitro* results of the *Ribes* species**

The *Ribes* species are found in temperate climates, and they are green shrubs during the year, with black or red fruits. Over time, the herbs have proven to treat various injuries, leading to numerous clinical studies based on their benefits. These species are also used in various preparations and beverages (Das et al., 2017).

Following several researches, Gulsen et al. (2019) discovered the importance of *Ribes* leaves, having anti-inflammatory effects studied by *in vivo* and *in vitro* methods (Tabart et al., 2012), antimicrobial (Paunović et al., 2017), but also high phenol content (Tian et al., 2017), anthocyanins and flavonoids (Paunović et al., 2017).

Very good results on *Ribes* species included tests of antimicrobial activity on microorganisms that cause infections (Bowler et al., 2001).

Gulsen et al. (2019) studied *Ribes* species following the HPLC method. Using this method, they found that *Ribes nigrum* has a high phenolic concentration and higher concentrations of gallic acid and quercetin. *Ribes rubrum* has the highest concentration of chlorogenic acid.

In several *in vitro* and *in vivo* studies, a variety of researchers have demonstrated the benefits of collagenic acid in treating various wounds and injuries (Almeida et al., 2012; Süntar et al., 2012; Chen et al, 2013), and in some *in vitro* tests have found that some phenols inhibit the action of enzymes that are closely related to the healing process of affected lesions (Higa et al., 2010).

Several scientific papers have been presented on phytochemical studies on Ribes species, including the rehydrated phenolic content of *Ribes nigrum* leaves. Gulsen et al. (2019) using the Ultra-Performance Liquid Chromatography (UPLC) method, determined the collage content. The most significant amount of collagenic acid was detected in *R. uva-crispa* (23.41 ± 0.45 µg/mg). Significant dominant concentrations of flavonol glycosides, especially quercetin, have been found in *Ribes nigrum* leaves (Cyboran et al., 2014; Paunović et al., 2017). Petunidine was detected as a major leaf anthocyanidin (1181 ± 115 µg/g) (Tabart et al., 2011).

Following research on the species *Ribes*, Gulsen et al. (2019) demonstrated that *Ribes nigrum* had the most significant effect of inducing collagen accumulation and reepithelialization, which suggested a possible migration and proliferation of epithelial cells. In this study, he compared the *Triticum vulgare* extract from FITO Krem® with the *Ribes* species extract, and the obtained results showed different degrees of healing of the parameters in favor of *Ribes nigrum* extract, obtaining more significant value, but on the other hand, analyzing the values partial reepithelialization *T. vulgare* had better results. These differences between the two extracts may be due to the various active ingredients found in them. *R. nigrum* leaf extracts have shown the best effect in wound healing.

CONCLUSIONS

The response surface methodology was adequate for modeling the extraction of phytochemical compounds and obtaining the in vitro bioactive characteristics for cornflower petal extract.

It has been observed that in species of the genus Lactarius, there are various bioactive compounds, such as:

- these depending on the species and are stored as esters of fatty acids in fungi;
- the biological activities of the compounds could be different;
- the nature of the fatty acids esterified are different.

The types and quantities of secondary metabolites formed in the fruiting bodies depending on the conversion of the original fatty acid ester (s). All these characteristics can be useful together with the morphological characteristics, and they can facilitate the valorization of the Lactarius species.

Extracts from *R. nigrum* leaves have shown the best effect in wound healing.

Wound healing activity can be attributed to phenolic compounds especially. However, it is still necessary to disclose the active substances by conducting a detailed isolation study guided by biological activity.

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