Bioactive Compounds with Antioxidant Activity Extracted from Lignocellulosic Biomass of *Galium verum*

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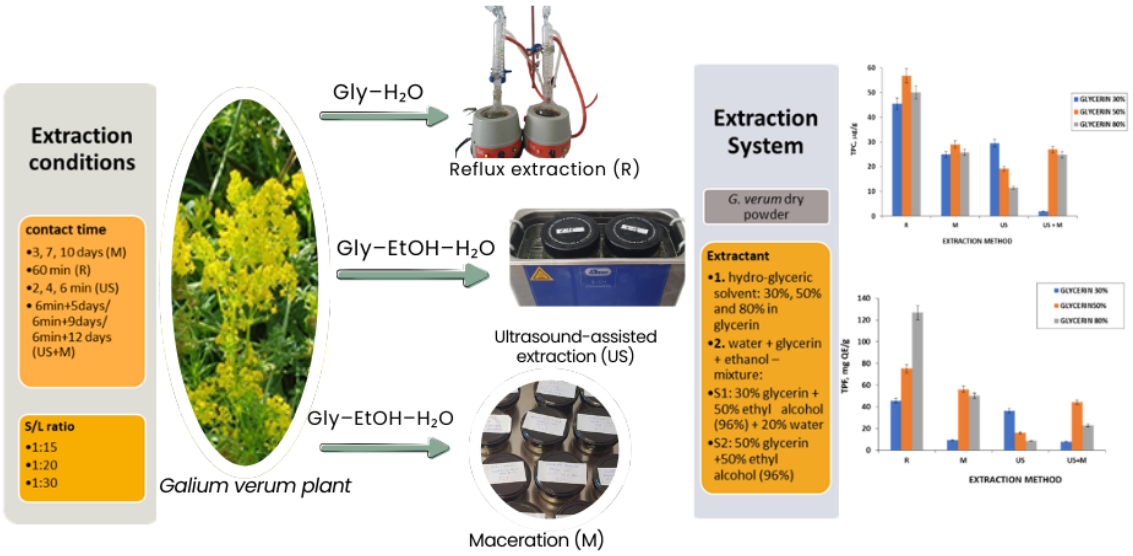
GRAPHICAL ABSTRACT



Bioactive Compounds with Antioxidant Activity Extracted from Lignocellulosic Biomass of *Galium verum*

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The active ingredients present in *Galium* species, especially *Galium verum*, are represented by antioxidant compounds in variable proportions. This study aimed to obtain, by solid-liquid extraction of *G. verum*, biologically active compounds with antioxidant properties, such as polyphenols and flavonoids. Four classic extraction techniques were used (maceration-M, refluxation-R, sonoextraction-US and a combined method: sonoextraction with maceration - US+M). In the extraction process, glycerin was used in different forms: hydro-glycerin; and water and glycerin-ethanol mixtures with different concentrations. Other monitored parameters were the solid-liquid ratio (S/L) and the extraction time. The best results were obtained using the hydro-glycerin solution as extraction solvent: 85.0 µg GAE/g polyphenols (R: 50% concentration, 60 min, S/L=1:15) and 117 mg QE/g flavonoids ((R: 80% concentration, 60 min, S/L=1:15). This study brings new data about obtaining plant extracts from *G. verum* with important antioxidant properties, based on the use of a green solvent extraction.

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Keywords: Antioxidant; Flavonoids; Galium verum extract; Hydro-glycerin extractant; Lignocellulose- vegetal biomass; Polyphenols

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INTRODUCTION

Plant biomass has long been recognised as a valuable renewable resource with a wide range of applications due to its rich chemical composition. Some of these applications can be listed as follows (Kabir *et al.* 2018; Omer 2018; Bedoic *et al.* 2019; Rodriguez-Rodriguez *et al.* 2020; Ferreira-Santos *et al.* 2020; Arias *et al.* 2021; Kee *et al*. 2021; Riva *et al.* 2021; Kozma *et al.* 2022):

(1) The chemical or biotechnological synthesis of biomaterials through various functionalisation methods that allow them to be used alongside or replace conventional materials, especially for biotechnological or medical purposes.

(2) The development of sustained release pharmaceutical forms and transdermal preparations allowing controlled release of active ingredients (*e.g*., drugs, vitamins, proteins, probiotics or other beneficial micro-organisms).

(3) The conversion of woody biomass into chemicals and energy through biorefinery processes.

(4) The extraction of bioactive compounds that are increasingly valued for their therapeutic potential.

(5) Alternative energy production, such as the production of biogas through anaerobic digestion.

(6) Soil improvers in horticulture that release nutrients to improve plant growth.

Natural compounds have a long-standing role in the pharmaceutical industry, and dermocosmetics is a particularly important area of application. The benefits of natural bioactive ingredients have led to a specialized product category called biocosmetics or cosmeceuticals, which acts as an intermediate between medicine and cosmetics (Roncea *et al.* 2016). These products contain biologically active ingredients and are considered to be similar to topical dermatological products. Natural active ingredients, such as hormones, vitamins, enzymes, and alkaloids, provide important biorevitalizing, protective, and trophic-supporting effects with significant pharmacological activity. Plants are a major source of these antioxidants, specifically primary and secondary metabolites that are continuously studied and used for their pharmacological and ecological benefits (Pietta *et al.* 2003). The key advantages of natural compounds include their small, non-protein molecular size, which ensures good penetrability (Turcov *et al.* 2020 a,b). They can be obtained through extraction methods that do not harm the quality of the ingredients and can be sourced from wild plants without incurring production costs. Many of these ingredients can be obtained by steam distillation from plant materials using organic or aqueous solvents, and they typically have a low molecular weight (Turcov *et al.* 2020).

The extraction and valorisation of bioactive compounds from plant biomass is becoming increasingly important. These compounds are crucial for a variety of applications, including dermatocosmetics, where they are valued for their antioxidant properties. Oxidative stress caused by UV radiation and pollutants has long been recognised as a key factor in skin ageing and various skin diseases. Despite extensive research on antioxidants, studies on more effective natural solutions, particularly from plants to combat oxidative damage, are still ongoing. The skin is highly susceptible to oxidative stress as it is constantly exposed to UV rays and environmental pollutants. Both UVB and UVA rays contribute to the breakdown of important skin structures, including DNA, proteins and lipids. UVB rays directly damage these structures, while UVA rays promote oxidative stress, that indirectly affects these structures and leads to cell damage and even cell death (Chien-Hsing *et al.* 2017; Li *et al.* 2022). These processes are the molecular basis for various diseases, such as cancer, neurodegenerative diseases, cardiovascular and autoimmune diseases and diabetes (Venkat *et al.* 2006).

The search for natural antioxidants that can be used in dermatocosmetic products to combat oxidative stress is rapidly increasing (Kumar and Pruthi 2015; Turcov *et al.* 2020). Polyphenols and synthetic compounds such as idebenone are among the best-studied antioxidants (Montenegro 2014; Turcov *et al.* 2021). Clinical studies have demonstrated the benefits of antioxidants in photoageing, especially for common skin problems such as wrinkles, hyperpigmentation, dehydration and loss of skin elasticity (Chien-Hsing *et al.* 2017; Soto *et al.* 2018; Ribet *et al.* 2019; Konisky *et al.* 2023; Turcov *et al.* 2023). Plant species that have long been used in traditional medicine are now the subject of modern research aimed at optimising their extraction and application methods.

Beyond their anti-aging effects on skin appearance, biocosmetics, and cosmeceuticals have proven effective in preventing or improving various dermatological diseases. They can simultaneously provide antioxidant, anti-inflammatory, and

anticarcinogenic effects. In addition, they can modulate cell proliferation, angiogenesis, melanogenesis, and protein synthesis. The dermatocosmetic industry is continually evolving, driven by the need for new, effective, and well-tolerated products, which fuels research into advanced formulas that combine different active ingredients (Tagami *et al.* 2006; Ndiaye *et al.* 2011). Extensive studies on oxidative stress are justified by a better understanding of how reactive oxygen and nitrogen species are produced, the identification of biomarkers for oxidative damage, and the direct link between oxidative stress and certain acute and chronic diseases. The primary benefit of combining specific bioactive compounds is the potential to increase or potentiate their core action, leading to better therapeutic results and greater patient satisfaction, while also offering a way to replace harmful synthetic chemical compounds.

Recent research from 2024 and 2025 continues to highlight the significant potential of natural extracts in dermatocosmetics, particularly for combating oxidative stress. A systematic review from 2025 focused on the efficacy and safety of topical plant-based products for skin aging, analyzing data from studies with 396 participants (Tomas *et al.* 2025). The findings indicated that these products are safe and effective, showing improvements in skin hydration and elasticity while also reducing melanin and erythema. However, a limitation noted was that these effects did not improve after eight weeks, suggesting the need for further research on long-term efficacy. Another study from 2025 investigated the photoprotective qualities of 10 various herbal plant extracts, showing an impressive *in vitro* Sun Protection Factor (SPF) value of 40.8 ± 0.2 (Le *et al.* 2025). The research supports the potential for natural antioxidants as a means of UV filtration, which can substantially decrease the reliance on conventional physical or chemical filters. Furthermore, a review from 2024 summarized the roles of plant-derived phytochemicals in anti-aging, emphasizing that they work by scavenging free radicals, modulating key enzymatic pathways, and promoting the skin's structural integrity (Cheng *et al.* 2024).

The field is also seeing innovative advancements in the application and sourcing of these natural compounds. For instance, a 2024 study demonstrated the beneficial effects of plant-derived extracellular vesicles (PDEVs) from fruits on human skin fibroblasts (Di Raimo *et al.* 2024). The research showed that these vesicles mitigated hydrogen peroxide-induced intracellular ROS, reduced melanin content, and exhibited anti-inflammatory effects. This antioxidant action was also linked to improved wound repair in a fibroblast monolayer. These developments underscore a growing focus on not only the efficacy of natural extracts but also on their sustainable sourcing and enhanced delivery methods.

Galium species, which belong to the Rubiaceae family and are part of the spontaneous flora, are easily accessible, safe, and frequently used in traditional medicine. Among the best known species are *G. verum*, which is characterised by its yellow inflorescences, and *G. mollugo* with its white flowers. Other species described in the scientific literature are *G. album*, *G. rival*, *G. pseudoaristatum*, *G. purpureum*, and *G. aparine* (Ghita *et al.* 2012; Mocan *et al.* 2016; Hanganu *et al.* 2018; Mocan *et al.* 2019). *Galium verum (G. verum)* (commonly known as bedstraw) has a long history of medicinal use, especially in traditional remedies (Bajpai 2016; Ji *et al.* 2018; Langsdorf *et al.* 2021; Hag *et al.* 2021; Borrero-Lopez *et al.* 2022; Velvizhi *et al.* 2022). It is particularly known for its high content of bioactive compounds and its significant biological effects, making it one of the most studied species of the *Galium* genus. Detailed studies have revealed a complex phytochemical profile in the flowers and leaves of *G. verum*, which includes

terpene iridoid glycosides, phenolic acids, flavonoids, polysaccharide complexes, aldehydes, alcohols, anthraquinones, acids, small amounts of tannins, saponins, waxes, pigments, vitamin C, and essential oils (Mocan *et al.* 2016; Al-Snafi 2018; Bradic *et al.* 2018; Farcas *et al.* 2018; Shynkovenko *et al.* 2018; Tava *et al.* 2020). These compounds are valued for their antioxidant, anti-inflammatory, and other beneficial properties, making G. verum a promising candidate for dermatocosmetic applications and a valuable resource for the development of products to combat oxidative stress and skin ageing.

Despite its promising potential, research on *G. verum* remains limited, particularly in terms of extraction methods and the full spectrum of bioactive compounds. Traditional methods such as solvent extraction are widely used, but often they are not sustainable and efficient (Garcia-Beltran *et al.* 2016; Besil *et al.* 2017; Walia *et al.* 2017; Mocan *et al.* 2018; Oreopoulou *et al.* 2021). Recent studies (Saxena *et al.* 2014; Oreopoulou *et al.* 2021) have emphasised glycerol, a "green" solvent, as an effective alternative to solvents such as ethanol and methanol. Both Apostolakis *et al.* (2014) and Huamán-Castilla *et al.* (2020) demonstrated that glycerol, when used as a co-solvent with water, outperforms ethanol in the extraction of polyphenols due to its ability to form hydrogen bonds. The non-toxicity of glycerol and its improved extraction efficiency make it a promising candidate for more sustainable, environmentally friendly extraction processes (Apostolakis *et al.* 2014; Huaman-Castilla *et al.* 2020; Nastasi *et al.* 2023).

The aim of this study was to evaluate different solid-liquid extraction methods for the recovery of bioactive compounds from *G. verum* biomass. Specifically, the study investigated the use of glycerol (in aqueous, alcoholic and hydroalcoholic solutions) as a solvent for the extraction of polyphenols and flavonoids. The extraction techniques investigated were hot reflux, maceration, ultrasound-assisted extraction (sonoextraction), and a combined method (maceration – sonoextraction). The efficiency of each method is evaluated based on the amount of extracted polyphenols and flavonoids and their antioxidant properties under different extraction conditions (type and concentration of solvent, solid-liquid ratio, extraction time and temperature). This preliminary study on *G. verum* extract contributes to the field of dermatocosmetics by investigating innovative extraction methods to obtain bioactive compounds from *G. verum*, with particular emphasis on glycerol as a sustainable and effective solvent. The results were aimed to advance the use of plant-based antioxidants in the fight against oxidative stress and skin ageing, which could lead to the development of more effective, natural skincare products.

EXPERIMENTAL

Materials

The *G. verum* plants were harvested in Rediu (Fig. 1), Iasi region, Romania (geographical coordinates 47°12′48″N 27°30′23″E). Young plants that had grown in the year of collection were harvested, and the maximum time of plant development was favoured (when they had reached maximum flowering and a height of about 60 cm, *i.e.* in June 2021). After harvesting the plant (or selecting only the inflorescences), it was protected from sunlight in a thin layer at room temperature (15 to 20 °C) and kept for one month for complete drying (humidity of 8.4 %, determined with a Kern DAB 100-3, thermobalance). Then it was crushed and sieved in the form of particles with a size of 3 to 5 mm and kept in brown containers for storage up to 4 weeks.

Fig. 1. Lady's bedstraw flowers (*G. verum*)

The reagents used in the experimental part were vegetable glycerin of p.a. purity (99.5% active ingredient, supplier Elemental SRL, Oradea, Romania), ethanol of 96% concentration, p.a. purity (manufacturer Chemical Company SRL, Iasi, Romania), Foling-Ciocalteu reagent of p.a. purity (Merk, supplier Chimexim SRL, Romania), and reactiv ROTH aluminium chloride 99 %.

As an extraction solvent, two types of glycerin-based solvent mixtures were used:
(i) Hydro-glycerin with the concentrations: 30%, 50%, and 80% (v/v) in glycerin.
(ii) Water + glycerin + ethanol mixtures with different concentrations. Two types of solvent mixtures based on glycerin, water, and 96% ethyl alcohol were considered, noted as follows: Mixture S1: 30% glycerin + 50% ethyl alcohol (96%) + 20% water; and Mixture S2: 50% glycerin +50% ethyl alcohol (96%).

Methods

Obtaining liquid extracts

The plant extracts were obtained from the *G. verum* plant by solid-liquid extraction with two types of glycerol-based extraction solvents, using a similar working protocol as in the authors' previous work (Turcov *et al.* 2022).

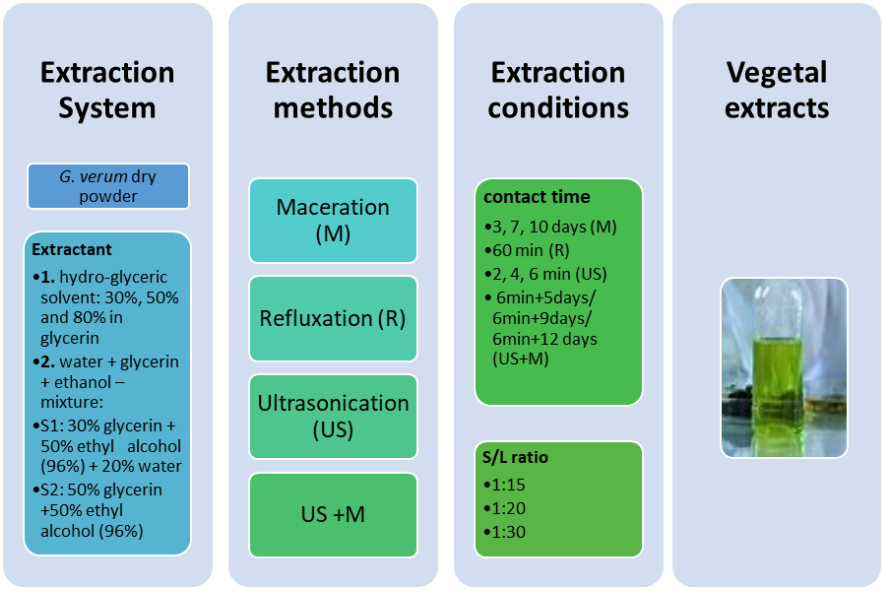

Fig. 2. The protocol for obtaining extracts from the *Galium verum* plant

The methods investigated were heat reflux extraction (refluxing - R), maceration (M), ultrasound-assisted extraction (sonoextraction - US), and the combined method of sonoextraction and maceration (US + M). The sonoextraction experiments were performed with a Sonorex RK 100H ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Berlin, Germany) at a frequency of 35 kHz, a maximum power of 80 W, extraction times of 2 min, 4 min and 6 min and extraction temperatures of 30, 50, and 80 °C (Fig. 2). The extraction was carried out taking into account a number of physical parameters: Type and concentration of extraction solvent, extraction time, ratio between solidand liquid phase (S/L) and temperature; these parameters were adjusted depending on the specific protocol of each extraction method (in particular the extraction time which is different for maceration (days) compared to the other methods used) (Fig. 2).

The performance of the extraction methods used was assessed by evaluating the quantities of the main extracted bioactive compounds.

Vegetal extract characterization

The assessment of the antioxidant activity of the obtained extracts was done by calculating the total amount of extracted polyphenols and flavonoids.

Total polyphenol content

Every experiment used to measure the amount of polyphenols in vegetal extracts was carried out twice. Gallic acid was employed as a standard reference in the Folin-Ciocalteu method introduced by Singleton *et al*. (1999), to assess the amount of polyphenolic components. The results are expressed in µg GAE/g.

The following steps were taken to prepare the samples for analysis: The system was kept at room temperature for 5 min after adding 0.5 mL of Folin-Ciocalteu reagent to 0.5 mL of *G*. *verum* sp. extracts. Then 8 mL of a 7.5% aqueous sodium carbonate solution was added, and the mixture has been sitting at room temperature in the dark for two hours. To find the concentration, the calibration curve approach was employed. Using a Shimadzu UV-1280 UV-VIS Spectro-photometer (Shimadzu Corporation, Kyoto, Japan), the standards and samples were recorded at 760 nm, the maximum wavelength (Turcov *et al.* 2022).

Total flavonoid content

All experiments for the flavonoids content determination in vegetal extracts were done in duplicate. The quantification of total flavonoids was made based on the reaction with 2% $AlCl_3$ in methanol, using the method proposed by Christ and Muller (1960) for the determination of flavanol derivatives in drugs. In this case the working protocol involved adding 1.0 mL of a 2% methanol solution of aluminum chloride to 1.0 mL of the vegetal extract sample, and the mixture was allowed to sit at room temperature for 15 minutes.

Next, a Shimadzu UV-1280 UV-VIS spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used to read the absorbance values at 510 nm. Quercetin (QE) served as the reference standard for flavonoid measurement, and the results are expressed as mg QE/g (Turcov *et al.* 2022).

RESULTS AND DISCUSSION

The Content of Polyphenols an in the Case of Extracts Based on Water-glycerin

The data obtained are shown in Fig. 3.

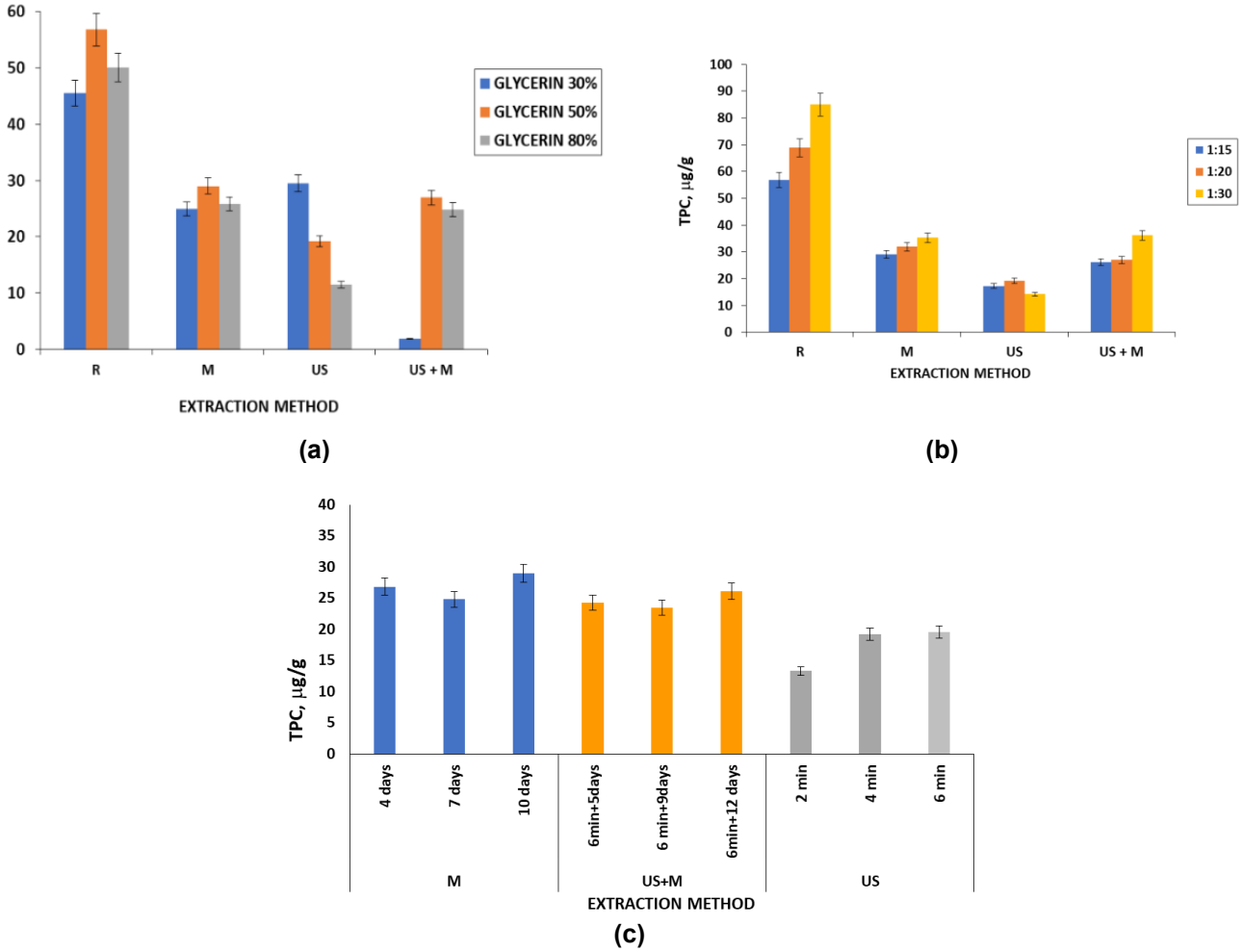

Fig. 3. Total polyphenols (TPC; µg GAE/g) content of plant extracts obtained from the dried plant *G. verum* with hydro-glycerin solution. Working parameters: (a) M: S/L = 1:15 and extraction time -10 days; US: S/L = 1:20 and extraction time - 4 min; US+M : S/L = 1:15 and extraction time-6min+12 days; (b) M: solvent concentration = 50% and extraction time -10 days; US: solvent concentration = 50% and extraction time - 4 min; US+M: Solvent concentration = 50% and extraction time-6min+12 days; (c) M: solvent concentration = 50% and S/L -1:15; US: solvent concentration = 50% and S/L -1:20; US+M: S/L = 1:1 and solvent concentration = 50%

The data in Fig.3 show that in the case of extraction with hydro-glycerin solution, the amount of polyphenols extracted from the dried plant of *G. verum* depended both on the type of extraction method used and on the conditions for their realization, as follows:

- Regardless of the glycerin concentration of the extraction reagent used, the highest amounts of polyphenols were obtained following simple refluxing: 45.5 to 56.8 µg GAE/g (extraction time of 60 min and S/L of 1:15, easily detaching with 50% concentration reagent).

- Using different S/L ratios, the best results in polyphenols, clearly superior to the other methods, were obtained also in the case of the extracts obtained by reflux method. The highest value was 85.0 µg GAE/g in condition of extraction time of 60 min and extraction reagent concentration of 50%, ratio S/L 1:30).
- The influence of extraction time was studied for three extraction methods: maceration, sonoextraction, and sonoextraction combined with maceration. The best results, 29.0 µg GAE/g, were obtained in the case of maceration, the variant performed under conditions of S/L ratio of 1:15, and extraction reagent concentration of 50%. These were, however, much smaller than those recorded in the case of reflux method.

The good results obtained in the case of reflux method, a hot extraction method, can be explained by the fact that the temperature changes the viscosity of the aqueous mixture, which makes glycerin a more efficient solvent, in agreement with a series of literature information (Apostolakis *et al.* 2014). In this regard, the authors conducted an additional study on the influence of temperature on hydro-glycerin extraction. Sonoextraction was performed using an S/L ratio = 1:20, hydro-glycerin solution of 50% concentration, an extraction time of 4 min and three temperature values: 30, 50, and 80 °C. The results are presented in Table 1.

In Table 1 it can be observed that the quantity of polyphenols extracted was maximum at 50 °C. Above this temperature, the results can be explained based on chemical instability and destruction of biologically active compounds.

Table 1. The Influence of Temperature on the Amount of Polyphenols Obtained from Sonoextraction in the Presence of Hydro-glycerin Solutions

Temperature (ºC)	Extract's Characteristics	Polyphenol Content (µg GAE/g)
30	S/L=1:20, 4 min, solvent concentration = 50%	19.231±0.133
50		21.309±0.006
80		19.089±0.0595

Content of Flavonoids in the Case of Extracts Based on Water-glycerin

The data obtained are represented in Fig. 4 and show that in the case of extraction with hydro-glycerin solution, the amount of flavonoids extracted from the dried plant of *G. verum* depends on the type of extraction method used and the conditions used, as follows:

- Depending on the concentration of the glycerin solution (extraction reagent), the refluxing led to the highest amounts of flavonoids, as in the case of polyphenols. About 117 mg QE/g flavonoids was achieved in the case of a glycerin concentration of 80%, at an extraction time of 60 min and using an S/L ratio of 1:30;
- Depending on the S/L ratio, it was found that in the case of reflow using an S/L ratio of 1:30 and 1:20, the best results (117 mg QE/g and 84.9 mg QE/g, respectively) were obtained, followed by maceration in all three ratios (1:15 - 56.1 mg QE/g; 1:20 - 53.55 mg QE/g; 1:30 - 51,800 mg QE/g) and the combined US+M method in the case of S/L ratio = 1:30 when flavonoids were obtained in the amount of 53.5 mg QE/g.
- The study of the influence of extraction time revealed that in each method, the time variable had no significant influence on the process of extraction of flavonoids. In maceration, the highest amounts of flavonoids were obtained (56.1 to 51.9 mg QE/g).

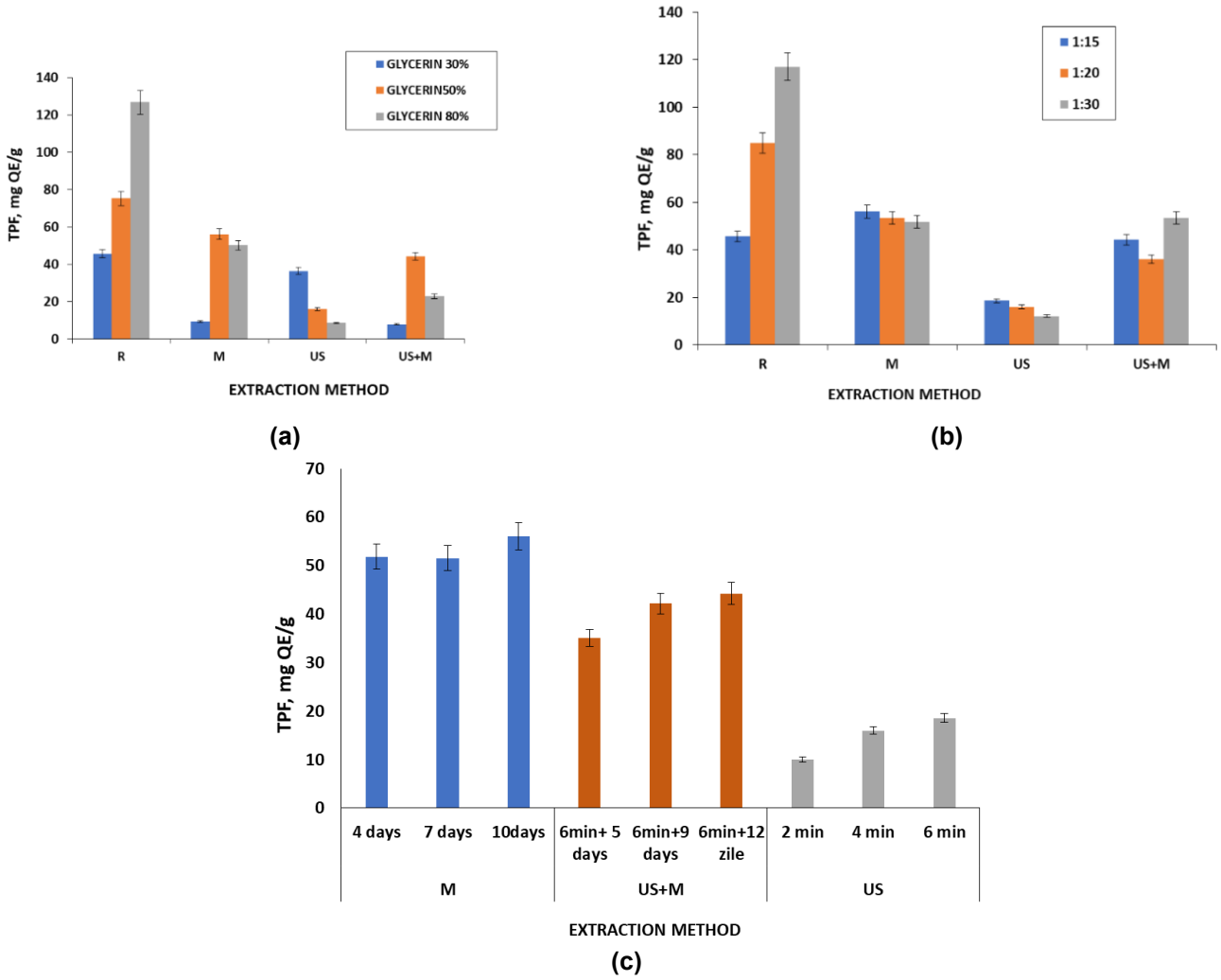

Fig. 4. Total flavonoid content (TFC) (mg QE/g) of plant extracts obtained from the dried plant *G. verum* with hydro-glycerin solution as extraction solvent. Working parameters: (a) R: S/L = 1:15 and extraction time-60 min; M: S/L = 1:15 and extraction time -10 days; US: S/L = 1:20 and extraction time - 4 min; US+M: S/L = 1:15 and extraction time - 6min+12 days; (b) R - solvent concentration = 50% and extraction time-60min; M: solvent concentration = 50% and extraction time -10 days; US: solvent concentration = 50% and extraction time - 4 min; US+M: solvent concentration = 50% and extraction time - 6min +12 days; (c) M: solvent concentration = 50% and S/L=1:15; US: solvent concentration = 50% and S/L=1:20; US+M: S/L=1:15 and solvent concentration -50%.

In order to study the influence of temperature, as with polyphenols, sonoextraction was performed with an S/L ratio 1:20, hydro-glyceric solution of 50% concentration, an extraction time of 4 min, and three temperature values: 30, 50, and 80 °C. The results are presented in Table 2.

Table 2. The Influence of Temperature on the Amount of Flavonoids Obtained from Sonoextraction in the Presence of Glycerin Aqueous Solutions

Temperature (ºC)	Extract's Characteristics	Flavonoid Content (mg QE/g)
30	S/L=1:20, 4 min, solvent concentration = 50%	16±0.0168
50		18.64±0.05
80		22.28±0.085

The analysis of the data in Table 2 shows that, under the selected extraction conditions, at a temperature of 80 °C the amount of extracted flavonoids was maximized.

The results obtained, both in the case of polyphenols and flavonoids, can be explained by the modification of the physical properties of the aqueous glycerin solution with temperature variation, which also affects its ability to extract the chemical compounds of interest, to which is added the thermal stability of the respective compounds.

In summary, when working in the extraction process with solvents based on hydro-glycerin solutions, the results depend on the extraction method and working parameters. Under the conditions employed, the highest amount of polyphenols (85.0 mg GAE/g) was obtained in the case of the extract resulting from the refluxing method with hydro-glycerin concentration of 50%, for 60 min and using a ratio S /L = 1:15, and the highest amount of flavonoids (117 mg QE/g) was found in the extract obtained by refluxing with hydro-glycerin concentration of 80%, for 60 min and using the ratio S/L = 1: 30.

Calculation of Polyphenols in the Case of Extracts Obtained with Glycerin-Water-Alcohol Solvents

Two types of mixtures of solvents were used: S1 mixture: 30% glycerin + 50% ethyl alcohol (96%) + 20% water; and S2 mixture: 50% glycerin +50% ethyl alcohol (96%). The methods were maceration (M), sonoextraction (US), and the combined method between sonoextraction and maceration (US +M). The polyphenols contents were calculated using standard protocols (Singleton *et al.* 1999). Results are given in Fig. 5.

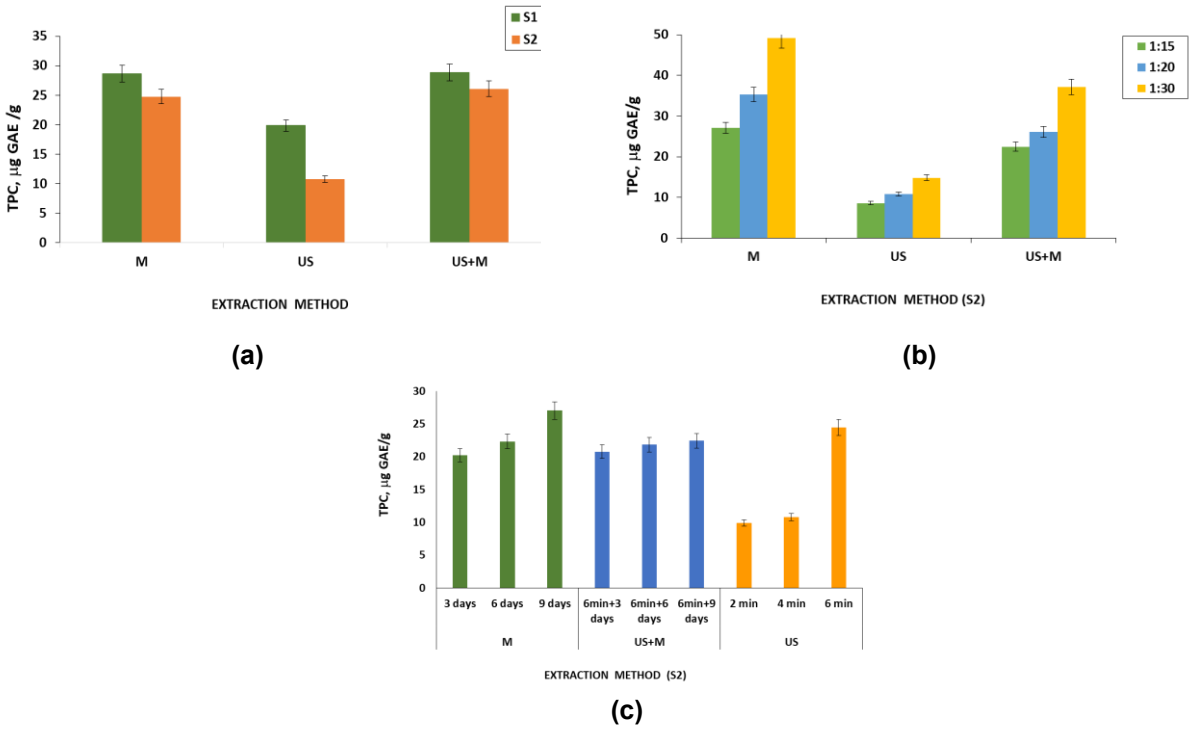

Fig. 5. Total polyphenols (TPC) µg GAE/g content of plant extracts obtained from the dried plant *G. verum* with glycerin + alcohol as extraction solvent (mixture S1 and S2). Working parameters: (a) M: S/L = 1:20 and extraction time - 9 days; US: S/L = 1:20 and extraction time - 4 min; US+M: S/L = 1:20 and extraction time – 6 min+9 days; (b) M: extraction time – 9 days and S2 mixture; US: extraction time - 4 min and S2 mixture; US+M: extraction time – 6 min + 9 days and S2 mixture; (c) M: mix S2 and S/L =1:15; US: mix S2 and S/L =1:20; US+M: mix S2 and S/L = 1:15.

The data in Fig. 5 show that in the case of extraction with solvents based on a mixture of glycerin-alcohol and water (S1), glycerin and alcohol (S2), the amounts of polyphenols extracted depended on the utilized method, the operational parameters, and the type of mixture used. In Fig. 5a it is apparent that regardless of the method used in the extraction with the S1 mixture (30% glycerin with 50% ethyl alcohol and 20% water), the highest amounts of polyphenols were obtained. Among the methods, maceration conditions of S/L=1:20 and 9 days extraction time led to 28.6 μg GAE/g, and the combined sonoextraction-maceration method under conditions of S/L=1:20 ratio and combined extraction time of 6 min + 9 days led to a polyphenol amount of 28.8 μg GAE/g. Working with the S2 extraction mixture, it can be seen from Fig. 5b that the S/L ratio = 1:30 gave the highest amounts of polyphenols when maceration was used (49.1 μg GAE/g), followed by the S/L ratio = 1:20 in which the best results were also obtained by maceration (35.3 μg GAE/g).

Analyzing the results according to extraction time (Fig. 5c), it is apparent that that variable had a relatively low influence in the case of maceration and the combined method in the presence of the S2 mixture, while sonoextraction was more influenced by its variation. Thus, after the extraction time it was observed that maceration for 9 days using a ratio S/L = 1:15 provided the largest amount of polyphenols extracted (27.0 μg GAE/g), followed by sonoextraction for 6 min and using a ratio S/L=1:20, which led to a polyphenol amount of 24.5 μg GAE/g.

Additional studies with the S1 mixture (Table 3) showed interesting results.

Table 3. The Influence of the Extraction Time on the Amount of Polyphenols Obtained from US Extraction in the Presence of Alcoholic Glycerin Solutions

Method of Extraction	Time	Solvent	The Amount of Polyphenols (μg GAE/g)	Solvent	The Amount of Polyphenols (μg GAE/g)
M	3 days	*Mixture S1* (S/L=1:15)	7.19±0.052	*Mixture S2* (S/L=1:15)	20.23±0.011
	6 days		24.56±0.058		22.32±0.055
	9 days		24.73±0.1		27.04±0.062
US+M	6 min+3 days		53.32±0.056		20.76±0.1
	6 min+6 days		51.62±0.015		21.83±0.075
	6 min+9 days		51.73±0.053		22.43±0.09

From the data organized in Table 3 it is notable that although there were no significant differences in the quantities of polyphenols obtained in maceration (except for 3 days extraction), in the case of the combined method in the presence of the solvent mixture S1 the quantities of polyphenols obtained were twice as high as in the case of the S2 mixture. The explanation could be given by the composition of the mixture of extraction solvents, namely S2, which consists of glycerin and alcohol compared to S1, which also contains a certain amount of water that changes the extraction capacity compared to a number of organic compounds. Viscous solutions, in the case of sonoextraction, prevent the propagation of ultrasound by reducing the mechanical effect of cavitation on the sample.

At the same time, the more viscous solutions in sonoextraction prevent the propagation of the ultrasound vibrations by reducing the mechanical effect of cavitation on the sample, which leads to lower results in polyphenols compared to those obtained by maceration.

Calculation of Flavonoids in the Case of Extracts Obtained with Glycerin-Water-Alcohol Solvents

Using the same two type of mixtures (S1 mixture: 30% glycerin + 50% ethyl alcohol (96%) + 20% water; and S2 mixture: 50% glycerin +50% ethyl alcohol (96%) as extraction agents and the same extraction methods (M, US and the combined method US +M), the flavonoids contents were calculated according to the standard protocols and the results are given in Fig. 6.

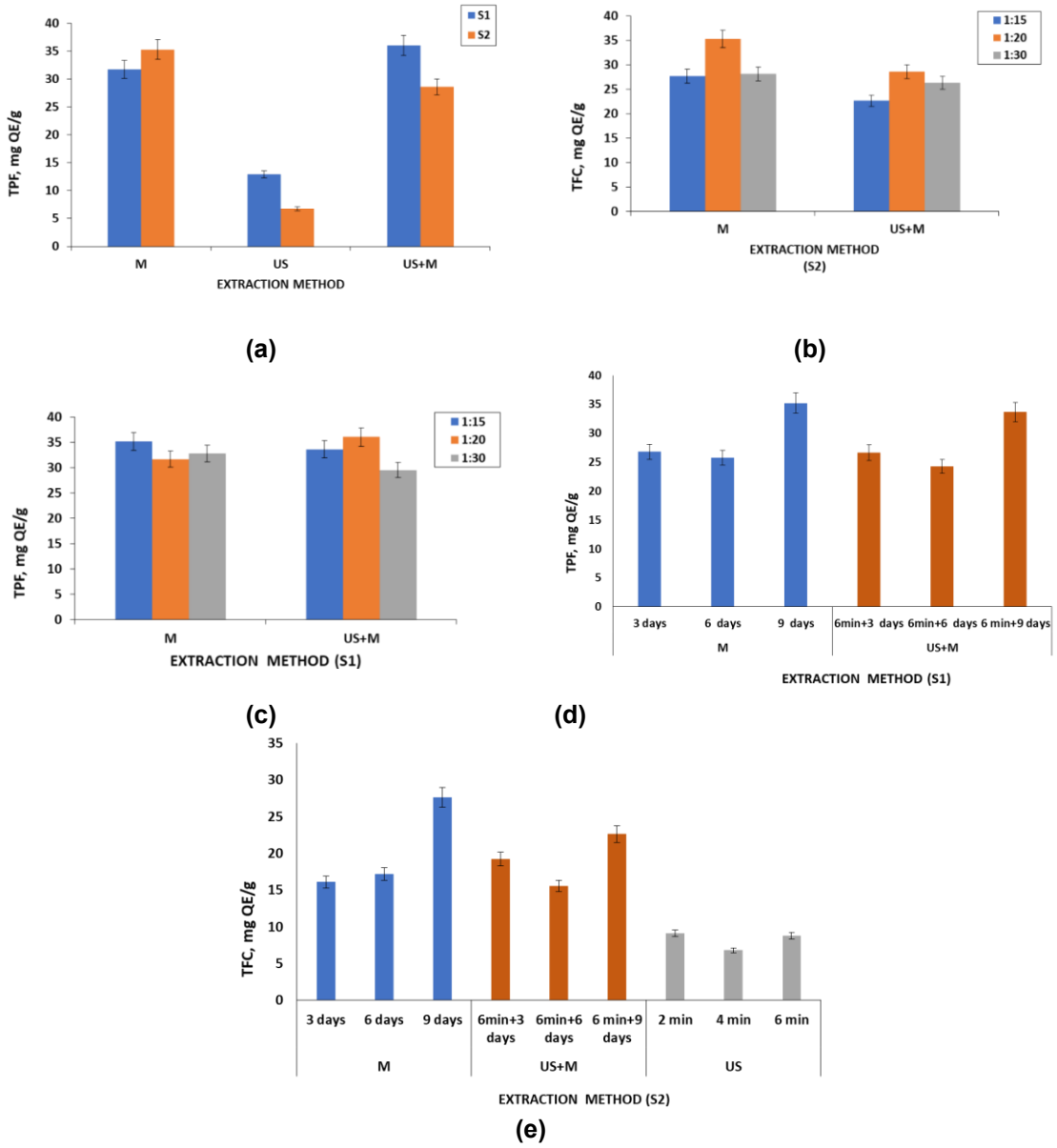

Fig. 6. Total flavonoid content (TFC) (mg QE/g) of plant extracts obtained from *G. verum* with glycerin + alcohol mixture as extraction solvent (solvent S1 and S2). Working parameters: (a) M: S/L = 1:20 and extraction time -9 days; US: S/L = 1:20 and extraction time - 4 min; US+M: S/L = 1:20 and extraction time – 6 min+9 days; (b) M: extraction time - 9 days and S2 mixture; US: extraction time - 4 min and S2 mixture; US+M: extraction time – 6 min+9 days and S2 mixture; (c) M: extraction time - 9 days and S1 mixture; US+M: extraction time – 6 min+9 days and S1 mixture; (d) M: mixture S1 and S/L =1:15; US+M: mix S1 and S/L=1:15; (e) M: mix S2 and S/L =1:15; US: mixture S2 and S/L=1:20; US+M: mix S2 and S/L=1:15.

The analysis of the data in Fig. 6 led to the following conclusions:

- A different behavior of the mixtures S1 and S2 was observed depending on the extraction method used (Fig.6a). If in the case of maceration, the mixtures have a similar behavior leading to comparable results (35.3 and 31.7 mg QE/g, respectively), in the case of sonoextraction and the combined method the mixture S1 leads to much better results than the mixture S2, by detaching the combined method leading with a flavonoid content of 36.1 mg QE/g.
- Using for extraction the S2 mixture (Fig. 6b) and various S/L ratios, the best results were obtained with maceration (35.3 mg QE/g) with work parameters of: extraction time of 9 days and using a S/L ratio of 1:20, followed by the combined method (28.6 mg QE/g) using the work parameters: extraction time 6 min + 9 days and a ratio S/L= 1:30 and maceration under conditions of ratio S/L = 1:30 and extraction time of 9 days (28.1 mg QE/g).
- Using the S1 mixture (Fig. 6c) and various S/L ratios for extraction, higher quantities of flavonoids were obtained than in the S2 solvent, and the best results are obtained in the combined method under S/L ratio = 1:20 and extraction time of 6 min + 9 days (36.1 mg QE/g) followed by maceration for 9 days at S/L ratio = 1:15, respectively 35.2 mg QE/g followed by the combined method at S/L = 1:15 ratio and extraction time of 6 min + 9 days (33.7 mg QE/g).
- Depending on the extraction time, when it was used the S1 mixture (Fig. 6d), the best results of about 35.2 mg QE/g were obtained by maceration in conditions of S/L=1:15 ratio 9 days as extraction time, followed by the combined method with 33.7 mg QE/g, achieved for 6 min+9 days extraction time and a S/L ratio of 1:15.
- Depending on the extraction time, when it was used the S2 mixture (Fig. 6e), the best results of 27.6 mg QE/g were obtained by maceration in conditions of a S/L=1:15 ratio and extraction time of 9 days and the combined method (22.62 mg QE/g) in conditions of 6 min+9 days extraction time and a S/L ratio of 1:15.

In conclusion, working in the extraction process with solvents based on alcoholic or hydro-alcoholic glycerin solutions, the results also depended on the extraction method, the composition of the extraction solvent, and working parameters.

Upon comparison of all the data obtained, the highest amount of polyphenols (53.3 µg GAE/g) was obtained in the extract from the combined method (sonoextraction + maceration) made with mix S1 (30% glycerin + 50% alcohol + 20% water), a time of 6 min + 3 days, and using S/L ratio=1:15, and the largest amount of flavonoids (36.1 mg QE/g) in the case of the extract resulting from the combined method (sonoextraction + maceration) made with extracting mix S1 (30% glycerin + 50% alcohol + 20% water) a time of 6 min + 9 days and using an S/L ratio of 1:20 .

Data summarized in Table 4 show that the solid-liquid extraction of dried *G.verum* plants by classical reflux method in the presence of hydro-glycerin solvent was the most effective. This efficiency is expressed by the polyphenols and flavonoids contents determined in the obtained extracts.

In order to appreciate the superiority of the glycerin, extracts compared to the hydroalcoholic ones were obtained and characterized in a previous study (Turcov *et al.* 2022), the data were presented in the form of Table 5.

Table 4. The Amount of Polyphenols and Flavonoids Obtained from Extraction with Glycerin-based Solutions – Water – Alcohol from *G. verum*

Extracted Compound	Type of Solvent/ Method Characteristics		
	Glycerin - Water	Glycerin-Alcohol	
		S1(30% Glycerin + 50% Alcohol + 20% Water)	S2 (50% Glycerin + 50% Alcohol)
Polyphenols (μg GAE/g)	85.0 (R: 50% concentration, 60 min, S/L=1:15)	53.3 (US+M: 6 min + 3 days, S/L=1:15)	49.1 (M: 9 days, S/L=1:30)
Flavonoids (mg QE/g)	117 (R: 80% concentration, 60 min, S/L=1:15)	36.1 (US+M: 6 min + 9 days, S/L= 1:20)	35.3 (M:9 days, S/L=1:20)

Table 5. Comparative Results between Hydroalcoholic and Hydroglycerin Solvent Extraction

Extracted Compound	Type of Solvent/ Method Characteristics	
	EtOH- Water (Turcov *et al.* 2022)	Glycerin – Water [this study]
Polyphenols (μg GAE/g)	78.9 (R: 50% concentration, 60 min, S/L=1:30)	85.0 (R: 50% concentration, 60 min, S/L=1:15)
Flavonoids (mg QE/g)	66.0 (Sx: 70% concentration, 90 min, S/L=1:20)	117 (R: 80% concentration, 60 min, S/L=1:15)

From the results in Table 4 and 5, it can be seen that the solid-liquid extraction using different hydro-glycerin solutions as extraction solvent led to higher amounts of polyphenols and flavonoids compared to the extraction using hydro-alcoholic solutions.

These research results are part of the broader field of valorization of bioactive compounds from plants, such as the *Galium verum* plant (Chita *et al.* 2012; Mocan *et al.* 2016; Al-Snafi 2018; Farcas *et al.* 2018; Hanhanu *et al.* 2018; Shynkovenko *et al.* 2018; Mocan *et al.* 2019; Tava *et al.* 2020; Bradic *et al.* 2023). In this regard, it is worth noting that phytochemicals are typically extracted from plant materials using organic solvents, often in varying proportions of water, depending on the properties of the target compounds. This variability in extraction solvents can complicate direct comparisons with previous studies, as different solvents may yield differing results for the same compounds. For instance, Lakić *et al*. (2010) observed variations in antioxidant activity, which were partially explained by the levels of phenolics (2.44–4.65 mg and 4.57–5.16 mg GAE/g dry extract), flavonoids (6.38–10.70 μg and 15.56–17.96 μg QE/g dry extract), and chlorophylls in methanolic extracts of *Galium verum* (Lakić *et al.* 2010). Similarly, Vlase *et al*. (2014) reported a DPPH free radical scavenging activity of 105 μg/mL for the 70% ethanolic extract of *G. verum*. Semenescu *et al*. (2023) analyzed the antioxidant capacity of the aqueous extract of *G. verum* at six different concentrations (1 mg/mL to 0.05 mg/mL), comparing it to an ascorbic acid ethanolic solution (AA), and found antioxidant activity ranging between 30% and 50%. Finally, Friščić *et al*. (2018) reported an extraction yield of 18%, total phenolics of 86.40 ± 1.74 mg GAE/g dry extract, and flavonoids at 23.11 ± 0.12 mg QE/g dry extract in their study of *G. verum*.

The extract of *G. verum* and the various compounds separated from it have found applications in medicine or in different phytopharmaceutical products. Less studied is the

cosmetic and dermato-cosmetic field where the authors have obtained a series of encouraging results (Turcov *et al.* 2022, 2023).

Directions for Further Research

Following the present results, some potential future research directions for building upon the successful use of glycerin for extracting bioactive compounds with potential antioxidant action, could be:

a) *Extract characterization and standardization*:
- Development of a standardized extract and establish criteria for the quality and consistency of the glycerin extract to ensure reproducible bioactivity.
- Formulation and stability studies.
- Dermato-cosmetic product development incorporating the glycerin extract and evaluate their stability and efficacy.
- *In vitro* and *in vivo* studies to evaluate the antioxidant, anti-inflammatory, and other beneficial effects of the formulated products on the skin.

b) *Green solvent exploration and comparison:*
- Extraction of polyphenols and flavonoids.

c) *Sustainability and life cycle assessment*:
- Environmental impact assessment of glycerin extraction and product formulation.

The conclusions of the analyses in this study highlight the possibility of developing new dermatocosmetic formulations that aim to address oxidative stress on the skin while also paving the way for further research on the extract and the emulsions made with it as a biologically active compound.

CONCLUSIONS

In order to combat oxidative stress at the skin level, this study demonstrated that *Galium verum* plants from the Romanian spontaneous flora represent a significant source of bioactive chemicals with potential antioxidant properties. As a result, they can be used in dermatocosmetic preparations.

1. This study investigated the use of glycerin (in aqueous, alcoholic, and hydroalcoholic solutions) as a solvent for extracting polyphenols and flavonoids, thereby contributing to a deeper understanding of the bioactive compounds in *Galium verum*.

2. By simple refluxing with hydro-glycerinic solvent, the highest amount of flavonoids (117 mg QE/g), twice the amount from using a hydro-alcoholic solvent, was obtained from the dried plant of *G. verum*, and the highest amount of polyphenols (85.0 µg GAE/g) was higher than that obtained with a hydro-alcoholic solvent with 50% and respectively 80% concentration.

3. In the case of extracts using glycerin-water-alcohol solvent, the highest amount of polyphenols (53.3 µg GAE/g) was obtained in the case of the extract resulting from the combined US+M method made with solvent mixture S1 (30% glycerin + 50% alcohol + 20% water), an extraction time of 6 min + 3 days and using S/L ratio = 1:15, and the largest amount of flavonoids (36.1 mg QE/g) in the case of the extract from the combined (US+ M) method made with S2 solvent solution (50% glycerin and 50% ethanol of 96%) an extraction time of 6 min + 9 days and using an S/L ratio of 1:20.

4. From the point of view of the antioxidant content, determined as total flavonoids and polyphenols the extract of dried *G. verum* plant material proved to be satisfactory and supports to the idea of replacing chemical actives with natural ones in the formulation of certain dermatocosmetic preparations. In this sense, this preliminary study is the basis for studying new directions for its in-depth characterization and inclusion in new dermatocosmetic preparations with their testing as formulations for combating oxidative stress on the skin.

REFERENCES CITED

Al-Snafi, A.E. (2018). "*Galium verum*–A review," *Indo Am. J. Pharm. Sci*. 05, 2142-2149.

Apostolakis, A., Grigorakis, S., and Makris, D. P. (2014). "Optimization and comparative kinetics study of polyphenol extraction from olive leaves (*Olea europaea*) using heated water/glycerol mixtures," *Sep. Purif. Technol.* 128, 89-95. DOI: 10.1016/j.seppur.2014.03.010 .

Arias, A., Rodriguez, S. G., Barros, M. V., Salvador, R., de Francisco, A. C., Piekarski, C. M., and Moreira, M. T. (2021). "Recent development in biobased adhesives from renewable natural resources," *J. Clean. Prod.* 314(1-2), article 1278972. DOI: 10.1016/j/jclepro.2021.127892

Bajpai, P. (2016). "Structure of lignocellulosic biomass," in: *Pretreatment of Lignocellulosic Biomass for Biofuel Production*, S. K. Sharma (ed.), Springer, Singapore. DOI: 10.1007/978-981-10-0687-6_1.

Bedoić, R., Čuček, L., Ćosić, B., Krajnc, D., Smoljanić, G. Z., Ljubas, D., Pukšec, T., and Duić, Ne. (2019). "Green biomass to biogas – A study on anaerobic digestion of residue grass," *J. Clean. Prod.* 213, 700-709. DOI: 10.1016/j. jclepro.2018.12.224.

Besil, M. N. A., Pequeño, F., Alonzo, N., Hladki, R., Cesio, M. V., and Heinzen, H. (2017). "Evaluation of different QuEChERS procedures for pesticide residues determination in *Calendula officinalis* (L.) inflorescences," *J. Appl. Res. Med. Aromat. Plants* 7, 143-148. DOI: 10.1016/j.jarmap.2017.09.001

Borrero-López, A. M., Valencia, C., and Franco, J. M. (2022). "Lignocellulosic materials for the production of biofuels, biochemicals and biomaterials and applications of lignocellulose-based polyurethanes: A Review," *Polymers* 14, article no. 881. DOI: 10.3390/polym14050881

Bradic, J., Petkovic, A., and Tomovic, M. (2018). "Phytochemical and pharmacological properties of some species of the Genus Galium L. (*Galium Verum* and *Mollugo*)," *Serb. J. Exp. Clin. Res*. 22(3), 187-193. DOI: 10.1515/SJECR-2017-0057

Bradic, J., Andjic, M., Novakovic, J., Kocovic, A., Tomovic, M., Petrovic, A., Nikolic, M., Mitrovic, S., Jakovljevic, V., and Pecarski, D. (2023). "Lady's bedstraw as a powerful antioxidant for attenuation of doxorubicin-induced cardiotoxicity," *Antioxidants (Basel)*12(6), article no.1277. DOI: 10.3390/antiox12061277

Cheng, F., Feng, J., Cao, Z., Duan, Q., and Li, H. (2024). "Efficacy and safety of topical application of plant-based products on skin aging in healthy individuals: A systematic review and meta-analysis of randomized controlled trials," *J. Cosmet. Dermatol.* 24(2), article e16710. DOI: 10.1111/jocd.16710

Chien-Hsing, L., Guan-Cheng, H., and Chung-Yi C. (2017). "Bioactive compounds from natural extracts in Taiwan cosmeceuticals – Mini review," *Biomed. Res.* 28(15), 6561-6566.

Christ, B., and Müller, K. H. (1960). "Zur serienm¨aßigen Bestimmung des Gehaltes an Flavonol-Derivaten in Drogen," *Archiv der Pharmazie* 293(12), 1033-1042.

Di Raimo, R., Davide Mizzoni, D., Aloi, A., Pietrangelo, G., Dolo, V., Poppa, G., Fais, S., Logozzi, M. (2024). "Antioxidant effect of a plant-derived extracellular vesicles' mix on human skin fibroblasts: Induction of a reparative process," *Antioxidants* 13(11), article no.1373. DOI: 10.3390/antiox13111373.

Farcas, D. A., Mot, C. A., Zagrean-Tuza, C., Toma, V., Cimpoiu, C., Hosu, A., Parvu, M., Roman, I., and Silaghi-Dumitrescu, R. (2018). "Chemo-mapping and biochemical-modulatory and antioxidant/ prooxidant effect of *Galium verum* extract during acute restraint and dark stress in female rats," *PLoS One* 13, article e0200022. DOI: 10.1371/journal.pone.0200022

Ferreira-Santos, P., Zanuso, E., Genisheva, Z. I., Rocha, C. M. R., and Teixeira, J. A. (2020). "Green and sustainable valorization of bioactive phenolic compounds from *Pinus* by-products," *Molecules* 25(12), article 2931. DOI: 10.3390/molecules25122931

Friščić, M., Baglama, M. Š., Milović, M., Pilepić, K. H., and Maleš, Ž. (2018). "Content of bioactive constituents and antioxidant potential of *Galium* L. species," *Croat. Chem. Acta* 91(3), 411-417.

García-Beltrán, J. M., and Esteban, M. Á. (2016). "Properties and applications of plants of *Origanum* Sp. Genus," *SM J. Biol*. 2, 1006-1015.

Ghita, G., Necula, R., Trifan, A., Gille, E., Zamfirache, M. M., and Stanescu, U. (2012). "Investigations regarding the phytochemical study of some samples of *Galium verum* L. and *Galium album mill*.," *Sci. Ann. Al. I. Cuza Univ. Iasi* 58, 45-50.

Hanganu, D., Burtescu, R. F., Petrescu, S., Pripon Furtuna, F. R., Chise, E., Turcus, V., Benedec, D., Oniga, I., and Olah, N. K. (2018). "*Galium* species–polyphenolic content and their antioxidant potential," *Hop Med. Plants* 1–2, 84-93.

Haq, I., Qaisar, K., Nawaz, A., Akram, F., Mukhta, H., Zohu, X., Xu, Y., Mumta, M. W., Rashid, U., Karim Ghani, W. A. W., and Choong, T. S. Y. (2021). "Advances in valorization of lignocellulosic biomass towards energy generation," *Catalysts* 11, article no.309. DOI: 10.3390/catal11030309

Huamán-Castilla, N. L., Mariotti-Celis, M. S., Martínez-Cifuentes, M., and Pérez-Correa, J. R. (2020). "Glycerol as alternative co-solvent for water extraction of polyphenols from *Carménère pomace*: Hot pressurized liquid extraction and computational chemistry calculations," *Biomolecules*. 10(3), article no. 474. DOI: 10.3390/biom10030474

Ji, H., Dong, C., Yang, G., and Pang, Z. (2018). "Valorization of lignocellulosic biomass towards multi-purpose fractionation: furfural, phenolic compounds and ethanol," *ACS Sustainable Chem. Eng*. 6, 15306-15315. DOI: 10.1021/acssuschemeng.8b03766

Kee, S. H., Chiongsoa, J. B. V., Saludes, J. P., Vigneswari, S., Ramakrishna, S., and Bhubalan, K. (2021). "Bioconversion of agro-industry sourced biowaste into biomaterials *via* microbial factories – A viable domain of circular economy," *Environ. Pollut.* 271, article no. 116311. DOI: 10.1016/j.envpol.2020.116311

Konisky, H., Bowe, P. W., Yang, P., and Kobets, K. (2023). "A clinical evaluation of the efficacy and tolerability of a novel topical antioxidant formulation featuring vitamin

C, astaxanthin, and fermented turmeric," *J.Cosmet. Dermatol.* 22, 3088-3094. DOI: 10.1111/jocd.15967

Kozma, M., Acharya, B., and Bissessur, R. (2022). "Chitin, chitosan, and manochitin: Extraction, synthesis, and applications," *Polymers* 14(19), article no. 3989. DOI:10.3390/polym14193989

Kabir, S. K. F., Sikdar, P. P., Haque, B., Bhuiyan, M. A. R., Ali, A., and Islam, M. N. (2018). "Cellulose –based hydrogel materials: Chemistry, properties and their prospective applications," *Progress in Biomaterials* 7, 153-174. DOI: 10.1007/s40204-018-0095-0

Kumar, N., and Pruthi, V. (2015). "Structural elucidation and molecular docking of ferulic acid from *Parthenium hysterophorus* possessing COX-2 inhibition activity," *3Biotech.* 5, 541-551. DOI 10.1007/s13205-014-0253-6

Lakić, N. S., Mimica-Dukić, N. M., Isak, J. M., and Božin, B. N. (2010). "Antioxidant properties of *Galium verum* L. (Rubiaceae) extracts," *Cent. Eur. J. Biol.* 5(3), 331-337. DOI: 10.2478/s11535-010-0022-4

Langsdorf, A., Volkmar, M., Holtmann, D., and Ulber, R. (2021). "Material utilization of green waste: A review on potential valorization methods," *Bioresour. Bioprocess*. 8, 19.

Le, T. X., Dang, P. Y., Tran, U. K. N., Nguyen, N. Q. T., and Tran, T. T. (2025). "High potential of herbal plant extracts for skin protection from ultraviolet radiation," *Indonesian Journal of Chemistry* 25(1), 190-201. DOI: 10.22146/ijc.99167.

Li, W., Mu, X., Wu, X., He, W., Liu, Y., Liu, Y., Deng, J., and Nie, X. (2022). "*Dendrobium nobile* Lindl. polysaccharides protect fibroblasts against UVA-induced photoaging via JNK/c-Jun/MMPs pathway," *J. Ethnopharmacol.* 298, article 115590.

Mocan, A., Crisan, G., Vlase, L., Ivanescu, B., Badarau, S. A. and Arsene, L. A. (2016). "Phytochemical investigations on four *Galium* species (Rubiaceae) from Romania," *Farmacia* 64, 95-99.

Mocan, A., Zengin, G., Mollica, A., Uysal, A., Gunes, E., Crișan, G., and Aktumsek, A. (2018). "Biological effects and chemical characterization of *Iris schachtii* Markgr extracts: A new source of bioactive constituents," *Food Chem. Toxicol*. 112, 448-457.

Mocan, A., Diuzheva, A., Badarau, S., Moldovan, C., Andruch, V., Carradori, S., Campestre, C., Tartaglia, A., De Simone, M., Vodnar, D. *et al*. (2019). "Liquid phase and microwave-assisted extractions for multicomponent phenolic pattern determination of five Romanian *Galium* species coupled with bioassays," *Molecules* 24, article 1226. DOI: 10.3390/molecules24071226

Montenegro, L. (2014). "Nanocarriers for skin delivery of cosmetic antioxidant," *J. Pharm. Pharmacogn. Res.* 2-4, 73-92. DOI: 10.56499/jppres14.033_2.4.73

Nastasi, J. R., Daygon, V. D., Kontogiorgos, V., and Fitzgerald, M. A. (2023). "Qualitative analysis of polyphenols in glycerol plant extracts using untargeted metabolomics," *Metabolites* 13, article no. 566. DOI: 10.3390/metabo13040566

Ndiaye, M., Philippe, C., and Ahmad, N. (2011). "The grape antioxidant resveratrol for skin disorders: Promise, prospects, and challenges," *Arch. Biochem. Biophys*. 508, 164-170. DOI: 10.1016/j.abb.2010.12.030

Omer, A. M. (2018). "Advanced in biomass and biogas energy," *CTBEB* 11(1), 21-23. DOI: 10.19080/CTBEB.2018.11.555804

Oreopoulou, A., Choulitoudi, E., Tsimogiannis, D., and Oreopoulou, V. (2021). "Six common herbs with distinctive bioactive, antioxidant components. A review of their

separation techniques," *Molecules* 26, article 2920. DOI: 10.3390/molecules26102920

Pietta, P., Minoggio, M. S., and Bramati, L. (2003). "Plant polyphenols: Structure, occurrence and bioactivity," in: *Bioactive Natural Products (Part I), Studies in Natural Products Chemistry*, A. Rahman (ed.), Elsevier Science B.V., Amsterdam, The Netherlands.

Ribet, V., Nobile, V., and Rossi, A. B. (2019). "*In situ* antioxidant activity of a dermato-cosmetic product: A randomized controlled clinical study," *Exp. Dermatol*. 28(11), 1219-1226. DOI: 10.1111/exd.14005

Rodriguez- Rodriguez, R., Espinosa-Andrews, H., Velasquillo-Martinez, C., and Garecia-Carvajal, Z. Y. (2020). "Composite hydrogel based on gelatin, chitosan and polyvinyl alcohol to biomedical application: A review," *Int. J. Polym. Mater. Polym. Biomater.* 69(1), 1-20. DOI: 10.1080/00914037.2019.1581780

Roncea, F., Miresan, M., Rosca, C., and Cazacincu, R. (2016). "Cosmetic vs. Cosmeceuticals," *Farmacist Ro* (Dermato-Cosmetology Supplement); Medichub Media, Bucharest, Romania, (in Romanian).

Riva, L., Fiorati, A., and Punta, A. (2021). "Synthesis and application of cellulose-polyethyleneimine composites and nanocomposites," *Materials* 14, article 473. DOI:10.3390/ma14030473

Saxena, H. O., Tripathi, Y. C., Pawar, G., and Kakkar, A. (2014). "Botanicals as biopesticides: Active chemical constituents and biocidal action," in: *Familiarizing with Local Biodiversity*, P. K. Khatri and P. B. Meshram (eds.), Tropical Forest Research Institute (ICFRE): Jabalpur, India, pp. 227-246.

Semenescu, A. D., Moacă, E. A., Chioibaş, R., Iftode, A. , Tchiakpe-Antal, D. S., Vlase, L., Vlase, A. M., Muntean, D., and Dehelean, C. A. (2023). "Phytochemical and biological screening of aqueous *Galium Verum* L. Extract," *The Annals of the University Dunarea de Jos of Galati (Romania)*. *Fascicle VI – Food Technology* 47(2), 172-189.

Shynkovenko, I. L., Ilyna, V. T., Kovalyova, M. A., Goryacha, V. O., Golembiovska, I. O, and Koshovyi, M. O. (2018). "Saponins of the extracts of *Galium aparine* and *Galium verum*," *Vìsnik Farm.* 4, 16-23.

Singleton, V., Orthofer, R., and Lamuela-Raventos, R. M. (1999). "Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent," *Methods Enzymol*. 299, 152-178. DOI: 10.1016/S0076-6879(99)99017-1

Soto, M. L., Parada, M., Falqué, E., and Domínguez, H. (2018). "Personal-care products formulated with natural antioxidant extracts," *Cosmetics* 5, article 13. DOI: 10.3390/cosmetics5010013.

Tagami, H., Kobayashi, H., O' Goshi, K., and Kikuchi, K. (2006). "Atopic xerosis: Employment of noninvasive biophysical instrumentation for the functional analyses of the mildly abnormal stratum corneum and for the efficacy assessment of skin care products," *J. Cosmet. Dermatol*. 5(2), 140-149. DOI: 10.1111/j.1473-2165.2006.00241.x

Tava, A., Biazzi, E., Ronga, D., and Avato, P. (2020). "Identification of the volatile components of *Galium verum* L. and *Cruciata leavipes* Opiz from the western Italian Alps," *Molecules* 25, article 2333. DOI: 10.3390/molecules25102333

Tomas, M., Günal-Köroğlu, D., Kamiloglu, S., Ozdal, T., Capanoglu, E. (2025). "The state of the art in anti-aging: plant-based phytochemicals for skin care," *Immunity & Ageing*. 22, article 5. DOI:10.1186/s12979-025-00498-9

Turcov, D., Zbranca, A., Horciu, I. L., and Suteu, D. (2020a). "Resveratrol in the prevention and treatment of oxidative stress," *The Buletin of Polytehnic Insitute from Iasi Chemistry and Chemical Engineering Section published by "Gheorghe Asachi" Technical University of Iasi, Romania* 66 (70) (2), 53-65.

Turcov, D., Rusu, L., Zbranca, A., Suteu, D. (2020). "New dermatocosmetic formulations using bioactive compounds from indigenous natural sources," *The Buletin of Polytehnic Insitute from Iasi Chemistry and Chemical Engineering Section published by "Gheorghe Asachi" Technical University of Iasi, Romania* 66, 67-76.

Turcov, D., Zbranca, A., Rusu, L, and Suteu, D. (2021). "Lycopene – background, perspectives and challenges in dermato-cosmetic formulas," *The Bulletin of Polytechnic Institute from Iasi Chemistry and Chemical Engineering Section published by "Gheorghe Asachi" Technical University of Iasi, Romania* 67 (71) (2), 9-20.

Turcov, D., Barna, A. S. , Trifan, A., Blaga, A. C., Tanasa, A. M., and Suteu, D. (2022). "Antioxidants from *Galium verum* as ingredients for the design of new dermatocosmetic products," *Plants- Basel*. 11(19), article no. 2454. DOI: 10.3390/plants11192454

Turcov, D., Butnaru, M., Zbranca-Toporas, A., and Suteu, D. (2023). "Biocompatibility investigation and preliminary tolerability observational study of the dermatocosmetic emulsions based on saffron (*Crocus sativus*) and lady's bedstraw (*Galium verum*)," in: *Proceedings of the 11th IEEE International Conference on E-Health and Bioengineering—EHB 2023*, Grigore T. Popa University of Medicine and Pharmacy Iasi, Bucharest, Romania, 9-10 November 2023.

Venkat Ratnam, D., Ankola, D. D., Bhardwaj, V., Sahana, D. K., and Ravi Kumar, M. N. V. (2006). "Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective," J. *Control. Release* 113(3), 189-207. DOI:10.1016/j.jconrel.2006.04.015

Velvizhi, G., Goswami, C., Shetti, N. P., Ahmad, E., Pant, K. K., and Aminabhavi, T. M. (2022). "Valorisation of lignocellulosic biomass to value-added products: Paving the pathway towards low-carbon footprint," *Fuel* 313(1), article 122678. DOI: 10.1016/J.FUEL.2021.122678

Vlase, L., Mocan, A., Hanganu, D., Benedec, D., Gheldiu, A., and Crișan, G. (2014). "Comparative study of polyphenolic content, antioxidant and antimicrobial activity of four G*alium* species (Rubiaceae)," *Dig. J. Nanomater. Biostruct.* 9(3), 1085-1094.

Walia, S., Saha, S., Tripathy, V., and Sharma, K. K. (2017). "Phytochemical biopesticides: Some recent developments," *Phytochem. Rev*. 16, 989-1007. DOI: 10.1007/s11101-017-9512-6

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