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Chemical profiling and biological activities of *Opopanax hispidus* extracts: A comparative insight on conventional and green extraction technologies

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Abstract

The plant species *Opopanax hispidus* is known in traditional Turkish medicine and nutrition. In addition to all the advantages that are known by the traditions of old generations, *O. hispidus* has been insufficiently examined, and its potential could be used in treatment in modern medicine and diet. In this paper, *O. hispidus* extracts obtained by conventional (maceration (MAC), and Soxhlet (SOX)) and contemporary (accelerated solvent extraction (ASE), homogenizer assisted extraction (HAE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE)) methods were examined in terms of their chemical composition and biological potential. The extract obtained by HAE extraction had the highest content of total phenolics and flavonoids (36.98 mg GAE/g and 15.04 mg RE/g, respectively). The highest antioxidant activity was recorded for HAE and ASE extracts. *O. hispidus* extracts achieved inhibitory activity against acetylcholinesterase (4.12–6.44 mg GALAE/g), tyrosinase (44.78–76.72 mg KAE/g), α-amylase (0.35–0.46 mmol ACAE/g), and α-glucosidase (1.67-1.77 mmol ACAE/g) enzymes. LC-MS technique was used for the quantitative analysis of the extracts, allowing the quantification of chlorogenic acid, neochlorogenic acid, and *p*-coumaric acid, rutin, luteolin, and kaempferol 3-*O*-glucoside as the most abundant phenolic components. Results suggested that the green extraction techniques for obtaining extracts that are rich in bioactive compounds have advantages compared to conventional extraction. Therefore, these extracts can be used as antioxidants, neuroprotective, antityrosinase, and antidiabetic agents while respecting the principles of sustainable development and green chemistry.

Keywords: *Opopanax*; traditional extraction; green technique; phenolics; pharmaceuticals

1. Introduction

The production and placement of products with natural constituents is on the rise, and the demand for these products has increased especially in the COVID-19 post-pandemic period. The reason for the high demand for these products is their effectiveness in the treatment and prevention of many serious medical conditions. Numerous products of the pharmaceutical industry as the main active principles contain secondary plant metabolites, which, unlike synthetic ones, have fewer side effects. In addition to the very strict requirements and criteria of the pharmaceutical industry, the production of such extracts increasingly requires the use of innovative environmentally friendly technologies. This includes the use of green solvents, as well as techniques capable of producing an extract of the desired characteristics in a time-effective and energy-effective way. Green extraction technologies that occupy a leading place are accelerated solvent extraction (ASE), homogenizer-assisted extraction (HAE), microwave (MAE), ultrasonic (UAE), and supercritical fluid extraction (SFE). Each of these techniques is based on a specific phenomenon that is responsible for the efficiency of the process: in the case of the ASE technique, the extraction is based on elevated pressure and temperature, which increases the infiltration of solvent into the plant while regulating the dielectric constant of the solvent (Cvetanović et al., 2018); in HAE extraction high rotation speed provides a high extraction yield without external heating (Eyiz, Tontul, & Turker, 2020); utilization of MAE technique is entrenched on the interaction between microwaves and solvent molecules, which causes very fast heating and increased process efficiency (Ng & Hupé, 2003) while in UAE the main phenomenon is cavitation, which leads to the penetration of solvents into plant cells, dissolving the components and transporting them outside the cell; In the case of SFE extraction, bringing the fluid into a supercritical state affects its physico-chemical and solvent characteristics. First of all, there is a change in its viscosity, and the fluid diffuses more easily into the matrix, which results in better mass transfer. Which

extraction technique will be applied depends on the plant material itself and the target components. Therefore, for each plant material, it is recommended to find optimal technique as well as extraction conditions.

Wild-growing plants are known as important source of bioactive molecules which, in addition to scientific studies, have a great industrial perspective (Cvetanović, 2020). The great advantage of wild-growing plant species lies in the fact that they do not require special growth conditions, in terms of providing nutrients to the soil, cultivation, or capital investments. These plant species are rich in secondary metabolites such as polyphenols, coumarins, alkaloids, terpenes, and other compounds (Pereira et al., 2020), that they synthesize increasingly in order to defend themselves against pest attacks and microbiological contamination.

The plant species *Opoponax hispidus* belongs to the Apiaceae family and is particularly important due to its great medicinal and economic potential. The plant species *Opoponax hispidus* is widespread in the Balkan Peninsula, the Aegean region, and especially in Turkey (Önder, Nahar, Nath, & Sarker, 2020). Opopanax species are known by the names Kaymakotu, Kaymacık, and Kirkozar in Turkey and with the name "Hercules' all-heal" in the world. This plant species is recognizable by its yellow flowers and has high value in traditional diet and medicine with a purpose to cure hemorrhoids, female infertility, and purification of blood, for preparing tea, and is also used as a spice (Çölçimen, 2020). Despite its exceptional medicinal value and traditional use, there are still no commercial products based on *O. hispidus* on the market. Utilization of the natural potential of *O. hispidus* can include the formulation of products not only for the pharmaceutical but also for the cosmetic and food industries.

On the other hand, scientific data about this plant in the literature are very scarce. Namewly, only two articles on extracts of *O. hispidus* were published so far. The paper published by

Gümüşok, SARIALTIN, ÇOBAN, and KILIÇ (2021) was focused on its antioxidant and anti-inflammatory activity, while in the study of Matejić, Džamić, Mihajilov-Krstev, Ranđelović, and Marin (2015), total phenolics compounds content, antioxidant, and antimicrobial properties were determined. Although its biological activity has been confirmed based on the abovementioned data, defining the procedure for obtaining highly valuable extracts is of crucial importance. This implies the selection of the most suitable extraction technique and an insight into the effects of all process parameters on the efficiency of the process. Accordingly, the aim of this paper was investigation and definition of the most suitable extraction technique for the isolation of bioactive components, primarily polyphenols, from *O. hispidus*. For this purpose, modern and conventional techniques were aplied, the influence of the technique on the content of individual polyphenolic components in the extracts, as well as on the biological activity, was determined. Moreover, data on the enzyme-inhibitory effect appear for the first time in this article.

2. Materials and methods

2.1. Collection and preparation of plant material

In the summer of 2021, aerial parts of *Opopanax hispidus* were collected (between Hadim and Taşkent villages, Konya, 1490 m) in Turkey. The plant specimens were identified by Dr. Evren Yildiztugay, and one specimen from the plants was deposited at the Selcuk University herbarium. Prior to extraction, the plant materials were carefully washed with tap and distilled water to eliminate any soil and contaminants. After being dried naturally, the aerial parts were ground to a powder form.

2.2. Extraction technologies

The powdered areal parts of *O. hispidus* were extracted using seven different techniques, devided in two groups: (1) modern and (2) traditional techniques. As modern techniques the

following ones were applied: Accelerated solvent extraction (ASE), homogenizer-assisted extraction (HAE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and supercritical fluid (carbon-dioxide) extraction (SFE). On the other hand, maceration (MAC) and Soxhlet extraction (SOX) were aplied as traditional techniques.

2.2.1. Modern extraction techniques

ASE was performed with ethanol (96%) at the temperature of 120°C and the pressure of 1500 psi following the procedure previously described in Sut et al. (2019). HAE was performed with 2 g of plant material and extracted with 20 mL of 96% ethanol following the procedure previously descibed in Dall'Acqua et al. (2020). UAE was done in a sonication bath while MAE was carried out in modified microwaved oven described in Cvetanović Kljakić et al. (2023) for 30 min at 600 W. In both MAE and UAE the sample-to-solvent ration was the same: 1:20. SFE was performed with 50g of plant material that was placed into extractor. Extraction was done at constant temperatures of 50°C and pressure of 300 bar during 5 hours. The conditions in the separator were 15 bar and 25 °C. Extracts were stored protected from the influence of temperature and light.

2.2.2. Traditional extraction techniques

In MAC process, plant material was mixed with solvent (96% ethanol) in a proportion of 1:20, while SOX was performed according to the standardized Soxhlet procedure for 6 hours. By the process of evaporating, the extracts were converted into a solid state and stored in the prescribed manner.

2.3. Determination of total bioactive molecules

Total bioactive molecules phenols and flavonoids were determined by spectrophotometric methods with Folin-Ciocalteu and $AlCl_3$, respectively (Uysal et al., 2017). The obtained results of analyzes were calculated and expressed as mg GAE/g for total phenolics, and mg Re/g for total flavonoids.

2.4. UHPLC–MS/MS analysis of polyphenolic compounds

UHPLC–MS/MS analysis was performed using methods previously described in detail in the paper (Nedić et al., 2022).

2.5. Determination of antioxidant and enzyme inhibitory effects

Biological and pharmacological potential which included examination of antioxidant and enzyme inhibitor potential of *O. hispidus* extracts were evaluated by *in vitro* assays. Antioxidant activity of the analyzed extracts was measured by DPPH, ABTS, FRAP, CUPRAC, total antioxidant activity (PBD) and metal chelating (MCA) assays (Uysal et al., 2017). In terms of enzyme excessive activity, neuroprotective, antityrosinase, and antidiabetogenic activity was estimated using enzymes cholinesterases (AChE and BChE), tyrosinase, α-amylase, and α-glucosidase. Obtained results were expressed in the way prescribed by official methods by using appropriate standards (Uysal et al., 2017).

2.6. Statistical analysis

All measurements, carried out in this research, were repeated three times, and the presented results are the mean values of three repetitions. All obtained data were statistically processed according to the method described in detail in our previous work (Nebrigić et al., 2023).

3. Results and Discussion

3.1 Content of total phenolics and flavonoids in *O. hispidus* extracts

The pharmaceutical and food industries are turning to the use of natural products, primarily plant extracts, in the formulation of their products. In the process of making extracts with high biological potential, the most important step is developing a procedure that would be enable to ensure maximal yield of bioactive ingredients and to ensure high-quality extracts. Therefore, this work is based on defining the most efficient extraction technique for the

extraction of polyphenols, as one of the main bioactives, from *O. hispidus*. The content of total phenols and flavonoids in the extracts obtained by modern (HAE, MAE, UAE, SFE) and traditional (MAC, SOX) extraction techniques is presented in the Table 1.

Except of SFE all modern techniques ensured higher content of total phenols and flavonoid than traditional ones. Applied SFE technique is selective towards volatile compounds, and not suitable for the extraction of polyphenolic compounds. However, although these compounds are predominantly moderately polar, their polarity can vary widely due to structural differences. Precisely because of this, the sample obtained by SFE technique contained both phenols and flavonoids, which is in line with previous research (Pavlić et al., 2021). The highest TP was observed in samples obtained by HAE (36.98 mg GAE/g), while all other modern techniques provided similar TP content. In terms of traditional techniques, MAC provided a higher TP content compared to SOX, which may be the influence of extraction time. SOX is a reference technique that runs for 6 hours, while MAC requires 24 hours. Longer contact of the solvent with the plant material can provide a higher yield of TP (Zengin, Cvetanović, et al., 2020).

The obtained data show that the proportion of flavonoids in extracts obtained by traditional techniques (43.69-43.89%) is higher than in the extracts prepared by modern techniques (6.21-41.68%). However, the total content of flavonoids is higher in extracts obtained in an unconventional way. As it was mentioned before, SFE exhibited poor selectivity towards flavonoids thus the lowest TF content was determined in SFE extract.

Comparing the obtained results with the study conducted by Matejić et al. (2015) it can be noticed that the extracts examined in this paper had a lower content of total phenolic molecules, which may be a consequence of the use of different solvents, extraction techniques, as well as the influence of environmental factors in which the plant grew. Determination of the content of total phenolic compounds by spectrophotometric methods cannot give a complete chemical characterization and cannot identified dominant

polyphenolic compounds that can give high contribution to the biological and pharmacological activity of *O. hispidus* extracts, which is why a detailed analysis of the phytochemical composition was determined using the LC-MS/MS method.

3.2 Phytochemical composition of *O. hispidus* extracts

Chemical profile of seven different *O. hispidus* extracts was defined by LC-MS/MS analysis. In investigated extracts, the presence of 18 phenolic compounds was identified, of which neochlorogenic acid was quantified in the highest concentration (Table 2).

Out of a total of 18 quantified compounds, 4 belong to the class of phenolic acids, and 15 to the class of flavonoids, of which 8 compounds are present in the form of aglycones, while 7 are present in bound forms. As it was expected, the lowest yield of individual components was in the extracts obtained by the SFE technique. The following compounds were quantified in the SFE extract: Chlorogenic acid, neochlorogenic acid, rutin, quercetin 3-*O*-glucoside, eriodictyol, pinocembrin. Interestingly, the yield of pinocembrin was higher in the SFE sample compared to that obtained by SOX extraction. This flavonoid is characterized by low solubility in water, so the non-polarity of supercritical CO_2 probably favors its extraction from natural sources, which is of great importance considering the high biological importance of this flavonoid. Pinocembrin is characterized by exceptional antioxidant, antibacterial, anti-inflammatory, and antineoplastic activities, and has great potential medicinal value for the treatment of ischemic stroke, neurodegenerative and cardiovascular diseases (Pei & Sun, 2018; Yan et al., 2014). In the observed SFE samples, the yield of this compound was higher than that of SOX extracts, and lower than that of UAE extracts, while other techniques were not able to ensure its isolation.

When it comes to flavonoid aglycones (Fig.1), ASE proved to be the technique that ensures their highest content in extracts, while the content achieved by the MAE technique was the

lowest. The reason for the low yield of aglycones in MAE extracts may be the increase in temperature under the influence of microwave irradiation, which can lead to overheating and degradation of agycones. In the case of the ASE technique, the aplied temperature was also high, but the extraction time was only a few minutes, which helped to avoid their thermo-degradation while taking advantage of the temperature effect. Another reason for the high efficiency of the ASE process is the fact that this process is performed at elevated pressure (1500 psi). Increased pressure during extraction improves solvent penetration inside plant pores, elevated temperatures in a short time interval favor their dissolution and extraction. Further, high pressure possesses positive effect on air bubbles in the extraction system. Namely, during the penetration of the solvent into the plant cell, air bubbles may appear, which slows down the mass transfer process. Applying high pressure during extraction this problem can be overcome (Cvetanović et al., 2018; Mustafa & Turner, 2011).

UAE is another modern extraction technique which, after ASE, has shown great advantage for the isolation of aglycones from *O. Hispidus*. In this technique, the cavitation effect helps to disrupt the cell wall, which facilitates the penetration of the solvent inside the cell. In this way, the strong effects of the plant matrix can be overcome. Based on the obtained results, it was shown that satisfactory results are also achieved by applying the MAC and SOX techniques, in which mild extraction conditions prevail.

UAE was observed to be the most effective in *C*-glucosides extraction (isoorientin and vitexin), followed by maceration and HAE, while microwave extraction resulted in their lowest content. According to their structure, both compounds represent bound, glycosidic, forms of flavones that require a more moderate temperature for their extraction (Cvetanović et al., 2019). Overheating of the extraction mixture in the MAE system can lead to the breaking of glycosidic bonds in glycoside molecules and the degradation of these molecules. This may be one of the reasons for their lowest content in MAE extracts. In addition to these two

compounds, other bound forms also showed a low degree of extraction by microwave extraction and were better extracted by ASE and UAE techniques.

In the case of phenolic acids, it was observed that the extraction techniques had a different effect on each of the individual phenolic acids. This shows that although they belong to the same class of compounds, each of the dominant extraction phenomena affects the individual molecule differently due to the structural differences between the molecules. In general, ASE proved to be a good option in all phenolic acids, as well as UAE and SOX. In the case of chlorogenic acid, MAE showed much better efficiency, but in the case of other phenolic acids, the use of this technique is not justified.

4. Biological and pharmacological potential of *O. hispidus* extracts

4.1 . Antioxidant activity

Antioxidant activity is the basis of the biological and pharmacological power of all natural products. It implies the ability of molecules to defend the cell from oxidative damage, as well as to neutralize free radicals. Applying different *in vitro* methods, the antioxidant activity of the *O. hispidus* extracts was determined. Each of the applied tests takes place according to a different mechanism, which is why their comparative analysis can provide a more realistic insight into the antioxidant capacity of the tested extract. In this regard, the ability of the extracts to neutralize free radicals (DPPH and ABTS), the reduction potential (FRAP and CUPRAC), the ability to chelate metal ions, and the total antioxidant capacity were determined.

In the case of anti-radical activity, it was observed that the extracts obtained by modern extraction techniques showed higher activity compared to those obtained by traditional ones. The effectiveness of the modern techniques was in the following order: HAE>MAE>UAE>ASE. Regarding the DPPH test, it was noticed that effectiveness of HAE

and MAE were similar, i.e. without statistically significant difference. Further, between UAE and ASE the results were close indicating the similar potential of these extracts against DPPH radicals. A similar situation was observed in the ABTS test where the activity of MAE, UAE and ASE extracts did not differ significantly.

In the case of reducing power, HAE extract showed the highest level of activity using both methods (CUPRAC and FRAP). It was observed that the ability of MAE and UAE extracts towards reduction was not significantly different, and these extracts showed similar activity in the case of both reduction tests. In terms of overall antioxidant activity and ability to chelate metal ions, all modern extraction techniques showed similar activity, that is, the achieved results did not differ statistically significantly (Table 3). It is worthy to point out that the extracts obtained by the SFE technique showed a lower level of activity compared to other extracts. This could be explained by poor selectivity and co-extraction of concomitant compounds (waxes and resin) which does not perform antioxidant action. When it comes to traditional extraction techniques, maceration has proven to be a more efficient technique compared to Soxhlet extraction.

The results presented in table 3 clearly show that this plant possess great potential to be used as source of natural antioxidants. The demand for compounds with extremely high antioxidant power is increasing due to their potential to be used in the pharmaceutical industry (for the production of dietary supplements that can be used to strengthen the body), in the cosmetic industry (for the production of creams, lotions, etc.) and in food industry (as functional food additives). Bearing this in mind as well as the results obtained in this study, it is clear that the extracts of this plant have great commercial potential. Extracts obtained by modern techniques, primarily by HAE, have an advantage over extracts obtained by traditional extraction techniques.

4.2 . Enzyme inhibitor activity

Diseases of modern times are most often the result of the influence of various factors of the external environment. Protection and improvement of health have become imperative in everyday life. Enzymes that regulate a series of biochemical reactions in the body play a key role in maintaining the balance between disease and health. A change in enzyme activity leads to the appearance of neurodegenerative disorders, cardiovascular diseases, the development of diabetes, and obesity. The use of synthetic drugs in solving health problems carries with it certain risks that can have unwanted effects on health. Therefore, the scientific community is focused on examining plants as natural sources of very powerful molecules that can prevent disease. What makes this research special are the results of inhibition of the excessive enzymatic activity of this plant species, because such analyzes have never been done on extracts of *O. hispidus*.

The investigation of the neuroprotective potential of *O. hispidus* involved the examination of the ability of its extracts to reduce the excessive activity of cholinesterase enzymes (AChE and BChE), as key enzymes in the functioning of the central nervous system (Akbar, Shabbir, Rehman, Akash, & Shah, 2021).

All obtained extracts showed a high degree of ability to regulate the activity of both enzymes. When it comes to the inhibition of the AChE enzyme, the activity of the extracts did not differ statistically significantly. In case of BChE the highest activity was expressed by SFE extract which imply that non-polar compounds contribute to this activity to a significant degree. In comparison to the literature data, extracts of *O. hispidus* was proven as better inhibitors of AChE and BchE than many other plants from the Apiaceae family (Zengin et al., 2019; Zengin, Sinan, et al., 2020).

An increasingly frequent problem in the modern lifestyle is excessive UV radiation, which, by triggering melanogenesis, leads to an excessive production of melanin, which in increased

amounts in the organism leads to the appearance of dark spots on the skin, but also a series of other negative effects such as neurodegenerative changes (Papaccio, D′ Arino, Caputo, & Bellei, 2022). One of the strategies that can be used to prevent these negative conditions is to control the activity of the enzyme tyrosinase, an important mediator in the biosynthesis of melanin (Seo, Sharma, & Sharma, 2003). Commonly used synthetic tyrosinase inhibitors have been associated with numerous side effects and toxicity, thus the focus of investigation has been in the discovery of natural bioactives as potential substitutes. *O.hispidus* extracts showed a satisfactory effect on the inhibition of tyrosinase, whereby the extracts obtained by MAC and ASE techniques showed the highest activity, while the SFE extracts showed the lowest affinity towards the inhibition of this important enzyme.

The third group of enzymes whose inhibition under the influence of extracts was examined are enzymes that play a key role in the metabolism of carbohydrates - alpha amylase and glucosidase. Carbohydrate metabolism can be disturbed by diet and lifestyle, which combined with genetic factors can lead to diabetes. This serious illness can often be accompanied by long-term complications (loss of vision, kidney failure, neuropathy and many others). Newer strategies to prevent and control the progression of diabetes include regulation of alpha amylase and glucosidase activity. Several important synthetic inhibitors of these enzymes are known so far, but their use can have side effects including toxicity. Therefore, the search for natural inhibitors is constantly increasing. In the framework of this work, it was shown that all extracts of *O. Hispidus*, regardless of the method of obtaining, show the ability to regulate the activity of both enzymes, with a higher affinity for glucosidase (1.67-1.77 mmol ACAE/g) compared to amylase (0.36- 0.46 mmol ACAE/g). In the case of amylase, the extracts obtained by HAE and SFE showed the highest efficiency, while the extracts obtained by ASE and SFE showed a higher affinity towards glucosidase compared to other extracts. This shows

that polyphenolic compounds and terpenoids, which are found in lipophilic extracts such as SFE, are of great importance in these activities.

Data mining

In recent years, multivariate analysis has become increasingly popular to understand the interrelationships between different parameters. In particular, this analysis can provide valuable insights into structure-activity relationships in phytochemical studies. To do this, we carried out a multivariate analysis of the tested extracts and parameters. First, we performed a Pearson correlation analysis between total and individual phenolic compounds and biological activities. Figure 2 shows a strong correlation ($R>0.8$) between total phenolic and flavonoid contents and antioxidant properties, except for metal chelating. However, among the enzyme inhibitory assays, only tyrosinase was positively correlated with total bioactive compounds. Individual components such as neochlorogenic acid and quercetin-3-O-glucoside were found to be the main contributors to the observed antioxidant effects, as supported by previous studies (Cheng et al., 2015; Park, Jin, Yi, Kim, & Park, 2018; Razavi, Zahri, Zarrini, Nazemiyeh, & Mohammadi, 2009; Zheng et al., 2017). The tyrosinase inhibitory effects were also associated with the presence of these compounds, which have been proven to be effective by several researchers (Jakimiuk et al., 2022; Liu et al., 2020). We performed PLS-DA analysis to classify the tested extraction methods based on biological activities and chemical composition (Figure 3). The extraction methods were classified into four groups. SFE was classified as Group 1, which had the lowest antioxidant properties. Both ASE and SOX were grouped in Group 2 (Figure 4), as they contained temperature in their extraction procedures and their enzyme inhibition properties were similar (Figure 5). Other techniques, namely HAE, UAE, and MAC, were classified as Group 3, as they did not involve any temperature applications. MAE was placed in Group 4, but it was closely associated with Group 3. Our findings provide insights into the effects of different extraction techniques on the bioactive

properties of *O. hispidus* extracts and can be used to guide the selection of appropriate extraction methods for specific applications.

Conclusion

Plant species *O. hispidus* collected in Turkey represent a very good and high-quality plant material for extraction of phenolic compounds. The effect of different extraction techniques, chemical composition, antioxidant, and enzyme inhibitory potential of obtained extracts was examined. The application of modern technologies in the process of extraction of bioactive compounds had a huge advantage compared with conventional technologies. The results of LC-MS/MS analysis indicate certain differences in the composition of the obtained extracts. In all samples, the predominant compounds were flavonoids. The high amount of these compounds in the extract produced by ASE extraction technique suggests that it provides an excellent alternative to conventional techniques for bioactive compounds isolation. For the formulation of the most potent extract in terms of strong natural antioxidant and enzyme inhibitor agents, it is recommended to apply ASE extraction techniques. This result is a direct consequence of the influence of phenolic molecules on the biological and pharmacological activity of the obtained extracts which suggests that for the realization of antioxidant and enzyme inhibitor potential, the mutual interaction of phenolic acids and flavonoids is necessary, as well as the influence of temperature for efficient isolation process of these metabolites. It has been shown that high-quality plant extracts could be obtained without creating a large amount of waste, using organic solvents, and consuming energy, which directly and positively affects the sustainability and environmental acceptability of the applied process. This paper presents the concept of using wild-growing plant species to obtain natural bioactive products by modern and green extractions which could potentially take their place on the shelves of pharmacies.

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Table 1. Total phenolic and flavonoid content[1] in the obtained extracts

Extracts	Total phenolic content (mg GAE/g)[2]	Total flavonoid content (mg RE/g)[3]
Modern techniques		
ASE	31.04±0.84[b]	12.76±0.50[b]
HAE	36.98±0.06[a]	15.04±0.52[a]
MAE	30.13±0.11[b]	12.56±0.08[b]
UAE	30.60±0.37[b]	12.52±0.23[b]
SFE	17.55±0.26[e]	1.09±0.02[d]
Traditional techniques		
MAC	27.14±0.18[c]	11.86±0.76[b]
SOX	23.83±0.32[d]	10.46±0.38[c]

[1]values are means ± SD of three measurements, means within each column with different letters (a–e) differ significantly ($p \leq 0.05$)
[1] mg Gallic acid equivalents per gram of extract
[2] mg Rutin equivalents per gram of extract

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Table 2. Polyphenolic profile[1] of *Opopanax hispidus* extracts (mg/kg)

Compounds	**Extraction techniques**						
	ASE	**HAE**	**MAE**	**UAE**	**SFE**	**MAC**	**SOX**
Chlorogenic acid	81.73±6.13^{b}	45.04±0.30^{d}	403.54±0.09^{a}	63.15±7.09^{c}	1.43±0.06^{e}	59.68±4.78^{c}	61.77±4.10^{c}
Neochlorogenic acid	2187.15±59.00^{d}	2839.73±32.28^{b}	1896.18±72.60^{f}	3091.19±107.84^{a}	1.36±0.01^{g}	2662.89±45.86^{c}	2026.07±30.73^{e}
Caffeic acid	29.20±0.96^{a}	11.48±1.48^{e}	10.49±0.32^{f}	15.54±0.49^{d}	< LoD*	16.66±0.05^{c}	18.57±1.17^{b}
p-Coumaric acid	297.15±9.33^{b}	133.18±1.22^{d}	136.60±8.69^{d}	257.26±4.25^{c}	< LoD*	296.63±5.83^{b}	337.63±5.51^{a}
Isoorientin	< LoD*	1.52±0.15^{c}	0.90±0.09^{d}	2.19±0.16^{a}	< LoD*	1.78±0.27^{b}	0.66±0.05^{e}
Rutin	66.71±0.95^{a}	44.57±0.73^{c}	37.09±1.62^{e}	< LoD*	0.27±0.02^{f}	55.06±1.75^{b}	39.61±0.73^{d}
Vitexin	2.33±0.01^{d}	2.78±0.02^{c}	1.66±0.05^{f}	3.41±0.11^{a}	< LoD*	3.13±0.05^{b}	1.85±0.02^{e}
Quercetin *3-O*-glucoside	37.93±0.13^{a}	32.94±1.23^{b}	18.89±0.18^{e}	31.46±0.12^{b}	0.70±0.06^{f}	29.23±0.16^{c}	22.20±0.49^{d}
Quercetin *3-O*-rhamnoside	84.85±1.60^{c}	94.59±0.68^{b}	38.92±0.68^{e}	121.53±6.96^{a}	< LoD*	98.84±1.07^{b}	76.49±2.60^{d}
Kaempferol *3-O*-glucoside	265.54±4.01^{a}	161.99±0.21^{d}	111.62±0.83^{e}	223.40±8.48^{c}	< LoD*	242.86±3.01^{b}	169.90±0.65^{d}
Eriodictyol	20.59±1.50^{a}	3.64±0.14^{e}	5.61±0.07^{c}	5.27±0.46^{c,d}	0.57±0.04^{f}	5.50±0.30^{c}	15.82±0.35^{b}
Luteolin	104.68±1.01^{a}	58.44±1.25^{c}	49.32±1.96^{d}	73.26±1.14^{b}	< LoD*	75.81±2.53^{b}	101.13±0.88^{a}
Quercetin	5.46±0.08^{a}	3.84±0.27^{d}	2.44±0.01^{e}	4.71±0.15^{b}	< LoD*	4.10±0.22^{c}	3.66±0.10^{d}
Naringenin	4.72±0.24^{a}	3.04±0.05^{d}	1.59±0.12^{e}	3.42±0.38^{c}	< LoD*	2.87±0.02^{d}	4.09±0.20^{b}
Apigenin	54.00±0.47^{a}	36.74±0.97^{e}	26.17±0.16^{f}	40.88±1.89^{d}	< LoD*	43.99±1.92^{c}	50.63±0.98^{b}
Hispidulin	36.62±0.29^{a}	29.29±0.94^{b,c}	16.68±0.22^{d}	31.47±1.52^{b}	< LoD*	31.01±0.26^{b}	29.29±0.60^{b,c}
Isorhamnetin	13.81±0.72^{a}	6.88±0.16^{c}	4.67±0.30^{d}	8.18±0.49^{b}	< LoD*	7.18±0.32^{c}	6.96±0.29^{c}
Pinocembrin	< LoD*	< LoD*	< LoD*	11.85±0.49^{a}	7.49±0.21^{b}	< LoD*	4.79±0.40^{c}

Table 3. Antioxidant properties[1] of the *Opopanax hispidus* extracts

Extracts	DPPH (mg TE/g)[2]	ABTS (mg TE/g)[2]	CUPRAC (mg TE/g)[2]	FRAP (mg TE/g)[2]	PBD (mmol TE/g)[3]	MCA (mg EDTAE/g)[4]
ASE	32.38±2.01^{b,c}	113.35±2.92^{b,c}	76.61±1.13^{b}	42.56±0.10^{b}	1.30±0.01^{a}	15.48±0.31^{a}
HAE	38.25±0.42^{a}	135.92±2.83^{a}	93.42±1.67^{a}	43.74±0.65^{a}	1.27±0.05^{a}	13.58±3.45^{a,b}
MAC	30.13±0.36^{c}	107.33±4.28^{c}	68.15±1.48^{c}	33.92±0.04^{e}	1.10±0.02^{b,c}	8.46±0.23^{c}
MAE	36.05±0.14^{a}	119.68±0.38^{b}	76.65±0.77^{b}	43.13±0.08^{a,b}	1.17±0.10^{a,b}	15.67±0.32^{a}
SFE	8.76±0.48^{e}	53.91±4.21^{e}	53.89±1.21^{e}	22.05±0.42^{f}	0.81±0.02^{d}	11.31±0.07^{b,c}
SOX	25.92±0.40^{d}	91.34±3.61^{d}	64.44±0.65^{d}	35.76±0.26^{d}	0.97±0.05^{c}	8.35±0.37^{c}
UAE	33.52±0.50^{b}	118.21±0.59^{b}	79.04±0.74^{b}	37.07±0.30^{c}	1.19±0.01^{a,b}	13.04±0.95^{a,b}

[1]values are means ± SD of three measurements, means within each column with different letters (a–f) differ significantly ($p \leq 0.05$)
[2]mg Trolox equivalents per g of extract
[3]mmol Trolox equivalents per g of extract
[4]mg EDTAE per g of extract

Table 4. Enzyme inhibitory potential[1] of the *Opopanax hispidus* extracts

Extracts	AChE (mg GALAE/g)[2]	BChE (mg GALAE/g)[2]	Tyrosinase (mg KAE/g)[3]	Amylase (mmol ACAE/g)[4]	Glucosidase (mmol ACAE/g)[5]
ASE	4.17±0.04[a]	5.35±0.13[b]	75.38±1.29[a,b]	0.39±0.01[b]	1.77±0.01[a]
HAE	4.13±0.04[a]	5.46±0.08[b]	71.49±1.76[b]	0.46±0.01[a]	1.67±0.02[c]
MAC	4.22±0.02[a]	5.29±0.23[b]	76.72±2.73[a]	0.41±0.01[b]	1.72±0.01[b]
MAE	4.13±0.06[a]	4.74±0.29[c]	64.63±0.64[c]	0.35±0.01[c]	1.74±0.01[a,b]
SFE	4.19±0.08[a]	6.44±0.08[a]	44.78±1.43[d]	0.45±0.01[a]	1.75±0.01[a]
SOX	4.18±0.03[a]	5.63±0.16[b]	64.92±1.02[c]	0.36±0.01[c]	1.75±0.01[a]
UAE	4.12±0.03[a]	5.69±0.10[b]	64.97±1.67[c]	0.40±0.01[b]	1.72±0.01[b]

[1]values are means ± SD of three measurements, means within each column with different letters (a–d) differ significantly ($p \leq 0.05$)
[2]mg Galatamine equivalents per g of extract
[3]mg Kojic acid per g of extract
[4]mmol Acarbose equivalents per g of extract

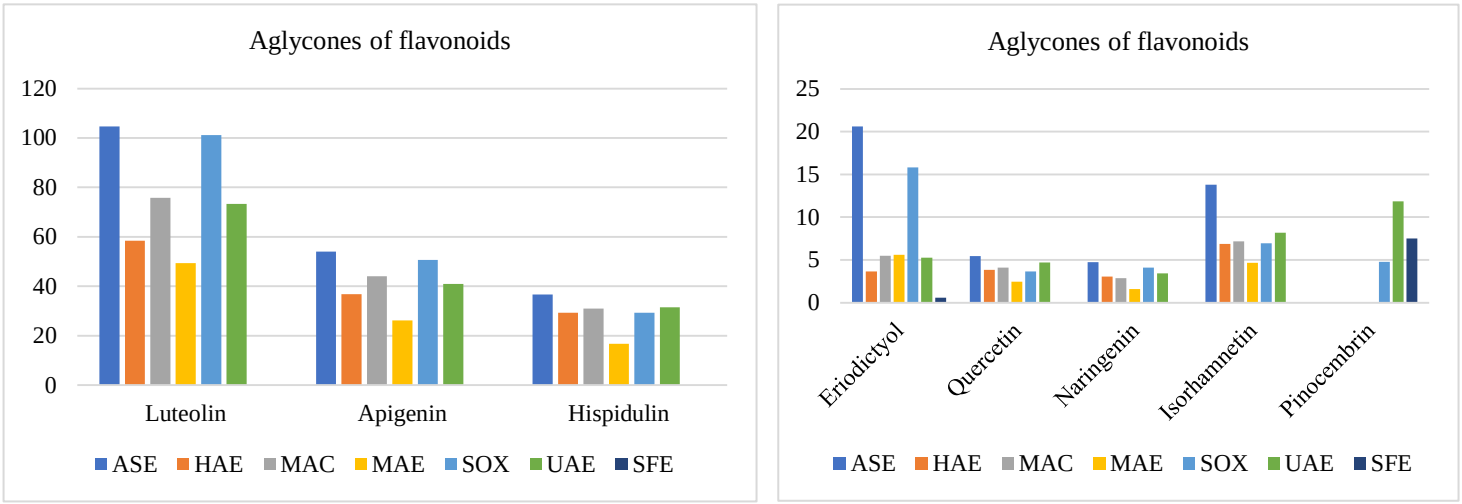


Fig 1. Influence of the extraction techniques on aglycone isolation from *O. Hispidus*

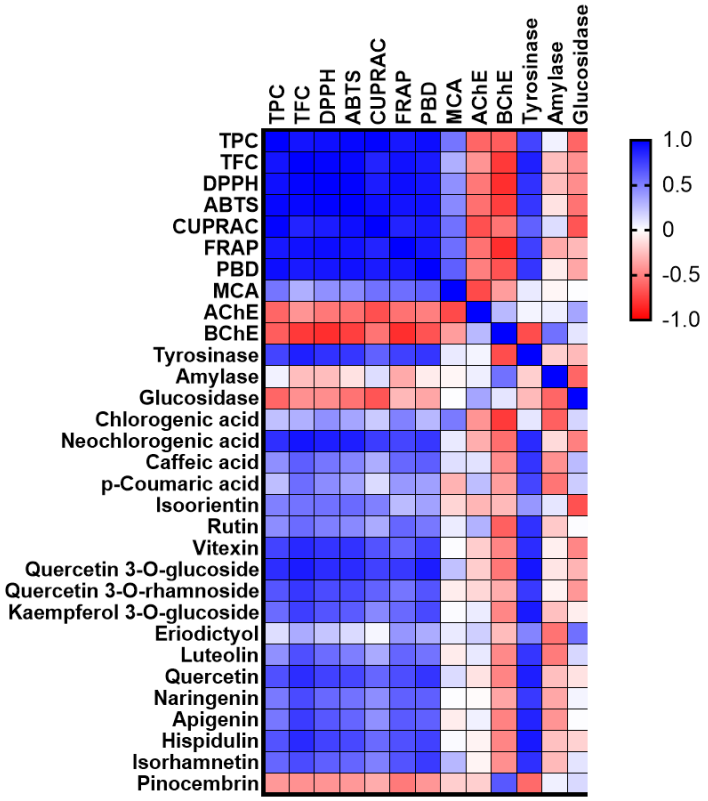


Figure 2. Pearson's correlation between total/individual compounds and antioxidant/enzyme inhibitory assays (MCA: Metal chelating assay; PBD: Phosphomolybdenum)

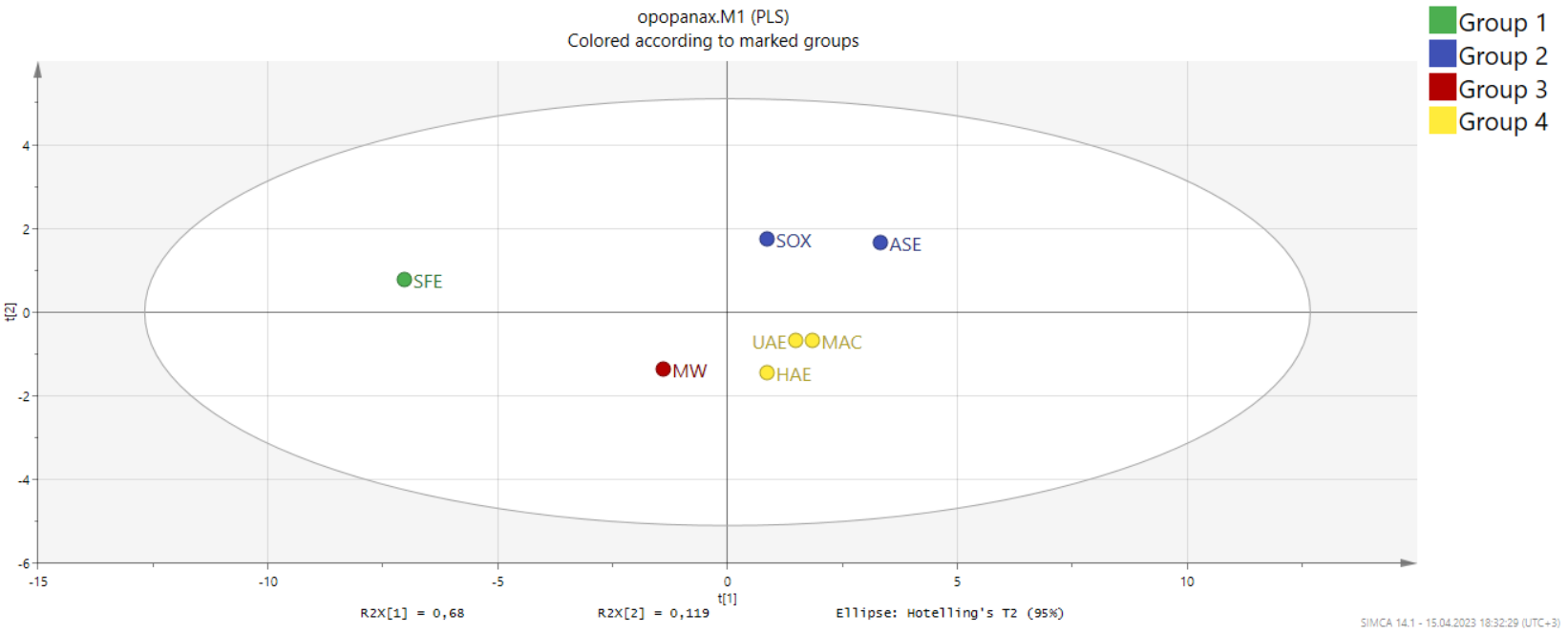


Figure 3. Relationship between the tested extraction methods through PLS analysis based on chemical composition and biological activities

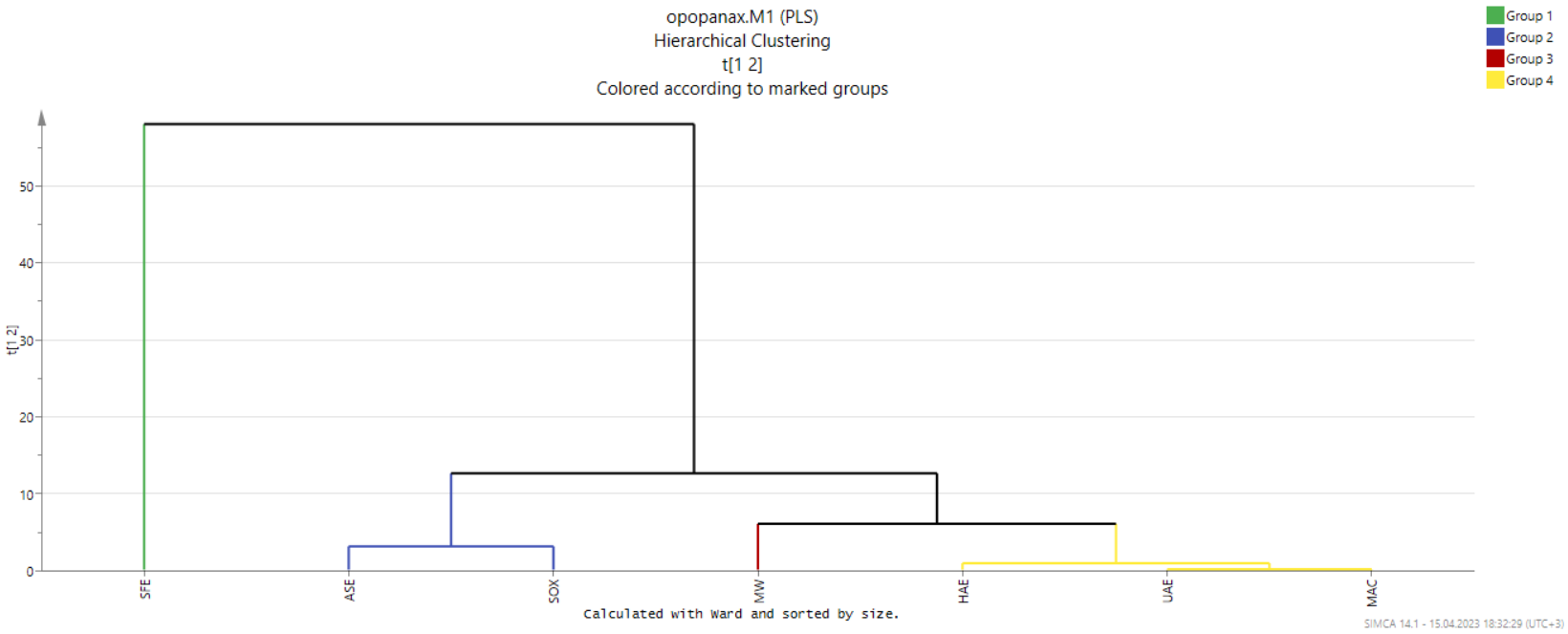


Figure 4. Hierarchical cluster analysis for the tested extraction methods based on the chemical profiles and biological activities.

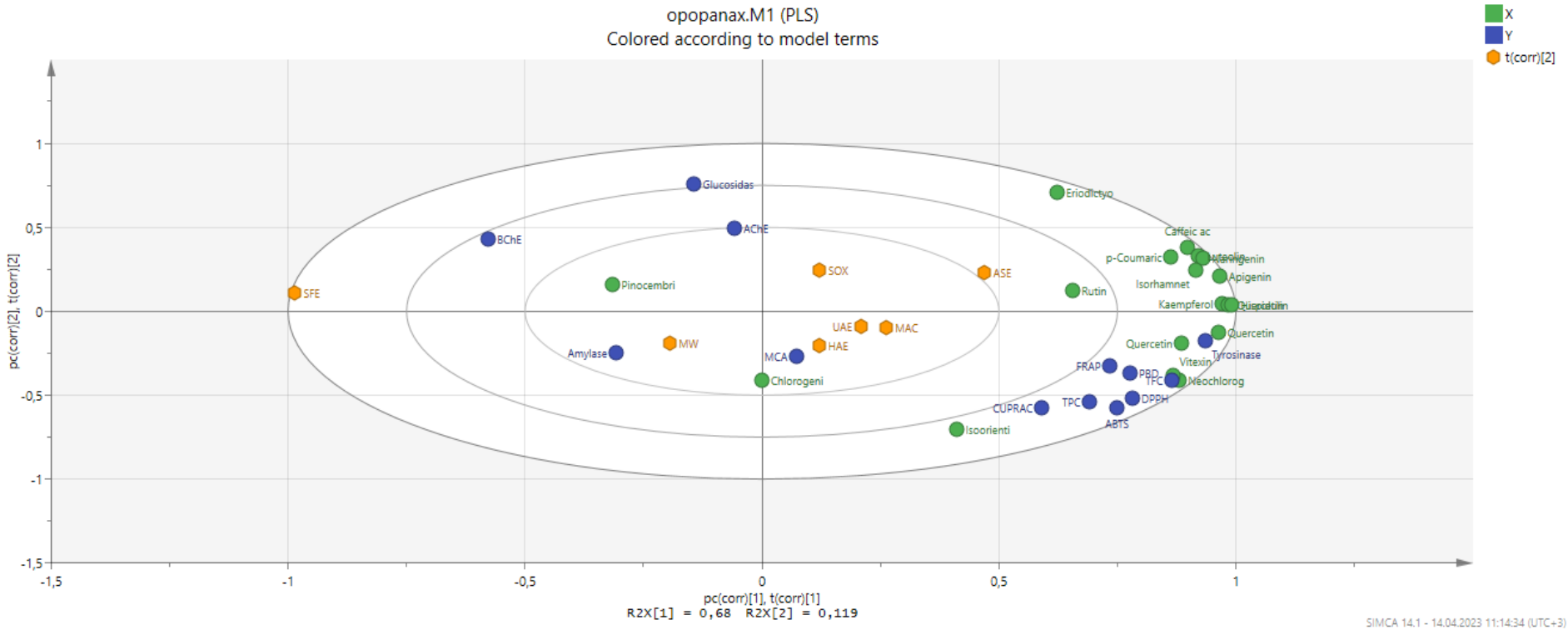


Figure 5. Biplot presentation for Partial least squared regression describing relationship tested extracts, chemical components and biological activity assays