Multifunctional Phyto-Pharmaceutical Properties and Metabolomic Profiling of *Pulicaria dysenterica* Extracts: Implications for Anti-Inflammatory and Antimicrobial

Drug Discovery
Dr. Lucas Meyer[1]*
Dr. Amira Hassan[2]
[1] University of Zurich, Institute of Pharmacognosy and Molecular Therapeutics, Zurich, Switzerland
[2] Alexandria University, Department of Pharmaceutical Sciences and Natural Products Chemistry, Alexandria, Egypt

Abstract

In this study, we aimed to evaluate the influence of different extraction procedures (decoction, homogenizer assisted extraction; infusion, maceration; soxhlet; and ultrasonication assisted extraction) on the phytochemical profiles and bioactivities of methanol and water extracts of *Pulicaria dysenterica* growing in Turkey. The chemical profiles of the extracts were evaluated by high-performance liquid chromatography coupled to electrospray ionisation and time-of-flight mass spectrometry (HPLC-ESI-TOF-MS), as well as by their total phenolic flavonoid, phenolic acid and flavonol contents. The antioxidant properties of the extracts were determined by using free radical scavenging, phosphomolybdenum, ferrous ion chelating, and reducing power assays. In addition, their inhibitory activities on cholinesterase, α-amylase, and α-glucosidase, tyrosinase and lipase were determined. The type of extraction and solvent were found to affect the concentration of phytochemicals. Homogenizer assisted extraction and ultrasonication assisted extraction. The methanolic extracts showed higher level of total phenolic flavonoid and phenolic acid content. Broadly, a total of 42 different compound were extracted from *P. dysenterica.* In relation to the potential *in vitro* anti-diabetic effects, the highest activity against the studied key enzymes was observed for the macerated extract (α-amylase: 0.58±0.03 and α- glucosidase: 1.65±0.03 mmol ACAE/g). The methanolic extract from homogenization (HAE-MeOH) was the most potent inhibitor of cholisterases where highest activities against tyrosinase were observed for ultrasonicated methanolic extract, followed by macerated and homogenized methanolic extracts. The inhibitory activity of the studied extracts against lipase were in the order: macerated > soxhlet > HAE-MeOH > UAE-MeOH. Multivariate Principal component and Hierarchical Cluster analysis showed a high significant correlation between phenolic compounds and antioxidant activity assays. Phenolic acids were found to be responsible for cupric reducing power and acetyl cholinesterase (AChE) inhibition, while flavonoid compounds were more related to inhibition of AChE, butyryl

cholinesterase (BChE), tyrosinase and amylase. In conclusion, this study provides promising baseline data pertaining to the therapeutic properties *P. dysentrica,* advocating the need for further investigation in an endeavour to develop novel phytomedicines and phyto-cosmeceuticals.

Keywords: ultrasonication; maceration; soxhlet; diabetes; phytomedicines; multivariate principal component; HPLC-MS; phenolic compounds; antioxidant

Introduction

Plants are considered as a repository of bioactive molecules, produced as secondary metabolites, known for being traditionally used for medical purposes since immemorial time. These bioactive compounds are often differentially distributed among groups of plants and only present in very low quantities in plants (Zullaikah et al., 2018). Natural bioactive compounds such as plants extracts, either as pure compounds or as standardized extracts, are of increasing interest for their versatile applications in pharmaceutical, nutraceutical and cosmetic industry. For this purpose, specific extraction techniques are essentially required to ensure isolation of specific bioactive constituents, to optimise the concentration of known constituents and also to maintain their biological activities (Dhanani et al., 2017).

Indeed, extraction is a crucial step in the itinerary of phytochemical analysis for the bioactive constituents of plants. The selection of a suitable extraction technique is also essential for removal of desirable soluble constituents, leaving out those, which are not wanted, with the aid of solvents and standardized process (Chaturvedi, 2018; Dhanani et al., 2017). Conventionally, secondary metabolites were extracted using techniques such maceration, percolation, soxhlet extraction and solvent extraction. In the past few decades, the green extraction techniques such as supercritical CO_2 extraction, instant controlled pressure drop (DIC), ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) has rapidly gained interest globally as these techniques are less-laborious, fast and have increased extraction yield as compared to the conventional methods (Ahmad et al., 2017; Armenta et al.,

2015; Dhanani et al., 2017; Sánchez-Valdepeñas et al., 2015). Also these techniques are environmentally friendly as it involve reduced solvent and energy consumption (Chaturvedi, 2018).

Pulicaria genus belongs to the family of the Compositae, tribe Inuleae, which is represented by *ca.* 100 species with a distribution from Europe to North Africa and Asia, particularly around the Mediterranean. Several member of this genus are used as traditional herbal medicines and on this basis, they have been subjected to biological and chemical investigations (Ezoubeiri et al., 2005). Phytochemical analysis of *Pulicaria* showed the occurrence of molecules of monoterpenes (El-Ghaly et al., 2016; Hussien et al., 2016), diterpenes (Ahmad et al., 2006; Hussien et al., 2016), sesquiterpenes (Elshamy et al., 2018; Hegazy et al., 2015; Hussien et al., 2016), triterpenes (Eshbakova and Saidkhodzhaev, 2001), flavonoids (El-Negoumy et al., 1982; Hussien et al., 2016; Pares et al., 1981; Williams et al., 2003) and steroids (El-Ghaly et al., 2016; Liu et al., 2010). Various biological activities have been reported for some species of *Pulicaria* such as antioxidant and cytotoxic activities for *Pulicaria jaubertii* E. Gamal-Eldin and *P. undulata* (Hussien et al., 2016), antimicrobial activity for *P. odora and P. inuloides,* antispasmodic activity of *P. glutinosa* (Tanira et al., 1996) and analgesic, antipyretic, anti-inflammatory, hepatoprotective and nephritic activities of *P. arabica* (Yusufoglu, 2014) .

P. dysenterica (L.) Bernh., commonly known as fleabane, is a perennial plant of up to 100 cm tall, with yellow flowers, growing on damp places and is widely spread in Europe, Anatolia, Iraq, Iran, Turcomania, Afghanistan, Pakistan, Caucasus and North Africa (Mumivand et al., 2010; Williams et al., 2003). *P. dysenterica* is one of the valuable medicinal plants used for the treatment of dysentery in the UK. In addition, the decoction of the aerial parts of this plant is used as an antidiarrhoeal agent in Iranian's folk medicine (Liu et al., 2010). The plant is also known to have insecticidal property (Basta et al., 2007; Mumivand et al.,

2010). Few research have been carried out concerning the chemical constituents of *P. dysenterica* including volatile oils (Basta et al., 2007; Mumivand et al., 2010), sesquiterpenes (Mumivand et al., 2010), flavonoids and caryophyllenes (Basta et al., 2007; Bohlmann and Zdero, 1981; Marco et al., 1992) .

Although chemical investigations have been carried out on *P. dysenterica,* there is no study attempted to investigate the antioxidant and enzyme inhibitory potentials of *P. dysenterica* extracts obtained via different extraction procedures. Based on the aforementioned facts, the present study focused on the potential usefulness of *P. dysenterica* growing in Turkey, based on the pharmacological evaluation of its different extracts by determining its antioxidant (free radical scavenging assays, reducing power, metal chelating, and phosphomolybdenum assays) and inhibitory potential against key physiological enzymes involved in common pathologies such as neurodegenerative diseases (cholinesterases – AChE and BChE), diabetes (α-amylase and α-glucosidase), hyper-pigmentation (tyrosinase) and obesity (lipase). HPLC-ESI-TOF-MS analysis was performed in order to provide detailed insights into the chemical profile of this species.

2. Materials and Methods

2.1. Plant material and preparation of extracts

Sampling of the plant species was done in Kastamonu (Hanonu) of Turkey in the year 2018. Botanical authentication of the plant was done by the botanist Dr. Ismail Senkardes (Marmara University, Faculty of Pharmacy, Turkey, Voucher Number: MARE-19136). The aerial parts were dried at room temperature (in shade, about 10 days). These materials were then powdered by using a laboratory mill.

The dried plant materials were extracted by different methods (decoction, homogenizer assisted extraction (HAE); infusion, maceration (MAC); soxhlet; ultrasonication assisted

extraction (UAE)) and they are summarized in Figure 1. All extracts were filtered and concentrated by using a rotary-evaporator. The obtained plant extracts were kept at +4ºC until further analysis.

2.2. HPLC-ESI-TOF-MS analysis

Dried extracts of *P. dysenterica* were analysed using a RRLC 1200 series (Agilent Technologies, Palo Alto, CA, USA), which comprises a vacuum degasser, an autosampler, a binary pump and a Diode Array Detector. The chromatographic separation was performed by a150 mm x 4.6 mm id, 1.8 µm Zorbax Eclipse Plus C18 column (Agilent Technologies, Palo Alto, CA, USA). The mobile phases employed to separate the phytochemicals were: water: acetonitrile 90:10 (v:v) acidified with 0.1% of formic acid (A) and acetonitrile (B). The following linear gradient was conducted during 45 minutes: 0 min, 5% B; 30 min, 60% B; 40 min 5% B. Finally, a conditioning cycle with initial conditions was applied for the next analysis. The flow rate was maintained at 0.5 mL/min and 10 µL of the sample were separated at 25 ˚C. The samples were dissolved using methanol or water at a concentration of 5 mg/mL.

The HPLC platform was coupled to a time-of-flight mass spectrometer (micrOTOF, Bruker Daltonics GmbH, Bremen, Germany) with an electrospray interface (ESI) (model G1607 from Agilent Technologies, Palo Alto, CA, USA) operating in negative ionization mode. In order to enhance the correct ionization of analytes, a “T” type splitter was used to decrease the flow rate towards mass spectrometer.

The source parameters were optimized and established as follow: capillary voltage of +4 kV; drying gas temperature, 210°C; drying gas flow, 9 L/min; and nebulizing gas pressure, 2.2 bar. The values of transfer parameters were: capillary exit, -120 V; skimmer 1, -40 V; hexapole 1, -23 V; RF hexapole, 80 Vpp; and skimmer 2, -22.5 V. The detection mass range was from 50 to 1000 m/z.

Additionally, an external calibration was conducted with a sodium acetate clusters solution in quadratic high-precision calibration (HPC) regression mode. This calibration solution was injected at the beginning of each run by a Cole Palmer syringe pump (Vernon Hills, Illinois, USA). All the spectra were calibrated prior to polar compounds characterization through Data Analysis 4.0 software (Bruker Daltonics, Bremen, Germany) which enabled a list of possible elemental formulas by a sophisticated CHNO algorithm.

2.2. Profile of bioactive compounds

By referring to our previous paper (Zengin and Aktumsek, 2014), the flavonoids (TFC), total phenolic (TPC), total phenolic acid (TPaC) and total flavonol (TFvlC) contents were determined on the basis of spectrophotometric assays. The results were expressed as equivalent of rutin (mg RE/g) for TFC, gallic acid equivalent (mg GAE/g) for TPC, caffeic acid (mg CAE/g) for TPaC and catechin (mgCE/g) for TFvlC.

2.3. Determination of antioxidant and enzyme inhibitory effects

The *in vitro* enzyme inhibitory effects of extracts on five enzymes, that are lipase, α-amylase, α-glucosidase, cholinesterases, and tyrosinase were evaluated, as previously reported (Grochowski et al., 2017). The enzyme inhibitory actions of extracts were assessed as equivalents of kojic acid (KAE) for tyrosinase, galantamine for acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE), orlistat for lipase, and acarbose for α-amylase and α-glucosidase.

Regarding antioxidant capacity of the extracts, different spectrophotometric experiments as ferrous ion chelating, phosphomolybdenum, reducing (FRAP and CUPRAC), and radicals scavenging tests (ABTS and DPPH) were performed as previously reported. The

findings were given as standard compounds equivalents of EDTA or Trolox (EDTAE/g and TE/g). The assay methods were given in our earlier work (Uysal et al., 2017).

2.4. Multivariate analysis

Data analyzing was performed under R (v. 3.5.1) and Xlstat (v. 2018) software through the One-way ANOVA test, Pearson's correlation coefficients and both multivariate Principal Component and Hierarchical Cluster analysis. One-way ANOVA was used to compare differences between the extracts on the estimated biological activities and phenolic classes. Then Tukey's test was performed to find out the difference between the different extracts in the event the (ANOVA) was significant (p<0.05). Pearson's correlation coefficients were calculated and representation of network was generated to recognize the relationship between phenolic classes and the studied biological activities. HCA Hierarchical cluster analysis using "ward" as linkage rule and the Euclidean similarity measure, was conducted for the classification of extracts based on biological activities and LC-MS results respectively. PCA allowed to pinpoint the biological activities describing the different groups obtained from HCA.

3. Results and Discussion

3.1 The total content of polyphenols, flavonoids, phenolic acids and flavonols

Biologically active compounds are generally present in low concentration in plants. A suitable extraction technique that produce optimal yields and with minimal changes to the functional properties of the compounds of interest is highly desirable. (Dhanani et al., 2017). The selection of extraction method to be employed is influenced by the chemical nature of the phyto-constituents, sample particle size, and also by the presence of interfering substances. Extraction time, temperature, solvent-to-feed ratio as well as extraction solvents to be used are the crucial parameters affecting the extraction yield (Do et al., 2014; Brglez Mojzer et al., 2016).

Several extraction techniques including decoction, infusion, maceration, soxhlet extraction and modern ones such as ultrasound-assisted extraction (UAE) and microwave-assisted extraction (MAE) are being employed to extract phyto-constituents from plants. However, extraction yield and bioactivities do not only depend on the extraction technique but also on the solvents used and their polarity. Studies have showed variations in the biological activities of extracts prepared using different extraction methods and solvents (Do et al., 2014; Muhamad et al., 2017; Złotek et al., 2016). The present study was undertaken to investigate the effect of extracts obtained using decoction, maceration, soxhlet extraction, homogenizer assisted extraction (HAE) and ultrasonication assisted extraction (UAE) technique, along with different solvents on the biochemical profile and bioactivity of *P. dysenterica*.

The amount of total phenolic content (TPC), total flavonoid content (TFC), total phenolic acid content (TPAC) and total flavonol contents of the *P. dysenterica* extracts were firstly assessed and the results are recorded in Figure 2. The results showed variations in the levels of TPC, TFC and TPAC content contents of all extracts. As shown, the phenolic content of the extracts ranged from 80.62±2.87 to 119.40±2.67 mg GAE/g and the highest amount was obtained for methanolic sample (HAE-MEOH) and lowest in water sample using homogenization technique. Similar, the highest values for TFC (UAE-MeOH: 50.60±0.15 and HAE-MeOH: 48.94±0.44 mg RE/g) and TPAC (UAE-MeOH: 8.35±0.14 and HAE-MeOH: 48.94±0.44 mg RE/g) were recorded for methanolic samples extracted using homogenization and ultrasonication techniques. In general, a low amount of flavonol was recorded for all the extracted samples, with a mean value ranging from 0.64±0.02 (HAE-MeOH) and 1.39±0.03 (HAE-Water). Overall, methanol was found to have a higher extractability, which may be caused by the possible complex formation of some phenolic compounds in the extracts that are soluble in methanol (Do et al., 2014). Furthermore, methanol has been generally found to extract lower molecular weight polyphenols more efficiently (Brglez Mojzer et al., 2016).

3.1. Phytochemical analysis of *P. dysenterica* extracts

The analytical platform provided eight different base peak chromatograms (BPC) corresponding to each extraction condition and they are shown in Figure 3. The characterization of detected compounds was based on the interpretation of MS spectra given by HPLC-ESI-TOF-MS equipment and the available information on the literature. Table 1 displays the most relevant information about proposed compounds including their retention time, experimental *m/z* and their occurrence in studied extracts. It is necessary to remark that the compounds were numbered and ordered according to their elution time.

Broadly, a total of 42 different compound were extracted from *P. dysenterica.* From them, a total of 34 were tentatively identified with the available tools which were classified in four different groups. Unfortunately, the rest of compounds were not possible to characterize.

Firstly, two organic acid were identified and related to peaks 1 and 2. Both, gave the same *m/z* at 191 but different molecular formula. In this sense, peak 1 was associate to quinic acid ($C_7H_{12}O_6$) and peak 2 was characterized as citric acid with a molecular formula $C_6H_8O_7$.

Overall, 9 phenolic acid were detected, most of them isomers. For instance, peak 3, 4 and 5 presented the same deprotonated molecular formula ($C_{16}H_{17}O_9$). These compounds were tentatively identified as chlorogenic acid and its isomers according to previous reports on *Pulicaria* plants (Eshbakova & Yili, 2014). It is necessary to clarify that only isomer 1 and 2 were found in all studied extracts whereas isomer 3 only was found in aqueous extractions. Other four isomers (peaks 8, 14, 16 and 17) were found in all studied extracts, they gave the same *m/z* at 515 and consequently, the same molecular formula ($C_{25}H_{24}O_{12}$) and characterized as dicaffeoylquinic acid and its isomers (Zhu, Li, Zhang, & Lin, 2017). Additionally, feruloylquinic acid was associated to peak 10 since it presented a deprotonated molecular formula $C_{17}H_{19}O_9$ and a retention time at 11.1 minutes. Peak 21 was characterized as ferulic

acid. These compounds were extracted only by soxhlet extraction and previously detected in different *Pulicaria* species (Triana et al., 2011).

The greater number of structures belong to flavonoids group. The first eluted flavonoid was peak 6 which gave a *m/z* at 305 and was obtained in all extracts. This phytochemical was found in infusion extracts from *P. incisa* (Elmann et al., 2016). Two flavonoid glycosides were also identified in this botanical matrix. The firs one (peak 12) was not recovered in methanolic conditions. According to its molecular formula ($C_{24}H_{22}O_{14}$) and *m/z* at 533 it was characterized as luteolin malonyl glucoside which was present in *Chrysanthemum,* a plant species closely related to *Pulicaria* species (Lin & Harnly, 2010). The other glycoside was peak 13 which was tentatively identified as quercetin glucoside, a vacuolar flavonoid previously detected in *P. dysenterica* (Williams, Harborne, & Greenham, 2000). This compound was retrieved from *P. dysenterica* leaves in all extraction methods applied. In addition, up to five different aglycones were detected. According to their elution order, the first aglycone eluted was eupatolitin (peak 23). However, this compound was obtained during methanolic ultrasound assisted extraction and decoction. Conversely, peak 26 revealed a molecular formula ($C_{15}H_{10}O_7$) which enabled its identification as quercetin also detected in *P. incise* (Elmann et al., 2016). Nevertheless, this aglycone only was found in aqueous homogenizer-assisted extraction. Peaks 29, 30 and 37 were detected in all methanolic conditions being the first, characterized as eupalitin ($C_{17}H_{14}O_7$) and the second one as eupatin ($C_{18}H_{16}O_8$). Finally, eupatilin displays a *m/z* at 343 and was also identified in decoction. The last flavonoid eluted was peak 39 which deprotonated molecular formula was $C_{26}H_{13}O_3$ and earlier described in *Pulicaria* species (Williams et al., 2003). This compound was identified as quercetagetin tetramethyl ether.

On the other hand, the rest of compounds were tentatively characterized and merger in this group. In this sense, peak 7 was identified as a plant hormone found in other plants and identified as tuberonic acid glucoside (Seto et al., 2011). Moreover, peak 22 was successfully

obtained in homogenizer-assisted extraction, infusion and ultrasound assisted extraction using water as solvent. This phytochemical also acts as plant hormone and was found in *Artemisia annua,* a botanical from *Asteraceae* family (Iqbal, Younas, Chan, Zia-Ul-Haq, & Ismail, 2012). This compound was associate to a jasmonate glucoside derivative. Four compounds (peaks 20, 25, 27 and 28) shown the same *m/z* at 747 and also the same molecular formula ($C_{37}H_{47}O_{16}$). For these reason, they were tentatively characterized as the sesquilignan glucoside alangisesquin and its isomers. A secoiridoid was associated to peak 24 which gave a deprotonated molecular formula ($C_{35}H_{53}O_{15}$) it was tentatively characterized as pteroceside A. In addition, another sesquiterpene with *m/z* at 559 and a molecular formula ($C_{29}H_{36}O_{11}$) was previously found in *Ixeris* species and tentatively characterized as prenantheside B (Zhang, Wang, Xu, Wang, & Zhao, 2014). Peaks 36 and 41 had the same *m/z* at 249 and the same molecular formula, and identified as deoxy-epipulchellin isomer 1 and isomer 2, respectively. These compounds were sesquiterpenoids found in *Inula Britannica* (Yang, Wang, Liu, & Shi, 2011)**.** Both phytochemicals, were found in all studied extracts, except the first isomer which was not found in aqueous homogenizer-assisted extraction. A terpenoid was found at 23.1 minutes and associated to peak 33. This compound was acamptoic acid (Jolad et al., 1988). Peak 32 was detected in both, methanolic and aqueous extracts. Its molecular formula ($C_{18}H_{36}O_5$) and its retention time at 22.7 minutes enabled its tentatively characterization as tianshic acid (Wu, He, Ma, Chen, & Aisa, 2017). Finally, other two isomer were found at 28.6 and 30.1 minutes, they were peaks 38 and 40, which were tentatively characterized as ilicic acid isomers 1 and 2 (Liu, Zhang, Zhang, Ye, & Li, 2010). The first isomer proved to have a complicated retrieval since it only was obtained after decoction and aqueous homogenizer assisted extraction whereas compound 40 was obtained after applying all extraction methods.

3.2 Antioxidant Properties

An increasing body of evidence supports the direct implication of reactive oxygen species (ROS) in the pathogenesis of oxidative stress-related complications. Various synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary butyl hydroquinone (TBHQ) are widely used worldwide to counteract the detrimental effects caused by ROS. However, as synthetic antioxidants have proved to pose side-effects, natural antioxidant from plants are increasingly sought in the food, cosmetic and therapeutic industries (Mehta and Gowder, 2015). Antioxidants have been reported to work through single or combined mechanisms and thus a multi-assay system is required to have a comprehensive prediction of the antioxidant potential of the plant extract from different perspectives. Thus, in the present study, the antioxidant activity of different extracts of *P. dysenterica* were evaluated using phosphomolybdenum, radical scavenging (DPPH and ABTS radicals) reducing power (FRAP and CUPRAC) and the metal chelating assays.

Total antioxidant capacity of the different extracts was assessed using phosphomolybdenum method. Based on the experimental results (Table 2), it can be noted that there was no significant divergence in the total antioxidant capacity of extracts for all the different extraction techniques. Radical scavenging assays are performed to evaluate using the DPPH and ABTS assay. For both assay, all the extracts have showed remarkable activities. Highest activities were observed for the methanolic extract (DPPH: 303.95±4.10 and ABTS: 348.47±9.72 mg TE/g) obtained using homogenization technique, followed by the infused sample (DPPH: 240.07±8.49 and ABTS: 334.96±3.92 mg TE/g).

The reducing power of a compound is considered as a significant indicator of its potential antioxidant activity (Güder and Korkmaz, 2012; Zengin et al., 2014). Therefore, FRAP and CUPRAC assays were employed to determine the reductive ability of the *P. dysenterica* extracts. Based on the data gathered, the methanolic extracts obtained using homogenization technique were the most potent reducing agent of iron (II). In contrast to the

free radical and reducing power assays, the decocted and ultrasonicated aqueous (UAE-Water) samples have showed highest metal chelating activity with a mean value of 18.92±1.81 and 18.05±0.50 mg EDTAE/g, respectively.

3.3. Enzyme Inhibitory Properties

Naturally-occurring compounds from medicinal plants such as secondary metabolites showed broad-spectrum enzyme inhibitory potential. Medicinal enzyme inhibitors are often mediated by its specificity and its effectiveness that designated the absorption desirable to inhibit the targeted enzyme. High specificity and potency suggest that drugs from natural source will have few side effects and possess low toxicity (Murray et al., 2013; Rauf and Jehan, 2017).

Alzheimer's disease is controlled by extending the action of acetylcholine (ACh) via acetylcholinesterase (AChE) inhibition. Current AChE inhibitors such as tacrine, donepezil, and the plant-derived alkaloids rivastigmine and galantamine used to cure early and moderate stages of AD by increasing the endogenous levels of acetylcholine to boost cholinergic neurotransmission. However, these drugs have showed limited efficacy and unfavorable effects such as hepatotoxicity and gastrointestinal disorder. Thus, there is a urge to find safer AChE inhibitors from natural sources (Machado et al., 2015; Murray et al., 2013). In this study, the methanolic extract obtained using homogenization (HAE-MeOH) was the most potent inhibitor of AChE and BChE (Table 3).

Diabetes mellitus (DM) is a serious metabolic disorder, which is primarily characterized by an abnormal postprandial increase of blood glucose level. Diabetes is associated with metabolic abnormalities and serious complications such as cardiovascular disorders, blindness, renal failure, neuropathies and increased risk of cancer. One of the therapeutic approaches to manage DM is to reduce postprandial hyperglycemia through inhibition of intestinal glucose absorption by altering the activity of carbohydrate hydrolyzing enzymes, such as α-glucosidase

and α-amylase (Min and Han, 2014; Szkudelski and Szkudelska, 2015). As for the potential *in vitro* anti-diabetic effects, the highest activity against the studied key enzymes was observed for the macerated extract (α-amylase: 0.58±0.03 and α- glucosidase: 1.65±0.03 mmol ACAE/g) (Table 3).

Tyrosinase is a key enzyme in synthesizing melanin through melanogenesis. Melanin is primarily responsible for the pigmentation of human skin, eye and hair. Over activity of tyrosinase leads to abnormal pigmentation which is related to several cosmetic and clinical conditions such as melasma, lentigo, age spots, inflammatory hypermelanosis and trauma-induced hyperpigmentation. Thus, because of its crucial role in melanogenesis, tyrosinase is considered as an attractive target in the search for various kinds of depigmenting agents (Nesterov et al., 2008). All the studied extracts have showed noteworthy inhibitory activity against tyrosinase with a mean value ranging from 63.46±1.42 to 126.91±1.04 mg KAE/g. Highest activities were observed for ultrasonicated methanolic extract, followed by macerated and homogenized methanolic extracts (Table 3).

The increasing prevalence of obesity and obesity-related diseases such DM and hypertension, has become a major concern across the world. Obesity is principally related to the lipid metabolism and the key enzymes involved with this metabolism can be targeted for developing anti-obesity therapeutics (Heck et al., 2000). In this study, the inhibitory capacity of the extracts, against lipase, one of the key enzymes associated with obesity was evaluated. Based on the data recorded in Table 3, the inhibitory activity of the studied extracts against lipase are in this order: macerated > soxhlet > HAE-MeOH > UAE-MeOH.

3.4. Multivariate analysis

In Figure 2, relationships between total bio-compounds and studied biological activities were shown as nodes in a network. A high significant correlation were found between phenolic

compounds and CUPRAC, FRAP, ABTS and DPPH (Figure 2B). In addition, the results showed that phenolic acids were responsible for cupric reducing power and AChE inhibition, while flavonoid compounds were more related to inhibition ability on AChE, BChE, tyrosinase and amylase.

Hierarchical clustering analysis of samples (based on biological activities) revealed two major clusters, segregated in terms of the solvent used for extractions (Figure 4A). The clusters I consolidated the samples obtained from decoction, infusion, HAE and UEA methods combined with the use of water, whereas the cluster II aggregated the extracts result from maceration, SE, HAE and UAE methods with the use of methanol. Another interesting reporting was observed within the clusters I, in particular, two subclasses were found in accordance with the temperature of extraction (Figure 4B). In fact, HAE, a cold extraction method, was distinguished from other method (UEA, decoction and infusion), described as hot extraction methods. Similarly, as regards the cluster II, HAE was clustered together in subclass IIA independently from SE, maceration and UEA (Figure 4B). In the next step, PCA was performed to identify the biological activities typifying the obtained cluster from HCA. The first component, summarizing 57% and 33,7% of the total variance respectively, displayed clearly separation of the samples in agreement with results of HCA statistical analysis (Figure 4C&D). This segregation was along the first component of the PCA. The water extracts within the cluster I showed negative score value along the axis1 while the methanol extracts were allocated on the negative side. The more homogeneous subclass IA belonging to the first group, showed higher radical scavenger activity whereas the less homogeneous subclass IIA within the second group, exhibited good enzymes inhibitory activities. Unlike to HAE-water (subclass IB, cluster I), HAE-MeOH extract was found to be the most active against all antioxidant assays (ABTS, DPPH, FRAP and CUPRAC). Finally HCA of LC-MS dataset of chemical profiles was

assessed (Figure 4). The dendogram-based on 42 identified components disclosed two group. This segregation depends on the solvents used for the extractions (Figure 4E).

Conclusion

In the present study, we evaluated the antioxidant and enzyme inhibitory potential of *P. dysenterica* extracts obtained from decoction, homogenization, maceration, infusion, soxhlet extraction, and ultrasonication using methanol, and water as extraction solvents. Overall, the homogenized and ultrasonicated methanolic extracts has showed high total phenolic, total flavonoid and total phenolic acid content. The extracts obtained using all the extraction techniques have showed to possess a low total flavanol content. The homogenized methanolic and infused extract have exhibited good reducing abilities, free radical scavenging activities as well as total antioxidant capacities, while the highest metal chelating activity was noted for the decocted and ultrasonicated water extracts. *P. dysentrica* extracts have showed promising results for the management of diabetes type II, Alzheimer's disease, and skin hyperpigmentation disorders and obesity. The presence of polyphenolics could justify the antioxidant and inhibitory capacity potential. This study provides promising baseline data pertaining to the therapeutic properties *P. dysentrica,* warranting the need of further investigation that could open avenues towards the development of novel phytomedicines and cosmeceuticals.

References

Ahmad, I., Yanuar, A., Mulia, K., Mun'im, A., 2017. Application of Ionic Liquid as a Green Solvent for Polyphenolics Content Extraction of Peperomia pellucida (L) Kunth Herb. Journal of Young Pharmacists 9, 486.

Ahmad, V., Rasool, N., Abbasi, M., Rashid, M., Kousar, F., Zubair, M., Ejaz, A., Choudhary, M., Tareen, R., 2006. Antioxidant flavonoids from Pulicaria undulata. Polish Journal of Chemistry 80, 745-751.

Armenta, S., Garrigues, S., de la Guardia, M., 2015. The role of green extraction techniques in Green Analytical Chemistry. TrAC Trends in Analytical Chemistry 71, 2-8.

Basta, A., Tzakou, O., Couladis, M., Pavlović, M., 2007. Chemical composition of Pulicaria dysenterica (L.) Bernh. from Greece. Journal of Essential Oil Research 19, 333-335.

Bohlmann, F., Zdero, C., 1981. Caryophyllene derivatives and a hydroxyisocomene from Pulicaria dysenterica. Phytochemistry 20, 2529-2534.

Brglez Mojzer, E., Knez Hrnčič, M., Škerget, M., Knez, Ž., Bren, U., 2016. Polyphenols: extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. Molecules 21, 901.

Chaturvedi, A.K., 2018. Extraction of Neutraceuticals from Plants by Microwave Assisted Extraction. Systematic Reviews in Pharmacy 9.

Dhanani, T., Shah, S., Gajbhiye, N., Kumar, S., 2017. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of Withania somnifera. Arabian Journal of Chemistry 10, S1193-S1199.

Do, Q.D., Angkawijaya, A.E., Tran-Nguyen, P.L., Huynh, L.H., Soetaredjo, F.E., Ismadji, S., Ju, Y.-H., 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. Journal of food and drug analysis 22, 296-302.

El-Ghaly, E.-S.M., Shaheen, U., Ragab, E., El-hila, A.A., Abd-Allah, M.R., 2016. Bioactive constituents of Pulicaria jaubertii: A promising Antihypertensive Activity. Pharmacognosy Journal 8.

El-Negoumy, S.I., Mansour, R.M., Saleh, N.A., 1982. Flavonols of Pulicaria arabica. Phytochemistry 21, 953-954.

Elshamy, A.I., Mohamed, T.A., Marzouk, M.M., Hussien, T.A., Umeyama, A., Hegazy, M.E.F., Efferth, T., 2018. Phytochemical constituents and chemosystematic significance of Pulicaria jaubertii E. Gamal-Eldin (Asteraceae). Phytochemistry Letters 24, 105-109.

Eshbakova, K., Saidkhodzhaev, A., 2001. Triterpenoids and sterols from three species of Pulicaria. Chemistry of natural compounds 37, 196-197.

Ezoubeiri, A., Gadhi, C., Fdil, N., Benharref, A., Jana, M., Vanhaelen, M., 2005. Isolation and antimicrobial activity of two phenolic compounds from Pulicaria odora L. Journal of ethnopharmacology 99, 287-292.

Grochowski, D.M., Uysal, S., Aktumsek, A., Granica, S., Zengin, G., Ceylan, R., Locatelli, M., Tomczyk, M., 2017. In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of Potentilla thuringiaca. Phytochemistry letters 20, 365-372.

Güder, A., Korkmaz, H., 2012. Evaluation of in-vitro antioxidant properties of hydroalcoholic solution extracts Urtica dioica L., Malva neglecta Wallr. and their mixture. Iranian journal of pharmaceutical research: IJPR 11, 913.

Heck, A.M., Yanovski, J.A., Calis, K.A., 2000. Orlistat, a new lipase inhibitor for the management of obesity. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy 20, 270-279.

Hegazy, M.-E.F., Nakamura, S., Tawfik, W.A., Abdel-Azim, N.S., Abdel-Lateff, A., Matsuda, H., Paré, P.W., 2015. Rare hydroperoxyl guaianolide sesquiterpenes from Pulicaria undulata. Phytochemistry Letters 12, 177-181.

Hussien, T.A., El-Toumy, S.A., Hassan, H.M., Hetta, M.H., 2016. Cytotoxic and antioxidant activities of secondary metabolites from pulicaria undulata. International Journal of Pharmacy and Pharmaceutical Sciences 8, 150.

Liu, L.L., Yang, J.L., Shi, Y.P., 2010. Phytochemicals and biological activities of Pulicaria species. Chemistry & biodiversity 7, 327-349.

Machado, L.P., Carvalho, L.R., Young, M.C.M., Cardoso-Lopes, E.M., Centeno, D.C., Zambotti-Villela, L., Colepicolo, P., Yokoya, N.S., 2015. Evaluation of acetylcholinesterase inhibitory activity of Brazilian red macroalgae organic extracts. Revista Brasileira de Farmacognosia 25, 657-662.

Marco, J.A., Sanz, J.F., Albiach, R., 1992. Caryophyllene derivatives from Pulicaria dysenterica. Phytochemistry 31, 2409-2413.

Mehta, S.K., Gowder, S.J.T., 2015. Members of antioxidant machinery and their functions, Basic Principles and Clinical Significance of Oxidative Stress. InTech.

Min, S.W., Han, J.S., 2014. Polyopes lancifolia extract, a potent α-glucosidase inhibitor, alleviates postprandial hyperglycemia in diabetic mice. Preventive nutrition and food science 19, 5.

Muhamad, I.I., Hassan, N.D., Mamat, S.N., Nawi, N.M., Rashid, W.A., Tan, N.A., 2017. Extraction Technologies and Solvents of Phytocompounds From Plant Materials: Physicochemical Characterization and Identification of Ingredients and Bioactive Compounds From Plant Extract Using Various Instrumentations, Ingredients Extraction by Physicochemical Methods in Food. Elsevier, pp. 523-560.

Mumivand, H., Rustaii, A.-R., Jahanbin, K., Dastan, D., 2010. Essential oil composition of Pulicaria dysenterica (L.) Bernh from Iran. Journal of Essential Oil Bearing Plants 13, 717-720.

Murray, A.P., Faraoni, M.B., Castro, M.J., Alza, N.P., Cavallaro, V., 2013. Natural AChE inhibitors from plants and their contribution to Alzheimer's disease therapy. Current Neuropharmacology 11, 388-413.

Nesterov, A., Zhao, J., Minter, D., Hertel, C., Ma, W., Abeysinghe, P., Hong, M., Jia, Q., 2008. 1-(2, 4-Dihydroxyphenyl)-3-(2, 4-dimethoxy-3-methylphenyl) propane, a novel tyrosinase inhibitor with strong depigmenting effects. Chemical and Pharmaceutical Bulletin 56, 1292-1296.

Pares, J.O., Oksuz, S., Ulubelen, A., Mabry, T., 1981. 6-hydroxyflavonoids from Pulicaria dysenterica (Compositae). Phytochemistry 20, 2057.

Rauf, A., Jehan, N., 2017. Natural Products as a Potential Enzyme Inhibitors from Medicinal Plants, Enzyme Inhibitors and Activators. InTech.

Sánchez-Valdepeñas, V., Barrajón, E., Vegara, S., Funes, L., Martí, N., Valero, M., Saura, D., 2015. Effect of instant controlled pressure drop (DIC) pre-treatment on conventional solvent extraction of phenolic compounds from grape stalk powder. Industrial Crops and Products 76, 545-549.

Szkudelski, T., Szkudelska, K., 2015. Resveratrol and diabetes: from animal to human studies. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1852, 1145-1154.

Tanira, M., Ali, B., Bashir, A., Wasfi, I., Chandranath, I., 1996. Evaluation of the relaxant activity of some United Arab Emirates plants on intestinal smooth muscle. Journal of pharmacy and pharmacology 48, 545-550.

Uysal, S., Zengin, G., Locatelli, M., Bahadori, M.B., Mocan, A., Bellagamba, G., De Luca, E., Mollica, A., Aktumsek, A., 2017. Cytotoxic and enzyme inhibitory potential of two Potentilla species

(P. speciosa L. and P. reptans Willd.) and their chemical composition. Frontiers in pharmacology 8, 290.

Williams, C.A., Harborne, J.B., Greenham, J.R., Grayer, R.J., Kite, G.C., Eagles, J., 2003. Variations in lipophilic and vacuolar flavonoids among European Pulicaria species. Phytochemistry 64, 275-283.

Yusufoglu, H.S., 2014. Analgesic, antipyretic, anti-inflammatory, hepatoprotective and nephritic effects of the aerial parts of Pulicaria arabica (Family: Compositae) on rats. Asian Pacific journal of tropical medicine 7, S583-S590.

Zengin, G., Aktumsek, A., 2014. Investigation of antioxidant potentials of solvent extracts from different anatomical parts of Asphodeline anatolica E. Tuzlaci: an endemic plant to Turkey. African Journal of Traditional, Complementary and Alternative Medicines 11, 481-488.

Zengin, G., Sarikurkcu, C., Aktumsek, A., Ceylan, R., 2014. Sideritis galatica Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's's and diabetes mellitus. Journal of Functional Foods 11, 538-547.

Złotek, U., Mikulska, S., Nagajek, M., Świeca, M., 2016. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves (Ocimum basilicum L.) extracts. Saudi journal of biological sciences 23, 628-633.

Zullaikah, S., Rachmaniah, O., Utomo, A.T., Niawanti, H., Ju, Y.H., 2018. Green Separation of Bioactive Natural Products using Liquefied Mixture of Solids, Green Chemistry. InTech.

Table 1. Proposed compounds of *P. dysenterica* found in different extracts.

Peak	RT (min)	Proposed compound	m/z Exp	Molecular formula (M-H)	Occurrence							
					A	B	C	D	E	F	G	H
1	3.4	Quinic acid	191.0557	$C_7H_{11}O_6$	X	X	X	X	X	X	X	X
2	3.7	Citric acid	191.0205	$C_6H_7O_7$	X	-	X	X	-	-	-	X
3	5.5	Chlorogenic acid isomer 1	353.0868	$C_{16}H_{17}O_9$	X	X	X	X	X	X	X	X
4	7.2	Chlorogenic acid isomer 2	353.0865	$C_{16}H_{17}O_9$	X	X	X	X	X	X	X	X
5	7.5	Chlorogenic acid isomer 3	353.0875	$C_{16}H_{17}O_9$	X	-	X	X	-	-	-	X
6	8.4	Epigallocatechin	305.0694	$C_{15}H_{13}O_7$	X	X	X	X	X	X	X	X
7	8.4	Tuberonic acid glucoside	387.1642	$C_{18}H_{27}O_9$	X	X	X	X	X	X	X	X
8	9.4	Dicaffeoylquinic acid isomer 1	515.1203	$C_{25}H_{23}O_{12}$	X	X	X	X	X	X	X	X
9	10.7	UK1	525.2338	$C_{26}H_{37}O_{11}$	-	-	X	-	-	-	-	X
10	11.1	Feruloylquinic acid	367.1023	$C_{17}H_{19}O_9$	-	-	-	-	-	X	-	-
11	11.5	UK2	289.0392	$C_7H_{13}O_{12}$	-	X	X	X	X	X	X	X
12	11.5	Luteolin malonylglucoside	533.0946	$C_{24}H_{21}O_{14}$	X	-	X	X	-	-	-	X
13	12.3	Quercetin glucuronide	477.0689	$C_{21}H_{17}O_{13}$	X	X	X	X	X	X	X	X
14	13.2	Dicaffeoylquinic acid isomer 2	515.1193	$C_{25}H_{23}O_{12}$	X	X	X	X	X	X	X	X
15	13.7	UK3	721.2736	$C_{35}H_{45}O_{16}$	X	-	X	X	-	-	-	X
16	14	Dicaffeoylquinic acid isomer 3	515.1182	$C_{25}H_{23}O_{12}$	X	X	X	X	X	X	X	X
17	14.6	Dicaffeoylquinic acid isomer 4	515.1187	$C_{25}H_{23}O_{12}$	X	X	X	X	X	X	X	X
18	15.1	UK4	671.3268	$C_{33}H_{51}O_{14}$	X	X	X	X	X	X	X	X
19	15.5	UK5	705.2779	$C_{35}H_{45}O_{15}$	X	X	X	X	X	X	X	X
20	16.8	Alangisesquin isomer 1	747.2869	$C_{37}H_{47}O_{16}$	X	X	X	X	X	X	X	X
21	17.2	Ferulic acid	193.0503	$C_{10}H_9O_4$	-	-	-	-	-	X	-	-
22	17.8	Jasmonic acid glucoside derivative	371.1692	$C_{18}H_{27}O_8$	-	-	X	X	-	-	-	X
23	17.9	Eupatolitin	345.0595	$C_{17}H_{13}O_8$	X	-	-	-	-	-	X	-
24	18.2	Pteroceside A	713.3385	$C_{35}H_{53}O_{15}$	X	X	X	X	X	X	X	X
25	19.1	Alangisesquin isomer 2	747.2889	$C_{37}H_{47}O_{16}$	X	X	X	X	X	X	X	X
26	19.7	Quercetin	301.0353	$C_{15}H_9O_7$	-	-	X	-	-	-	-	-
27	20	Alangisesquin isomer 3	747.2896	$C_{37}H_{47}O_{16}$	X	X	X	X	X	X	X	X
28	20.8	Alangisesquin isomer 4	747.2884	$C_{37}H_{47}O_{16}$	X	X	X	X	X	X	X	X

29	21.3	Eupalitin	329.0658	$C_{17}H_{13}O_7$	-	X	-	-	X	X	X	-
30	21.9	Eupatin	359.0768	$C_{18}H_{15}O_8$	-	X	-	-	X	X	X	-
31	22.0	Prenantheside B	559.2199	$C_{29}H_{35}O_{11}$	X	-	X	X	-	-	-	X
32	22.7	Tianshic acid	329.2288	$C_{18}H_{33}O_5$	X	X	X	X	X	X	X	X
33	23.1	Acamptoic acid	463.2718	$C_{26}H_{39}O_7$	X	-	X	-	-	-	-	X
34	24.4	UK6	281.1768	$C_{16}H_{25}O_4$	-	X	-	-	X	X	X	-
35	26.9	UK7	505.2775	$C_{28}H_{41}O_8$	-	-	X	-	-	-	-	X
36	28.3	Deoxy-epipulchellin isomer1	249.1473	$C_{15}H_{21}O_3$	X	X	-	X	X	X	X	X
37	28.3	Eupatilin	343.0803	$C_{18}H_{15}O_7$	X	X	-	-	X	X	X	-
38	28.6	Ilicic acid isomer1	251.1631	$C_{15}H_{23}O_3$	X	-	X	-	-	-	-	-
39	28.8	Quercetagetin tetramethyl ether	373.0897	$C_{26}H_{13}O_3$	X	X	-	X	X	X	X	-
40	30.1	Ilicic acid isomer2	251.1631	$C_{15}H_{23}O_3$	X	X	X	X	X	X	X	X
41	30.6	Deoxy-epipulchellin isomer 2	249.1487	$C_{15}H_{21}O_3$	X	X	X	X	X	X	X	X
42	32.2	UK 8	293.1757	$C_{17}H_{25}O_4$	-	-	-	X	X	X	-	-

*A: Decoction; B: HAE methanol; C: HAE water; D: Infusion; E: maceration methanol; F: Soxhlet methanol; G: UAE methanol; H: UAE water. X: presence, -: non detected.

Table 2. Extraction yields and antioxidant properties of the tested extracts.

Decoction	16.9	2.72±0.09^{b}	220.43±9.42^{c}	315.29±3.18^{b}	574.91±8.05^{b}	316.88±2.59^{b}	18.92±1.81^{a}
HAE-MeOH	11.2	2.83±0.06^{ab}	303.95±4.10^{a}	348.47±9.72^{a}	710.57±11.03^{a}	367.49±5.16^{a}	8.50±0.45^{c}
HAE-Water	12.8	2.19±0.03^{c}	190.17±3.39^{f}	235.76±7.11^{d}	458.22±4.73^{e}	260.63±8.39^{e}	12.23±1.22^{b}
Infusion	17.9	2.59±0.08^{b}	240.07±8.49^{b}	334.96±3.92^{a}	541.23±9.76^{c}	325.41±7.59^{b}	17.41±0.46^{a}
MAC	9.3	2.33±0.13^{c}	192.56±2.35^{ef}	204.43±9.28^{e}	539.68±8.95^{c}	281.28±8.04^{d}	10.74±0.45^{b}
SE	13.9	2.10±0.03^{c}	187.25±4.90^{f}	197.63±7.18^{e}	499.94±20.37^{d}	233.99±5.14^{f}	8.58±1.21^{c}
UAE-MeOH	6.4	2.97±0.28^{a}	216.64±4.44^{cd}	240.35±10.04^{d}	568.75±13.00^{b}	274.84±3.24^{d}	10.86±0.80^{b}
UAE-Water	20.0	2.68±0.09^{b}	206.08±6.48^{de}	275.22±5.50^{c}	488.75±4.07^{d}	294.55±1.60^{c}	18.05±0.50^{a}

* Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent. HAE: Homogenizer assisted extraction; MAC: Maceration; SE: Soxhlet; UAE: Ultrasonication assisted extraction. Different letters indicate significant differences in the tested extracts ($p< 0.05$).

Table 3: Enzyme inhibitory activity of the tested extracts

Decoction	1.03±0.04[d]	na	0.10±0.01[d]	0.63±0.05[d]	71.50±1.95[e]	na
HAE-MeOH	3.97±0.05[a]	2.30±0.47[a]	0.56±0.05[ab]	1.49±0.05[b]	120.05±1.12[c]	55.69±0.87[b]
HAE-Water	0.48±0.04[e]	na	0.18±0.01[c]	0.78±0.07[c]	63.46±1.42[f]	na
Infusion	0.94±0.03[d]	na	0.09±0.01[d]	na	75.26±0.82[d]	na
MAC	3.17±0.16[c]	1.54±0.44[c]	0.58±0.03[a]	1.65±0.03[a]	126.42±1.09[ab]	61.09±2.96[b]
SE	3.50±0.15[b]	1.98±0.22[ab]	0.50±0.02[b]	1.60±0.02[a]	123.84±0.99[b]	73.50±7.01[a]
UAE-MeOH	3.47±0.22[b]	1.67±0.06[bc]	0.56±0.05[ab]	1.46±0.05[b]	126.91±1.04[a]	55.32±5.27[b]
UAE-Water	0.54±0.04[e]	na	0.10±0.01[d]	0.69±0.05[d]	73.00±2.03[de]	na

[*] Values expressed are means ± S.D. of three parallel measurements. GALAE: Galatamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; OE: Orlistat equivalent; na: not active. HAE: Homogenizer assisted extraction; MAC: Maceration; SE: Soxhlet; UAE: Ultrasonication assisted extraction. Different letters indicate significant differences in the tested extracts ($p < 0.05$).

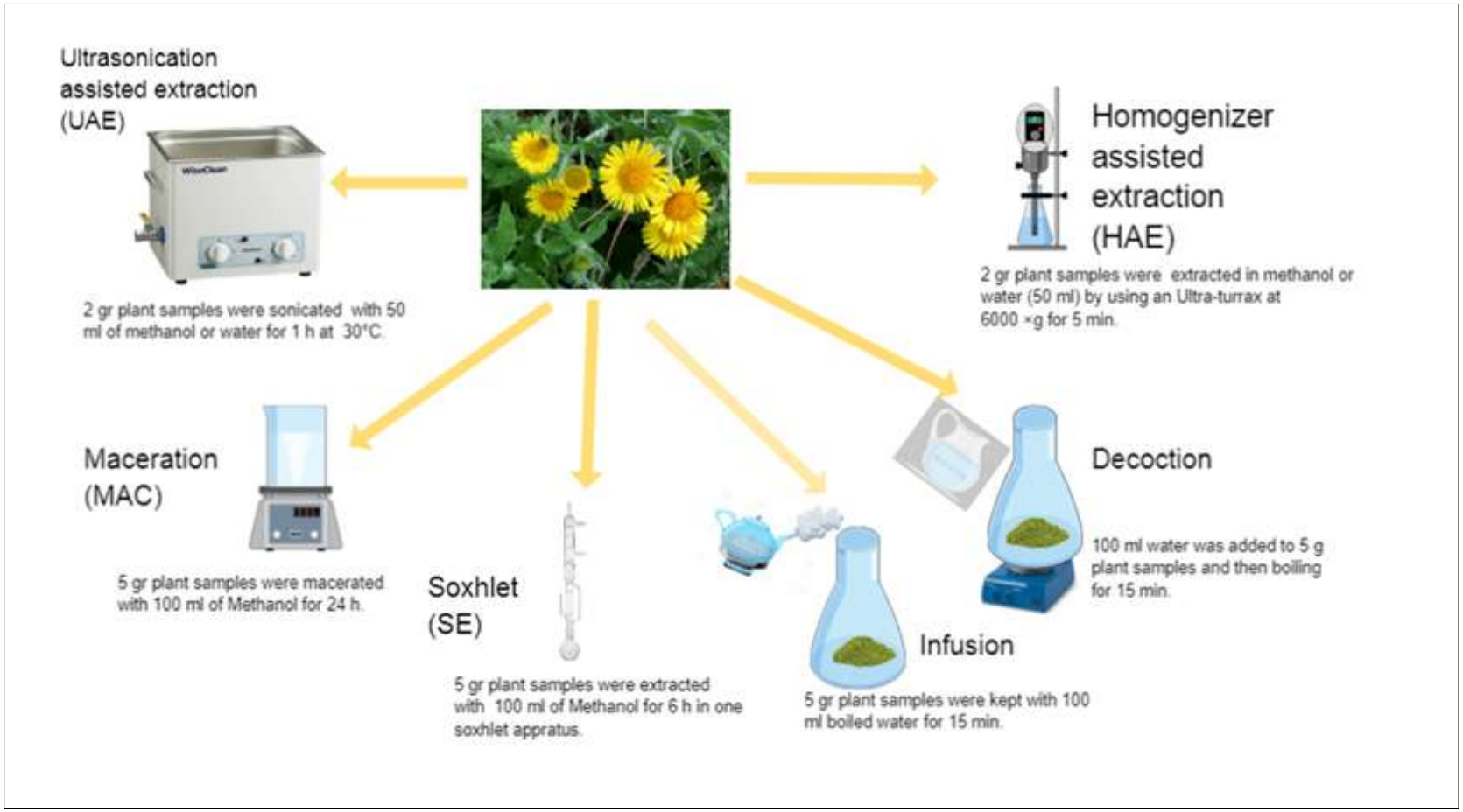

Figure 1. The extraction methods performed in the present study.

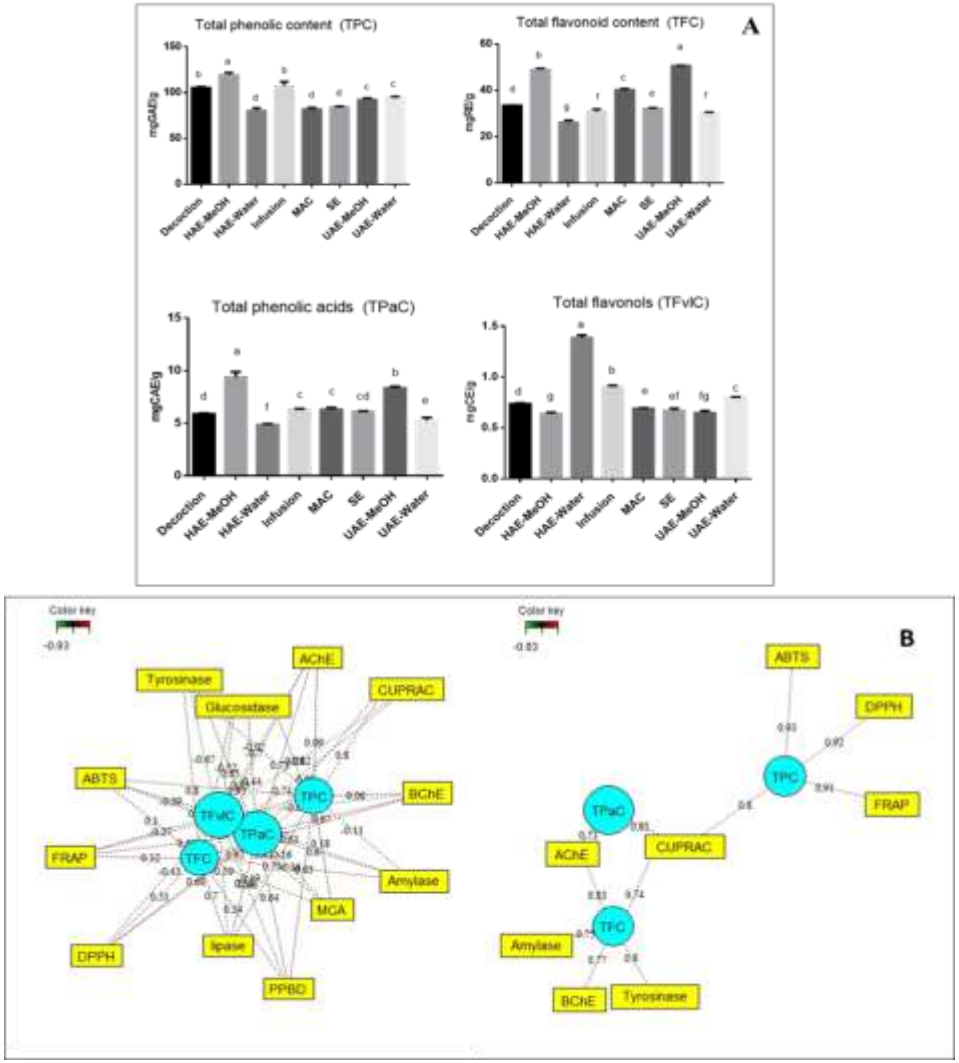

Figure 2. Total bioactive components (A) and relationship between these components and biological activities (B). Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent; CAE: Caffeic acid equivalent; CE: Catechin equivalent; HAE: Homogenizer assisted extraction; MAC: Maceration; SE: Soxhlet; UAE: Ultrasonication assisted extraction. Different letters indicate significant differences in the tested extracts ($p < 0.05$).

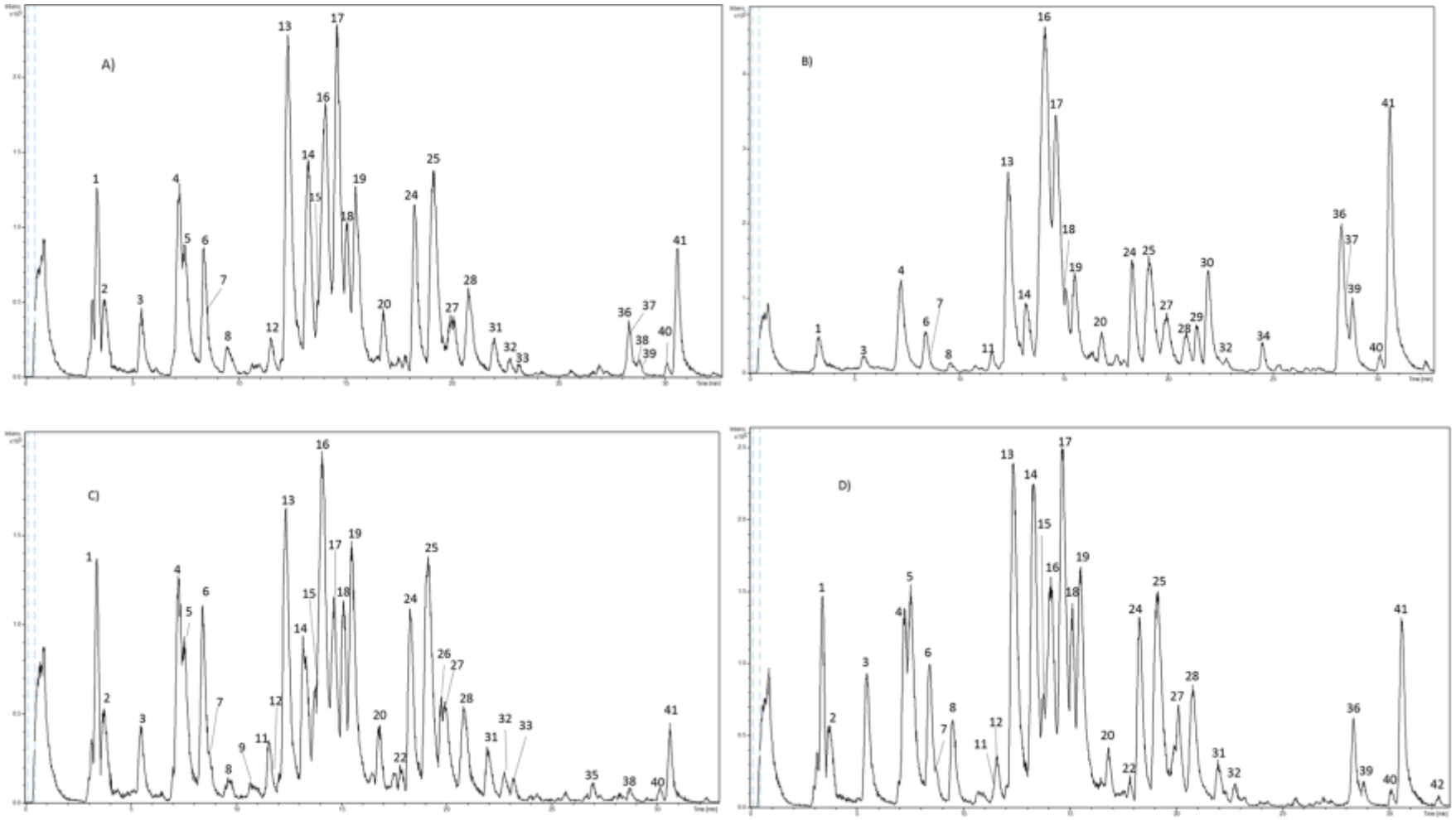

Figure 3. Base peak chromatograms of the tested extracts. A: Decoction; B: HAE methanol; C: HAE water; D: Infusion; E: maceration methanol; F: Soxhlet methanol; G: UAE methanol; H: UAE water.

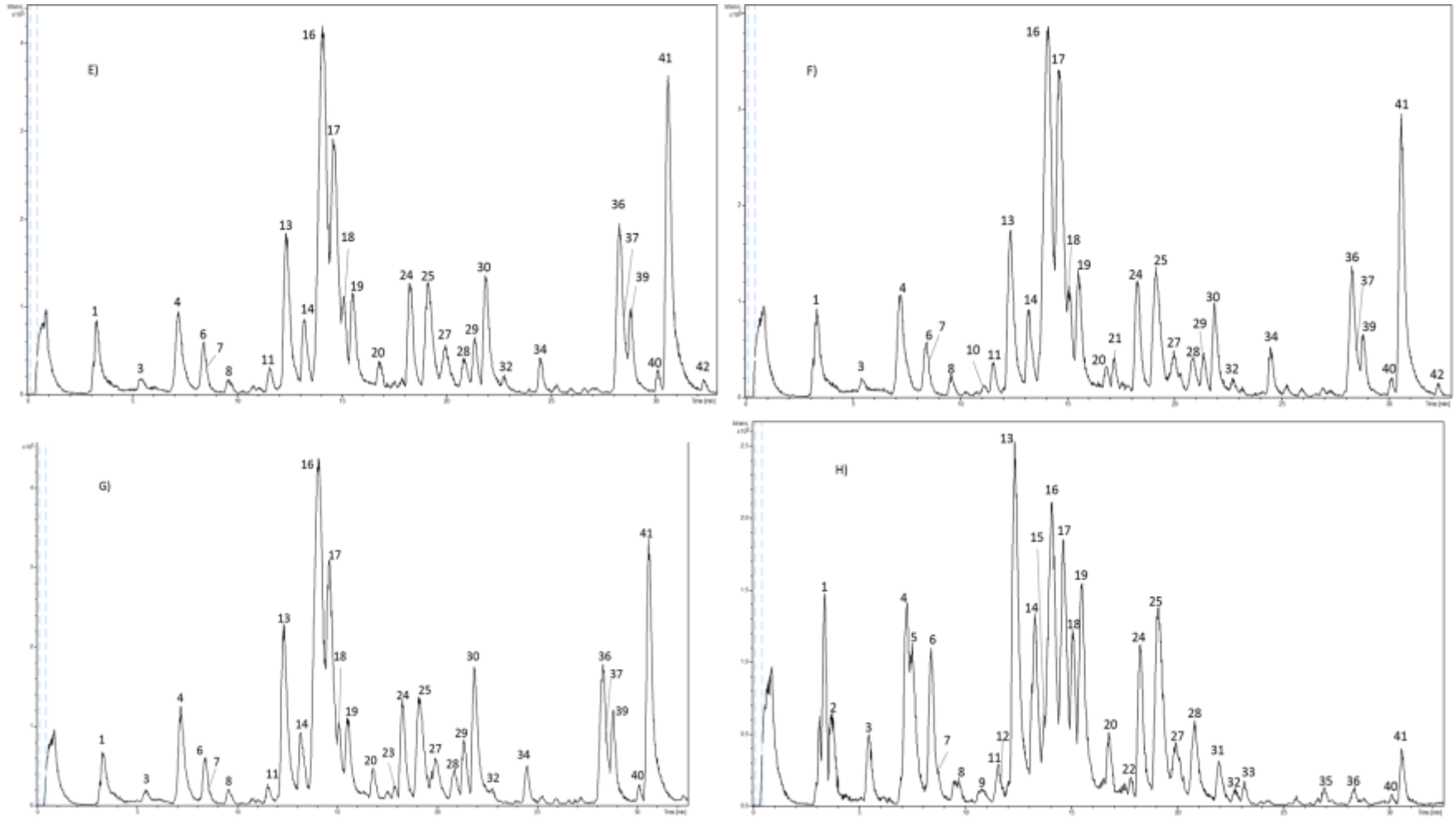

Figure 3 (continued).

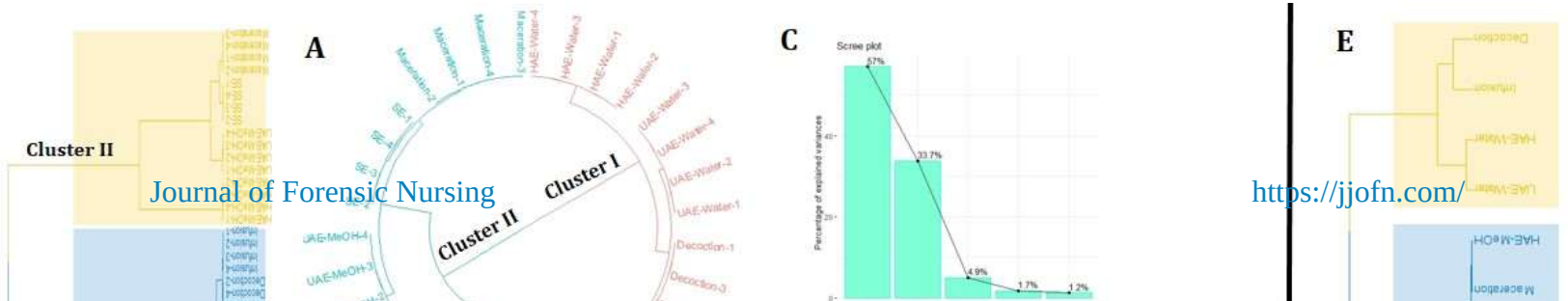

Figure 4. Multivariate statistical analysis on the tested extracts biological activities and compounds from according to different extraction methods. A&B: HCA plots of biological activities from the tested extracts displaying two major clusters and four subclasses respectively. C: Scree plot of PCA showing total variance explained by each component. D: PCA score plot of PC1 vs PC2. E: Hierarchical clustering analysis plot of compounds from *t*he tested extracts (from LC-MS results).