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PLANTS' PHYTOCHEMICALS – A CRITICAL DISCUSSION ON THEIR POTENTIAL TO PROTECT CULTURAL HERITAGE OBJECTS

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Abstract
Since ancient times, plants were used for different purposes: even we talk about medicine, construction materials, food or feed. In modern times, plants are a huge factory of compounds which can replace synthesis and hazardous compounds, being appreciated like raw materials in different fields. In the field of cultural heritage protection there is an urgent need to develop innovative, eco-sustainable, and safe solutions, so, plants' constituents can be an excellent choice. In this context, our short review intends to present a critical opinion on aspects regarding obtaining methods of plants extract, some aspects regarding chemical active compounds, and their application in the cultural heritage domain, being a starting step for a future trans disciplinary study, which offers links between multy disciplinary sciences: chemistry, biology, restoration and conservation.

Keywords: extraction methods, cultural heritage, plants extract.

1. INTRODUCTION

Since ancient times, plants were used for different purposes: even we talk about medicine, construction materials, food or feed. In modern times, plants are a huge factory of compounds which can replace synthesis and hazardous compounds, being appreciated like raw materials in different fields. In the field of cultural heritage protection there is an urgent need to develop innovative, eco-sustainable, and safe solutions, so, plants' constituents can be an excellent choice (Palla et al., 2020). In the form of extracts or essential oils, they can be used for removing biodeterioration (for stone heritage) (Bruno et al., 2019), or corrosion inhibitors for metallic objects (Cano and Lafuente, 2013).

Cultural heritage can be defined in a large sense, as a connection between the past and the future (Indrie et al., 2019). Cultural heritage consists in stone and wooden objects, paintings or photographs, parchments, papers and metallic objects (Gutarowska, 2020). While for wooden, paper or stone objects we deal with biodeterioration caused by microorganisms, for metallic objects we have damages caused by corrosion products due to the burial processes, or to the unproper restoration treatments.

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Wood artefacts - Due to the composition based on cellulose (40–50%), hemicellulose (15–25%), and lignin (15–25%) the risk of fungal attack is maximum (Singh, 2012). For example, degradation with *Flavobacteria* and *Cytophaga* is often associated with buried and waterlogged archaeological wood (Bjordal, 2012); *Clostridium xylanolyticum* affects lignin to a limited extent, in the presence of higher amounts of oxygen (Clausen, 1996).

Stone artefacts - *Fischerella*, *Eucapsis*, *Leptolyngbya* are phototrophic microorganisms that form biofilms and can damage stone artefacts (Crispim and Gaylarde, 2005); *Halobacterium, Halococcus* can grow in a salty environment (Mazzoli et al., 2018) and *Cladosporium*, *Penicillium* or *Trichoderma* can penetrate into stone (Cutler and Viles, 2010).

Paper artefacts - Paper is made of vegetable fibers with cellulose as their main compound; it is often attacked by *Aspergillus*, *Paecilomyces*, *Chrysosporium*, *Penicillium* and *Cladosporium* in the presence of temperature and humidity conditions (Okpalanozie et al., 2018); meanwhile *Stachybotrys* spp., *Chaetomium* spp. or *Epicoccum* spp. need moisture conditions (Sterflinger and Pinzari, 2012).

The use of traditional biocides is reduced in the last decades, and there is an interest in finding new “green” alternatives which can replace hazardous chemicals. So, plants extracts or nanoarchitectures obtained through “green technologies” was carried out to explore their antimicrobial potential. Due to the complex chemical compositions which is highly variable due to factors, such as age of plant, season variations and modality of extraction, there is a wide range for obtaining active principles.

In this context, our short review intends to present a critical opinion on aspects regarding obtaining methods of plants extract, some aspects regarding chemical active compounds, and their application in the cultural heritage domain, being a starting step for a future trans disciplinary study, which offers links between multy disciplinary sciences: chemistry, biology, restoration and conservation.

2. MATERIALS AND METHODS

Our short review is a critical discussion which cover some important aspects regarding the obtaining of active antimicrobial compounds used for cultural heritage protection, from vegetal materials. Our opinion is based on studying the articles published in the last decade, through consultation Scopus Data base.

3. RESULTS AND DISCUSSIONS

Obtaining plants extracts

Extraction is an essential feature in natural product research. For obtaing increased yelds of active compounds, some aspects must be discussed, starting with the choice of the solvent and finishing with the extraction methods. Depending of the solubility of the target compounds can be used different solvents, with different pollarities (Fierascu et al., 2019). Some of them are soluble in polar solvents (phenols, carotenoids, etc.), some in non-polar (Strati and Oreopoulou, 2011). Classical (traditional) methods are based on combining the extraction capacity of solvents with different thermal factors.

The main conventional techniques include Soxhlet extraction, maceration, percolation and hydrodistillation. For Soxhlet extraction, the main advantage of this method is the use of small amounts of solvent for the recovery of bioactive compounds from numerous series of plant materials. However, the extraction process requires a long time, with the risk of thermal degradation of the compounds of interest. The Soxhlet extractor is also frequently used because it is

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simple, can be used in multiple extractions, and does not require a filtration step (Siddique et al., 2020). Maceration is a fairly old method (used since ancient times), initially used in domestic use, later becoming a cheap technique for extracting bioactive compounds and essential oils. Its main disadvantage is related to the solvents and energy requirements (Sagar et al., 2018). It can be applied, in the recovery of polyphenols, using as solvent dichloromethane, ethanol, methanol or water/methanol mixture (Teixeira and Da Costa, 2005). In the hydro-distillation process, water is used as a solvent. Its working mechanism is similar to that of the Soxhlet extractor (Vankar, 2004). The use of water as a solvent in the extraction process is the main advantage, as it is inexpensive and environmentally friendly. However, the longtime it requires, leads to the thermal degradation of the compounds of interest.

The efficient extraction of bioactive compounds directly depends on both temperature, pressure, extraction time, pH and the chosen solvent (for the modern extraction techniques). The main advanced techniques for the extraction of bioactive compounds, from vegetal materials, are: microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), along with eutectic solvent extraction (DES). Their advantages relie in the use of reduced solvent and energy consumption, providing significantly higher recovery yields for target compounds (Fierascu et al., 2019).

Microwave-assisted extraction (MAE) can be considered as the "green" extraction technique, because compared to other techniques, it shortens the extraction time and reduces the solvent consumption. Within it, microwave electromagnetic radiation heats a dielectric material, through the rotation of the dipole moment, of the polar components present in the matrix (Ran et al., 2019). Ethanol itself or diluted with water can absorb microwave energy and is frequently used in MAE extractions, being a proper method to extract phenolic compounds (Fierascu et al., 2020). By optimizing the operational parameters of the MAE method at: a solid/solvent ratio (47% ethanol) of 45 g/L, 200W microwave power, 180°C extraction temperature, for 20 minutes, an extraction yield of 76% is obtained from tomatoes, with a total polyphenol content of 43.9 mg GAE/g and a total flavonoid content of 3.5 mg CE/g (Pinela et al., 2017). Bioactive compounds can also be extracted from grape skins. Thus, using the MAE method, the extraction process of the compounds of interest takes place quickly (83 seconds, with a microwave power of 900 W) (Pedroza et al., 2015).

Ultrasound-assisted extraction (UAE) also allows reducing the time and amount of solvent required for efficient extraction of compounds. This type of extraction also allows reducing the time and amount of solvent required for efficient extraction of compounds, being an efficient alternative as an extraction method (Garcia-Salas et al., 2010). Jang and coworkers extracted quercetin (11 mg/g dry weight) from onion waste by the UAE method, reporting the optimal conditions as: 1% solvent (ethanol 59%) and extraction temperature 49°C. Moreover, they demonstrated that both pH, extraction temperature and solid/solvent ratio do not significantly affect the final yield (Jang et al., 2013). Chlorogenic acids, caffeic acid, catechin and epicatechin, can be efficiently recovered from carrot pomace using optimized UAE with response surface methodology (Jabbar et al., 2015).

Super critical fluid extraction (SFE) uses supercritical fluids (systems consisting of one or more compounds, at conditions above their critical values of pressure and temperature) as an extraction solvent for the separation different components from the matrix. Supercritical fluid is used as an alternative to traditional organic liquid solvents (Sairam et al., 2012). The addition of a co-solvent (ethanol, an ecofriendly solvent) can change the polarity of supercritical fluid, allowing the extraction of more polar molecules (Pereira și Meireles, 2010). Ferrentino and coworkers obtained active compounds from raw material apple pomace (fresh, oven-dried, and lyophilized), variable

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pressures (20 or 30 mPa) and temperatures (45°C or 55°C), in the absence/presence of ethanol as co-solvent (5%, v /v) (Ferrentino et al., 2018).

Different chemical compounds with antimicrobial role (some examples)

Phenolic compounds – are the most common compounds found in extracts composition, phenolics globally account for 8,000–10,000 molecules (Brglez Mojzer et al., 2016). Phenolic compounds can be divided into four large clases: phenolic acids, flavonoids, stilbenes and tannins (Gil-Martín et al., 2020). The concentration of these compounds is different for species and plants depending on different factors such as, climate, soil quality, topography and cultivar (Perussello et al., 2017). A large part of the extraction protocols includes the use of solvents such as ethanol or glycerol, in order to replace petroleum-derived solvents (Manousaki et al., 2016). Deep eutectic solvents (DES) are a new modern altrnative to replace toxic solvents for the extraction of phenolics (Socas-Rodríguez et al., 2021). Flavonoids, catechins and phenolic acids delay lipid oxidation and inhibit the growth of many food microorganisms (Sorrentino et al., 2018). Furthermore, gallic or ferulic acid inhibits the formation of biofilms on food surfaces. Moreover, its formation is a key factor in triggering toxin production and microorganism pathogenicity (Papuc et al., 2017). Betanin, lutein, riboflavin and anthocyanins, which are found in the residues of fruit peels (Rodriguez-Amaya, 2016).

Terpenes – are one of the most abundant compounds, divided into two main categories: terpene hydrocarbons and oxygenated compounds (Moghaddam and Mehdizadeh, 2017). Are present in essential oils from plant resins (Morsy, 2017). These compounds oxidize rapidly due to their fast reaction to heat and air sources (George et al., 2015). Essential oils obtained from cinnamon, oregano and thyme, based on sesquiterpenes, monoterpenes have antimicrobial effect against *Listeria monocytogenes*, *Escherichia coli*, *Bacillus thermosphacta* and *Pseudomonas fluorescens* (Beggas and Bendoukhane, 2017). *Rosmarinus officinalis* L. contain compounds such as phenolic diterpenes (Irmak et al., 2010); thyme has as main contituients thymol, carvacrol, gamma-terpinene, p-cymene and linalool (Fonseca et al., 2020).

Alkaloids are heterocyclic nitrogen compounds, the first medically useful example of an alkaloid was morphine, isolated in 1805 from the opium poppy *Papaver somniferum* (Cowan, 1999). Methanol and ethanol are the most commonly used as solvents for the extraction of alkaloids (Sun et al., 2012). Among classical methods maceration, Soxhlet and percolation, are suitable in the extraction of alkaloids (Doughari, 2012). From modern techniques, microwave extraction method generates pressure inside the cells, facilitating the cell rupture and increasing the extraction yield (Chan et al., 2011).

Application of „green alternatives" in cultural heritage protection

Frequent application of classical biocides (Isothiazolinones, quaternary ammonium compounds, etc.) which are hazardous substances, is costly and dangerous to both humans and the environment. Finding new alternatives is a new challenge for the researchers in trans disciplinary domains (chemists, restorer, archaeologist, biologist). In some cases, some properties of „greener alternatives", such as high volatility, different solubilities in water, and thermal properties of biologic active compounds could make them difficult to apply to cultural heritage (Romano et al., 2020). Appropriate methodologies have been developed in the last decades, in order to use these

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„green alternatives”. This is the case of controlled release system based on chitosan nanoparticles., loaded with thymol for the limestone-built areas of Feilaifeng (introduced in the World Heritage List by UNESCO in 2011) (Wang et al., 2022). Thymol encapsulated in chitosan nanoparticles (encapsulation range 15.22 to 33.90%, antimicrobial activity against *Aspergillus niger*), not only improves the thermal stability of the formulation and its antimicrobial properties, but also obtains the ability of a controlled release. *Anethum graveolens* L., *Cymbopogon citratus*, *Juniperus oxycedrus* L. were succesfully used against fungal deterioration of stucco ornaments in the Mihrab of Mostafa Pasha Ribate, Cairo, Egypt (Afifi, 2012). *Calamintha nepeta* and *Allium sativum* extracts were used against fungal colonies of *Bacillus subtilis*, *Micrococcus luteus* and *Penicillium chrysogenum*, *Aspergillus* spp (Rotolo et al., 2016). Stupar and coworkers used *Origanum vulgare*, *Rosmarinus officinalis* and *Lavandula angustifolia* against fungi isoletd from wooden and stone artefacts from Serbia (Stupar et al., 2014), where carvacrol (64.06%) and linalool (37.61%) were the main constituients from these plants. Sakr and coworkers used essential oils of lemon, spearmint, fennel, marjonam and rosemary against strains isolated from Egypitan tombs (*C. lipolytica*, *C. albicans*, *L. elongspous*), obtaining inhibition zones from 2,5 to 6 mm (Sakr et al., 2012). The deterioration of the Royal Tombs of the Joseon Dynasty in South Korea was studied by Jeong and coworkers. They tested eugenol against 19 types of molds, obtaining 80% reduction in microorganisms (Joeng et al., 2018).

Another alternative to control deterioration are dedicated coatings for different supports. This can be the case of coatings based silver nanoparticles obtained by phytosinthesis with *Schinus molle*, *Equisetum giganteum*, and *Ilex paraguariensis Saint Hilaire* which have the property to inhibit fungal and bacterial growth (Barberia-Roque et al., 2019). Caffeine and nicotine increased the properties of comercial product Paraloid B-72 in the case of iron artefacts conservation, in 98% relative humidity, nicotine presenting a high degree of protection, superior to the caffeine protection (Roncagliolo Barrera et al., 2019). The extract of *Punica granatum* can decrease the damage caused by corrosion in 2 M HCl and 1 M H_2SO_4 solutions. Weight loss of the samples varied between 1,47 to 2,91 mgcm^{-2} , thus demonstrating the beneficial effect of the extract (Behpour et al., 2012).

4. CONCLUSIONS

In order to highlight the importance of „ecofriendly” solutions used as alternatives to toxic chemical compounds used for the protection of cultural heritage, this paper was developed as a critical discussion to be a „start point” for future practical works. It was presented that plants can be a useful and inexhaustible source of active compounds with potential to obtaing practical solutions to remove (bio)deterioration and to stop future damages.

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