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STATEMENT OF INTEGRITY

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

EXPLORAÇÃO DE EXTRATOS DE PLANTAS PARA A COSMÉTICA E INDÚSTRIA TÊXTIL

RESUMO

Os extratos de plantas e outras fontes naturais contêm metabolitos com atividade biológica benéfica para aplicações nas mais diversas áreas, incluindo Saúde, Cosmética e Têxtil. A extração destes metabolitos é uma etapa crítica, pois envolve processos exaustivos e demorados de isolamento e purificação. Estes processos são considerados "limpos" quando comparados com as indústrias químicas, mas o seu impacto ambiental continua a ser considerável. Há assim necessidade de desenvolver técnicas ecológicas e eficientes, para substituir os métodos e solventes convencionais. Pela sua biodegradabilidade e não toxicidade, os extratos obtidos de fontes naturais representam uma ótima alternativa para substituir ingredientes tóxicos e não ecológicos não só na Indústria Cosmética como também na Têxtil na substituição dos corantes sintéticos. As questões ambientais associadas a esta indústria têm levado à procura de métodos alternativos que permitam a redução do consumo de água e de produtos químicos nos processos de tingimento e na remoção de efluentes poluentes. Nesta dissertação, foi efetuada a extração de compostos bioativos de oito fontes naturais (bagas de azereiro, grãos de cacau, carqueja, canta viril, damiana, donzela, alfavaca e pólen) com o auxílio de solventes eutécticos profundos (DES) e solventes convencionais (água e dioxano) e três métodos de extração (extração assistida por ultrassom (UAE), extração assistida por misturadora (MAE) e extração por "sealed system" (SSE)). Os extratos foram posteriormente caracterizados quanto ao conteúdo total em fenólicos (TPC), atividade antioxidante e composição, bem como citotoxicidade e atividade antimicrobiana. Os extratos obtidos com DES foram também aplicados no tingimento do algodão, tendo sido estudadas as condições de processo como pré-tratamento e pré-mordentes. Além disso, os DES foram aplicados no tingimento do poliéster e no tratamento e destoxificação de efluentes. Após extração com DES e solventes convencionais, os resultados demonstraram que os solventes eutécticos promoveram maiores rendimentos de extração do que os solventes convencionais, e que os métodos de extração UAE e MAE demonstraram elevado potencial extrativo. Os extratos obtidos apresentaram alto teor em fenólicos, destacando-se os extratos obtidos da carqueja, e com elevado poder antioxidante, principalmente os extratos obtidos da carqueja, dos grãos de cacau e do canta viril. Os extratos obtidos de bagas de azereiro, alfavaca e pólen não apresentaram atividade citotóxica. Os extratos obtidos pela extração com DES de grãos de cacau, bagas de azereiro e pólen apresentaram atividade antimicrobiana contra *E. coli* e *S. aureus*. Os extratos naturais à base de DES demonstraram capacidade para tingir algodão, produzindo cores claras, sendo que não foram verificadas diferenças notórias de intensidade de cor, independentemente do pré-tratamento e pré-mordente realizado. O tingimento de poliéster na presença de DES resultou em tecidos tingidos com força colorística similar ou superior à obtida com um solvente convencional (água). A capacidade dos DES sequestrarem corantes de efluentes têxteis também foi estudada e os resultados revelaram que a concentração de corante, a dose de DES e o pH do DES limitam a eficiência do processo de extração. A destoxificação de efluente de curtumes foi também efetuada na presença de DES com o auxílio de ultrassons e centrifugação, e os resultados demonstraram que o pH do eutéctico é um dos fatores que mais influencia a sequestração do corante da solução de efluente. A caracterização do efluente após tratamento com DES revelou que este foi capaz de remover a maioria dos poluentes e compostos tóxicos das águas residuais de uma indústria de curtumes. O trabalho desenvolvido revelou diferentes potencialidades dos solventes eutécticos que, para além das suas capacidades extrativas, revelaram capacidade de, quando incluídos na mistura extraída, fazer parte de formulações cosméticas e conferir capacidade antioxidante e antimicrobiana, sem adicionar toxicidade. Ao mesmo tempo, estes solventes podem ser utilizados como solventes em processos de tingimento para obtenção de níveis de força colorística similares aos usados como solventes convencionais, mas com redução da quantidade de água no processo. A capacidade de sequestração e destoxificação de efluentes das indústrias Têxtil e Curtumes revelou-se promissora para futura otimização e aplicação no tratamento de efluente destas indústrias.

Palavras-chave: Extratos naturais; solventes eutécticos profundos; compostos bioativos; extração; tingimento; tratamento de efluentes.

EXPLORING PLANT EXTRACTS FOR COSMETIC AND TEXTILE INDUSTRY

ABSTRACT

Plant and other natural sources extracts contain metabolites with beneficial biological activity for application in the most diverse areas, including Health, Cosmetics, and Textile. The extraction of these metabolites is a critical step as it involves lab-intensive and time-consuming isolation and purification processes. These processes are considered "clean" compared with the heavy chemical industries, but their environmental impact is far greater than expected. Hence, there is a need to develop ecological and efficient techniques while replacing harsh solvents. Due to their biodegradability and non-toxicity, extracts from natural sources represent a great alternative to replace toxic and non-environmentally friendly ingredients in the Cosmetic Industry and Textile, replacing synthetic dyes. The environmental issues associated with this industry are increasingly demanding new alternative methods that allow the reduction of water and chemicals consumption in dyeing processes and the removal of polluting wastewaters. In this dissertation, was performed the extraction of bioactive compounds from eight natural sources (cherry bay berries, cocoa beans, "carqueja", "canta viril", damiana, "donzela", African basil and pollen), with deep eutectic solvents (DES) and conventional solvents (water and dioxane) and three extraction technologies (ultrasound-assisted extraction (UAE), Mixer-assisted extraction (MAE) and sealed system extraction (SSE)). The extracts were posteriorly characterized in terms of total phenolic content (TPC), antioxidant activity and composition, as well as cytotoxicity and antimicrobial activity. The extracts obtained with eutectic solvents were also applied in cotton dyeing, and the process conditions such as pre-treatment and pre-mordants were studied. In addition, DES were applied in the dyeing of polyester and the treatment and detoxification of effluents. After extraction with DES and conventional solvents, the results showed that eutectic solvents promoted higher extraction yields than conventional solvents, and that the extraction methods, UAE and MAE, showed high extractive potential. The extracts obtained showed a high content of phenolics, highlighting the extracts obtained from "carqueja", and high antioxidant power, especially the extracts obtained from "carqueja", cocoa beans and "canta viril". The extracts obtained from cherry bay berries, African basil and pollen did not show cytotoxic activity. The extracts obtained with DES from cocoa beans, cherry bay berries and pollen showed antimicrobial activity against *E. coli* and *S. aureus*. Natural extracts based on DES demonstrated the ability to dye cotton, producing light colours, and no differences in colour intensity were observed, regardless of the pre-treatment and pre-mordant performed. Dyeing of polyester in the presence of DES resulted in dyed fabrics with similar or superior colour strength to that obtained with a conventional solvent (water). The ability of DES to sequester dyes from textile effluents was also studied and the results revealed that dye concentration, DES dose and DES pH limit the efficiency of the extraction process. The detoxification of the effluent from tanneries was also carried out in the presence of DES, with the aid of ultrasound and centrifugation, and the results showed that the pH of the DES is one of the factors that most influences the sequestration of the dye from the effluent solution. The characterization of the effluent after treatment with DES revealed that it was able to remove most pollutants and toxic compounds from the wastewater of a tannery industry. The developed work revealed different potentialities of eutectic solvents which, in addition to their extractive capacities, revealed the capacity, when included in the extracted mixture, to be part of cosmetic formulations and to confer antioxidant capacity without adding toxicity. At the same time, these solvents can be used as solvents in dyeing processes to obtain levels of colour strength similar to those with conventional solvents but with a reduction in the amount of water in the process. The capacity for sequestration and detoxification of effluents from the Textile and Tannery industries has shown to be promising for future optimization and application in the treatment of effluents from these industries.

Key words: Natural extracts; deep eutectic solvents; bioactive compounds; extraction; dyeing; effluent treatment.

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LIST OF ABBREVIATIONS

%rDPPH	Percentage of reduced DPPH
Al	Aluminium
ANOVA	Analysis of variance
BSTFA	N,O-Bis(trimethylsilyl)trifluoroacetamide
CFUs	Colony forming units
ChCl	Choline chloride
COD	Chemical oxygen demand
CS	Copper sulphate
DB124	Disperse blue 124
DB134	Disperse blue 134
DES	Deep eutectic solvents
DLV	Discharge limit values
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ELV	Emission limit values
FBS	Fetal bovine serum
FTIR	Fourier transform infrared spectroscopy
GA	Gallic acid
GAE	Gallic acid equivalents
GC-MS	Gas chromatography-mass spectrometry
H_2O	Water
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
HCl	Hydrochloric acid
IC_{50}	Half maximal inhibitory concentration
ICP	Inductively Coupled Plasma
IL	Ionic liquids
K/S	Colour strength
LOD	Limit of detection

LOQ	Limit of quantitation
MAE	Mixer-assisted extraction
NADES	Natural deep eutectic solvents
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
NMR	Nuclear magnetic resonance
owf	on weight of fabric
PBS	Phosphate-buffered saline
PLE	Pressurized liquid extraction
ROS	Reactive oxygen species
SD	Standard deviation
SS	Sealed system
SSE	Sealed system extraction
$T_{5\%}$	Temperature at which 5% weight loss occurs
TA	Tannic acid
TGA	Thermogravimetry analysis
T_{dmax}	Temperature with maximum degradation rate
TPC	Total phenolic content
TCr	Total chromium
TN	Total nitrogen
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TS	Total solids
TSA	Tryptic-soy agar
TSB	Tryptic-broth agar
UAE	Ultrasound-assisted extraction
US	Ultrasound
UV-Vis	Ultra-violet - visible

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1. INTRODUCTION

1.1. Plant extracts

Plants have been used for centuries for various applications, as it is recognized that they represent a source of many compounds with beneficial activities. Using plants as medicines, such as teas, is one of the oldest forms of healthcare (Veiga et al., 2020).

Plant extracts correspond to complex mixtures that contain substances with desirable properties. Extracts are obtained from plant tissues, such as leaves or roots. These extracts contain various chemicals with biological properties (bioactive compounds), including antioxidant, antibiotic and anticancer activity (Armendáriz-Barragán et al., 2016).

1.1.1. Composition of the plant extracts

Plants produce a large volume of compounds classified as primary and secondary metabolites. From the water, mineral elements, carbon dioxide, and the sunlight captured, plants synthesize primary metabolites. These are pivotal for cell maintenance and for plants to complete their lifecycle, performing photosynthesis and respiration. Moreover, primary metabolites are highly conserved in their structure and comprise the highest proportion of the metabolome (sugars and amino acids) (Beckles & Roessner, 2012; Vuolo et al., 2019).

On the other hand, secondary metabolites do not participate directly in plant growth but are likewise essential for survival. These compounds are synthesized in smaller quantities, often synthesized in specific cells or a particular group of plants, and can accumulate in high concentrations in specialized cells or specific tissues (de Brito Francisco & Martinoia, 2018; Sangwan et al., 2018). Currently, it is recognized that these metabolites play essential functions in plants' life. Secondary metabolites are responsible for conferring competitive advantages over other plants, protection from predatory attacks, the attraction of pollinating agents or seed dispersers, protection from ultraviolet radiation, and others are involved in plant-to-plant communication (Böttger et al., 2018; Vuolo et al., 2019). Secondary metabolites can be divided into four major groups: phenolic compounds, terpenes, alkaloids, and sulphur-based compounds (Figure 1).

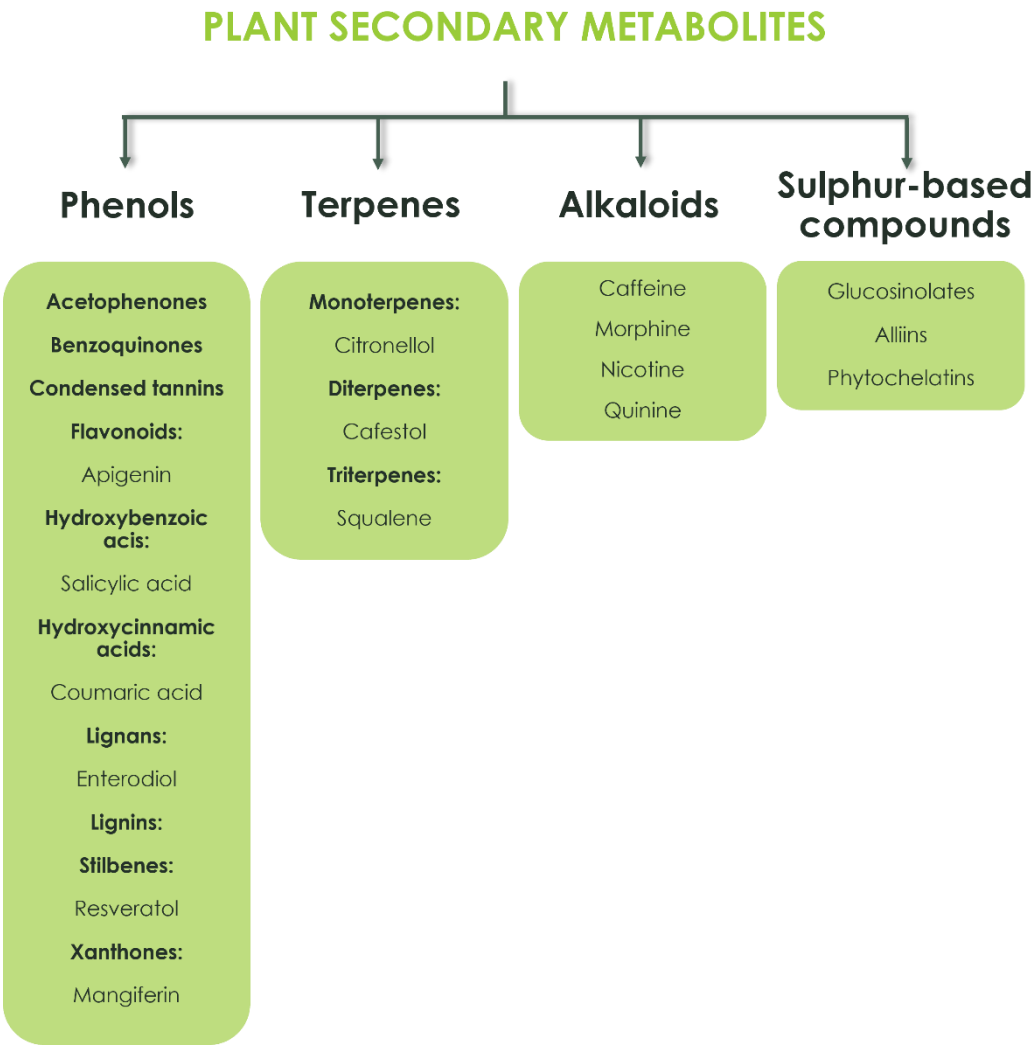


Figure 1: Plant secondary metabolites classification and some examples. (Aldred, 2009; Raab & Feldmann, 2019; Schläger & Dräger, 2016; Vuolo et al., 2019)

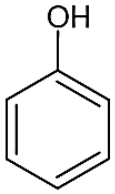
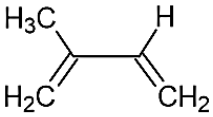
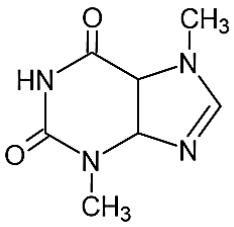
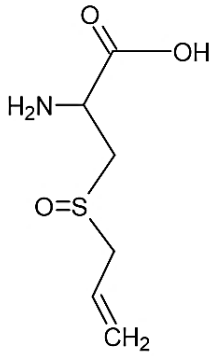
Phenolic compounds are chemical compounds consisting of a hydroxyl group (—OH) bonded directly to an aromatic ring (Table 1). These metabolites constitute a large group of secondary plant metabolites responsible for the defence against predators and pathogens and UV radiations (Daglia, 2012; Veiga et al., 2020). Additionally, phenolic compounds are responsible for colour and flavour assignment and have substantial beneficial properties, including antioxidant, anti-inflammatory, and antimicrobial (Daglia, 2012; Manousi et al., 2019; Vuolo et al., 2019). Phenolic compounds can be classified into two major groups: polyphenols and simple phenols, subdivided into nine classes: benzoquinones, hydroxybenzoic acids, hydroxycinnamic acids, acetophenones, xanthones, stilbenes, flavonoids, lignans, lignins, and condensed tannins (Vuolo et al., 2019). Due to their antioxidant activity, these compounds can prevent cells' oxidative stress, associated with several diseases (Manousi et al., 2019).

Terpenes represent a set of natural hydrocarbons that are derived from isoprene units (molecular formula: $(C_5 H_8)_n$, where *n* represents the number of isoprene molecules) (Table 1). According to the number of isoprene units, they are classified as monoterpenes, sesquiterpenes, diterpenes, triterpenes, and tetraterpenes (2, 3, 4, 6 and 8 isoprene units, respectively) (Aldred, 2009). Terpenes can also be subdivided into acyclic or cyclic, the first ones are linear, and the others form a ring (Canhoto, 2010). These compounds constitute the essential oils of plants and play differentiated roles either in primary or secondary metabolism of plants that include hormones, protein modification agents, antioxidants and defence against herbivores and pathogens, and signals to beneficial organisms, such as pollinators (Pichersky & Raguso, 2018). The antimicrobial properties of the extracts have the potential for several applications, namely in the cosmetic and textile industries. The essential oils from eucalyptus have antimicrobial activity against Gram-positive and Gram-negative, and it is due to the presence of aromatic terpenes (e.g., α-pinene) (Faccio, 2020).

Alkaloids are nitrogen-containing compounds frequently derived from amino acids (Schläger & Dräger, 2016). These compounds are classified according to their chemical structure and are divided into several groups: indole, purine, quinoline, isoquinoline, tropane, imidazole, and others (Dey et al., 2020). These compounds are alkaline, water-soluble, and present strong biological activity. Moreover, some of the most important drugs, resultant from plants, are alkaloids. Alkaloids interfere with neurotransmitters activity and, in plants, one of the functions is to provide protection, as they are toxic for herbivores (Canhoto, 2010). Because of the properties of these substances, namely antimalarial, antibacterial and anticancer, plant-based alkaloids are still used in modern medicine (Dey & Mukherjee, 2018).

Sulphur-based compounds are a vast group of secondary metabolites derived from sulphur-containing amino acids (cysteine) or contain a sulphate, sulphonate-group, or a thiol-ring in their structure. They are involved in plant defence mechanisms against different microbial pathogens and include glucosinolates and alliins (Raab & Feldmann, 2019).

Table 1: Chemical structures of secondary metabolites classes.

Secondary metabolites	Compound's structure	References
Phenolic compounds	 parent compound – phenol	
Terpenes	 isoprene unit	
Alkaloids	 alkaloid structure	(Aldred, 2009; Raab & Feldmann, 2019; Schläger & Dräger, 2016; Vuolo et al., 2019)
Sulphur-based compounds	 alliin structure	

Due to their beneficial properties, secondary metabolites have been used in various products, including resins, essential oils, dyes, medicines, spices, cosmetics, and food additives (Canhoto 2010; Veiga et al. 2020).

1.2. Plants and natural sources used for extraction

Plants traditionally used as infusions and medicines most likely owe their activity to many bioactive compounds in their composition. Therefore, it is crucial to study their properties for their potential application in different areas, including the cosmetic and textile industries.

"Acantes" (Figure 2A), *Ptychopetalum uncinatum*, belongs to the *Olacaceae* family and is native to the Amazon rainforest. Although data on its phytochemical composition is not yet fully clarified, this plant has been used, in the form of tea, to treat numerous disorders as neuromuscular disorders, sexual impotence, rheumatism, and influenza (Pedrollo et al., 2016).

African basil (Figure 2B), *Ocimum gratissimum*, is a perennial plant that belongs to the family *Lamiaceae*, native from Africa and Asia. It is used as a medicinal plant in many countries (Soumanou & Adjou, 2016). It is used in traditional medicine due to its composition, which includes tannins, saponins, flavonoids, phenols, and anthraquinone glycosides. Moreover, the plant owns beneficial biological activities such as anti-inflammatory, anti-oxidative, and antibacterial (Alabi et al., 2018).

"Carqueja", *Pterospartum tridentatum* (Figure 2C), is a small shrub with yellow flowers from the *Fabeaceae* family, used in traditional medicine and culinary. The bioactive compounds found in this plant include alkaloids and flavonoids, owning antioxidant and antimicrobial properties. It is used in conventional medicine to treat head and stomach aches, colds, and diabetes (Coelho et al., 2011).

Cocoa beans (Figure 2D), from *Theobroma cacao L.*, *Malvaceae* family, is a known source of bioactive compounds. Cocoa contains polyphenols, phenolic acid derivatives, flavonoids, alkaloids and has antimicrobial and antioxidant activity (Garcia et al., 2021; Ramos-Escudero et al., 2021).

Cork (Figure 2E) is the outer shell of *Quercus suber* from *Fagaceae* family. The removed bark from this oak has several applications. However, it is also a resource from which to extract bioactive compounds. Cork has in its chemical composition different compounds, namely friedelin, suberin, lignin, and other minor components. Some of these compounds have recognized anti-tumour, anti-inflammatory, analgesic, and antipyretic activities (Pinto et al., 2019; Vieira et al., 2020).

Damiana (Figure 2F), *Turnera diffusa*, is a small shrub of the *Tuneraceae* family. It is a medicinal plant traditionally used as a stimulant, diuretic, and aphrodisiac, and it is also used in the production of cosmetics (Soriano-Melgar et al., 2012). Phytochemical studies have shown flavonoids, phenolic glycosides, terpenoids, and other secondary metabolites as constituents of this plant (Zhao et al., 2008).

Bee pollen (Figure 2G) is a combination of mainly floral pollen with some nectar or honey, enzymes, wax and bee secretion. It has gained attention as a functional food due to its high content of compounds

with health-promoting effects. Pollen comprises fatty acids, free amino acids, vitamins, carotenoids, and flavonoids (Thakur & Nanda, 2020). Therapeutical activities include antimicrobial, antifungal, antioxidant, and anti-inflammatory activities (Pascoal et al., 2014).

Portuguese cherry laurel (Figure 2H), *Prunus lusitanica,* is a species of the *Rosaceae* family. It is a small tree where the fruit is a small dark purple cherry. There aren't many chemical studies of this plant, however, extracts of *P. lusitanica* are composed of terpenes, being friedelin identified as its primary component. This compound has anti-inflammatory, antimicrobial , and antioxidant activities (Costa et al., 2015).

Quinine (Figure 2I), *Cinchona calisaya,* is a species of shrub or tree in the family *Rubiaceae*, native to Bolivia, Ecuador, and Peru. The genus *Cinchona* has been used as a cure for malaria due to its antimalarial activity, and it is a source of various alkaloids, mostly quinine, quinidine, cinchonidine, and cinchonine (Murauer & Ganzera, 2018).

Sage (Figure 2J), *Salvia officinalis,* is a perennial plant cultivated in central Europe and the USA, belonging to the *Labiatae* family. It is used for culinary and medicinal purposes (Giacometti et al., 2018). Carnosic acid, carnosol, and rosmarinic acid are the main antioxidant compounds in sage, which is also a source of di- and triterpenoids, phenolic acids, and flavonoids (Kontogianni et al., 2013).

Stone pine (Figure 2K), *Pinus pinea*, is a tree from the *Pinaceae* family, widely planted in the Mediterranean, especially in Spain, Portugal, Italy, Greece, Tunisia, and Turkey (Kemerli-Kalbaran & Ozdemir, 2019). Extracts from its leaves are composed of a variety of terpenes, including limonene and α-pinene (Saroj et al., 2020).

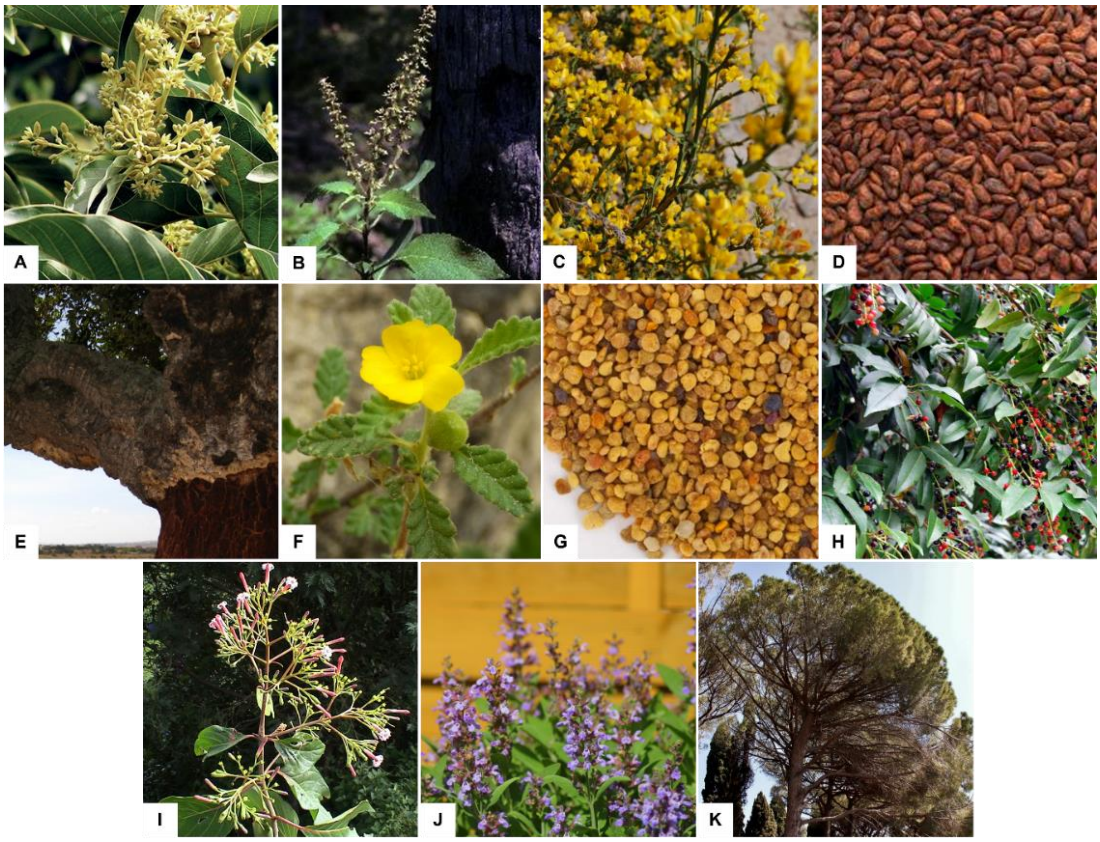


Figure 2: Natural sources used for extraction: A) *Ptychopetalum uncinatum*; B) *Ocimum gratissimum*; C) *Pterospartum tridentatum*; D) Cocoa beans; E) Cork; F) *Turnera diffusa*; G) Pollen; H) *Prunus lusitanica*; I) *Cinchona calisaya*; J) *Salvia officinalis* and K) *Pinus pinea.* Information from where the images were adapted in Annex VIII.

1.3. Extraction Processing

1.3.1. Solvents

Solvents dissolve, extract, or suspend substances to form a solution being the component in excess. Considering their chemical bonds, solvents can be classified as molecular, ionic, and atomic. Molecular solvents include organic solvents (e.g., alcohols) and ionic liquids (IL) mainly composed of molten salts, and atomic liquids comprise low-melting metals (e.g., liquid mercury) (Reichardt & Welton, 2010).

Organic solvents are conventionally used for bioactive compounds extraction, however, many of these solvents present high volatility, toxicity, and flammability, and thus hazardous for health and the environment (Benvenutti et al., 2019). Green solvents have emerged as an alternative to replace the conventional hazardous solvents used in the industry, and IL began to gain plenty of attention (Zainal-Abidin et al., 2017).

1.3.2. Ionic liquids

IL are composed of organic cations and organic/inorganic cations in the liquid form with melting points below 100°C and have recognized remarkable properties, including chemical and thermal stability, low vapor pressure, and non-flammability (Yavir et al., 2019). These solvents can be designed to optimize a specific reaction, for example, to provide maximum yield and purity of the isolated product (Nasirpour et al., 2020). IL have other applications beyond its use for extraction solvents, like mobile or stationary phase in high-performance liquid chromatography (Tang et al., 2012). IL association with green chemistry arises from the fact that they have a low vapor pressure. However, it is not enough to categorize IL as green solvents since most are not biodegradable and have a multi-step synthetic route (Nasirpour et al., 2020). Although the release of the solvent into the atmosphere is prevented by negligible vapor pressure, which reduces pollution and the risks involving working with the solvent, the possible release into aquatic environments may lead to water pollution (Romero et al., 2008). For this reason, these solvents have been replaced by deep eutectic solvents (DES).

1.3.3. Deep Eutectic Solvents

Another type of solvents, similar to IL, has also emerged in the last years. DES are obtained by mixing compounds, usually, a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), resulting in a eutectic mixture with a melting point that is lower than that of any of its components (Manousi et al., 2019; Santana-Mayor et al., 2021). DES are composed of several compounds, including the low cost and non-toxic choline chloride (ChCl), usually combined with carboxylic acids (e.g., amino acids) and polyols (e.g., glycerol) (Zhang et al., 2012). Glycerol is a non-toxic solvent used in the food and pharmaceutical industries, and it is used as HBD in DES composition (AlOmar et al., 2016; El Kantar et al., 2019). Lactic acid has been used as a substitute for ChCl. It is a natural organic acid that can act as HBA and HBD (Macchioni et al., 2021).

Despite having similar physicochemical properties to IL, DES present some advantages. These solvents have high biocompatibility and biodegradability, inexpensive and straightforward preparation, and high accessibility of raw materials (Santana-Mayor et al., 2021; Zhang et al., 2012). The synthesis of these solvents is achieved by simply mixing the components and heating them without further purifying the resulting DES (Cunha & Fernandes, 2018).

A new term recently introduced: natural deep eutectic solvents (NADES), designates solvents obtained by mixing natural primary metabolites such as sugars, amino acids, and amines, and, often, water in

specific molar ratios. These solvents offer several advantages, including biodegradability, low cost, and simple preparation, making them suitable for health-related applications, such as pharmaceuticals, foods, and cosmetics (Dai et al., 2015).

This type of solvent has already been successfully applied to extract bioactive compounds from several natural sources. ChCl and menthol based DES were used to extract bioactive compounds from *Curcuma longa* L, showing superior extraction yield than ethanol (Oliveira et al., 2021). Another ChCl and lactic acid-based DES was applied to extract bioactive compounds from *Lippia citriodora* leaves, where higher extraction yields than methanol were obtained (Ivanović et al., 2018).

1.4. Extraction techniques

Extraction is essential in isolating, identifying, and using the chemical compounds present in plant extracts. There are several extraction methods, and all of these techniques have the same purposes: (1) to extract the desired bioactive compounds, (2) to increase the selectivity of analytical techniques, (3) to increase the concentration of bioactive compounds, thus improving the sensitivity of bioassays (4) to convert the compounds into a more appropriate form for detection and separation, and (5) to provide reproducible methods independent of variations in the sample matrix (Azmir et al., 2013). In addition, the choice of the extraction method and the optimization of its operation parameters is fundamental to obtain high-quality and yield of the extracts.

Conventional extraction methods include water distillation, steam distillation, maceration, and *enfleurage* (Giacometti et al., 2018). *Enfleurage*, also known as cold fat extraction, uses fat to extract volatile chemical compounds, mainly from flowers, and it is commonly used to produce essential oils. The method has to be performed in cold rooms, and the fat chosen must be odourless and allow the formation of a semi-solid surface to facilitate the removal of the plant material after the extraction process (Manousi et al., 2019). Solid-liquid extraction, commonly known as maceration, is one of the oldest used methods (Lefebvre et al., 2021). In this process, the natural material is placed in a closed vessel along with the solvent and allowed to stay at room temperature for at least three days with agitation (Manousi et al., 2019). Water and steam distillation are two types of hydrodistillation techniques. In water distillation, the plant material is combined with water in a flask connected to a condenser. The mixture is brought to boil, and the distillate is collected separately from the steam. In steam distillation, the plant is not in direct contact with the heat source, avoiding charring the material, but instead supported above the steam inlet (Manousi et al., 2019). However, conventional extraction techniques imply long extraction times, large amounts of solvent, and high-energy inputs (Belwal et al., 2020; Ojha et al., 2020).

Therefore, there is a need to incorporate alternative techniques that are more economical, ecological, and efficient than the methods commonly used. Green extraction processes aim to reduce energy while allowing the use of alternative solvents and ensuring a safe and high-quality extract (Giacometti et al., 2018). The alternative techniques include ultrasound, microwaves, high-pressure liquid, and enzyme-assisted extraction (Belwal et al., 2020). The techniques using ultrasounds and high-pressure liquid will be further explained.

1.4.1. Ultrasound-assisted extraction

Ultrasound-assisted extraction (UAE) is an inexpensive and efficient method representing an alternative to conventional extraction methods (Ojha et al., 2020). The sound waves create the cavitation phenomenon, including the creation, growth, and collapse of bubbles (Lefebvre et al., 2021; Manousi et al., 2019). This causes cavitation damage in the cells through increased cell wall permeability and improves heat and mass transfer (Barba et al., 2016; Sasidharan et al., 2018).

UAE has been used to extract bioactive compounds from plants (Trojanowska et al., 2019; Wang et al., 2020), as it presents many advantages compared with conventional techniques, such as better solvent penetration, shorter processing and residence time, higher yields, low solvent consumption, and high processing throughput (Barba et al., 2016).

1.4.2. Pressurized liquid extraction

Pressurized liquid extraction (PLE) uses high temperatures and pressure to extract solid matrices keeping the solvent at a liquid state above its boiling point. The extraction conditions increase solubility and mass transfer rates, and the solvent easily penetrates the solid matrix due to decreased viscosity (Alvarez-Rivera et al., 2020).

PLE has been widely applied for the extraction of bioactive compounds from several vegetable matrices, including green coffee (Belandria et al., 2016), truffles (Tejedor-Calvo et al., 2020), olive leaves (Lama-Muñoz et al., 2020), and lemon verbena leaves (Leyva-Jiménez et al., 2018).

Compared to conventional extraction methods, PLE allows higher yields with reduced extraction time and solvent consumption (Gilbert-López et al., 2017; Manousi et al., 2019).

1.5. Extract forms

Plant extracts can be classified into four categories: liquid extract, solid extract, oleoresin, and dry extract. The liquid extracts consist of a liquid preparation obtained using water, alcohol, or another extraction solvent (boiling water, aqueous ethanol, or glycerol). Soft extracts are semi-solid preparations obtained by partial or total evaporation of the solvent from a liquid extract. Oleoresins are semi-solid materials composed of a resin in solution in an essential and/or fatty oil obtained by evaporating the excess solvent. Dry extracts are solid preparations obtained by the total evaporation of the solvent from a liquid extract. It can also be prepared by spray-drying or drying and milling to produce a powder. It may be further processed by compression or using a binding agent or granulation liquid to produce multiparticulate granules (World Health Organization, 2018).

1.6. Extracts Characterization

After extraction, it is imperative to quantify and identify the bioactive compounds present in the plant extracts through different analytical methods. Gas chromatography-mass spectrometry (GC-MS) is one of the techniques used to analyse metabolites. This method is divided into two steps, GC considered the pre-separation, followed by the second, the separation process occurring in the mass analyser. The samples subjected to GC-MS analysis become gaseous through the heated injector of the GC, and the compounds are separated as they migrate in the chromatographic column. The different volatility and polarity of the compounds drive the separation, and metabolites become dispersed between the mobile phase (carrier gas) and the stationary phase (column). The last part of the column is linked to the entrance of the MS ion source, and, commonly, two ionization methods are applied, namely, electron impact ionization and chemical ionization. The software for data analysis allows handling the obtained data, being loaded with spectral libraries to help identify the molecules (Beckles & Roessner, 2012). Therefore, compound identification is usually achieved by comparing a query mass spectrum with reference mass spectra in a library via spectrum matching (Koo et al., 2014).

1.7. Application of the extracts

1.7.1. Cosmetic Formulations

Cosmetic products are part of daily routines not only for personal hygiene but also for personal care. A cosmetic product is defined, according to the European Regulations, as "any substance or mixture intended to be placed in contact with the external parts of the human body (epidermis, hair system, nails, lips, and external genital organs), with the teeth and the mucous membranes of the oral cavity with aiming for cleaning, perfuming, changing appearance, protecting, keeping in good condition or correcting body odours" (Regulation (EC) 1223/2009).

Cosmetics contain several chemical compounds in their composition. Some of these ingredients are essential for the formulation's performance, while others make it more pleasant and attractive to the customers (Table 2).

Table 2: Cosmetic ingredients of a cosmetic formulation and respective functions.

Ingredient	Functions	Reference
Water	Solvent and moisture content.	(Costa & Santos, 2017)
Oils	Improve elasticity, prevent water loss, and enhance skin barrier function.	
Emollients	Restore natural skin lipids, increase the spreadability of the formulations, increase penetration of active ingredients and act as emulsifiers.	
Humectants	Improve skin hydration.	
Preservatives	Prevent the growth of microorganisms.	
Surfactants	Prevent the separation of the aqueous and lipid miscible components.	
Thickeners	Increase the viscosity of the formulation, enhance sensory properties and stabilize emulsions and suspensions.	
Active ingredients	Offer therapeutic benefits to the skin (anti-aging and antioxidant activities).	
Fragrance	Improve sensory properties.	

Nowadays, there is a trend in the cosmetic industry towards developing new products of natural origin, replacing synthetic chemical compounds and animal substances with natural and eco-friendly alternatives. Therefore, plant extracts have emerged as promising alternatives to synthetic chemicals.

The skin is constantly exposed to UV radiation and environmental pollutants, and it responds to these aggressions by developing inflammatory responses that generate reactive oxygen species (ROS), leading to premature aging and skin cancer. The application of antioxidants could control ROS generation and prevent premature aging (Sajna et al., 2015). Bioactive compounds derived from plant extracts can be potentially applied as biologically active ingredients in cosmetic formulations, as reported by many researchers. For example, applying phenols to the skin can induce effects on skin aging and attenuation of inflammation signals (Wagemaker et al., 2017). Aloe vera leaves are the foundation of aloe vera gel incorporated in cosmetics, acting at different levels in photoaged skin, improving the elasticity and wrinkles, collagen production, and helping in cellular repair (Kapoor et al., 2009; Long, 2016). Algaroba, *Prosopis juliflora,* extracts containing α-glucan and phenolic derivatives are used to develop cosmetic formulations, being considered a new ingredient with potential for moisturizing and anti-aging cosmetics (Damasceno et al., 2020).

Bioactive compounds often have antimicrobial activity, which is extremely important since many bacteria and fungi can cause skin infections, being *Staphylococcus* and *Streptococcus* the most common ones (Kaneria et al., 2017). The essential oils from three basil varieties showed antibacterial and antifungal activity, specifically *Ocimum citriodorum* and *Ocimum thyrsiflora* revealed high inhibition rates against *Staphylococcus aureus* (Avetisyan et al., 2017). Their antimicrobial properties also make these natural compounds alternatives for replacing or reducing the usage of common preservatives.

The choice of solvents used for extraction is a key factor from a cosmetic perspective. The solvents may also be incorporated onto cosmetic formulations, so it is vital to choose safe compounds. Taking ChCl as an example, despite being highly used in DES development, it is prohibited in cosmetics in Europe (Regulation (EC) 1223/2009, annex II). Besides the safety aspects of the solvent, the cosmetic properties may also be considered. Several compounds used as DES components are used in different cosmetic formulations (Table 3).

Table 3: Examples of chemical compounds used in cosmetic formulations and respective functions.

Compound	Function	Reference
Lactic acid	Buffering, humectant, and skin conditioning.	(*CosIng - Cosmetics - GROWTH - European Commission*, n.d.)
Glycerol	Hair conditioning, humectant, oral care, perfuming, skin conditioning and protection, and viscosity controlling.	
Diethylene glycol monoethyl ether	Hair dye products, rinse-off products other than hair dye, non-spray and spray cosmetic products: fine fragrances, hair sprays, antiperspirant, and deodorant.	

1.7.2. Textile Industry

1.7.2.1. Natural dyeing

The dyeing of textiles with natural dyes represents another promising application for plant extracts. Natural dyes have been used since ancient times throughout the world, but their use has decreased due to synthetic dyes. These dyes allow the production at a large scale and economical prices with consistent colour quality, being the first choice of the textile industry (Zerin et al., 2020).

However, synthetic dyes are slow- or non-biodegradable, owning some undesirable properties, including carcinogenicity, allergenicity, and dermatics effects. The textile industry is responsible for discharging these dyes to waste waters in large amounts. In dyeing processes, dye waste is between 5 and 50%, depending on the type of fabric and dye, which results in approximately 200 billion litres of effluents per year (Tkaczyk et al., 2020).

Recently with the worldwide requests over eco-friendly and biodegradable materials, natural dyes have emerged as an essential alternative to synthetic dyes. Natural dyes, derived from plants, are composed mainly of phenolic compounds, being flavonoids the most abundant species. These colourants can be found in flowers, leaves, fruits, and seeds (Zerin et al., 2020).

Natural colourants can be classified according to their origin, application method, or chemical structure. Classification according to their chemical structure can be given as follows:

Table 4: Classification of natural colourants.

Class	Info	Reference
Carotenoids	Belong to the class of isoprenoid lipids and are extracted from leaves, flowers, and fruits of plants and vegetables (e.g., carrots and tomatoes). These compounds owe their colour to the presence of conjugated double bonds.	(Ghosh et al., 2022; Nambela et al., 2020; Uddin & Sayem, 2020)
Indigoids	Blue aromatic compounds extracted from the leaves of *Indigofera tinctoria*. Indigo plants are the best source of colour-fast blue dyes that provide different shades of blue due to their different chemical compositions.	
Quinonoids	These are fused benzoic ring system compounds found in flowering plants. Quinonoids colorants are further classified into benzoquinones, napththoquinones, anthraquinones, and other quinonoids.	
Flavonoids	Phenolic compounds responsible for vivid colours in fruits and vegetables. Flavonoid colorants are classified into flavones, flavonol, flavanone, isoflavonoid, anthocyanidin, aurone, and chalcones.	
Tannins	Water-soluble phenolic compounds found in many plants (gallotannins and ellagitannins).	
Betalains	Nitrogen containing compounds derivatives of the betalamic acid chromophore, being classified into red violet betacyanins and yellow orange betaxanthins. These compounds are extracted from plants' roots, fruits, and flowers (e.g. beetroot, cactus fruits).	

There are several reports on the potential use of natural dyes extracted from different plant sources, such as fruits of trees. Arjun tree, *Terminalia arjuna,* and Indian tulip, *Thespesia populnea*, give bright yellow and pale-yellow shade to different fabrics (Amutha et al., 2020). *Croton urucurana Baill* bark extract, composed of tannins, lignin, cellulose, and hemicellulose gives beige to reddish-brown colour to dyed fabrics (Figure 3) (Silva et al., 2020).

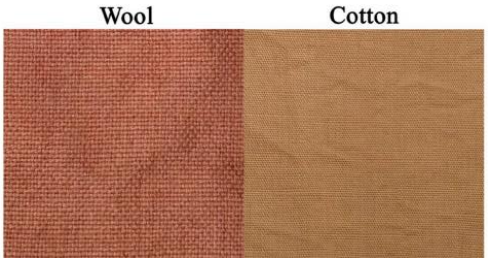


Figure 3: Coloration of wool and cotton fabric after dyeing with the extract of *C. urucurana.* Adapted from (Silva et al., 2020).

Natural dyes present several advantages compared to synthetic ones, being biodegradable and noncarcinogenic, extensively available from natural sources, and allowing the disposing of natural dye waste as a biofertilizer for agriculture. Besides the medicinal properties, there is a demand for eco-friendly and non-toxic dyes, specifically for health-sensitive applications such as dyeing textiles for children's wear (Vankar & Shukla, 2019).

Textile processes consume high levels of energy and water (200-400 L per kg of the finished product) (Buscio et al., 2019; Haji & Naebe, 2020), thus developing techniques that reduce energy and water consumption is crucial. Solvent dyeing is an approach to reduce water consumption and reduce the number of chemicals involved. Within this strategy, different solvents are used, instead of water, as a dyeing medium. Decamethyl-cyclopentasiloxane was used as a dyeing medium for dyeing polyester. The dyeing process was performed with the addition of water to the dyeing solutions, from 0 to 200% on weight of fabric (owf). The colour strength of the dyed polyester samples in the D5 non-aqueous medium containing water is significantly higher than without water, reaching a maximum at 50% owf water content. The higher solubility of disperse dyes in this non-aqueous medium delivers good dyeing levelness, which avoids dispersant usage (An et al., 2021). For replacing the usual auxiliary agents used in the dyeing process, a single IL, 1-(2-hydroxyethyl)-3-methylimidazolium chloride, was used in the dyeing of polyester, cotton, and wool. The addition of a concentration 2 g/L of IL was found to be sufficient to the dye being absorbed by the fibre, providing bright and intense coloration (Bianchini et al., 2015).

Moreover, selecting a solvent should focus on the matters related to dyeing performance combined with the environmental, health, and safety impacts (Xu et al., 2016). In this context, DES can represent a possible alternative to reduce water consumption and auxiliary chemicals for their properties. A glycerine, urea, and ChCl based DES was used as a dyeing medium to minimize water consumption in polyester with disperse dyes. For an optimized process at 130°C for with 1% of dye owf DES produced increased colour strength in comparison with aqueous dyeing (Pawar et al., 2019). Although these are promising results temperature optimization for energy consumption reduction is still necessary.

1.7.2.2. Wastewater treatment

Another major problem associated with the textile industry is the large amount of wastewater discharged that represents a source of pollution. Due to its toxicity, it is thought crucial to remove synthetic dyes and other substances from textile wastewaters. The removal of dyes can be achieved by different procedures, namely membrane filtration, adsorption, precipitation, and many others. Nevertheless, these methods present some disadvantages, including high cost, low removal efficiency, and labour-intensive operation (Ferreira et al., 2014). Liquid-liquid extraction is a preferred alternative technique for dye separation, however, these techniques require the use of organic solvents, which, besides their toxicity, involves the use of large volumes and longer extraction time (Kemerli-Kalbaran & Ozdemir, 2019). The green properties of DES makes them, once again, great candidates to replace the solvents generally used in liquid-liquid extraction for wastewaters treatment.

Tannery wastewater represents a serious environmental challenge due to the elevated concentrations of pollutants with low biodegradability. It contains several chemicals, such as acids, alkalis, chromium salts, tannins, solvents, sulphides, dyes, auxiliaries, which are not entirely fixed to the substrates, remaining in the effluent. For instance, the current chrome tanning method gives only about 50–70% chromium uptake (Hansen et al., 2020; Lofrano et al., 2013).

1.7.2.3. Cosmetotextiles

Cotton is the most important natural textile fibre and one of the most used globally. Its structure consists of cellulose, a polymer constructed of monomeric glucose units, between 86 and 96% by dry weight. Besides, it contains other components such as pectin, proteins, waxes, and minerals. Cotton is a unicellular fibre that grows from the epidermis cells on the surface of cotton seeds (Gordon, 2009). Characteristics like natural comfort, appearance and excellent performance such as alkali resistance, hygroscopicity and moisture retention contribute to the widespread use of this fibre. However, cotton fibre has some properties that still need improvement, namely weak crease recovery, low dye fixation, poor colour fastness, and bacterial growth (Verma et al., 2021). The use of plant extracts for dyeing can also confer antimicrobial activity to cotton fabrics, as confirmed by others. The aqueous extracts of olive tree leaves showed a bacterial reduction of both Gram-positive and Gram-negative bacteria in dyed fabrics (Yılmaz & Bahtiyari, 2020). Moreover, natural extracts can also provide ultraviolet protection to the dyed fibres (Silva et al., 2018).

The term cosmetotextiles has been used to designate textiles with cosmetic properties. Because clothes act as a second skin, textiles are suitable platforms for delivering cosmetic products to the skin. The active principles are encapsulated in microcapsules which are dispersed inside the fibres or as a coating. Friction, chemical reaction with sweat, or changes in pH, may cause the degradation of the microcapsules and the release of the active ingredients at a constant and regular rate (Decaens & Vermeersch, 2018). The encapsulation of extracts in nanoparticles is widely used. It presents a solution for direct application on the skin since they can penetrate the intra and intercellular spaces and through the hair follicles. Incorporating extracts in the nanoparticles increases the number of bioactive compounds in the system, increasing their biological activity, ensuring protection and stability against external factors (Armendáriz-Barragán et al., 2016).

2. MOTIVATION AND AIMS OF THIS WORK

Plant extracts contain a variety of metabolites that have beneficial biological activities. Extraction is critical for isolating, identifying, and using the bioactive compounds from plant extracts. Therefore, there was a need to develop economic, ecological, and efficient techniques while replacing harsh solvents with environmentally friendly solvents.

The cosmetic industry has been incorporating new products of natural origin, aiming to replace toxic and non-environmentally friendly ingredients. For this, the use of extracts from plants is the ideal solution. The natural ingredients would offer therapeutic benefits to the skin due to their antioxidant and antimicrobial activities, controlling reactive oxygen species, and inhibiting bacteria and fungi from causing skin infections. Another promising application for plant extracts is the natural staining of textiles. Together with their biodegradability and non-carcinogenicity and the worldwide concerns about the use of non-environmentally friendly chemicals, the dyes derived from natural sources have emerged as a promising alternative to synthetic dyes.

Environmental demands for greener alternatives to minimize the environmental impact have been growing in the last years. Specifically, the development of methods that allow the reduction of water and chemicals consumption in dyeing processes and the removal of wastewaters generated by the textile industry are increasingly pertinent.

Considering this, the main aims of this work are:

- The exploitation of different methods and solvents to extract bioactive compounds from natural sources;
- The characterization of the extracts for their application in two fields: cosmetics and textile dyeing;
- The use eutectic mixtures to optimize the dyeing of polyester: water and temperature reduction;
- The use of eutectic mixtures for wastewater treatment: dye extraction from aqueous solutions and dyeing effluent.

3. MATERIALS AND METHODS

3.1. DES characterization

3.1.1. Thermogravimetry analysis (TGA)

The TGA was performed using a TGA 4000 (Perkin Elmer, Waltham, MA, US) and acquired using the Pyris software (version 13). DES (8 - 10 mg) were analysed from 25 to 600 °C at 10 °C/min under a nitrogen atmosphere (flow rate: 20 mL/min). Weight loss, in percentage, and its derivative were represented as a function of temperature. The temperature calibration was done by Curie temperatures of reference materials: alumel, nickel, and perkalloy, in the same conditions.

3.1.2. Density

Density was measured by weighing 5 ml of DES inside a 5,00 ± 0,02 ml volumetric flask. DES weight was measured in triplicate, and density was calculated using the quotient between mass and volume of the solvent.

3.1.3. Viscosity

Viscosity was measured using a rotatory viscometer (DV-II +, Ametek Brookfield). The spindle (S1) was dipped into the sample to the spindle depth mark, and the measurement was recorded upon reading stabilization. The viscosity values were expressed in mPa.s.

3.1.4. Conductivity

Conductivity was measured using a Thermo Scientific Benchtop Meter (Orion Versa Star Pro).

3.1.5. pH

pH was measured using a pH bench meter (HI5221, probe HI1131, Hanna Instruments).

3.1.6. Fourier transform infrared spectroscopy (FTIR)

FTIR was performed to study chemical interactions on DES. The spectra were obtained using a Bruker Alpha II (Massachusetts, USA) and acquired by Opus 8.22.28. DES were placed directly over the crystal, and the spectra were acquired between 400 and 4000 cm^{-1} wavenumbers with a 2 cm^{-1} resolution.

3.2. Extraction of bioactive compounds from the natural sources

3.2.1. Plant materials

Regional teas were purchased in San Tome and Prince. These include "Canta viril", composed of *C. calisaya*, *P. uncinatum,* and sprouts of pine; "Donzela", composed of *S. officinalis* and *T. diffusa*; Damiana tea (*T. diffusa*), and African basil tea (*O. gratissimum*). The teas were obtained in the form of dried leaves and used as received. “Carqueja” (*P. tridentatum*) was collected from home harvest in 2021 and was left to air dry prior to use. Cherry bay (*P. lusitanica*) berries were collected on the campus of Universidade do Minho in 2021 and were lyophilized prior to use. Pollen collected at Serra de Agra, Portugal, and the Cocoa beans were a gift from an NGO from San Tome and Prince, and both natural materials were used as received.

All the reagents used were purchase from Sigma-Aldrich, TCI, Fisher Scientific or Labkem, and utilized without further purification. The extraction methods and extraction conditions were chosen based on previous experiments performed in the lab group.

3.2.2. Preparation of DES

DES were prepared using an alcohol, an acid, a diethylene glycol monoethyl ether. The components were measured in the desired molar ratio (Table 5) in a flask and stirred at elevated temperature (≈80 °C) until a clear homogeneous liquid was obtained.

Table 5: Composition of DES used for the extraction of bioactive compounds.

Abbreviation	Composition	Molar ratio
DES 1	Alcohol : Acid 1	1:1
DES 2	Alcohol : Diethylene glycol monoethyl ether	1:1

3.2.3. Ultrasound-assisted extraction (UAE)

The UAE was performed in an ultrasonic bath (USC600TH, VWR International) with an ultrasonic input power of 400 W and a frequency of 45 kHz. 1 g of ground plant material were combined with 20 mL of DES (1 and 2), water, or dioxane in flasks. The extraction process was carried out at 50 °C for 120 min. After extraction, the solution was centrifuged (Multifuge X3R, Thermo Scientific) for 40 min at 10 000 rpm to collect the supernatant.

3.2.4. Sealed system extraction (SSE)

The SSE was performed using a liquid paraffin bath. 0,25 g of ground plant material was combined with 5 mL of DES (1 and 2), water, or dioxane in vials closed with a metallic capsule under pressure. The extraction process was carried out at 100 °C for 6 h, with stirring. After extraction, the solution was centrifuged (Multifuge X3R, ThermoScientific) for 40 min at 10 000 rpm to collect the supernatant.

3.2.5. Mixer-assisted extraction (MAE)

The MAE was performed using a planetary centrifugal mixer (Thinky Mixer, ARE-250). 2,5 g of ground plant material were combined with 50 mL of DES (1 and 2), water, or dioxane in a proper container. The extraction process was carried for a total of approximately 30 min, following the steps: 1) 5 min at 1000 rpm; 2) 5 min at 2000 rpm; 3) 5 min at 2000 rpm; 4) 5 min at 2000 rpm; 5) 5 min, 1000 rpm and a defoaming step at the end of each phase: 1 min., 2200 rpm. After extraction, the solution was centrifuged (Multifuge X3R, ThermoScientific) for 40 min at 10 000 rpm to collect the supernatant.

3.2.6. Extraction yields

After the extraction processes, DES and water extracts were filtered with paper filters (20-25 µm, PRAT DUMAS, France) and lyophilized. The dioxane extracts were filtered and then evaporated. All the remaining natural materials were washed with water to remove the adsorbed solvent. It was then collected and dried in the oven overnight to remove the water content. The extraction yields were calculated by the difference of the initial plant weight and the weight obtained after the extraction process.

3.3. Characterization of the extracts

3.3.1. Absorption UV-Visible (UV-Vis) spectra of the extracts

The obtained extracts were diluted with distilled water and analysed in a spectrophotometer (Synergy Mx, BioTek), for measurement of the respective UV-Vis spectra in the region of 230 - 700 nm, aiming to evaluate if different solvents and different extraction methods would conduct to extracts with different UV-Vis absorption.

3.3.2. Total Phenolic Content (TPC)

The TPC in the extracts was determined spectrophotometrically according to the Folin-Ciocalteau method. A 0,1 mL aliquot of a certain extract concentration was mixed with 0,5 mL of Folin-Ciocalteu's reagent (Sigma-Aldrich, Merck, Germany) in 6 mL of distilled water. The mixture was vortexed for 1 min, and then 2 mL of Na_2CO_3 (15% w/v) were added. The mixture was again vortexed for 1 min and allowed to incubate for 2 h at room temperature. The absorbance was measured, in triplicate, at 750 nm using a microplate reader (Synergy Mx, BioTek). The blanks had the same constituents except that the respective solvents replaced the extract. Gallic acid (Sigma-Aldrich, Merck, Germany) was used as a standard for preparing the calibration curve, and different concentrations were prepared (Annex I). The total phenolic content (TPC) was expressed as mg gallic acid equivalents (GAE) per g of extract: $\mathbf{TPC= C_{GA}xV.m^{-1}}$, where C_{GA} is the concentration in GA equivalents, from the GA calibration curve (mg/mL), V is the volume of extract (mL), and m is the weight of the plant extract (g).

3.3.3. DPPH radical-scavenging activity

The antiradical activity of the extracts was evaluated using the assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. To 10 μL of extract sample, in ethanol or water, was added 140 μL of the DPPH (Sigma-Aldrich, Merck, Germany) solution (200 μM, in ethanol). For each sample, different concentrations were tested (1; 0,5; 0,25 and 0,1 mg/mL). The decrease in absorbance was measured continuously, in triplicate, for 60min, at 515 nm using a microplate reader (Synergy Mx, BioTek). Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard to prepare the calibration curve of DPPH reduction. The DPPH radical scavenging activity of each sample was calculated as the percentage of reduced DPPH: $\mathbf{\%rDPPH=(A_0-A_1)/A_0}$, where A_0 is the initial absorbance, and A_1 is

the absorbance at steady state. The radical scavenging activity of each sample was expressed as an IC_{50} value, defined as the concentration that reduces DPPH by 50%, and was calculated from the concentration-effect linear regression curves (Annex II).

3.3.4. GC-MS analysis

GC-MS analysis was performed in cherry bay berries, African basil, and pollen extracts. These extracts were chosen based on their extraction yields, different TPC and antioxidant activity. Moreover, only the extracts obtained with dioxane and DES 1 were analysed, to compare the composition of extracts obtained with a conventional solvent in comparison with a DES. GC–MS analysis were performed using a gas chromatograph (SCION 436 SQ1, Bruker), equipped with a Rxi-5Sil MS column (Restek; 30 m, internal diameter 0.25 mm, film thickness 0.25 μm). The chromatographic conditions were as follows: initial temperature of 70 °C for 1 min, temperature rate of 5 °C/min up to 260 °C, for 5 min, and up to 300 °C; injector and detector temperature of 280 °C and 270 °C, respectively. The MS was operated in the electron impact mode with an electron impact energy of 70 eV, and data were collected in full scan mode, over a range of m/z 35 – 600. Before being submitted to GC–MS analysis, the samples were derivatized. The derivatization method is a transesterification process promoted by the mixture of pyridine and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA). To 1 mg of extract were added 100 μL of pyridine (Sigma-Aldrich, Merck, Germany) and 100 μL of BSTFA (TCI, America) and the mixture was incubated at 60 °C for 25 min. Compounds were identified by comparing the mass spectra with the GC–MS spectral library, NIST/EPA/NIH 2020 (National Institute of Standards and Technology, Gaithersburg, MD, USA). The relative abundance of the compounds was calculated from the peak areas in the total ion gas chromatogram.

3.3.5. Cytotoxicity evaluation

The *in vitro* cytotoxicity of cherry bay berries, African basil and pollen extracts, obtained using water or the DES 1 and 2 as solvents, was evaluated through the effect of their contact on the metabolic viability of immortalized human fibroblasts (BJ-5ta). The selection of these natural sources was due to their different composition, confirmed by GC-MS, and their different values of TPC and antioxidant activity, which would be reflected in different biological effects. The metabolic viability of the cells exposed to the extracts and to the eutectic solvents was assessed by the MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) method. The BJ-5ta cells were maintained

according to ATCC recommendations, four parts Dulbecco MEM medium (DMEM) containing 4 mmol/L-glutamine, 4,5 g/L sodium bicarbonate, and 1 part 199 medium, supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin/streptomycin and 10 µg/ml hygromycin B. The cells were maintained at 37 °C in a 5% CO_2 atmosphere, and the culture medium was renewed every two days.

The day before the experiments, the BJ-5ta cells were plated at a density of 1 x 10^4 cells/well in polystyrene 96-well plates (TCPS) for adherent cells (TPP, Trasadingen, Switzerland). To assess the effect of the extracts and the DES on the metabolic viability of the fibroblasts, the cells were exposed to four concentrations (1%; 0,5%; 0,25%, and 0,1%) of the cherry bay berries, African basil, and pollen extracts, and the DES 1 and 2. The extracts and DES were prepared in PBS (pH 7, 0.1 M), and were incubated for 24 hours. A negative control (culture medium) and positive control (30% dimethyl sulfoxide, DMSO) were also included. At the end of 24 hours of contact, the metabolic viability was evaluated by MTS. The reduction of MTS by viable cells was measured with a SpectraMax Plus microplate reader (Molecular Devices) at 490 nm. All conditions were tested in triplicate, and the results were expressed in percentage of cell viability relative to the negative control.

3.3.6. Antimicrobial activity

3.3.6.1. Organisms and growth conditions

The antimicrobial activity of cocoa, cherry bay berries and pollen extracts was evaluated against Gram-positive, *Staphylococcus aureus* (*S. aureus*; ATCC 6538) and Gram-negative *Escherichia Coli* strain (*E. Coli*; CECT 434), using the disc diffusion assay (qualitative method) and the broth microdilution method (quantitative method). The same extracts evaluated on cytotoxicity assays were chosen, with the exception of African basil extract, which revealed, in preliminary experiments, no antimicrobial activity, being replaced in this study by cocoa beans extracts.

For all experiments, the bacteria strains were subcultured on Tryptic Soy agar (TSA; Merck, Germany) and incubated for 24 h at 37 °C. Cells were then inoculated in Triptych Soy Broth (TSB; Merck, Germany) and incubated overnight at 37 °C, 120 rpm. After incubation, the cells' suspensions were centrifuged for 10 min, at 3000 g and 4°C, and washed twice with phosphate-buffered saline (PBS; pH 7; 0,1 M). Pellets were suspended in 5 ml of PBS, and the cellular density was adjusted for 0,5 of optical density, that corresponds approximately to 1x10^5 cells mL^{-1}.

3.3.6.2. Extracts preparation

To determine the antimicrobial activity, the extracts were prepared in the following concentrations in PBS (pH 7; 0,1 M): cocoa extracts at 8 mg/mL containing DES 1 and DES 2; pollen extracts at 15 mg/mL containing DES 1 and DES 2; and cherry bay berries extracts at 11 mg/mL containing DES 1 and DES 2. The selection of testing concentrations was based on the extraction yields (which allows the calculation of the concentration of each extract) for each source (see section 4.2). Simultaneously, lyophilized water extracts of these natural sources were prepared in PBS (pH 7; 0,1 M) under the same concentrations of the DES containing extracts. As a control, DES were also diluted using PBS (pH 7, 0.1 M) at the same concentration as present in the extract samples.

3.3.6.3. Disc diffusion assay

The antibacterial activity was first evaluated using the disc diffusion halo test (NCCLS, 2000). For that, an aliquot of each species (300 µL) was spread in TSA petri dishes. Then, an aliquot of 50 µL of each sample previously prepared, was placed on sterile blank disc. PBS (pH 7, 0.1 M) was used as negative control. The plates were incubated at 37 °C, during 24-48 h, followed by measuring the diameters of the inhibition zones (mm).

3.3.6.4. Broth microdilution assay

The antibacterial activity was secondly evaluated using the broth microdilution method performed according to the guidelines from the Nature Protocols (Wiegand et al., 2008). An aliquot of bacterial cells from the colonies in TSA Petri dishes was suspended in 5 mL of PBS and mixed for 15 s with a vortex. The resulting suspension was adjusted by a spectrophotometric method, adding PBS to reach the value of the 0,5 McFarland scale. The extract sample and the respective controls were prepared in TSB medium, and aliquots of each extract (100 µL) were dispensed at twice of concentration in the 96-well plates (Orange Scientific, Braine-l'Alleud, Belgium). Furthermore, aliquots (100 µL) of bacteria suspensions were also added to each well plate. Sample-free and bacteria controls were also included. The 96-well plates were incubated at 37 °C for 24 h. Then, the number of viable cells was assessed by the determination of the number of colonies forming units (CFUs) through several dilutions. After 24h of incubation at 37 °C, the number of colonies formed was counted. The results were presented as the total of CFUs (Log CFUs), and the experiments were repeated in triplicate on three different occasions.

3.4. Dyeing of cotton fibres with plant extracts

Cork was supplied by Cork Supply Portugal, S.A., and has a grain size of 0,5 to 1 mm. Cotton fabric (100% cotton, 65 g/m^2, 37 x 37 yarns/cm) was obtained from a Textile Company.

3.4.1. Cotton pre-treatment and mordanting procedures

Cotton fibres were pre-treated as follows: the fabrics were immersed in water at 60 °C for 5 h, with and without tannic acid (Sigma-Aldrich, Merk, Germany) (4% owf); afterwards, the fabrics were washed with running water and left to air-dry overnight. Mordanting of pre-treated fabrics (with and without tannic acid) was performed at 50 °C, for 1 h, using copper sulphate (Sigma-Aldrich, Merk, Germany) (2% owf) and aluminium (Sigma-Aldrich, Merk, Germany) (4% owf). Afterwards, the fabrics were washed with running water and left to air dry.

3.4.2. Preparation of DES and extraction of dyes from natural sources

DES was prepared using an acid and a sodium salt. The components were measured in the desired molar ratio (Table 6) in a flask and stirred at elevated temperature (≈80 °C) until a clear homogeneous liquid was obtained. This DES was selected based on previous knowledge acquired during textile dyeing assays.

Table 6: Composition of DES used for extraction.

Abbreviation	Composition	Molar ratio
DES 3	Acid 1 : Sodium salt 1	4:1

MAE was performed using a planetary centrifugal mixer (ARE-250, Thinky Mixer). For this, 10 g of ground plant material (African basil and cherry bay berries) and 5 g of cork were combined with 50 mL of DES 3 in a proper container. The extraction process was carried for a total of approximately 30 min, using the same extraction program (section 3.2.5). After extraction, the extracts were filtered with paper filters. The remaining natural material was washed with water to remove the adsorbed solvent. It was then collected and dried in the oven overnight to remove the water content. The extraction yields were obtained by weight loss between the initial plant weight and plant weight after the extraction process.

3.4.3. Dyeing of cotton with extracts solutions

The cotton fabric was cut into squares (1,5x1,5 cm) and weighted (11 mg). The dyeing procedure was performed using the sealed system (SS) in a thermostatic bath, with African basil, cherry bay berries, and cork extracts obtained by extraction with DES 3 using MAE. Fabric and dyeing solutions were placed in vials closed with a metallic capsule under pressure. The dyeing was carried out with 2 mL of the extract with 50% water content, for 45 min at 90 °C, with stirring. After the dyeing, the fabric was washed with Diadavin UN (Tanatex Chemicals, Netherlands), a non-ionic washing agent, at 40 °C for 30 min, then with running water, and left to air-dry overnight.

3.4.4. Colour strength evaluation

Determination of colour strength (K/S) of dyed cotton was done using a Datacolor apparatus, Spectraflash 600 Plus, from Datacolor International, at standard illuminant D65. Kubelka–Munk theory gives the following relation between reflectance and absorbance: $K/S=(1-R) \times 2R^{-1}$, where K stands for the absorbance, S stands for the scattering, and R is the reflectance.

The colour intensity (I) of the samples was also determined, using the following equation, as described by Štěpánková et al. (2011): $\mathbf{I}=\sum_{\boldsymbol{\lambda}=\mathbf{400\ nm}}^{\mathbf{700\ nm}} \mathbf{K/S}\ (\lambda) \times \Delta\boldsymbol{\lambda}$, where $\Delta\lambda=10$.

3.5. Optimization of polyester dyeing with DES

100% polyester fabric (96 g/m^2, 30 x 30 yarns/cm) was obtained from a Textile Company. All the reagents used were purchased from Sigma-Aldrich, TCI, Fisher Scientific or Labkem, and utilized without further purification.

3.5.1. Preparation of DES

The DES were prepared using an alcohol, acids, diethylene glycol monoethyl ether, and sodium salts. The components were measured in the desired molar ratio (Table 6) in a flask and stirred at elevated temperature (≈80 °C) until a clear homogeneous liquid was obtained.

Table 7: Composition of DES used for polyester dyeing.

Abbreviation	Composition	Molar ratio
DES 3	Acid 1 : Sodium salt 1	4:1
DES 4	Acid 1 : Acid 2	1:1
DES 5	Alcohol : Sodium salt 2	1:1
DES 6	Sodium salt 2 : Diethylene glycol monoethyl ether	1:1
DES 7	Acid 1 : Diethylene glycol monoethyl ether	1:1

DES dyeing solutions were prepared with different percentages of water (0, 25, and 50%). Due to the use of non-pure reagents, DES have a portion of water from the composition of the starting materials, and though the dyeing solutions containing 0% water considered the original water content of DES. The water content of each DES was considered for water addition.

3.5.2. Dyeing of polyester – Laboratory dyeing machine

Aiming to reduce the amount of water on the polyester dyeing procedure, the dyeing was performed with two commercial dyes using the DES containing different water percentages. For this, the polyester fabric was cut into rectangles (1x4 cm) and weighted (40 mg). The dyeing was performed in an Ibelus C-720 (Pregitzer&Ca, Lda.) with two disperse dyes, which are non-ionic dyes used in the dyeing of non-ionic fibres, such as polyester. Disperse Blue 124 (DB124; 2-(N-Ethyl-4-((5-nitrothiazol-2-yl)azo)-m-toluidino)ethyl acetate, Sigma-Aldrich, Merk, Germany) and Disperse Blue 134 (DB134; 1,4-Bis(isopropylamino)anthraquinone, Sigma-Aldrich, Merk, Germany) with a fabric-bath ratio of 1:320, using the DES with different water percentages as solvent. The dyeing was carried out with 3% owf of dye and 3% owf of levelling agent (Levegal UNI, Tanatex Chemicals, Netherlands), following the all-in procedure:

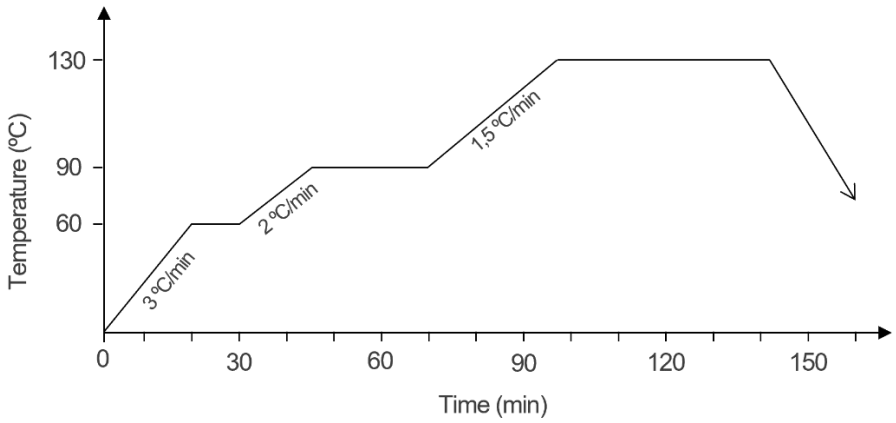


Figure 4: Schematic representation of the polyester dyeing procedure in the laboratory dyeing machine.

After dyeing, the fabric was washed with Diadavin UN (Tanatex Chemicals, Netherlands), and running water and dried in the oven at 40 °C.

3.5.3. Dyeing of polyester – Sealed system (SS)

The optimization of the polyester dyeing process was also tested using a SS in a thermostatic bath. The polyester fabric was cut into squares (1,5x1,5 cm) and weighted (21 mg). The dyeing was performed with DB124 and DB134 (Sigma-Aldrich, Merk, Germany) with a fabric-bath ratio of 1:250, with 3% owf of dye and 3% owf of levelling agent (Levegal UNI, Tanatex Chemicals, Netherlands). Fabric and dyeing solutions were placed in vials closed with a metallic capsule under pressure. The dyeing process was carried out at 130 °C for 120 min, with stirring.

The dyeing was performed with different DES at different pH values, using NaOH and acetic acid for pH adjustment. Firstly, a dyeing was performed without pH correction with DB124, other dyeing was conducted at pH 3-4 with DB124 and a dyeing at pH 4-5 with DB134. After the dyeing procedures, the fabrics were washed with Diadavin UN (Tanatex Chemicals, Netherlands) at 50 °C for 30 min, then with running water, and left to air-dry overnight.

3.5.4. Optimization of dyeing temperature – SS

Aiming to reduce the temperature of dyeing experiments were conducted at 120 °C, temperature bellow the optimum polyester dyeing temperature (130 °C). For this, the polyester fabric was cut into squares (1,5x1,5 cm) and weighted (21 mg). The dyeing was performed with DB124 and DB134 with a material-bath ratio of 1:250, with 3% owf of dye and 3% owf of levelling agent (Levegal UNI, Tanatex

Chemicals, Netherlands). Fabric and dyeing solutions were placed in vials closed with a metallic capsule. The extraction process was carried out at 120 °C for 120 min, with stirring. After dyeing, the fabric was washed with Diadavin UN at 50 °C for 30 min, then with running water, and left to air-dry overnight.

3.5.5. Colour strength evaluation

Determination of K/S of dyed polyester was performed as described in section 3.4.4.

3.6. Dye extraction wastewater effluents using DES

Aiming to understand the ability of DES to sequester dyes from wastewater effluents, DES 4 (Acid 1 : acid 2) was tested for the extraction of two dyes (DB124 and DB134, Sigma-Aldrich, Merk, Germany) varying several conditions like dye concentration, DES dosage, and pH (DES and dye). This DES was chosen due to its potential extractive capacity that was noticed during polyester dyeing assays.

3.6.1. Effect of DES dosage

10 mL of DB124 and DB134 solutions (1 mg/mL) were prepared using distilled water and ethanol (80/20% v/v). After preparation, different DES dosages (0,004; 0,008; 0,016; 0,024; 0,032 and 0,040 mol) were added to the tubes. The dye solutions were left to rest 1h. After resting, the two phases of DES-extracted dye and clear aqueous solution were separated using a decanting funnel. For DB124 solutions, the process was repeated with the following DES dosages: 0,040; 0,064; 0,088; 0,112; 0,136, and 0,160 mol. The solutions were left to rest 1h, centrifuged (K3, Centurion Scientific) for 30 min at 4000 rpm, and left to rest overnight. Afterwards, the two phases of DES-extracted dye and clear aqueous solutions were separated using a decanting funnel.

3.6.2. Effect of dye concentration

10 mL of DB124 and DB134 solutions were prepared in falcon tubes at different concentrations (0,1; 0,3; 0,5; 0,7 and 1 mg/mL) using distilled water and ethanol (80/20% v/v). After preparation, a 0,016 mol dosage of DES was added to DB134 solutions and 0,160 mol to DB124 solutions. Both dye solutions were left to rest 1h, and afterwards the DB124 solutions were centrifuged (K3, Centurion Scientific) for 30 min at 4000 rpm and left to rest overnight. After resting, the two phases of DES-extracted dye and clear aqueous solution were separated using a decanting funnel.

3.6.3. Effect of DES pH

10 mL of DB124 and DB134 solutions (1 mg/mL) were prepared using distilled water and ethanol (80/20% v/v). Afterwards, the pH of DES 4 was adjusted with NaOH to 2, 4, 6 and 7, and 0,016 mol dosage of each solution were added to DB134 solutions and 0,160 mol to DB124 solutions. The final dye solutions were left to rest 1h, and after, DB124 solutions were centrifuged (K3, Centurion Scientific) for 30 min at 4000 rpm and left to rest overnight. After resting, the two phases of DES-extracted dye and clear aqueous solution were separated using a decanting funnel.

3.6.4. Effect of dye pH

10 mL of DB124 and DB134 solutions (1 mg/mL) were prepared using distilled water and ethanol (80/20% v/v). After preparation, NaOH and HCl were used to adjust the pH of the solutions (2 ,4, 6, 7, 8, and 10). Then, 0,016 mol of DES 4 was added to DB134 solutions and 0,160 mol to DB124 solutions. The solutions were left to rest 1h, and after, DB124 solutions were centrifuged (K3, Centurion Scientific) for 30 min at 4000 rpm and left to rest overnight. After resting, the two phases of DES-extracted dye and clear aqueous solution were separated using a decanting funnel.

3.6.5. Extraction efficiency

After the two phases separation, the phase of DES-extracted dye was analysed for dye concentration. DB124 and DB134 were used for preparing calibration curves, and different concentrations were prepared with DES 4 (Annex VI). The absorbance was measured at 590 nm for DB124 and 644 nm for DB134, using a microplate reader (Synergy Mx, BioTek). The following equation was applied to calculate the extraction efficiency of the dyes: $\mathbf{\%Extraction\ efficiency=(M_o-M_f)/M_o\ x\ 100}$, where M_o and M_f represent initial dye mass and final dye mass, respectively. In addition, the aqueous phase was analysed by measuring the visible spectra in comparison with the initial dye solutions, using a microplate reader (Synergy Mx, BioTek).

3.6.6. Nuclear magnetic resonance spectroscopy (NMR)

To evaluate possible hydrogen bonding between DES 4 and the dyes, proton nuclear magnetic resonance spectroscopy (^{1}H NMR) was performed, using a Bruker Avance III 400 (400 MHz), using DMSO-d6 (Eurisotop, CIL) as deuteride solvent, and the peak solvent used as internal reference.

3.6.7. FTIR-ATR

Chemical interactions of the DES 4 and the dyes were studied by FTIR-ATR. The spectra were obtained using a Bruker Alpha II (Massachusetts, USA) and acquired by Opus 8.22.28. DES solutions were placed directly over the crystal, and the spectra were acquired between 400 and 4000 cm^{-1} wavenumbers with a 2 cm^{-1} resolution.

3.7. Tannery effluent degradation and detoxification using DES

Aiming to understand the role of DES 4 on the degradation and detoxification of tannery wastewater effluent, provided by Fábrica de Curtumes de Roldes, Guimarães, several conditions were tested. Namely, different apparatus (ultrasound and centrifuge), the number of cycles of ultrasound (US) and centrifugation, different effluent dosages, different DES doses dosages, and different pH (DES and effluent). Furthermore, the effluent was characterized before and after the degradation process to perceive the role of DES on detoxification and degradation.

3.7.1. Effect of US and centrifugation cycles

0,040 mol of DES 4 were added to different effluent dilutions (10 mL). Afterwards, the solutions were placed in an ultrasonic bath (USC600TH, VWR International) for 30 min and centrifuged for 30 min at 4000 rpm (K3, Centurion Scientific). This procedure was repeated twice and three times, in order to understand the influence of the number of cycles. It was immediately perceived by the appearance of the solutions that one cycle alone would not be enough to treat the effluent. Further, the two phases of DES and clear aqueous solution were separated using a decanting funnel. The visible spectra of initial and final effluent solutions were measured using a microplate reader (Synergy Mx, BioTek).

3.7.2. Effect of effluent concentration

0,040 mol of DES 4 were added to different effluent dilutions (10 mL). Afterwards, the solutions were placed in an ultrasonic bath (USC600TH, VWR International) for 30 min and centrifuged for 30 min at 4000 rpm (K3, Centurion Scientific). This procedure was repeated twice. Further, the two phases of DES and clear aqueous solution were separated using a decanting funnel. The visible spectra of initial and final effluent solutions were measured using a microplate reader (Synergy Mx, BioTek).

3.7.3. Effect of DES pH

0,040 mol of DES solutions with different pH values (2 ,4, 6 and 7), adjusted with NaOH, were added to 10 mL of effluent. After, the solutions were placed in an ultrasonic bath (USC600TH, VWR International) for 30 min and centrifuged for 30 min at 4000 rpm (K3, Centurion Scientific). This procedure was repeated twice. Further, the two phases of DES and clear aqueous solution were separated using a decanting funnel. The visible spectra of initial and final effluent solutions were measured using a microplate reader (Synergy Mx, BioTek).

3.7.4. Effect of effluent pH

Different solutions (10 mL) of effluent with different pH values were prepared (2 ,4, 6, 7, 8 and 10). Then, 0,040 mol of DES 4 was added to each solution. After, the solutions were placed in an ultrasonic bath (USC600TH, VWR International) for 30 min and centrifuged for 30 min at 4000 rpm (K3, Centurion Scientific). This procedure was repeated twice. Further, the two phases of DES and clear aqueous solution were separated using a decanting funnel. The visible spectra of initial and final effluent solutions were measured using a microplate reader (Synergy Mx, BioTek).

3.7.5. Physicochemical characterization of the effluent

The effluent before and after detoxification with DES was characterized in terms of pH, conductivity, total solids content, oils and greases, chemical oxygen demand (COD), and total chromium.

3.7.5.1. Total solids (TS) content

For determining the TS concentration, the samples were placed in pre-weighted falcon tubes and lyophilized to remove the water content. Afterwards, the residues were weighed and the difference in weight is equivalent to TS in the samples.

3.7.5.2. Oils and greases determination

A liquid-liquid extraction with hexane was carried out to quantify the oil and greases amount in the samples. 10 mL of sample was poured into a separating funnel, and 15 mL hexane was added. Two immiscible liquid layers were obtained, with hexane forming the upper layer. The aqueous layer was collected through the tap of the separating funnel while the organic phase (hexane) was poured into a pre-weighted flask. The sample was sequentially extracted with three aliquots of hexane in the separating funnel. The solvent extracts were collected together and evaporated to dryness at ambient temperature in a fume cupboard. The difference in weight is equivalent to oil and grease in the samples.

3.7.5.3. Chemical oxygen demand (COD)

The determination of COD involves the reaction of the sample with a strong oxidizing agent which oxidizes the organic matter. COD of the samples was obtained using a COD determination kit (LCK 514, Hach Lange). 2 mL of the sample was added to the sample tube and incubated (LT200, Hach Lange) at 148 °C for 2 h. The oxidizable substances react with the sulphuric acid and potassium dichromate solution. After the incubation, the test tube was allowed to cool to room temperature. The resultant green coloration was evaluated spectrophotometrically (DR2800, Hach Lange) and the result is expressed in mg/L O_2.

3.7.5.4. Total Nitrogen (TN)

TN was measured using a TN determination kit (LCK 338, Hach Lange). 0,2 mL of the sample was added to a test tube, and 2,3 mL of reagent A (sodium hydroxide) and a tablet of reagent B (potassium peroxodisulfate, sodium tetraborate, and sodium metaborate) were added. Then, the sample was incubated (LT200, Hach Lange) at 120 °C for 30 min. After the incubation, the tube was allowed to cool to room temperature, and 0,5 mL of this solution was added to the sample tube along with 0,2 mL of

reagent D (2-propanol). The solution was left to react for 15 min and was evaluated spectrophotometrically (DR2800, Hach Lange) with the result expressed in mg/L N.

3.7.5.5. Total chromium (TCr)

To determine the TCr in the tannery effluent, the samples were first digested using a Berghof microwave digestion system. 350 µL of the sample were added into the digestion vessel, with 3 mL of HNO_3, 1 mL of HCl, and 3 mL of H_2SO_4. The mixture was shaken, and the vessels closed after 10 min. The vessels were then heated in the microwave with the following program:

Table 8: Microwave digestion program.

Step	T (ºC)	P (bar)	Time (min)	Power (%)
1	160	30	5	80
2	200	35	15	90
3	50	25	10	0

The vessels were cooled until room temperature before opening. The digested samples were then diluted with ultra-pure water (1:2) and filtered with 0,22 µm pore size, polyether sulfone (PES) syringe filters (FilterBio). The samples were posteriorly analysed by ICP (Inductively Coupled Plasma) optical emission spectrometer (Optima 8000, PerkinElmer). Firstly, a calibration curve was done with different concentrations of a standard solution in HNO_3. Afterwards, the samples were analysed. The spectrometer software automatically does duplicate readings and calculates total chromium in the samples.

3.8. Statistical analysis

Statistical analysis of data was performed using the GraphPad Prism 8 software. Results are shown as mean values ± standard deviation (SD), and statistical comparisons were calculated with a 95% confidence interval. One-way or two-way analysis of variance (ANOVA) was used to examine the influence of one or two independent variables, respectively, on one continuous variable, followed by Tukey's multiple comparison test. Of each test result a p-value indicates the significance value of each tested sample. This significance ($p < 0.05$) is shown in the figures with letters.

4. RESULTS AND DISCUSSION

4.1. DES characterization

This first section will present the characterization results of the different DES used throughout the work.

4.1.1. Physicochemical and thermal properties

The physicochemical characterization of the DES has considered the following properties: pH, density (ρ), viscosity (μ), and conductivity (σ), which are represented in Table 9.

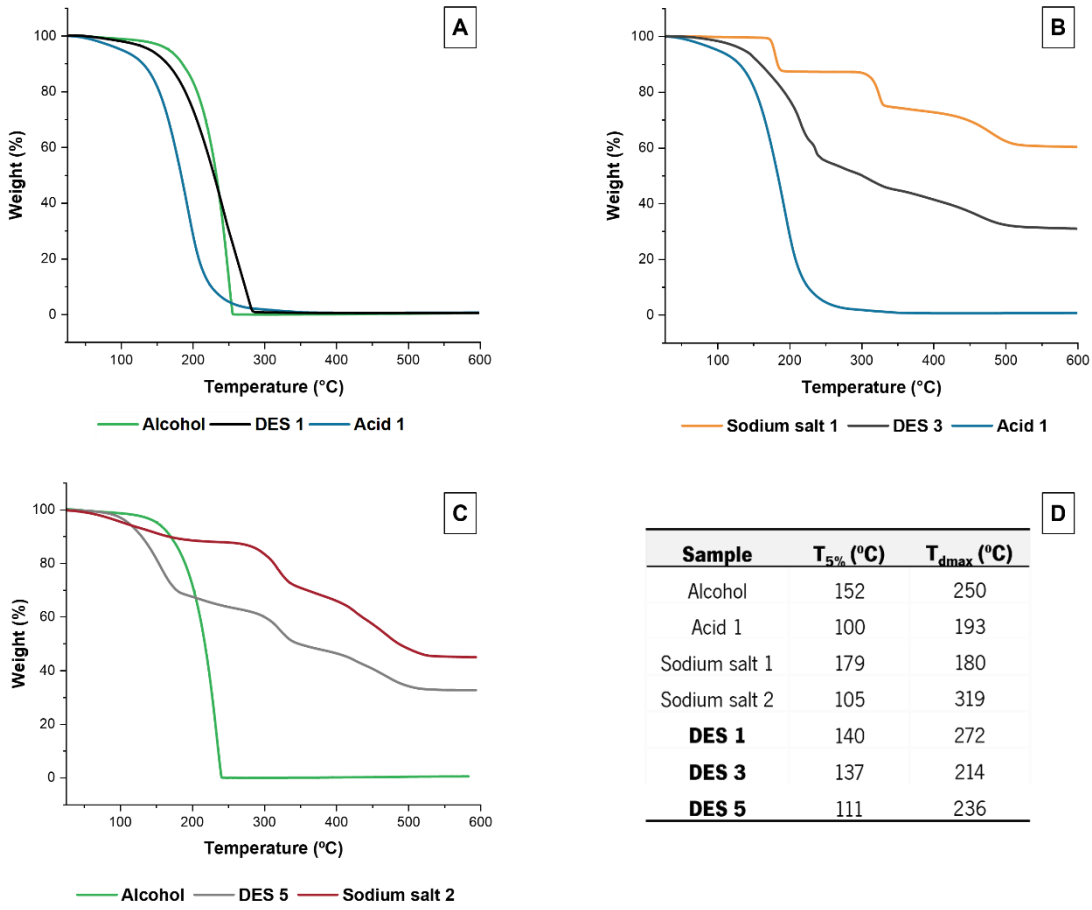
Table 9: Physicochemical properties of DES: molar ratio, pH, density (ρ), viscosity (μ) and conductivity (σ).

Abbreviation	Composition	Molar ratio	pH	ρ (g/mL)	μ (Pa.s)	σ (μS/cm)
DES 1	Alcohol : Acid 1	1:1	0,9	1,242	1,033	0,873
DES 2	Alcohol : Diethylene glycol monoethyl ether	1:1	7,0	1,082	0,0824	0,062
DES 3	Acid 1 : Sodium salt 1	4:1	4,0	1,392	10,11	346,8
DES 4	Acid 1 : Acid 2	1:1	0,2	0,985	0,320	0,057
DES 5	Alcohol : Sodium salt 2	1:1	7,4	1,366	1,608	708,2

DES physicochemical properties change according to their composition, and thus their application will depend on these properties. For example, solvents with low viscosity are preferred for extraction purposes, as it enables higher diffusion rates and different density from the matrix to facilitate the posterior separation (Cunha & Fernandes, 2018; Macchioni et al., 2021). All the DES tested present higher density and viscosity than water, being DES 3 the most viscous solvent and DES 2 the less viscous. Generally, DES present low conductivity due to their high viscosity. However, conductivity increases with the increase of temperature due to the reduction of DES viscosity (Cunha & Fernandes, 2018; Zhang et

al., 2012). Nevertheless, it is possible to observe that the more viscous the DES, the higher its conductivity. Based on the DES properties, DES 1 and DES 2 were chosen to extract bioactive compounds from several natural sources. The two DES present distinct low viscosities, which are vital for the extraction processes and obtaining high extraction yields. Moreover, the two DES present other distinct properties, namely pH, which will allow understanding the influence of different DES properties in the extraction efficiency. Furthermore, these DES are composed of safe compounds often used in cosmetic formulations.

Thermal stability is a fundamental property of DES, as it provides the range of temperatures in which these solvents can be used. TGA was used to evaluate the degradation temperature of the DES. The results for DES 1, DES 3, and DES 5 are shown in Figure 5 and expressed as weight loss (%) vs temperature. All the DES presented a thermogram with a hybrid profile, in agreement with previous studies that reported TGA analysis of ChCl based DES (Delgado-Mellado et al., 2018).



D

Sample	$T_{5\%}$ (°C)	T_{dmax} (°C)
Alcohol	152	250
Acid 1	100	193
Sodium salt 1	179	180
Sodium salt 2	105	319
DES 1	140	272
DES 3	137	214
DES 5	111	236

Figure 5: TGA data of DES and respective components. (A) DES 1 (B) DES 3, (C) DES 5, and (D) $T_{5\%}$ and T_{dmax} of DES and components.

Relevant data, such as $T_{5\%}$ and T_{dmax}, indicating the temperature at which 5% weight loss occurs and the temperature with maximum degradation rate, is summarized in Figure 5D. $T_{5\%}$ gives information about the initial weight loss of the materials. Thus, it is possible to perceive the temperatures at which the decomposition processes begin. DES 1 and DES 3 have higher $T_{5\%}$ than DES 5. Accordingly, both eutectic mixtures can be used up to temperatures of 140 °C without decomposition. DES 5 has a lower T_{dmax} than its components, suggesting that the solvent formed is less stable than its precursors. On the contrary, the T_{dmax} of DES 1 and DES 3 is higher than its constituents separately, indicating a possible interaction between the mixture's components, leading to increased stability and consequently increased T_{dmax}. Several factors influence the thermal stability of DES. For instance, stability increases with the use of HBD compounds with larger alkyl chains or compounds rich in hydroxyl and carboxyl groups in their molecular structures, as this enables the formation of a larger number of hydrogen bonds between components (Guimarães et al., 2022).

4.1.2. Chemical structure – FTIR-ATR

FTIR-ATR is an essential technique for the characterization of intermolecular interactions between DES constituents. It measures the vibrational intensities of the chemical bonds of the compounds, which can be influenced by intra/intermolecular interactions. The interactions between DES constituents may cause shifts in the spectral bands compared with the chemical compounds of the starting materials (Banjare et al., 2018).

The FTIR-ATR characterization of the synthesized DES was conducted to explore the functional groups involved in the interactions and analyse possible changes in their structure. The FTIR spectra of DES 1 and its pure components (Alcohol : Acid 1) are represented in Figure 6A. The alcohol spectrum shows a vibrational band at 3276 cm^{-1} related to the presence of the O-H group and two peaks in the region of 2810-2950 cm^{-1} due to the C-H stretching. Bending of O-H group can be observed in the region of 1400 to 1420 cm^{-1}, and C-O stretching of the primary alcohol is shown at 1107 cm^{-1}. The acid 1 spectrum shows the presence of O-H and alkyl groups in the form of a large band around 3300 cm^{-1}. The characteristic peak of C=O stretching is presented at 1719 cm^{-1}, while O-H bending from the carboxylic and hydroxyl groups appears at 1454 and 1375 cm^{-1}, respectively. Acid 1 characteristic C-O stretching of the carboxylic group appears at 1201 cm^{-1} and of the secondary alcohol at 1120 cm^{-1}. FTIR results confirm the formation of DES 1, as all the functional groups of both constituents were identified in the IR spectrum. The vibrational bands at 3600 to 3000 cm^{-1} and 2850 to 2950 cm^{-1} refer to the hydroxyl and aliphatic C-H alkyl group. The peak at 1729 cm^{-1} is related to the presence of C=O (shifted comparative to the same

acid 1 peak). The peaks at 1453 and 1373 cm^{-1} are relative to O-H bending from the carboxylic and hydroxyl groups and the C-O stretching bands at 1206 and 1122 cm^{-1} from carboxylic and hydroxyl groups.

The FTIR spectrum of DES 2 is represented in Figure 6B, together with the spectra of its pure components (Alcohol : Diethylene glycol monoethyl ether). Diethylene glycol monoethyl ether spectrum shows a vibrational band at 3421 cm^{-1} related to the presence of the O-H group and three peaks in the region of 2974 - 2867 cm^{-1} due to the C-H stretching. Bending of C-H group can be observed in the region of 1450 cm^{-1}, and C-O stretching of the primary alcohol and ether groups is shown at 1108 and 1065 cm^{-1}. The FTIR also results confirmed the formation of DES 2, since the functional groups of both constituents were identified in the IR spectrum with shifts in some of their bands. The vibrational bands at 3600 to 3000 cm^{-1} and 2974 to 2867 cm^{-1} refer to the hydroxyl and aliphatic -CH alkyl group. The peaks at 1102 and 1040 cm^{-1} are relative to the C-O stretching bands of the hydroxyl (primary and secondary) and ether groups.

The FTIR spectra of the pure acid 1 and sodium salt 1, and DES 3 are shown in Figure 6C. Sodium salt 1 spectrum shows a peak at 3444 cm^{-1}, corresponding to O-H group, and two major peaks at 1579 and 1416 cm^{-1} that refer to COO^- stretching bands of carboxyl groups of the sodium salt. DES 3 presents peaks of both constituents, the presence of O-H and alkyl groups in the form of a large band around 3390 cm^{-1}; the peak of C=O from acid 1 at 1711 cm^{-1}; the peak from COO^- stretching of the sodium salt 1 at 1583 cm^{-1}, and finally, the C-O stretching of the carboxylic group appears at 1229 cm^{-1} and of the secondary alcohol at 1123 cm^{-1}, from acid 1.

The FTIR spectra of pure acid 1, acid 2, and DES 4 are represented in Figure 6D. Acid 2 spectrum shows two bands at 2923 and 2855 cm^{-1} relative to C-H stretching of the alkyl group. The peak of C=O stretching appears at 1704 cm^{-1}, and the peak of O-H bending at 1412 cm^{-1}. Finally, C-O stretching of the carboxylic group appears at 1274 cm^{-1}. DES 4 spectrum presents the characteristic peaks of both components, and the peaks are shifted, confirming the formation of the solvent. Bands at 2925 and 2855 relative to C-H stretching; the peak at 1707 relative to the C=O stretching, the peaks at 1457 and 1410 cm^{-1} referring to the O-H bending; C-O stretching peaks of the carboxylic groups appear at 1265, 1222 and of the secondary alcohol of the acid 1 at 1123 cm^{-1}.

The FTIR spectra of DES 5, pure alcohol, and sodium salt 2 are represented in Figure 6E. Sodium salt spectrum shows the presence of O-H group in a large band at 3280 cm^{-1} and at 2976 cm^{-1} a peak relative to C-H stretching. The characteristic peak of COO- stretching is presented at 1575 cm^{-1}. C-H bending vibration appears at 1453 cm^{-1}, and O-H bending and C-O stretching of the secondary alcohol peaks show up at 1416 – 1360 cm^{-1} and 1117 cm^{-1}, respectively. The FTIR results confirm the formation

of DES 5, as there is observable the presence of peaks from both components. Is it possible to observe in the spectrum a large band around 3270 cm^{-1} corresponding to O-H group; two peaks in the region of 2970-2933 cm^{-1} due to the C-H stretching from the alcohol; the peak of COO- stretching from the sodium salt is presented at 1585 cm^{-1}. Bending of O-H group can be observed at 1416 cm^{-1}, and C-O stretching is shown at 1121 cm^{-1}.

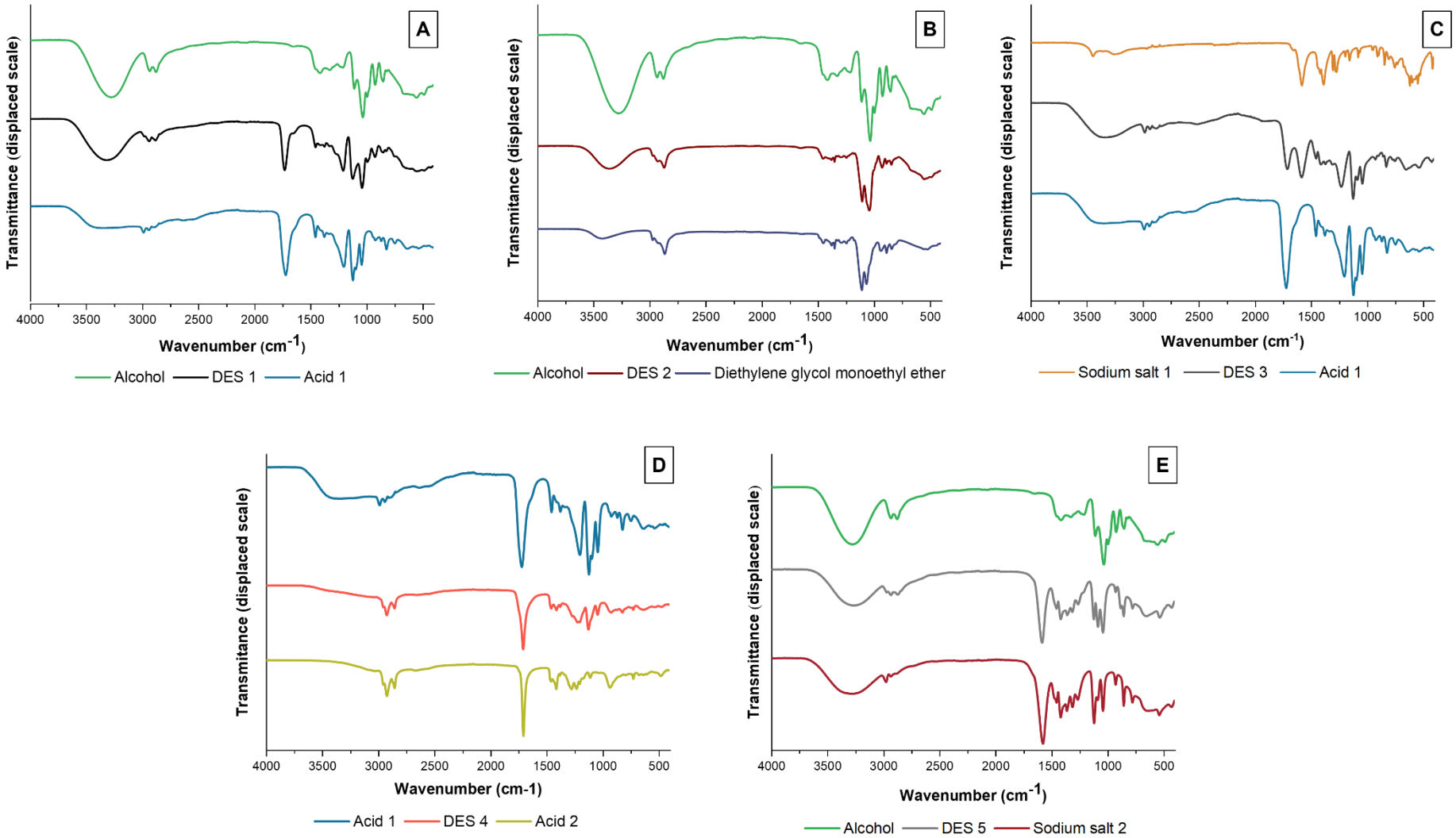


Figure 6: FTIR-ATR spectra of DES and respective components. (A) DES 1, (B) DES 2, (C) DES 3, (D) DES 4, and (E) DES 5.

4.2. Extraction of bioactive compounds from natural sources

4.2.1. Extraction yields

The extraction of bioactive compounds from natural sources was performed with four different solvents (water, dioxane, DES 1, and DES 2) and three extraction methods (UAE, SSE, and MAE). The extraction yield is an essential parameter for establishing the effectiveness of an extraction process. Hence, the influence of the extraction method and the solvent used in the extraction efficiency was evaluated (Figure 7). In general, extraction with DES leads to similar or higher yields than water or dioxane, regardless of the method used. Accordingly, DES might be an excellent alternative to replace water and organic solvents in extraction processes. Water, unlike organic solvents, is, of course, a green solvent. However, as an extraction solvent, it presents some disadvantages. It can promote the growth of bacteria and moulds, hydrolyse the extracted compounds, and requires elevated temperatures for the concentration of the extracts (Manousi et al., 2019).

For all the natural materials used, the extraction yields obtained with DES were similar or superior to those obtained with water and dioxane, at least for one of the extraction methods, except for cocoa beans. For cocoa beans, dioxane was the solvent that promoted the highest extraction yield. Dioxane was possibly the only solvent that was able to extract most of the cocoa fat, which usually comprises 40 - 50% of cocoa butter (used for the development of chocolate) (Ramos-Escudero et al., 2021).

DES 1 shows, in many cases, superior extraction yields compared to DES 2. DES extraction efficiency is influenced by its physical-chemical properties, such as polarity, pH, and viscosity. For instance, low viscosity solvents are preferred for extraction processes. Although DES 1 has superior viscosity when compared with DES 2, this may not influence the extraction capacity, as the viscosity of solvents can be reduced by the extraction temperature (Cunha & Fernandes, 2018). DES can establish H-bonding interactions with, for example, phenolic compounds, being the reason for their higher extraction efficiency (Macchioni et al., 2021). Hence, different extraction efficiencies between DES should be attributed to the ability of hydrogen bonds formation (Fu et al., 2021).

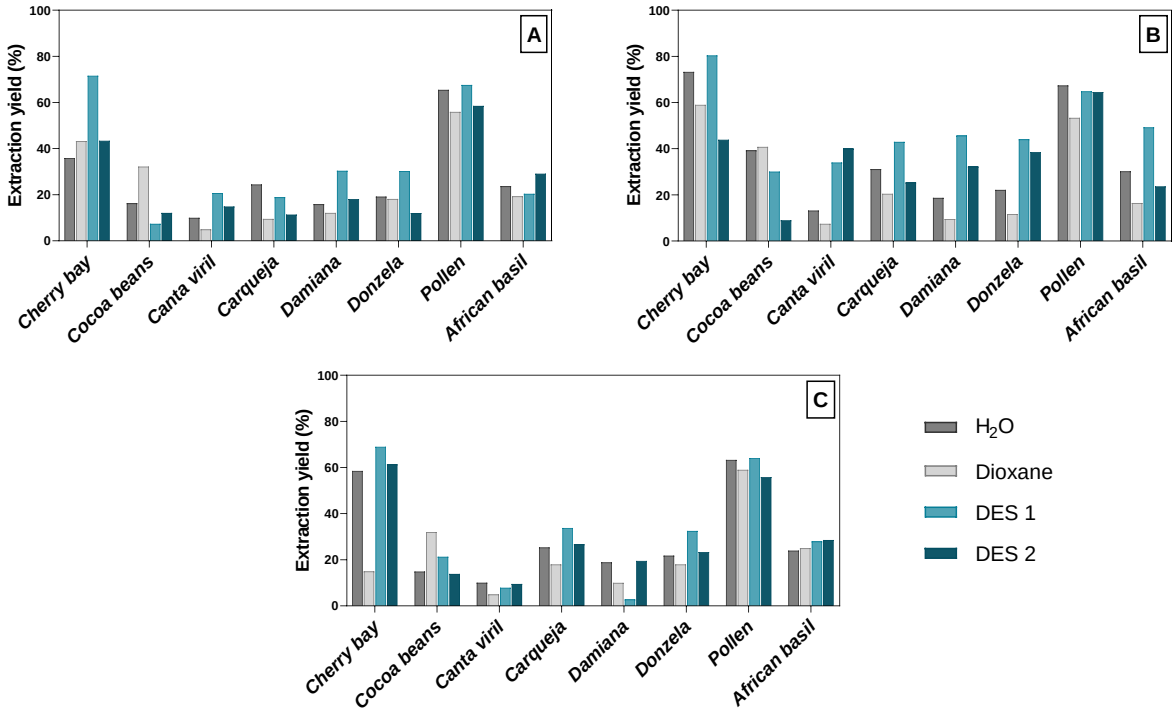


Figure 7: Extraction yields (%) obtained by different extraction methods. **(A)** UAE, **(B)** SSE, **(C)** MAE.

Regarding the extraction method, SSE was the method that led to the highest extraction yields in the majority of the extractions studied. These results are in accordance with the expected, since this methodology, besides pressure, also involves higher temperatures and longer extraction periods than the other methods. However, the extraction yields obtained when using UAE and MAE methods are, in some cases, close to the ones obtained with SSE. Considering that both extraction methods involve the use of lower temperatures and shorter extraction time than SSE, these would represent appealing methodologies for extracting bioactive compounds. Furthermore, increasing the extraction time, such as conducting two cycles in MAE instead of one, could possibly lead to greater yields in less time, with less energy consumption.

Concerning all the natural sources tested, the highest extraction yields were obtained for cherry bay and pollen. The lowest extraction yield (36%) obtained for cherry bay was when using the UAE method with water as solvent, while the best extraction efficiency (80%) was obtained when the SSE method was used with DES 1 as solvent. For pollen, independently of the extraction method, the extraction yields were very similar (≈60%). The lowest extraction yields were obtained for "canta viril" tea when using MAE as the extraction method for all the solvents used (≤10%).

4.2.2. UV-Visible absorption spectra of the extracts

The UV-Vis spectra of cherry bay and pollen extracts obtained with the different extraction methods and solvents are shown in Figure 8 and Figure 9, respectively. By analysing the obtained spectra, it is possible to infer that, for the extracts of cherry bay berries, the highest absorbance value is observed for the extracts obtained using SSE, indicating a higher concentration of compounds in solution and, consequently, greater efficiency of the extraction process.

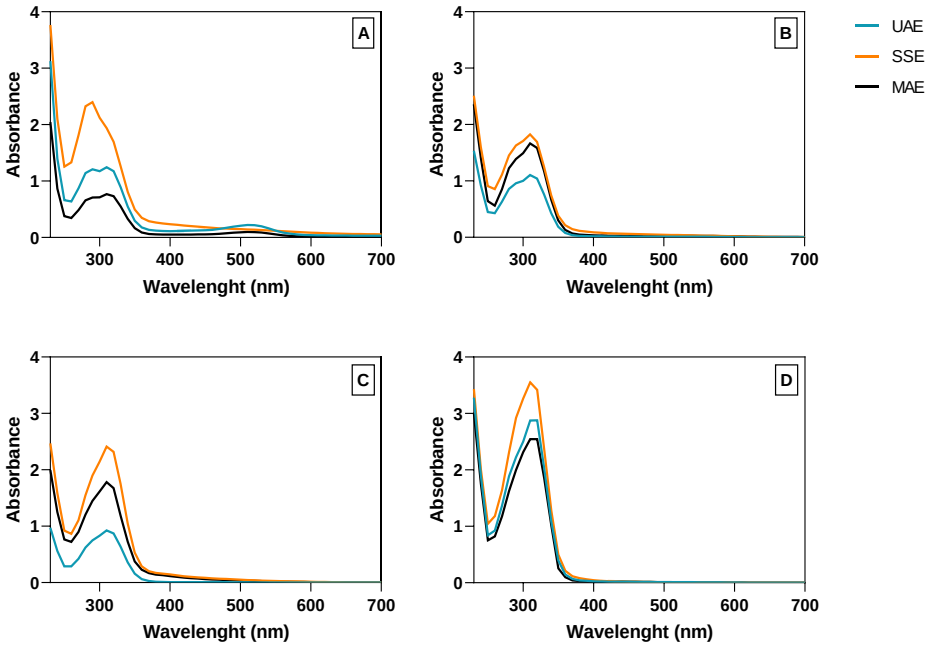


Figure 8: Absorption UV-Vis spectra of cherry bay extracts. (A) DES 1, (B) DES 2, (C) H_2O and (D) Dioxane.

The results are in concordance with the obtained extraction efficiencies. It is also possible to notice that the spectra of the extracts obtained with other extraction methods are, in some cases, very similar to the ones of SSE extracts. The same pattern can be observed for the pollen extracts. It is noteworthy that the spectra profile is different according to the solvent used for extraction. Extracts spectra from the other natural sources are shown in Annex IV.

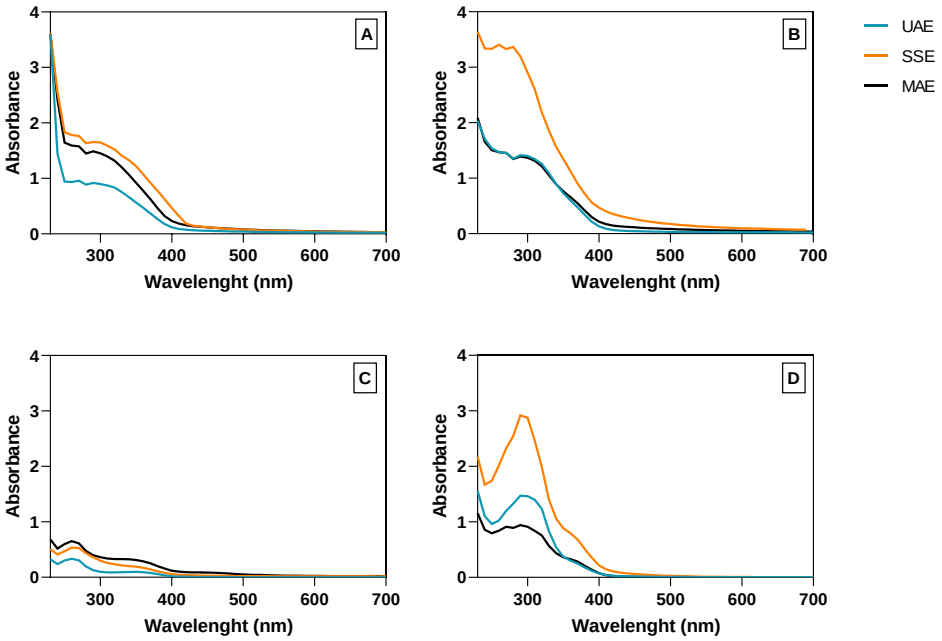


Figure 9: Absorption UV-Vis spectra of pollen extracts. (A) DES 1, (B) DES 2, (C) H_2O, and (D) Dioxane.

4.2.3. Total phenolic content and antioxidant activity

The influence of the extraction methods and solvents on the composition of the extracts was also evaluated. Phenolic compounds are of particular interest because of their antioxidant activity. The extracts' TPC determination was carried out by colorimetric analysis using the Folin-Ciocalteau method. In the presence of phenols, the Folin reagent (yellow coloured) forms a blue complex when reduced, which is quantified spectrophotometrically between 620 and 760 nm. An increase in absorbance is correlated with higher phenolic content (Margraf et al., 2015). TPC values of all the extracts are shown in Figure 11. The TPC of the extracts is not proportional to the extraction values obtained by weight evaluation. “Canta viril” extracts, which gave rise to the lowest extraction yields, presents higher TPC than pollen, one of the natural materials with higher extractability efficiencies. In fact, pollen extracts have the lowest TPC, with a maximum of 46,1 ± 0,8 mg GAE/g extract. On the other hand, “carqueja” extracts present high phenolic content, with a maximum value of 230,8 ± 4,5 mg GAE/g extract when extracted with dioxane using the SSE method. This is in accordance with other studies performed by Coelho et al. (2011) which revealed that the phenolic content for this plant ranged from 270,7 to 402,9 mg GAE/g dry material, depending on the harvest location and season.

TPC obtained with the different extraction methods and solvents are variable according to the natural material. For instance, TPC is higher for cherry bay extracts when using DES 2 and UAE or SSE methods.

In comparison, dioxane gave rise to the highest TPC values for "carqueja" extracts, for all the extraction methods, and water for "donzela" extracts for all the extraction methods. Although DES 1 has generally promoted the highest extraction yields, it is not the solvent that gave rise to solutions with the highest TPC in almost all cases. The dissolution of bioactive compounds is strongly related to the polarity of the solvent used. Dai et al. (2013) studied the extraction of aromatic pigments from *Carthamus tinctorius L* with a varied range NADES with different polarities, showing that the NADES with the lowest polarity promoted the lowest extraction efficiency for polar and the highest extractability for non-polar compounds. Phenolic compounds are generally polar (Lefebvre et al., 2021), being the use of more polar solvents, like water, the most efficient way to proceed to their extraction. However, different phenolic compounds may present distinct polarities (Figure 10). Therefore, other solvents may be more suitable for extraction depending on the plant's composition, which might explain the differences obtained.

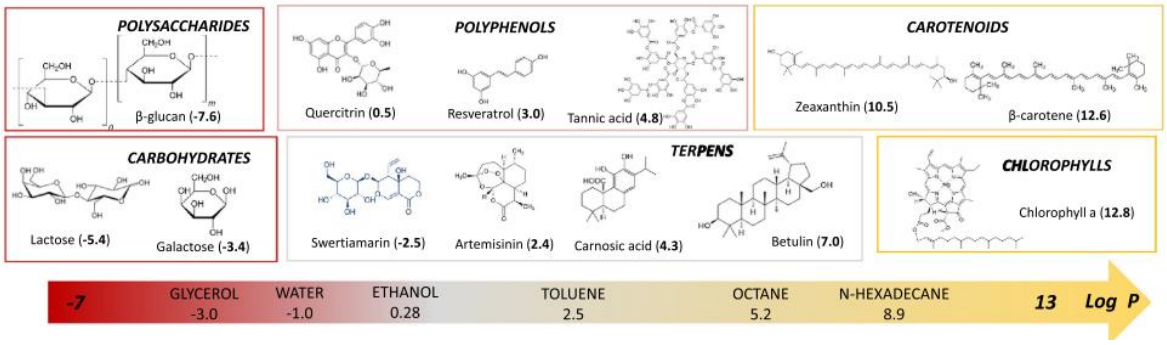


Figure 10: Compounds polarity based on log Ko/w scale. Adapted from (Lefebvre et al., 2021).

Concerning the extraction method, extracts obtained by the SSE method showed the highest TPC values in most cases. However, the TPC of the extracts obtained with UAE and MAE are, in general, similar, or in some cases higher. Once again, considering that both extraction methods are less time-consuming than SSE, these methods represent excellent alternatives for extracting bioactive compounds from natural sources. Even so, it is important to mention that TPC evaluated through this method does not correspond to an absolute measurement of the amount of phenolic compounds, but instead, their reducing ability relativity to an equivalent reducing capacity of gallic acid.

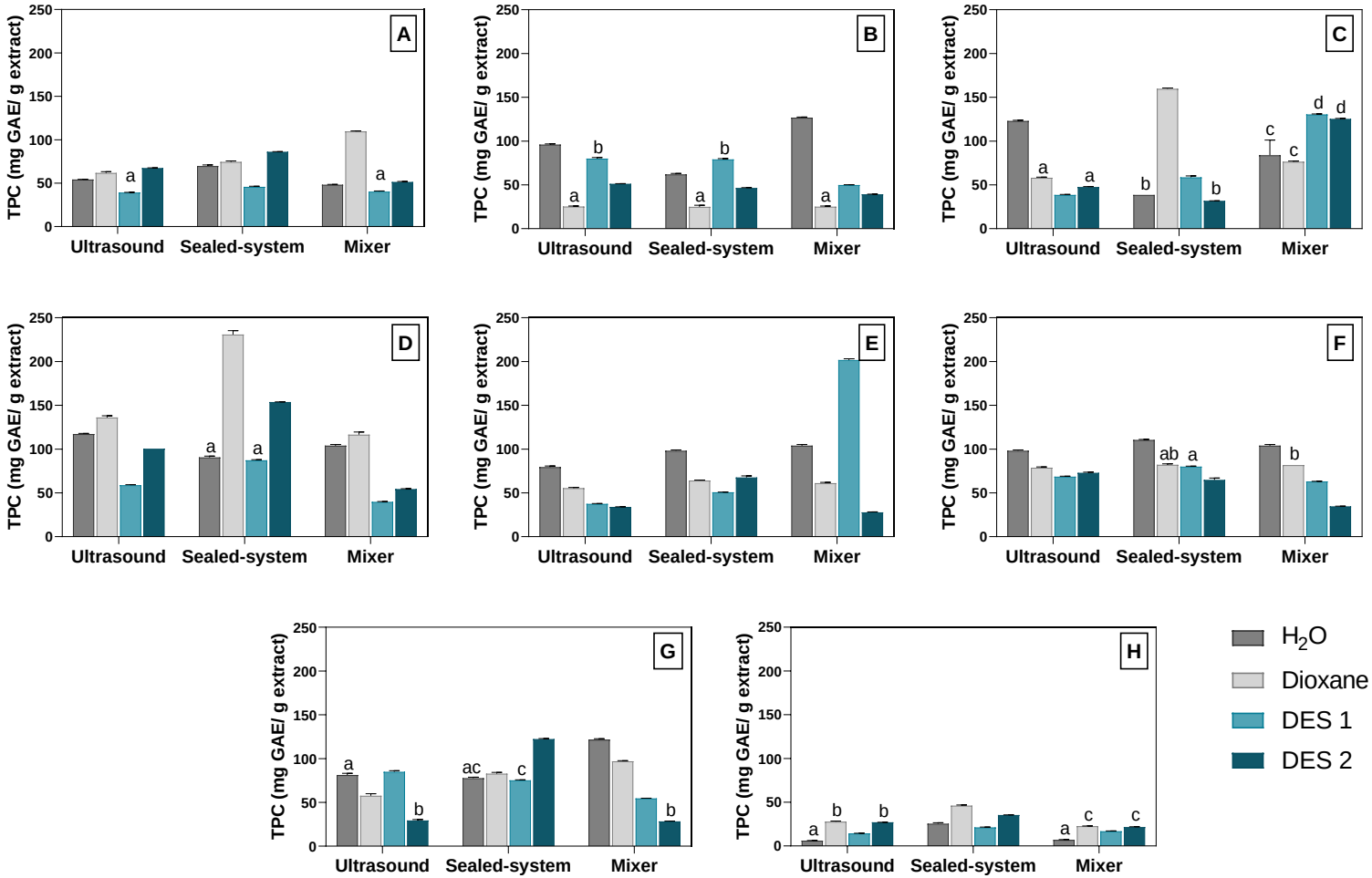


Figure 11: TPC of plant extracts obtained with different solvents and extraction methods. (A) Cherry bay, (B) Cocoa beans, (C) Canta viril, (D) Carqueja, (E) Damiana, (F) Donzela, (G) African basil and (H) Pollen. Triplicate readings were performed for each condition, and data is expressed as mean ± SD. The results were statistically analysed by two-way ANOVA, followed by Tukey's multiple comparison test. Equal letters represent no statistically significant difference.

The antioxidant potential of the extracts is an important parameter when considering its application in cosmetic products to help control ROS generation. DPPH scavenging assay is a method that measures the scavenging capacity of natural antioxidants. In the presence of antioxidants, the nitrogen atom of DPPH is reduced, losing its purple colour, and becoming yellow. The measurement of absorbance between 515 and 520 nm allows the determination of the scavenging capacity of the extract based on DPPH discoloration (Vuolo et al., 2019). The percentage of reduced DPPH (% rDPPH) increases with the decrease in absorbance. Radical scavenging activity values of all the extracts are represented as IC_{50}, which is the concentration needed to reduce 50% of DPPH in Figure 12. As for the TPC data, the results obtained with the different extraction methods and solvents are variable according to the natural material. However, the results are mostly in accordance with the TPC data. That is, the natural extracts with the highest TPC values, present higher DPPH scavenging activities. Cocoa beans and “canta viril” extracts present the highest DPPH scavenging activity, presenting the lowest IC_{50} values, followed by “carqueja”, and pollen extracts with the lowest TPC values also showing the lowest scavenging activity.

As for the extraction method, it is also possible to consider that both UAE and MAE methods represent good alternatives for extracting bioactive compounds from natural sources.

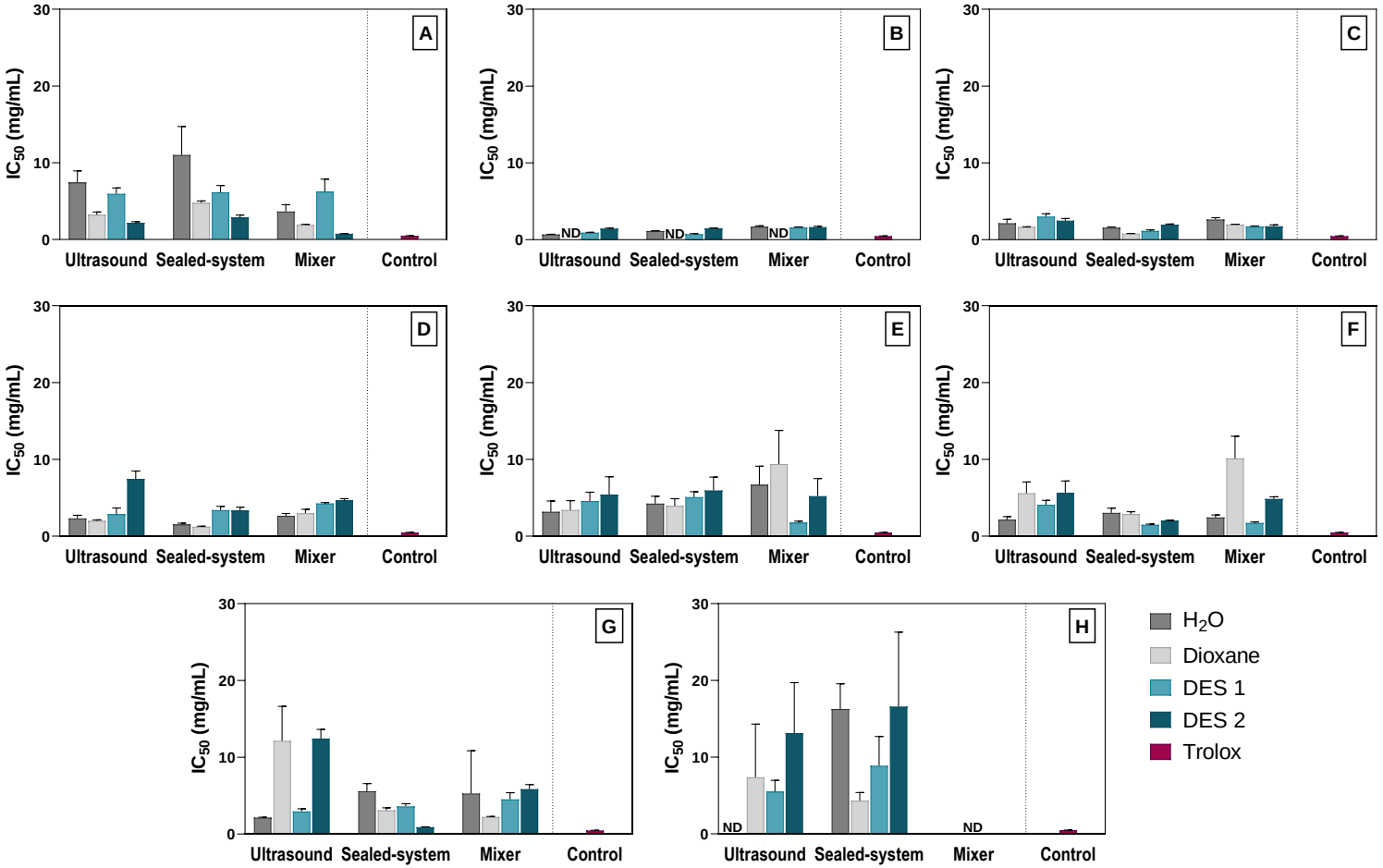


Figure 12: IC_{50} results of DPPH scavenging activity of plant extracts obtained with different solvents and extraction methods. (A) Cherry bay, (B) Cocoa beans, (C) Canta viril, (D) Carqueja, (E) Damiana, (F) Donzela, (G) African basil and (H) Pollen. Triplicate readings were performed for each condition, and data is expressed as mean ± SD. The results were statistically analysed by two-way ANOVA, followed by Tukey's multiple comparison test. Results of this analysis are represented in Annex VII for better clarification. *ND: Non-determined.

4.2.4. GC-MS analysis of the extracts

Plant extracts combine various bioactive compounds with different polarities, so their separation and identification are a big challenge. Cherry bay, African basil, and pollen extracts, obtained using dioxane and DES 1 as solvents, and the three different extraction systems (UAE, SSE, and MAE) were characterized by GC-MS. The use of GC-MS allowed a qualitative and quantitative analysis of the samples. This analysis made it possible to compare the compositions of the extracts obtained with different solvents and extraction methods. The identification and quantification data (expressed as a percentage of composition of compound classes in the extracts) are summarized in Table 10.

Table 10: Composition of cherry bay berries, pollen, and African basil extracts by GC-MS analysis.

Natural material	Compounds classes	UAE		SSE		MAE	
		Dioxane (%)	DES 1 (%)	Dioxane (%)	DES 1 (%)	Dioxane (%)	DES 1 (%)
Cherry bay berries	Alcohols and Small acids	23,62	53,63	28,09	31,23	24,43	55,16
	Fatty acids and derivatives/Oils	1,06	0,38	1,44	0,02	1,09	0,05
	Phenolic compounds	0,78	-	0,59	-	1,97	-
	Steroids	-	2,81	-	1,69	0,03	1,04
	Sugars	74,29	43,06	68,42	66,99	72,15	42,18
	Others	0,06	0,04	0,12	0,03	0,20	0,03
African basil	Alcohols and Small acids	20,86	50,95	25,69	50,19	14,77	52,28
	Carotenoids	6,89	-	-	-	-	-
	Fatty acids and derivatives/Oils	21,43	0,02	22,29	0,01	26,78	0,03
	Phenolic compounds	8,37	-	10,21	-	5,51	-
	Steroids	0,78	3,51	3,19	3,24	7,42	2,86
	Sugars	27,61	45,27	18,73	46,30	23,50	44,61
	Terpenes	4,50	-	14,51	-	10,55	-
	Others	9,35	0,12	4,44	0,20	10,76	0,11
Pollen	Alcohols and Small acids	1,90	52,86	3,54	50,98	4,10	48,11
	Fatty acids and derivatives/Oils	1,20	0,02	0,68	0,02	11,23	0,01
	Steroids	-	8,02	0,03	9,82	0,2	3,03
	Sugars	96,62	38,74	91,49	38,97	81,63	48,69
	Others	0,11	0,21	3,88	0,12	2,21	0,14

Analysing the data, it is possible to observe some differences in the composition of the extracts obtained using dioxane and DES1 as solvents. The content in alcohols and small acids is higher for the extracts containing DES 1 due to its components (Alcohol : Acid 1) peaks in the spectrum. Steroids content also increases, for cherry bay and pollen extracts, when using DES 1. On the other hand, phenolics and fatty acids content is zero or decreases, respectively, for all the natural materials when using DES 1 as extracting solvent. And the same happens with terpenes in African basil extracts. Although the chromatograms are a good indicator of the composition of the extracts, this does not necessarily mean that these compounds are not present in the extracts. The chromatograms of extracts with DES 1 (Annex III) are very distinct due to the presence of the eutectic solvent at a high concentration. The large peaks of the eutectic end up overlapping other peaks and making the peaks' areas of other compounds very small, making hard their identification. Regarding the extraction method, it is possible to observe that extracts present slight differences between each other.

Cherry bay berries and pollen are mainly composed of sugars. The composition of pollen is quite variable and may contain carbohydrates (13–55%), proteins (10–40%) and lipids (1–13%) (Thakur & Nanda, 2020). Cherry bay extract (leaves and steams) has friedelin, a terpenoid, as predominant component (Costa et al., 2015). The obtained extracts of cherry bay berries do not contain terpenes in their composition, but it is only natural that the fruit of cherry bay presents a different composition from the aerial parts of the plant. African basil has in its composition a variety of different types of compounds, including phenolic compounds and terpenes as confirmed previously Kumar et al. (2019) in their studies.

4.2.5. Extracts cytotoxicity

The addition of plant extracts in cosmetic products implies the need to evaluate their cytotoxic potential to ensure their safety in future applications. The results of *in vitro* cytotoxicity, evaluated as metabolic viability, are presented as cell survival after 24 h of incubation with various concentrations (0,1 – 1%) of DES and aqueous extracts of cherry bay, African basil, and pollen. The DES used for extraction were also evaluated regarding their potential cytotoxic effect. The results of cellular viability are illustrated in Figure 13.

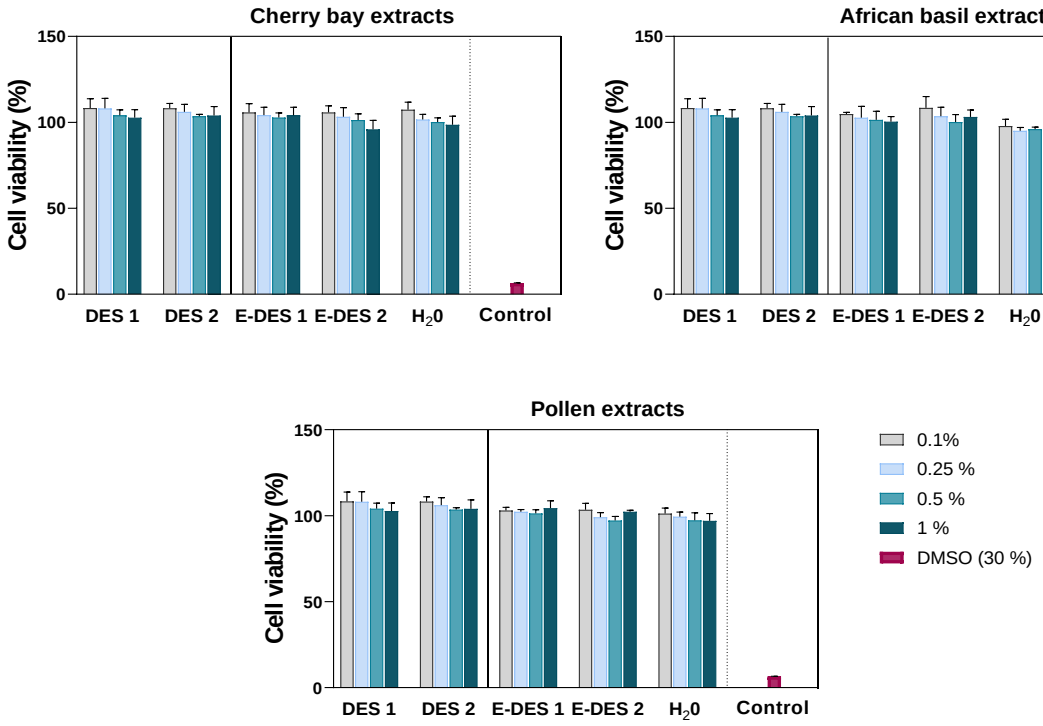


Figure 13: Effect of DES and cherry bay berries, African basil, and pollen extracts (DES and water-based), at different concentrations, on cellular viability. Triplicate experiments were performed for each condition, and data is expressed as mean ± SD. The results were statistically analysed by two-way ANOVA, followed by Tukey's multiple comparison test.

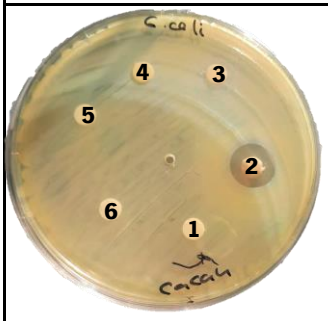
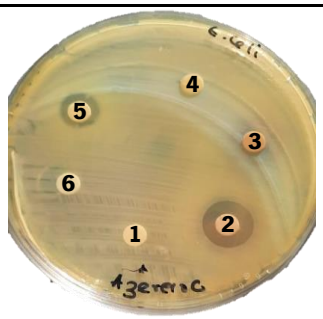
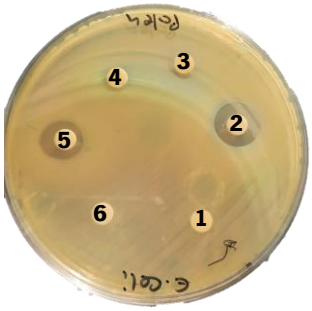
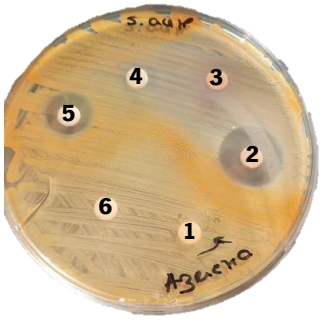
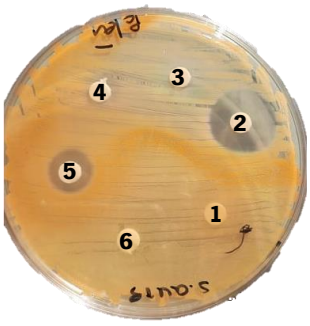
DES 1 and DES 2 do not affect the cellular viability at any tested concentrations. The same trend was observed for the cherry bay berries extracted with DES 1, DES 2, and water. Regarding African basil, both DES-African basil extracts do not affect cell viability at any tested concentrations, while the extracts obtained with water present cell viability values slightly below 100%. None of the pollen extracts seems to affect negatively cell viability for all the tested concentrations. The results suggest that the application of these plant extracts containing DES in topical application cosmetic formulations for daily use would be safe, as they do not present cytotoxic activity in a 24 h incubation period, even at the highest concentration analysed (1%).

4.2.6. Antimicrobial activity of the extracts

Bioactive compounds often reveal antimicrobial activity, which is particularly important since they can help prevent skin infections when added to cosmetics. The antimicrobial activity of cocoa beans, cherry bay berries, and pollen extracts was evaluated based on the determination of *S. aureus* and *E. coli*

susceptibility, using diffusion disc. The concentrations of extracts and respective controls used for this experiment were chosen based on a pre-screening of various concentrations. The results are shown in Table 11. Water-based extracts do not present an inhibitory effect against the bacteria for all the natural materials. On the contrary, both bacteria are susceptible to all DES 1-based extracts. DES 1 also presents antibacterial activity, but inhibition halos are smaller when compared to the halos of DES 1-based extracts, indicating lower antimicrobial activity. Thus, the antimicrobial activity incremented by the extracts is likely due to the bioactive compounds present in their composition.

Table 11: Antimicrobial activity of cocoa beans, cherry bay berries, and pollen extracts by diffusion discs assay.

	Cocoa beans (8 mg/mL)	**Cherry bay berries** (11 mg/mL)	**Pollen** (15 mg/mL)
E. coli			
Halos diameter (mm)	**1:** nd **2:** 15 **3:** 8 **4:** nd **5:** nd **6:** nd	**1:** nd **2:** 14 **3:** nd **4:** nd **5:** 10 **6:** nd	**1:** nd **2:** 15 **3:** 10 **4:** nd **5:** 12 **6:** nd
S. aureus			
Halos diameter (mm)	**1:** nd **2:** 20 **3:** nd **4:** nd **5:** nd **6:** nd	**1:** nd **2:** 20 **3:** nd **4:** nd **5:** 12 **6:** nd	**1:** nd **2:** 22 **3:** nd **4:** nd **5:** 12 **6:** nd

***1:** Water extract in PBS, **2:** DES 1-based extract, **3:** DES 2-based extract, **4:** DES 2, **5:** DES 1 and **6:** PBS.
nd: non-determined.

Pollen and cocoa DES 2-based extract also showed inhibitory effects, but only against *E. coli*. Compared with DES 1-based extract, the inhibition halo is smaller, indicating less bacterial susceptibility, probably

due to differences in the extract's composition. For DES 2 alone, no formation of inhibition halos is observed, implying an inferior antimicrobial activity when compared to DES 2-based extracts, reinforcing the idea of increased antimicrobial activity due to the bioactive compounds present in the extracts. Cocoa beans extracts show higher antibacterial activity from the three natural sources, presenting similar inhibition halos at a lower concentration. These results could be explained by the different bioactive compounds of each plant. Cocoa beans have between 12 – 18% of polyphenols (Ramos-Escudero et al., 2021), while cherry bay berries and pollen have a lower or no percentage of phenolic compounds in their composition, according to GC-MS performed analysis. The obtained data also shows that extracts have higher antibacterial activity against *S. aureus* when compared to *E. coli* once inhibition halos are larger. This behaviour has been observed previously. Todorovic et al. (2017) studied the antimicrobial activity of cocoa extracts, observing that the extracts had higher potential against Gram-positive bacteria. This could be attributed to the difference in the composition of the cell wall of Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thick peptidoglycan layer which allows the penetration of small molecules, while Gram-negative bacteria have thin polysaccharide walls overlaid by a thin layer of lipopolysaccharides that modulates the accessibility of the cells to small molecules (Zhou et al., 2022).

The antimicrobial activity was also evaluated based on colony forming units (CFUs) reduction after bacterial growth in the presence of the extracts. The results obtained for *E. coli* and *S. aureus* are represented in Figure 14. For cocoa beans extracts, DES-based extracts present a higher reduction in CFUs than the water-based extracts (4 log reduction vs 1 log reduction, for *E. coli* and 2 log vs 1 log reduction for *S. aureus* in relation to PBS control). Comparing DES-based extracts with the respective solvent control, it is also possible to infer those extracts have higher antimicrobial activity (2 log reduction), reinforcing the idea of the antimicrobial activity of the bioactive compounds. Catechins, which are phenolic compounds, are some best-known bioactive components in cocoa (Ramos-Escudero et al., 2021). Zhou et al., (2022), demonstrated an improved antimicrobial activity of catechins in a ChCl and glycerol-based DES compared to water. The authors attributed this improvement to the stabilizing ability of DES, due to hydrogen bond formation. However, unlike in the susceptibility diffusion tests, there is no significant difference between the antimicrobial activity of DES 1 and DES 2-based extracts.

For cherry bay berries and pollen, water-based extracts do not present antimicrobial activity. On the other hand, DES-based extracts present antimicrobial activity (between 2 – 4 log reduction in relation to PBS control), but there is no significant difference relative to the respective solvent control. Besides, there is also no significant difference between the antimicrobial activity of DES 1 and DES 2-based extracts.

Furthermore, there is no difference in the susceptibility between the two bacteria in this experiment, contrary to what was observed with the inhibition halos test.

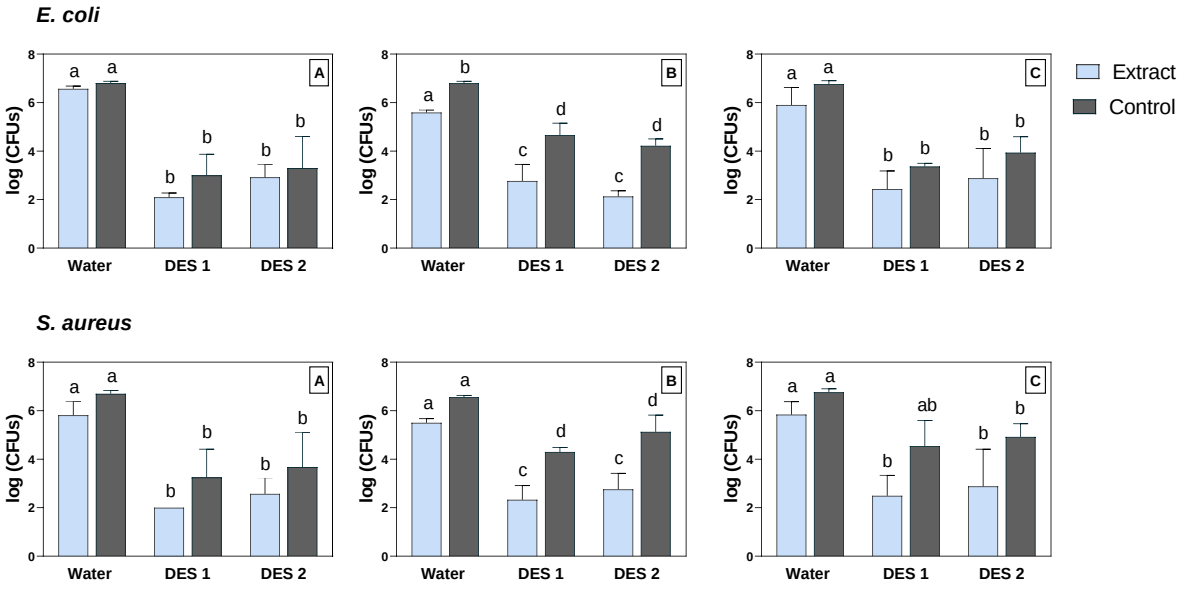


Figure 14: Antimicrobial activity of extracts against *E. coli* and *S. aureus*. (A) Cherry bay berries, (B) Cocoa beans and (C) Pollen, results are presented as log (CFUs). Triplicate experiments were performed for each condition, and data is expressed as mean ± SD. The results were statistically analysed by one-way ANOVA, followed by Tukey's multiple comparison test. Different letters represent a statistically significant difference ($p<0,05$).

4.3. Dyeing of cotton fabrics with the extracts

Hydroxyl groups present in cellulosic fibres are naturally oxidized, forming carboxylic groups, resulting in a slight negative charge of the fibres. Hence, natural dyeing of cotton is difficult because of the electrostatic repulsion between anionic moieties present in the cellulose structure and in natural dyes, which results in a low dye uptake and poor colour strength (Pisitsak et al., 2016; Rattanaphani et al., 2007). To improve textile fibres and natural dyes affinity, pre-treatment with cationic agents or mordanting processes are applied, using metallic salts as mordants. The most used mordants nowadays are copper sulphate, zinc sulphate and alum (Souissi et al., 2018). In this study, two mordants were tested, copper sulphate and aluminium, using a pre-mordanting method to assess its influence on the dyeing process. Dyeing of cotton fabric was performed with three different extracts, from cherry bay berries, African basil, and cork, obtained with DES 3. These extracts were chosen from the variety of extractions tested due to their strong coloration. The concentration of the extracts was calculated through the extraction yield and is presented in Table 12.

Table 12: Concentration of the extracts used for cotton dyeing.

Extract	Concentration (mg/mL)
Cherry bay berries	136,6
African basil	47,3
Cork	11,9

The dyeing was performed in pre-treated fabrics in two different ways, with and without tannic acid. Tannic acid is used to increase the affinity of the substrate through the creation of van der Waals forces and hydrogen bonds resulting from the large molecular size and good availability of hydroxyl groups (Phan et al., 2020). The obtained results are represented in Figures 15, 16 and 17. Cherry bay berries extract produced the samples with higher colour intensities, being the most concentrated extract (Figure 15).

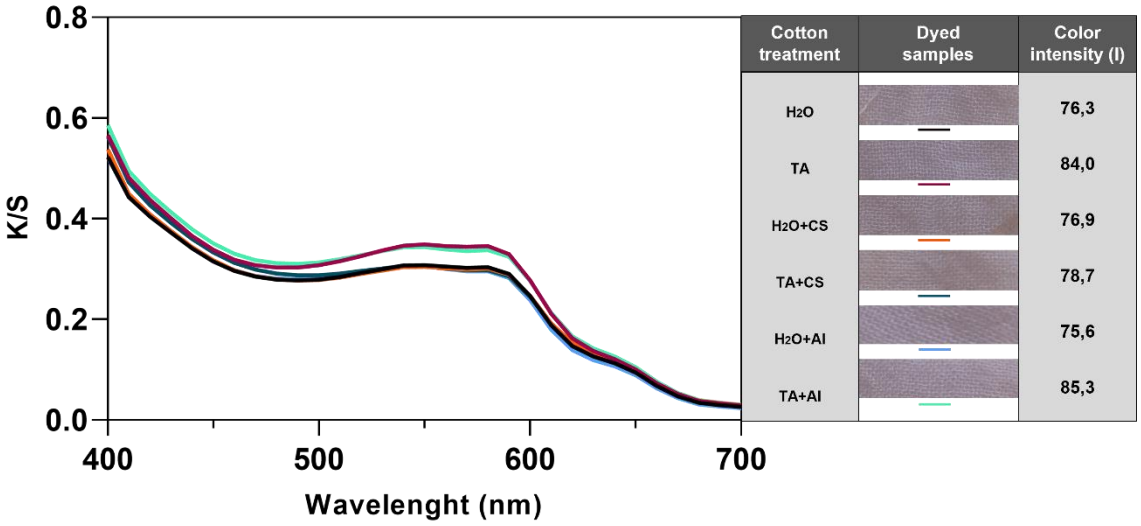


Figure 15: Cotton dyeing with cherry bay berries extracts. K/S Curves, samples dyed with pre-treatments and pre-mordants and respective colour intensity (I). *H_2O: Cotton pre-treated without tannic acid; TA: cotton pre-treated with tannic acid; H_2O+CS: Cotton pre-treated without tannic acid mordanted with copper sulphate; TA+CS: cotton pre-treated with tannic acid mordanted with copper sulphate; H_2O+Al: Cotton pre-treated without tannic acid mordanted with aluminium and TA+Al: Cotton pre-treated with tannic acid mordanted with aluminium.

The pre-treatment with tannic acid resulted in an increase in the colour intensity in comparison with the pre-treatment without this compound. Besides, the samples pre-treated with tannic acid also produced higher colour intensities when using both pre-mordents. The combination of pre-treated cotton with aluminium as pre-mordent produced the highest colour intensity.

Despite being the less concentrated one, cork extract produced the greatest colour intensity (Figure 16), right after the cherry bay berries extracts. For these extracts, the sample with the highest colour intensity was the sample pre-treated with tannic acid and with copper sulphate as pre-mordent. Pre-

treatment with tannic increased colour intensity in relation to the pre-treated sample without TA, and its use in combination with copper sulphate further improved colour intensity. The use of aluminium with both pre-treated samples not only did not increase but decreased the colour intensity of the respective samples.

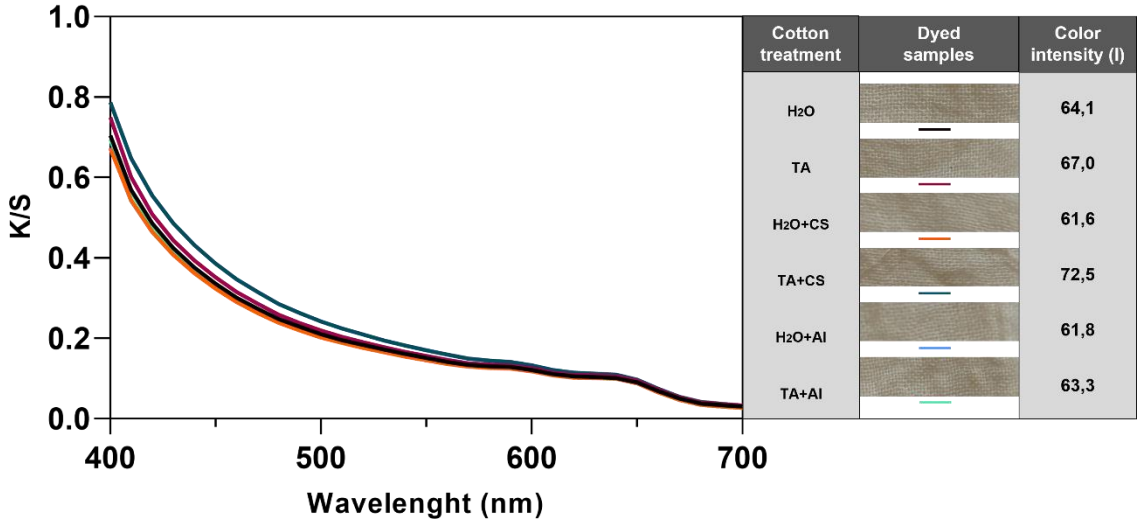


Figure 16: Cotton dyeing with cork extracts. K/S Curves, samples dyed with pre-treatments and pre-mordants and respective colour intensity (I). *H_2O: Cotton pre-treated without tannic acid; TA: cotton pre-treated with tannic acid; H_2O+CS: Cotton pre-treated without tannic acid mordanted with copper sulphate; TA+CS: cotton pre-treated with tannic acid mordanted with copper sulphate; H_2O+Al: Cotton pre-treated without tannic acid mordanted with aluminium and TA+Al: Cotton pre-treated with tannic acid mordanted with aluminium.

Despite their medium extract concentration, African basil extracts produced the lowest colour intensity (Figure 17). The sample with the highest colour intensity was also the sample pre-treated with tannic and with cooper sulphate as pre-mordent. The pre-treatment with tannic acid did not increase colour intensity in relation to the pre-treated sample without tannic acid. The use of pre-mordants in the samples pre-treated without tannic acid decreased colour intensity of the samples. The opposite can be observed for the use of both pre-mordants with samples pre-treated with tannic acid.

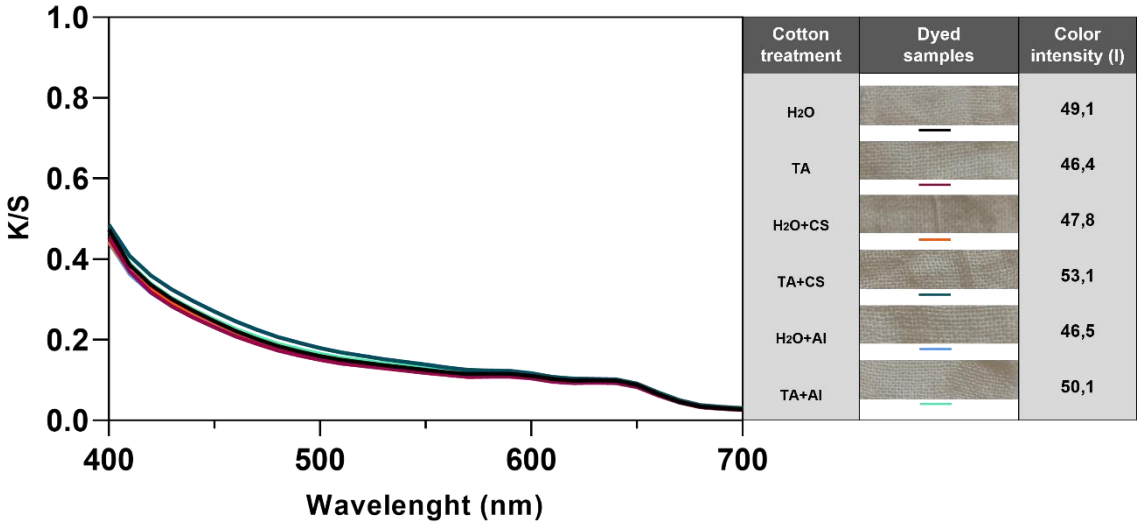


Figure 17: Cotton dyeing with African basil extracts. K/S Curves, samples dyed with pre-treatments and pre-mordants and respective colour intensity (I). *H_2O: Cotton pre-treated without tannic acid; TA: cotton pre-treated with tannic acid; H_2O+CS: Cotton pre-treated without tannic acid mordanted with copper sulphate; TA+CS: cotton pre-treated with tannic acid mordanted with copper sulphate; H_2O+Al: Cotton pre-treated without tannic acid mordanted with aluminium and TA+Al: Cotton pre-treated with tannic acid mordanted with aluminium.

For all the three extracts, the differences between the dyed samples are not considerably notorious. These particular pre-treatments and pre-mordents may have almost no influence on the dyeing process when using these plant extracts. Higher concentrations of these agents might have to be used to modify the fabrics to have a greater affinity for the natural dyes. Ben Ticha et al. (2016) demonstrated that the dyeing performance of the fabrics increased when tannic acid and other cationising agents´ concentration increased from 5 to 10 %.

4.4. Optimization of polyester dyeing process with eutectic mixtures

The dyeing of polyester was carried out with two different synthetic dyes, DB124 and DB134, using five different DES (3, 4, 5, 6 and 7). DES were used with distinct water content percentages, and the results were compared with the conventional aqueous-dyed polyester dyeing. First, dyeing was performed in a dyeing laboratory machine. Only two of the five DES tested successfully dyed the fabric (Table V1, Annex V). The results are presented in Figures 18 and 19. When using DB124 with DES 3 (Figure 18) without water addition, the same maximum K/S value was obtained as aqueous dyeing. When using this same DES with 25 or 50% of water content, higher K/S values were obtained.

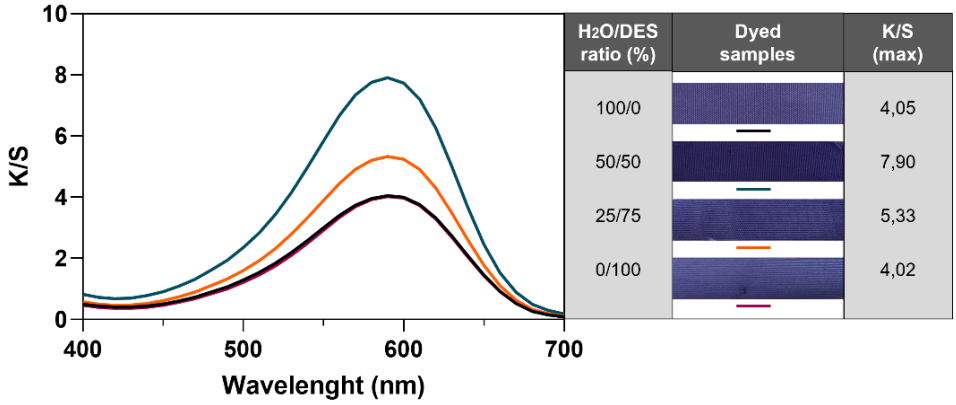


Figure 18: Polyester dyeing, in dyeing machine, with DB124 using DES 3. K/S Curves, samples dyed with different water contents, and respective maximum K/S values.

When using DB134 with DES 3 and DES 5 (Figure 19), samples present higher maximum K/S, at least two-fold, for all the conditions of water content tested, than aqueous polyester dyeing. For this dye, the highest maximum K/S values were obtained, in both DES, for 25% of water content.

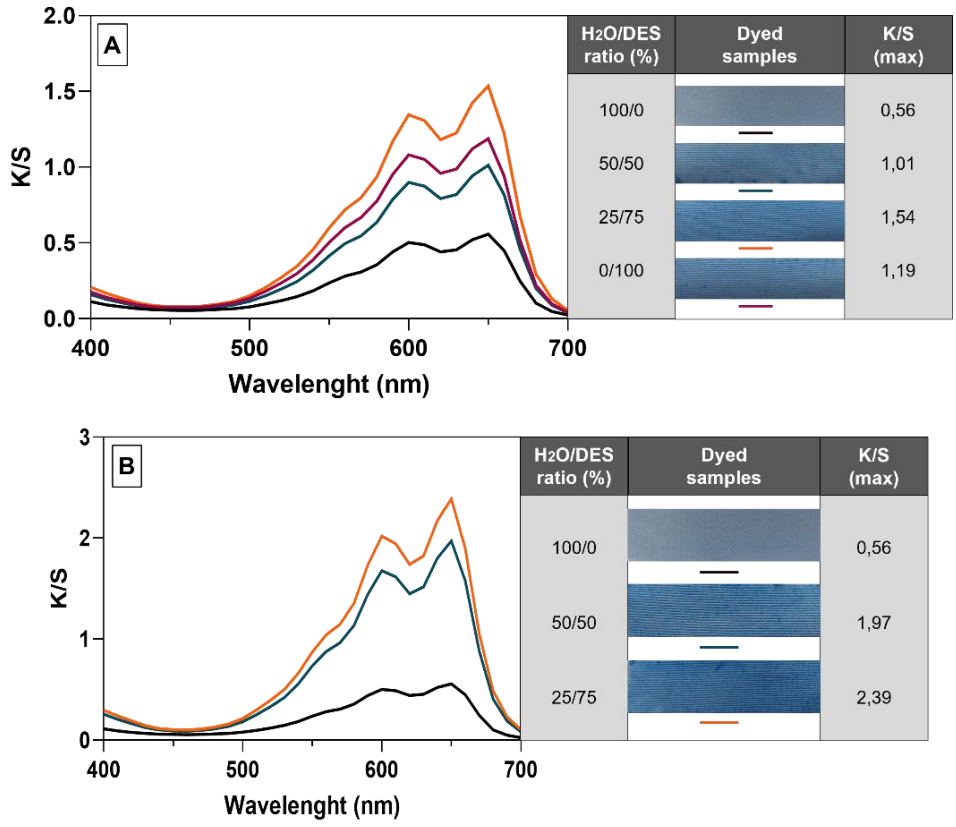


Figure 19: Polyester dyeing, in dyeing machine, with DB134 using (A) DES 3 and (B) DES 5. K/S Curves, samples dyed with different water contents, and respective maximum K/S values.

Posteriorly, dyeing was performed using SS in a thermostatic bath in order to try to optimize the dyeing conditions (i.e. reduce water and dyeing temperature). The dyeing conditions were adjusted to be as similar as possible to the conditions used in the dyeing machine. Dyeing was first performed with DB124, and only DES 3 was able to successfully dye the polyester fabric (Table V2A, Annex V), and the results are presented in Figure 20. In this process, only the sample dyed with DES 3 with 50% water content had a maximum K/S higher than the aqueous dyeing sample.

Afterwards, the dyeing was performed using DES with the pH adjusted to 3 - 4, and then 4 - 5 since the dyeing of the polyester takes place in a mildly acidic medium (Chakraborty, 2014a). The pH correction was only done in DES with pH values out of these ranges.

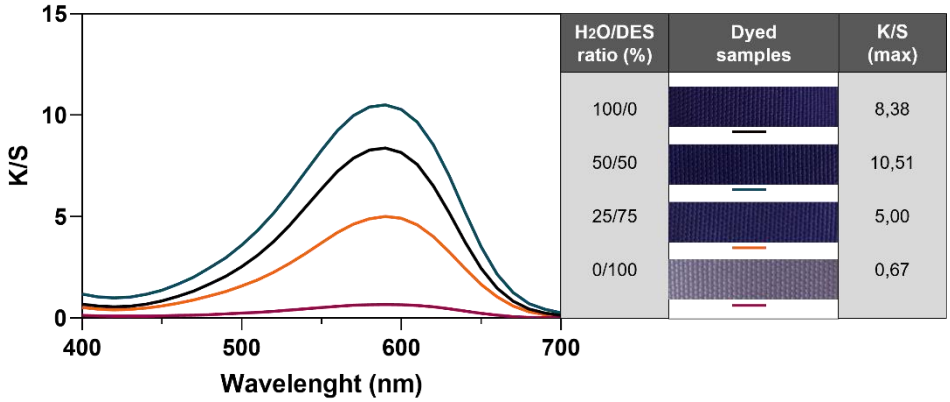


Figure 20: Polyester dyeing, in SS, with DB124 using DES 3. K/S Curves, samples dyed with different water contents, and respective maximum K/S values.

When using DB124, the dyeing of the polyester did not occur, except using DES 5. The results are represented in Figure 21. Aqueous dyed samples still had the highest K/S, and in comparison, DES 5 dyed samples presented less than half of K/S.

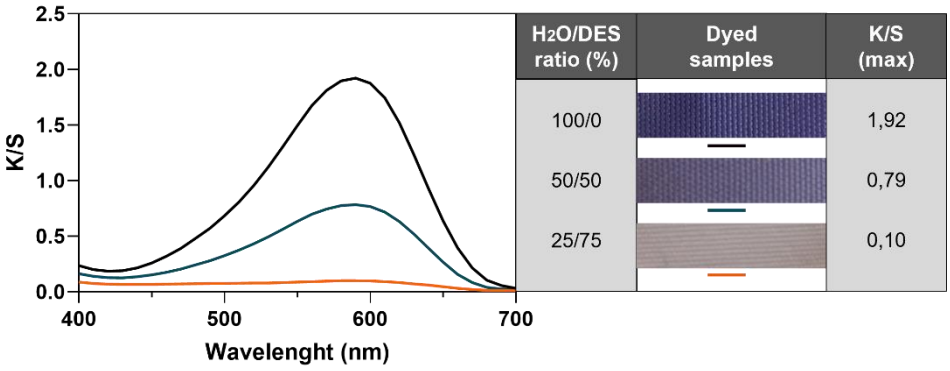


Figure 21: Polyester dyeing, in SS, with DB124 using DES 5. K/S Curves, samples dyed with different water contents and respective maximum K/S values.

When using DB134, DES 3 and DES 5 were still the only solvents able to dye the fabric (Table V2B, Annex V). Results are shown in Figure 22. Both DES dyed samples have lower K/S values than the aqueous dyed samples for all the conditions tested.

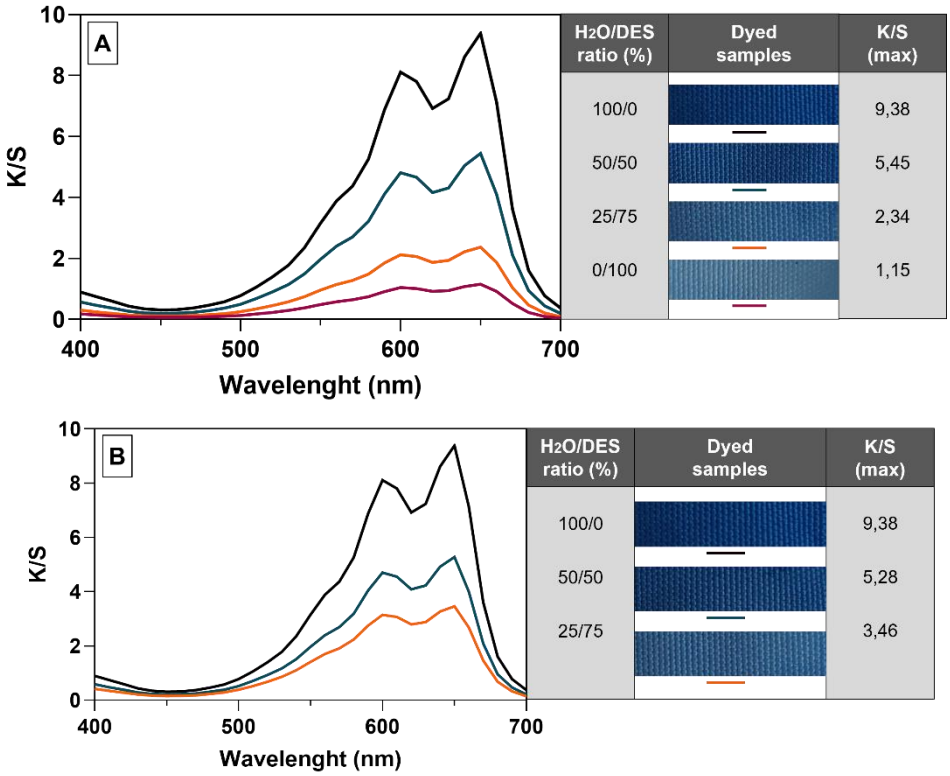


Figure 22: Polyester dyeing, in SS, with DB134 using (A) DES 3 and (B) DES 5. K/S Curves, samples dyed with different water contents, and respective maximum K/S values.

The application of DES in polyester dyeing, using a laboratory dyeing machine, produced good results compared to conventional dyeing. Therefore, the use of DES can reduce/minimize water consumption. Disperse dyes are anionic compounds, insoluble in water, used in the dyeing process of non-ionic fibres, such as polyester. Due to the extremely low water solubility, these dyes need to rely on dispersants for uniform dispersion in water or/and the dyeing process occurring at a high temperature for the breakdown dye clusters. When the dye is added to the dyeing bath, it stays in two forms. A small part is in completely soluble form, and the remaining dye is in a dispersed insoluble form. When the fibre is added to the dyebath, dissolved dye molecules are deposited and adsorbed on the fibre surface (Chakraborty, 2014c). The applied DES enhanced the solubility of the used dyes compared to water, which could have been beneficial to improving the levelness of polyester dyeing. The addition of water to the DES dyeing mediums

improved colon strength for both dyes. It reduces DES viscosity, which might help with the improvement of the diffusion rates.

The results obtained when using the SS for polyester dyeing differed from those obtained with the laboratory dyeing machine. Although conditions were adjusted to be similar to the dyeing machine process, it is not possible to exactly replicate dyeing conditions, namely, gradual increase and decrease of temperature, and temperature plateaus. This can affect dye uptake and, consequently, colour strength of dyed samples. There are three steps for dyeing of polyester with disperse dyes: (i) deposition of dye on the surface at lower temperature, (ii) diffusion at the surface layers with increase in temperature, and (iii) diffusion at the interior at a higher temperature (Chakraborty, 2014b).

Subsequently, to study the effect of temperature on the dyeing process, it was performed with DES 3 and DES 5 at 120 °C using both disperse dyes. The obtained results are illustrated in Figures 23 and 24. With temperature decrease, there is also a decrease in the colour strength of the dyed fabric samples, including the samples dyed in the aqueous medium. In a dyeing process, the fibre structure opens up at higher temperatures enabling the deposition of dyes on its surface, being this opening proportional to temperature increase. The necessary opening is obtained just above 120 °C for the efficient dye diffusion (for most dye molecules). However, dyeing temperature is usually maintained at 130 °C for complete distribution of dye through migration, required to produce levelled shades (Chakraborty, 2014b).

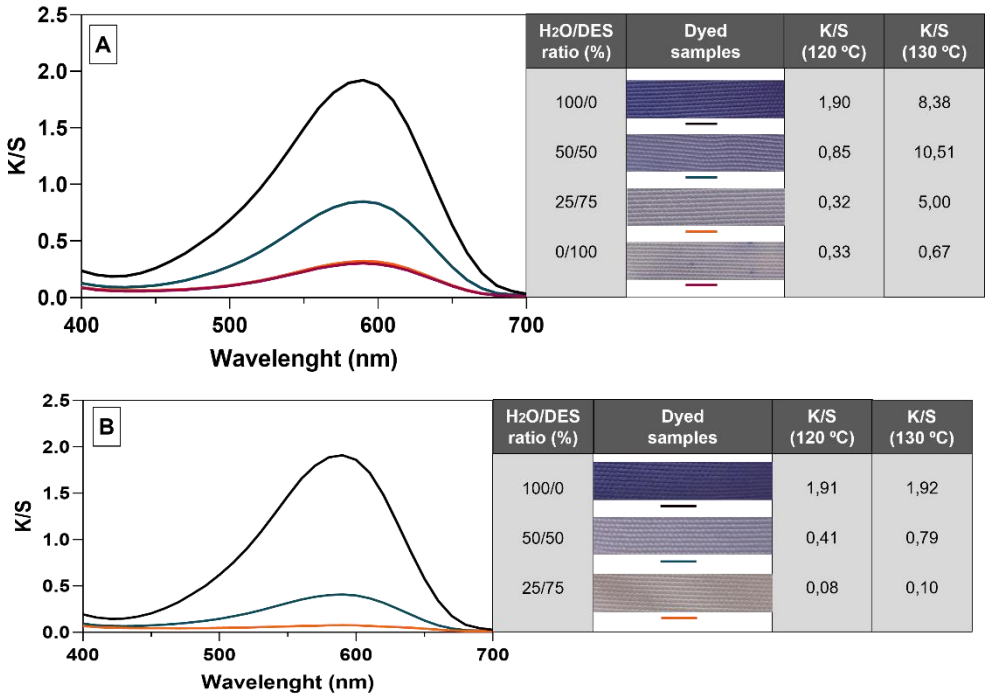


Figure 23: Polyester dyeing at 120 °C, in SS, with DB124 using (A) DES 3 and (B) DES 5. K/S Curves, samples dyed with different water contents, and respective maximum K/S values.

In a study done by Pawar et al. (2019), a ChCl, urea, and glycerine-based DES was used as dyeing medium, optimization of temperature was performed at a temperature range of 100 -150 °C. It was found that the increase of temperature up to 130 °C increases the colour strength of the dyed samples.

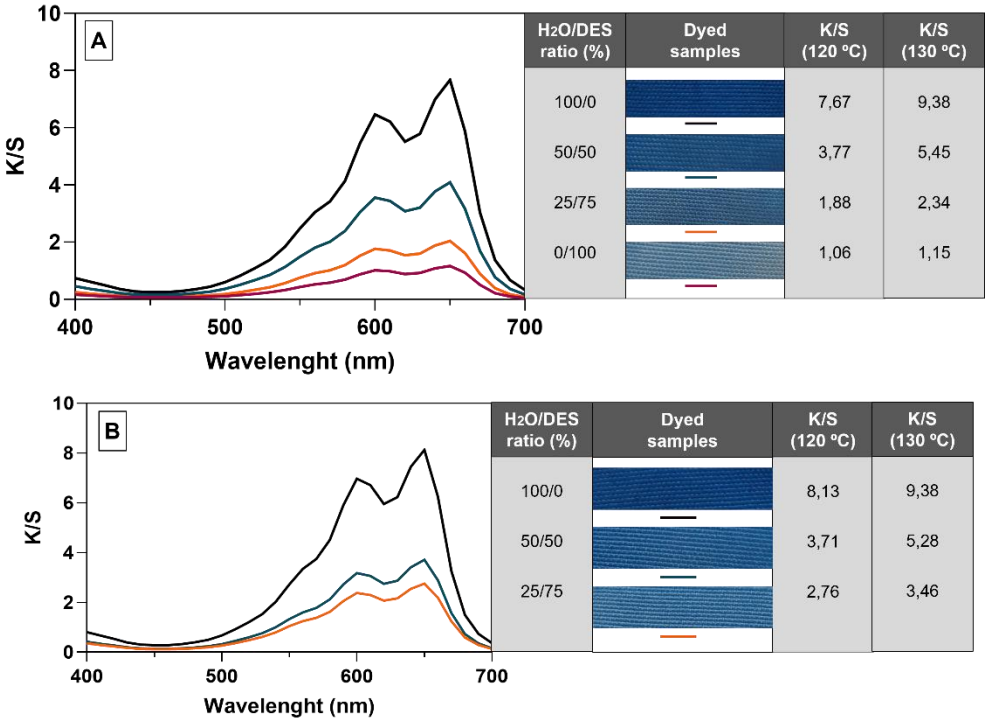


Figure 24: Polyester dyeing at 120 °C, in SS, with DB134 using (A) DES 3 and (B) DES 5. K/S curves, samples dyed with different water contents, and respective maximum K/S values.

4.5. Dye extraction from wastewater using DES

Developing efficient, economic, and environmentally friendly processes to removing dyes from wastewaters is of great importance. In this work, DES 4 (Acid 1 : Acid 2) was used to extract dyes (DB124 and DB134) from aqueous solutions (Figure 25). Different conditions were tested to evaluate their influence on extraction efficiency.

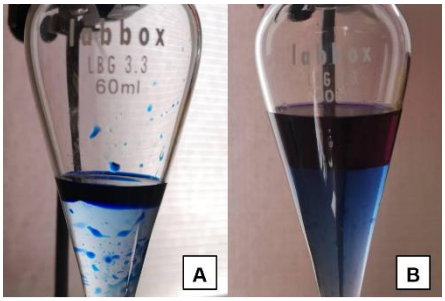


Figure 25: DES 4 and dye solution after extraction and before the two phases separation. (A) DB134 and (B) DB124.

4.5.1. Effect of DES dosage

The effect of DES 4 dosage was analysed by varying the addition from 0,004 to 0,040 mol in dye solutions of 1 mg/mL, and the results are represented in Figure 26. Regarding DB134 solutions, the extraction efficiency only increases until 0,016 mol of DES. With higher DES dosage, the extraction efficiency remains constant. For DB124, the extraction efficiency increases proportionally with the DES dosage, however without reaching the levels of extraction obtained for DB134. These results indicate that the extraction process is limited by the limiting DES dosage and that different dosages are necessary depending on the type of dye to extract.

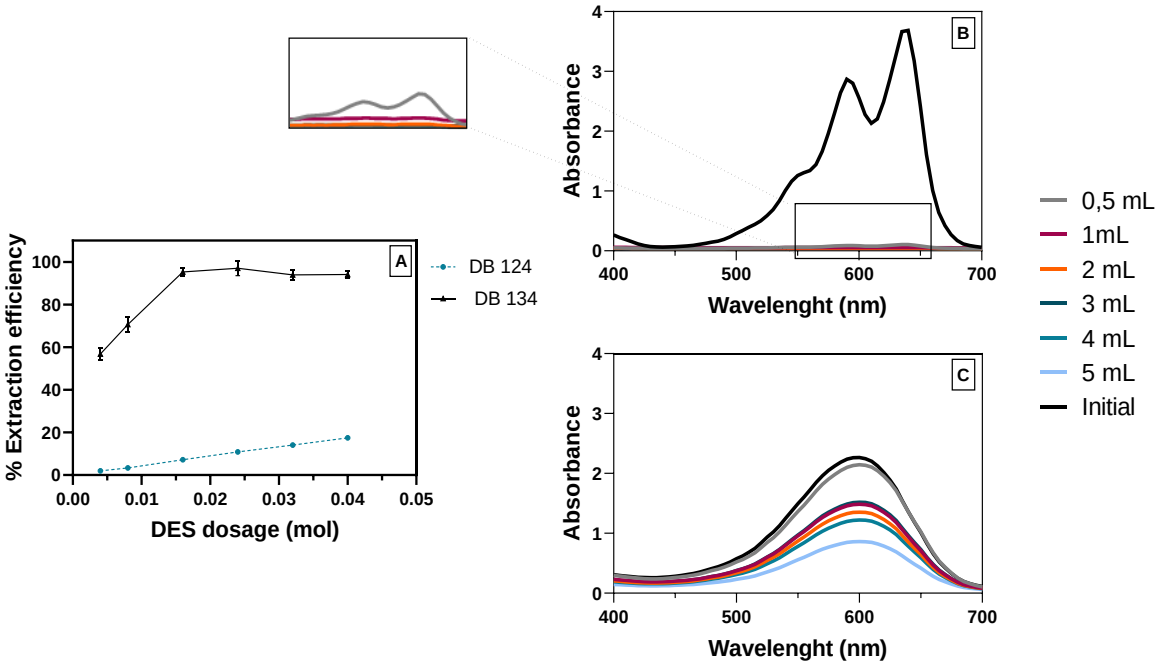


Figure 26: Dye extraction with different doses of DES 4. (A) Extraction efficiency of DB124 and DB134, (B) Absorption visible spectra of dye solutions (DB134) before and after extraction, and (C) Absorption visible spectra of dye solutions (DB124) before and after extraction.

The visible spectra of the dye solutions are in concordance with the obtained extraction efficiencies, and it is possible to observe a high absorbance of the DB124 solutions in the analysed wavelengths, which indicates that there is still a high non-extracted concentration of dye in the solution.

Since the extraction efficiency was very low for DB124, the effect of DES dosage was once more evaluated by varying DES dosages from 0,040 to 0,160 mol. Aiming to increase extraction efficiency, a centrifugation step was added before separating the two phases. The results are presented in Figure 27. Extraction efficiency increases with superior DES dose, and the highest efficiency was obtained for a

0,160 mol DES dosage. Also, it is possible to observe a decrease in absorbance of the DB124 solutions, indicating that there is a decrease in the concentration of the dye in solution. The centrifugation also incremented extraction efficiency, from 17,5 ± 0,5% to 46,5 ± 1,3% comparing the DES dosage of 0,040 mol. For the following studies, the DES dosage chosen for DB124 and DB134 were 0,160 and 0,016 mol, respectively. Also, a centrifugation step was included in the extraction processing of DB124 solutions.

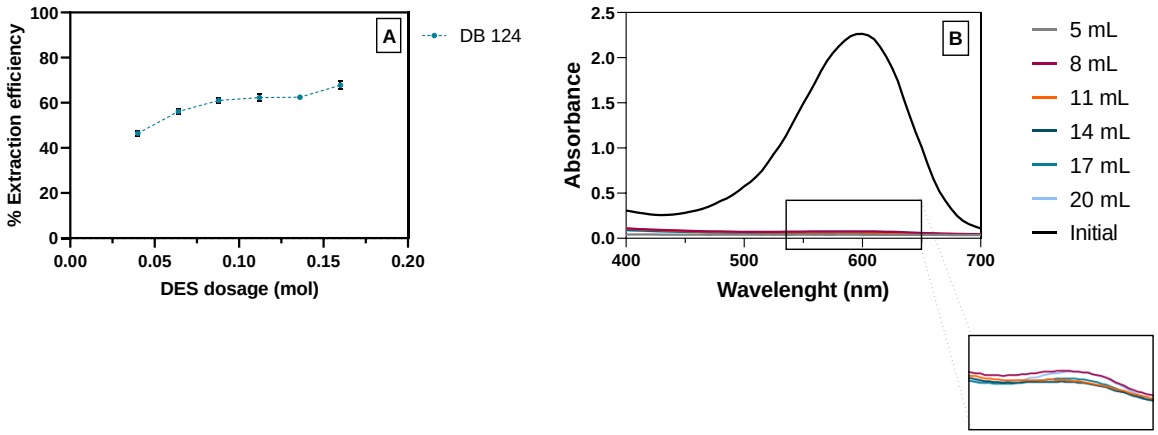


Figure 27: Dye extraction with different doses of DES 4. (A) Extraction efficiency of DB124, and (B) Absorption visible spectra of dye solutions (DB124) before and after extraction.

4.5.2. Effect of initial dye concentration

The effect of initial dye concentration on the extraction efficiency was studied by varying concentrations of both dyes, (DB124 and DB134) from 0,1 to 1 mg/mL, and the results are represented in Figure 28. As the initial dye concentration increases, the extraction efficiency for DB124 decreases to less than 50%, whilst it remains almost constant for DB134, indicating that DES 4 has a higher extraction capacity for higher concentrations of DB134 in comparison with DB124. It is observed that the extraction process is effectively affected by the initial dye concentration in the solution.

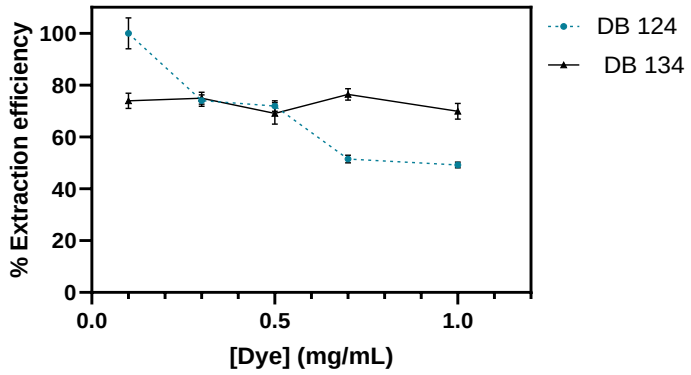


Figure 28: Dye extraction efficiency of DES 4 of different dye concentrations of DB124 and DB134.

The visible spectra of all the solutions were measured before and after the extraction process. The obtained spectra for DB134 e DB124 (Figure 29 and 30, respectively) allow to observe the reduction of dye in the solutions related to the decrease of the absorption curve.

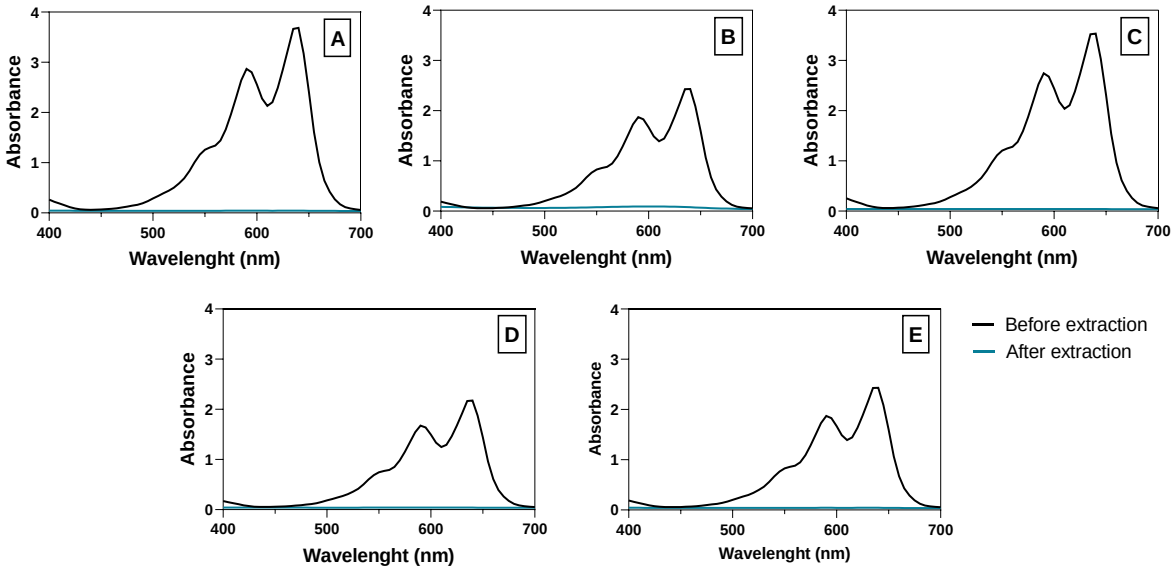


Figure 29: Absorption visible spectra of dye solutions (DB134) before and after dye extraction. (A) 1 mg/mL, (B) 0,7 mg/mL, (C) 0,5 mg/mL, (D) 0,3 mg/mL and (E) 0,1 mg/mL).

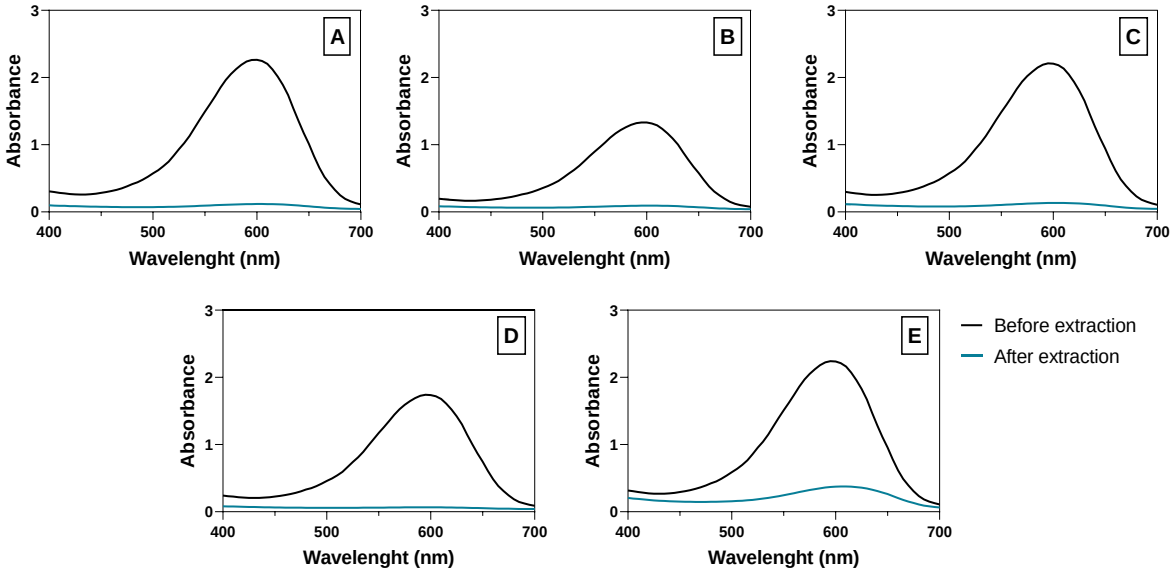


Figure 30: Absorption visible spectra of dye solutions (DB124) before and after dye extraction. (A) 1 mg/mL, (B) 0,7 mg/mL, (C) 0,5 mg/mL, (D) 0,3 mg/mL and (E) 0,1 mg/mL).

4.5.3. Effect of dye pH

The effect of dyes solutions pH on the extraction efficiency was evaluated by varying the pH of the solutions between 2 and 10. The results are represented in Figure 31. It can be seen that, as the pH of the dye solution increases from 2 to 10, the extraction efficiency for DB124 remains constant, except for pH=7. For DB134, pH= 2 and 7 presents the lowest extraction efficiencies. Therefore, by maintaining the original pH of the aqueous dye solutions of 8, higher extraction efficiencies are obtained compared to lower pHs.

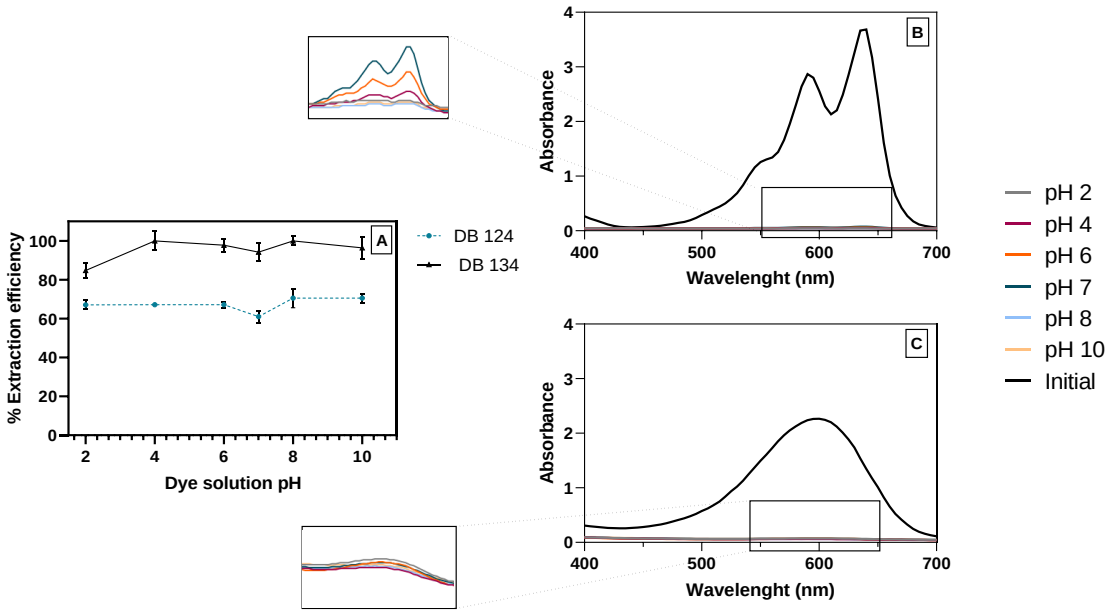


Figure 31: Dye extraction with different pH solutions. (A) Extraction efficiency of DB124 and DB134, (B) Absorption visible spectra of dye solutions (DB134) before and after extraction, and (C) Absorption visible spectra of dye solutions (DB124) before and after extraction.

4.5.4. Effect of DES pH

The effect of DES 4 pH on the extraction efficiency was evaluated by varying the pH between 2 and 7, using HCl and NaOH solutions to adjust it. The results are represented in Figure 32. The extraction efficiency of DB124 decreases using DES pH= 4 and 6, and slightly increases at using DES at pH=7. For DB134, the extraction efficiency remains constant from pH 2 to 6, but there is a marked decrease at pH 7. By increasing the DES pH, there is the formation of the carboxylate salts of the acids composing the solvent and the water content increases. This process possibly disrupts hydrogen bonds between DES components and decreases the solvent availability to form hydrogen bonds with the dyes. At pH=7, DES viscosity substantially increased, becoming a white pasty substance. Hence, the solubility of dyes decreased, and extraction efficiency for DB124 could have been improved by the centrifugation step and not by the DES 4 action itself.

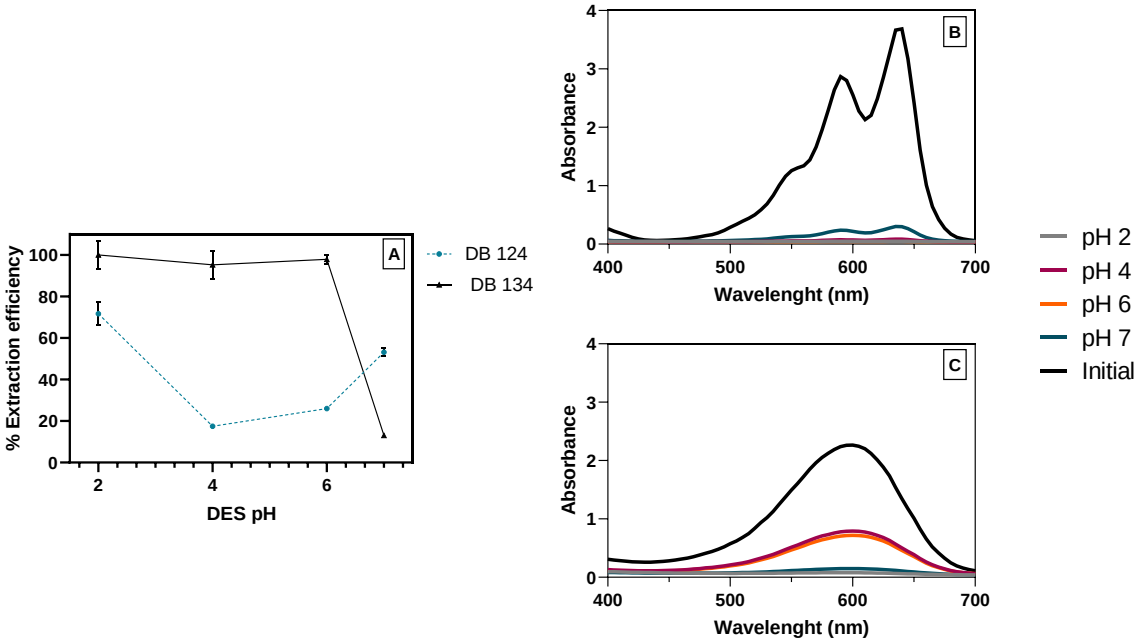


Figure 32: Dye extraction with DES 4 at different pHs. (A) Extraction efficiency of DB124 and DB134, (B) Absorption visible spectra of dye solutions (DB134) before and after extraction, and (C) Absorption visible spectra of dye solutions (DB124) before and after extraction.

The extraction of the dyes with DES 4 is expected to occur due to the hydrogen bonding between the dye molecule and the DES, which can be verified by FTIR and NMR analysis. FTIR spectra of DES solutions with DB124 and DB134 are shown in Figure 32. In the DES 4 + DB124 spectrum there is not possible to observe any peak corresponding to the dye. Regarding DES 4 + DB134 spectrum, the presence of the dye is visible in the region of 1500 to 1600 cm^{-1}. The peaks are barely noticeable, one at 1564 cm^{-1} and the other at 1519 cm^{-1} and should correspond to the peaks at 1558 and 1515 cm^{-1} from DB134 spectra that refer to C=C stretching. The dyes are probably not in sufficient concentration to be visible along with DES 4 spectrum.

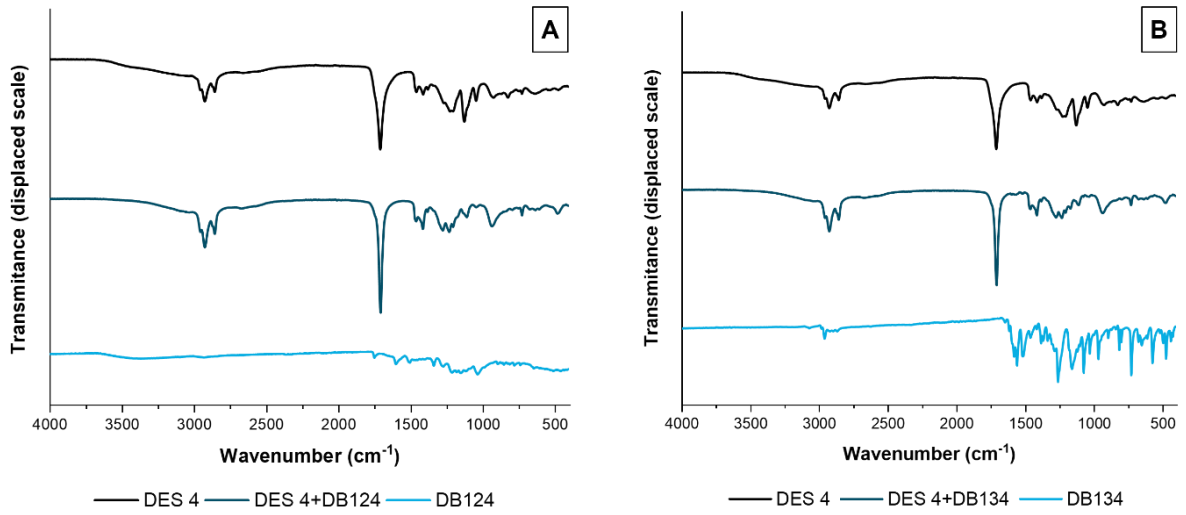


Figure 33: DES 4 and dye solutions FTIR spectra. (A) DB124 and (B) DB134.

^{1}HNMR spectra of the DES 4 solutions containing DB124 and DB134 are represented in Figures 34. Similarly to what succeeds with the FTIR spectra, there are no DB124 signals in the NMR spectrum, only the eutectic peaks appear. Concerning DB134, it is possible to observe the dye presence in DES 4 + DB134 spectrum. The spectrum does not present any change compared to the dye before being mixed with DES 4. Therefore, contrarily to the expected, it was not possible to confirm hydrogen bonding between DES 4 and the dyes.

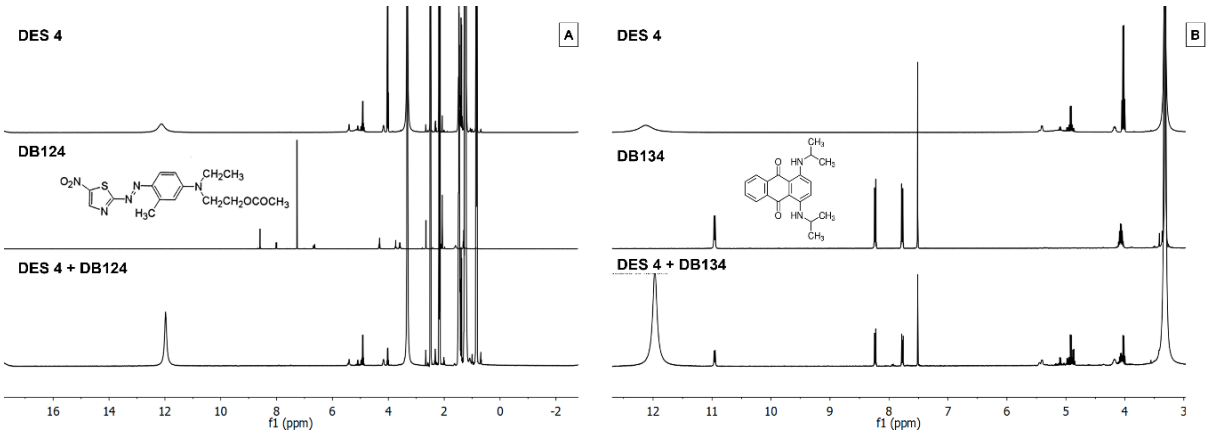


Figure 34: DES and dye solutions ^{1}H NMR spectra. (A) DB124 and (B) DB134.

4.6. Wastewater effluent detoxification and degradation using DES

The textile wastewaters contain, besides dyes, other substances in their composition, which may alter the extraction performance of DES. In order to study the performance of DES 4 (Acid 1 : Acid 2) on the extraction, degradation, and detoxification of effluent (Figure 35), the experiments were done with tannery dyeing wastewater effluent collected from Fábrica de Curtumes de Roldes, Guimarães. The composition of tannery dyeing wastewater is variable depending on the conditions and processes used, but besides dyes these effluents may contain deacidulants, retanning agents, natural and synthetic oils, surfactants, chemical auxiliaries, acids and metals (Hansen et al., 2020; Ortiz-Monsalve et al., 2019). The effluent composition used in this study consisted of dyes, deacidulants, retanning agents, naturals and synthetic oils, surfactants, auxiliary chemicals, acids, and metals. Different conditions for degradation and detoxification were tested.

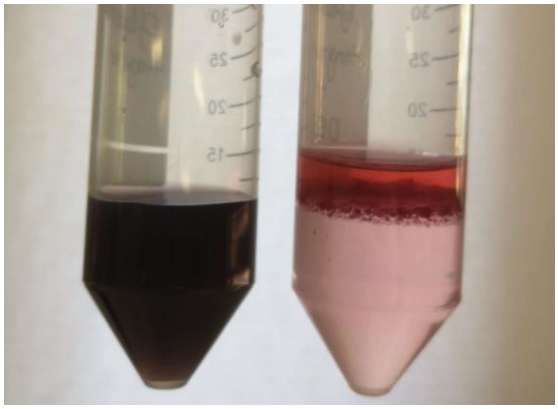


Figure 35: Tannery effluent (left) and DES 4 + effluent after extraction and before the two phases separation (right).

4.6.1. Effect of Ultrasound and Centrifugation cycles

Combined cycles of US and centrifugation (30 min in US bath + 30 min centrifugation at 4000 rpm) were used to improve the extraction process, and the influence of the number of cycles was investigated. The application of ultrasound can result in intensified processes with improved productivity. Organic pollutants can be degraded, by oxidation, due to the production of free radicals through the cavitation phenomena (Saxena et al., 2018). The results are illustrated in Figure 36. For the effluent samples, it was not possible to calculate the extraction efficiency as dye concentration on the sample was not exactly known, so efficiencies were evaluated through visible spectrum changes between treated and non-treated samples.

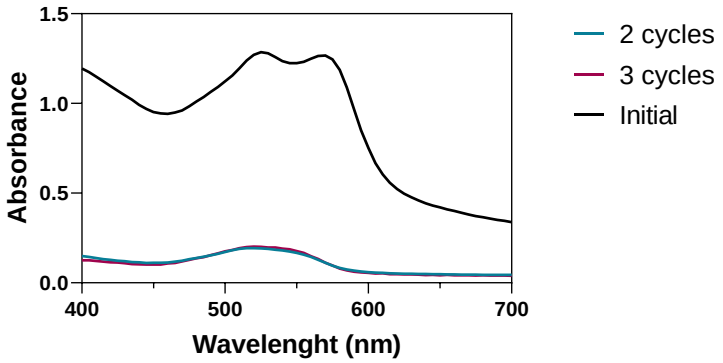


Figure 36: Absorption visible spectra of effluent solutions before and after US and centrifugation cycles.

As it is possible to observe from the obtained spectra, there are practically no differences between the two- and three-cycle spectrum, and thus, the two-cycle process was chosen for the subsequent studies.

4.6.2. Effect of wastewater effluent dilution

The effluent was prepared with different water percentages, namely 10, 20, 30, 50, 70, and 80% of water, to evaluate DES 4 extraction performance of low concentrated samples. The results are represented on Figure 37. The spectra of the samples are practically identical, independently of the percentage of water. After treatment, all spectra revealed lower absorbance values indicating that DES 4 is capable of extracting effluent components even at high concentrations and that diluting the samples is not beneficial for the extraction processing of the wastewater.

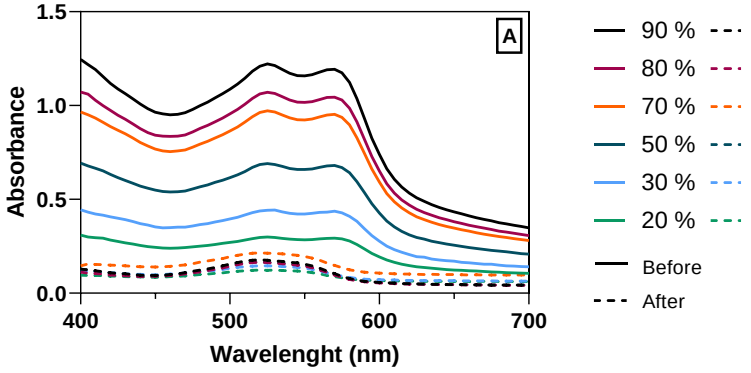


Figure 37: Absorption visible spectra of effluent at different dilutions. (A) Before treatment and (B) After treatment with DES 4.

4.6.3. Effect of effluent pH

To study the effect of effluent pH on DES 4 extraction capacity, the pH of the effluent was adjusted to values between 2 and 10, using HCl and NaOH solutions. The results are shown in Figure 38. The spectra of the treated samples are very similar, indicating that independently of the pH of the effluent, the DES reveals a similar extraction capacity.

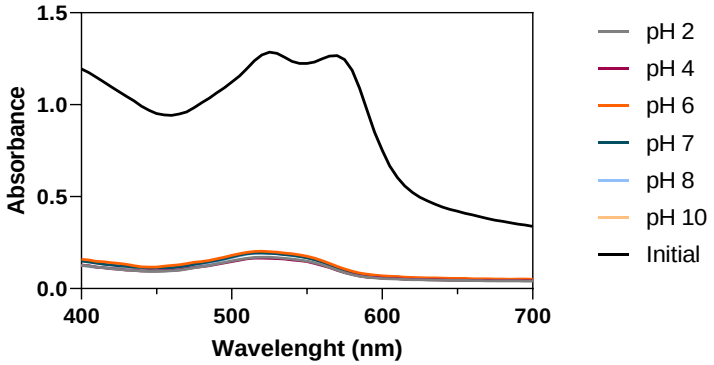


Figure 38: Absorption visible spectra of effluent with different pHs before and after treatment with DES 4.

4.6.4. Effect of DES pH

To analyse the effect of DES 4 pH, it was adjusted to values between 2 and 7, using HCl and NaOH solutions. The results are shown in Figure 39. The spectra of the treated samples are very similar, with the exception of pH=2. Higher pH values of DES favour the extraction of the dye from the effluent.

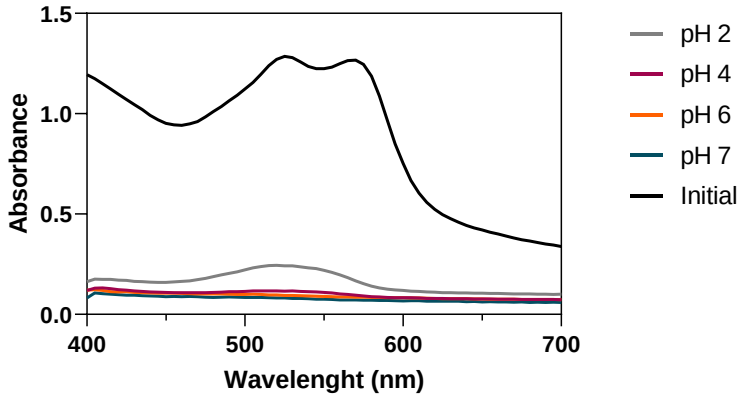


Figure 39: Absorption visible spectra of effluent before and after the treatment with DES 4 with different pHs.

4.7. Physicochemical characterization of the products of effluent extraction/degradation/detoxification

The effluent treatment with DES 4 was performed aiming to minimize the pollutants in the effluent of the tannery wastewater. For this reason, various effluent quality parameters have been evaluated in the study. Due to the impacts it can produce on the environment, industrial effluents are subject to laws that play a preponderant role in protecting available resources. Decree-law 236/98 of 1 August establishes the emission limit values (ELV) for many substances and parameters. This concept is explained as the mass, concentration, or emission level of a substance that should not be exceeded by an installation in the discharge in the aquatic environment and on the ground for one or more specified periods of time. More specifically, limit values are presented "as emission limit value, understood as a monthly average, defined as the arithmetic average of the daily averages referring to the working days of a month, which should not be exceeded". Municipalities have defined discharge limit values (DLV) for wastewaters that flow into the municipal wastewater drainage system. Table 13 presents the DLV for the municipality of Braga and the ELV according to the decree-law 236/98 of 1 August for some effluent characterization parameters.

Table 13: Discharge and emission limit values for industrial wastewater.

Parameters	DLV	ELV
pH	6 - 9	6 - 9
Total suspended solids (mg/L)	1000	60
Oils and greases (mg/L)	150	15
COD (mg O_2/L)	1000	150
TCr (mg Cr/L)	2	2
TN (mg N/L)	40	15

The results for all the parameters analysed in tanner dyeing effluent, before and after the treatment process, are presented in Table 14. The pH of the effluent decreases after the extraction because some of the acid 1 of the DES 4 passes into the aqueous phase. Thus, pH stays way below the VLE and VLD established. TS of the effluent are within the permitted values for discharge. TS of the treated effluent becomes zero, which indicates that all solids present in the sample are removed by DES 4. The same

occurs with oils and greases. This value decreases substantially indicating that DES 4 also removed oils and greases from untreated effluent. The concentration of "oxygen-demanding" substances is an important parameter for measuring organic contaminants in a water source, being total organic carbon (TOC), biochemical oxygen demand (BOD), and COD commonly used indicators. COD analysis measures the consumed oxygen correlating to the reduction of strong oxidizing agents under highly acidic conditions at high temperatures (Ma, 2017). COD removal with DES 4 was of 100% as well as chromium removal, which makes its values fall within the legal limits established for the discharge of wastewaters.

Table 14: Physicochemical parameters of tannery dyeing effluent, before and after the treatment with DES 4.

Parameters	Effluent	
	Before treatment	**After treatment**
pH	7,721	1,490
Conductivity (μS/cm)	3,893	6,013
TS (mg/L)	0,004	0
Oils and greases (mg/L)	0,060	0,001
COD (mg/L)	11750 ± 70	0
TCr (mg Cr/L)	0,690 ± 0,002	0
TN (mg N/L)	101,9 ± 8,6	97,0 ± 25,2

TN values do not decrease in treated effluent, and in both effluent samples, values are above the fixed limits. Although some parameters on treated effluent do not meet the legal requirements for discharge, DES 4 was able to treat and detoxify the effluent and reduce dye, TS, oils and greases, and TCr in solution. A great part of the effluent components remains in the DES fraction which is easily separated from the other phase. The process needs further optimization to minimize the passage of acid 1 to the effluent phase, which influences pH and COD values, and to reduce the values of TN. For this, DES 4 with a different molar ratio could be used. Optimization processes are also necessary to recover and separate DES from the extracted compounds.

5. CONCLUSION AND FUTURE PERSPECTIVES

The experimental results of this study revealed that the physicochemical characteristics of DES differ according to their components, and their applications can be defined accordingly. TGA analysis revealed hybrid thermograms and DES more stable than its individual components. DES 1 and 3 have a wide range of temperatures in which they can be used (up to 140 °C) without starting the decomposition process. FTIR analysis confirmed the formation of all the DES analysed.

DES 1 and DES 2 promoted good extraction yields comparable or superior to conventional solvents. Despite SSE being the best extraction method in most cases, it was possible to demonstrate that UAE and MAE represent viable alternatives with extractions yields close to SSE. Solvent and method of extraction affect plant extracts composition, including the total phenolic content (TPC). TPC was very variable depending on the natural source. Still, in general, all the natural materials presented high TPC, being "carqueja" one of the best, and pollen extracts the ones with the lowest TPC. Consequently, pollen extracts had the lowest scavenging activity, and "carqueja", along with cocoa and "canta viril" extracts the ones with the highest scavenging activity.

DES 1 and 2 did not present cytotoxicity, as well as cherry bay berries, African basil and pollen extracts. Additionally, cocoa beans, cherry bay berries, and pollen DES-based extracts presented antimicrobial activity against Gram-positive and Gram-negative bacteria.

Cotton dyeing showed promising result, being achieved with cherry bay berries, African basil and cork extracts containing DES 3. However, there were no major differences between the dyed samples with different pre-treatments and pre-mordants and the process has to be further exploited.

Polyester dyeing using DES was also successful. When using the laboratory dyeing machine to dye polyester with DB124 and DB134, DES 3 and DES 5 were able to produce equal and superior colour strength than aqueous dyeing. Besides, the addition of water to DES solutions further increased colour strength. Dyeing using SS was possible, but only DES 3 with 50% water content was able to surpass aqueous dyeing colour strength. When reducing the dyeing temperature (120 °C) DES dyed samples did not reach the same colour strength as the aqueous dyed samples. Nevertheless, both DES can be used to replace/reduce water consumption and auxiliary chemicals in polyester dyeing. Moreover, DES can be used to achieve different shade levels from aqueous dyeing.

Dye extraction with DES 4 revealed that dye concentration in solution, DES dosage, and DES pH limit the extraction process, affecting extraction efficiency. DES dosage depends on the type of dye to extract, and DES lower pHs favour the extraction process. Higher extraction efficiencies were obtained for DB134.

Also, extraction of this dye does not require any additional steps to the addition of DES. On the contrary, the extraction efficiency of DB124 increases with the additional centrifugation step. By analysing the solutions, it was not possible to confirm the formation of hydrogen bonding between the DES and the dyes.

Regarding tannery dyeing wastewater treatment with DES 4, 2 cycles of ultrasound and centrifugation incremented the extraction process. In this process, the treatment is influenced by DES pH, being pH 2 the responsible for the lowest dye uptake. The physicochemical characterization of the effluent revealed that DES 4 can remove the solids, oils and greases, and all the chromium from the wastewater, representing, this way, an alternative method for the treatment of polluting wastewaters.

In conclusion, this work demonstrated the efficiency of new DES, in conjugation with alternative extraction methods, in the extraction of bioactive compounds from different natural sources, some of which few explored. The obtained extracts, namely DES-based extracts, showed great potential for their application in the cosmetic industry due to their antioxidant, antimicrobial and non-cytotoxic properties. Additionally, although the process still needs to be optimised, it was possible to dye cotton fabric with DES-based extracts. It was also possible to use the new DES in the development of promising and environmental-friendly alternative to conventional polyester dyeing, which allows the reduction of water usage in the dyeing processes, and also to detoxify a tannery effluent, allowing the removal of pollutants and toxic components present in the wastewater.

Based on the results obtained, the following suggestions are presented for future work:

- Incorporation of the extracts in cosmetic formulations and testing of their final performance (stability tests, antimicrobial and antioxidant properties);
- Optimize the developed process of cotton dyeing in order to improve colour strength (other mordants, different concentrations);
- Assess the antimicrobial activity and protective capacity anti-UV of dyed cotton samples with the natural extracts;
- Determine colour fastness of dyed polyester samples (washing resistance);
- Determine if DES polyester dyeing solutions are reusable;
- Evaluate the possibility of dyes recovery from DES solutions after the extraction;

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ANNEXES

ANNEX I – Total Phenolic Content: Gallic acid calibration curve

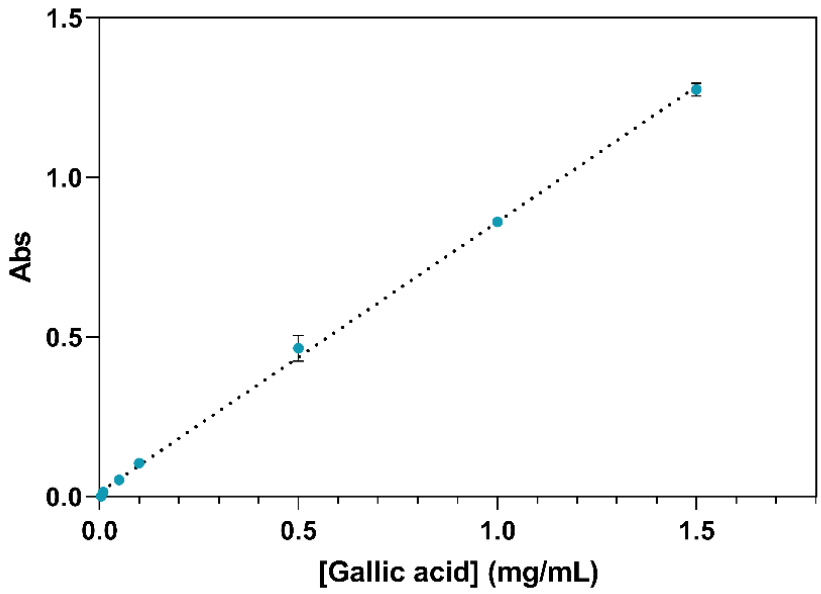

Figure 1I: TPC calibration curve using gallic acid as standard, where **Absorbance (760nm)=(0,85±0,03) x [Gallic acid] (mg/ml) + (0,01±0,02)**, R^2=0,9992. The calibration curve has a limit of detection (LOD) of 0,04 mg/mL and a limit of quantitation (LOQ) of 0,17 mg/mL.

ANNEX II – DPPH radical-scavenging assay: concentration-effect linear regression curves

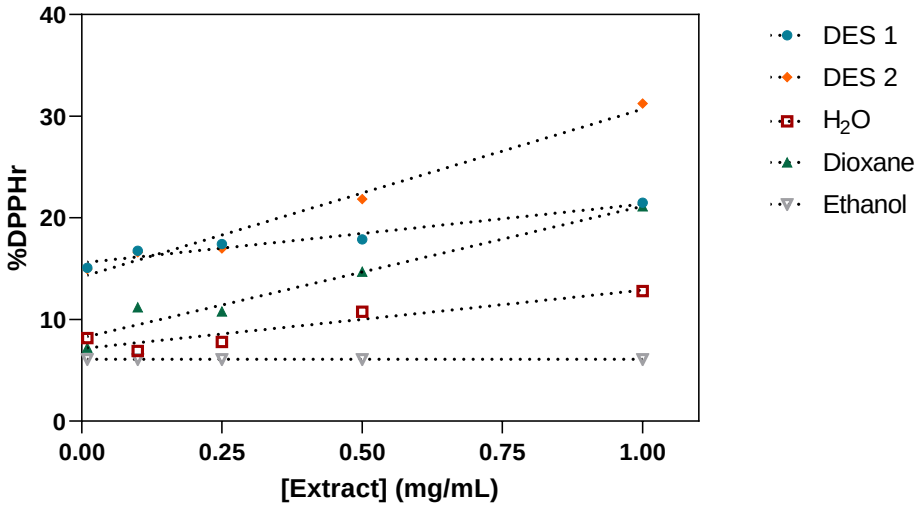

Figure 1II: Cherry bay berries UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(5,7±2,5) x [Extract] (mg/ml) + (15,5±1,3)**, R^2=0,9472. The curve has a LOD of 3,02 mg/mL and a LOQ of 3,78 mg/mL. **DES 2: %DDPHr=(16,5±4,2) x [Extract] (mg/ml) + (14,8±2,2)**, R^2=0,9807. The curve has a LOD of 1,05 mg/mL LOQ of 1,50 mg/mL. **H_2O: %DDPHr=(5,8±9,9) x [Extract] (mg/ml) + (7,1±2,0)**, R^2=0,8821. The curve has a LOD of 1,74 mg/mL and a LOQ of 2,92 mg/mL. **Dioxane: %DDPHr=(13,0±4,9) x [Extract] (mg/ml) + (8,2±2,5)**, R^2=0,9588. The curve has a LOD of 0,92 mg/mL and a LOQ of 1,58 mg/mL.

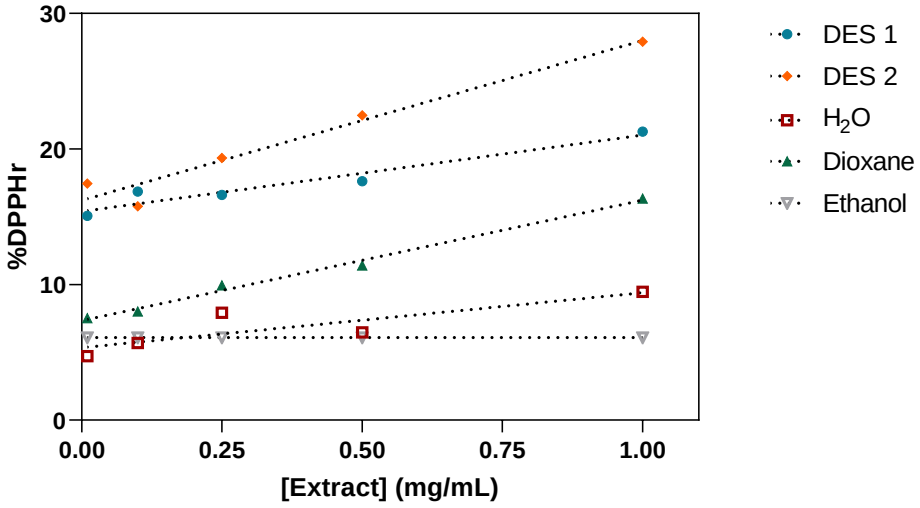

Figure 2II: Cherry bay berries SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(5,6±2,7) x [Extract] (mg/ml) + (15,4±1,4)**, R^2=0,9342. The curve has a LOD of 3,10 mg/mL and a LOQ of 3,95 mg/mL. **DES 2: %DDPHr=(11,8±4,6) x [Extract] (mg/ml) + (16,2±2,4)**, R^2=0,9560. The curve has a LOD of 1,67 mg/mL and a LOQ of 2,36 mg/mL. **H_2O: %DDPHr=(4,1±4,5) x [Extract] (mg/ml) + (5,3±2,3)**, R^2=0,7360. The curve has a LOD of 2,14 mg/mL and a LOQ of 4,06 mg/mL. **Dioxane: %DDPHr=(8,9±1,4) x [Extract] (mg/ml) + (7,3±0,7)**, R^2=0,9930. The curve has a LOD of 0,94 mg/mL and a LOQ of 1,21 mg/mL.

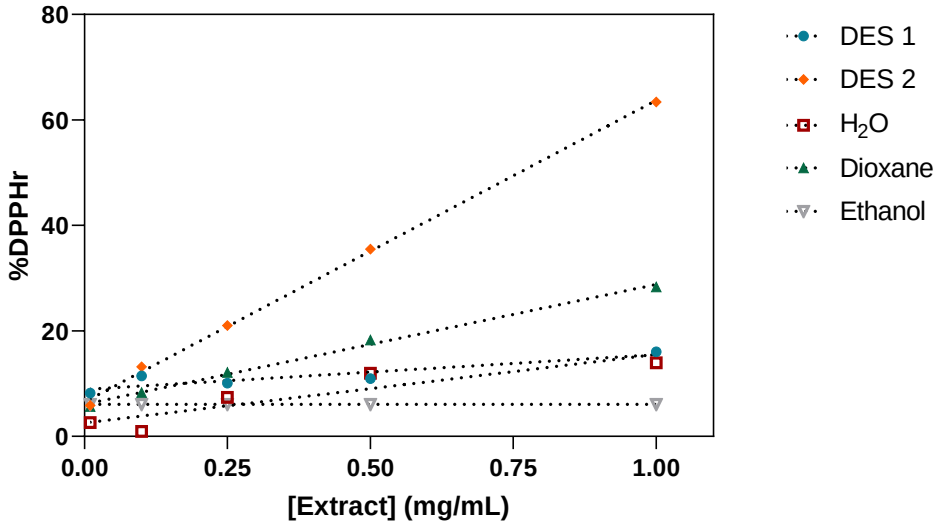

Figure 3II: Cherry bay berries MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(6,6±5,8) x [Extract] (mg/ml) + (8,9±3,0)**, R^2=0,8151. The curve has a LOD of 2,01 mg/mL and a LOQ of 3,54 mg/mL. **DES 2: %DDPHr=(57,2±3,8) x [Extract] (mg/ml) + (6,5±1,9)**, R^2=0,9987. The curve has a LOD of 0,16 mg/mL and a LOQ of 0,28 mg/mL. **H_2O: %DDPHr=(13,0±10,9) x [Extract] (mg/ml) + (2,6±5,6)**, R^2=0,8272. The curve has a LOD of 0,83 mg/mL and a LOQ of 2,29 mg/mL. **Dioxane: %DDPHr=(22,7±2,8) x [Extract] (mg/ml) + (6,1±1,4)**, R^2=0,9955. The curve has a LOD of 0,36 mg/mL and a LOQ of 0,57 mg/mL.

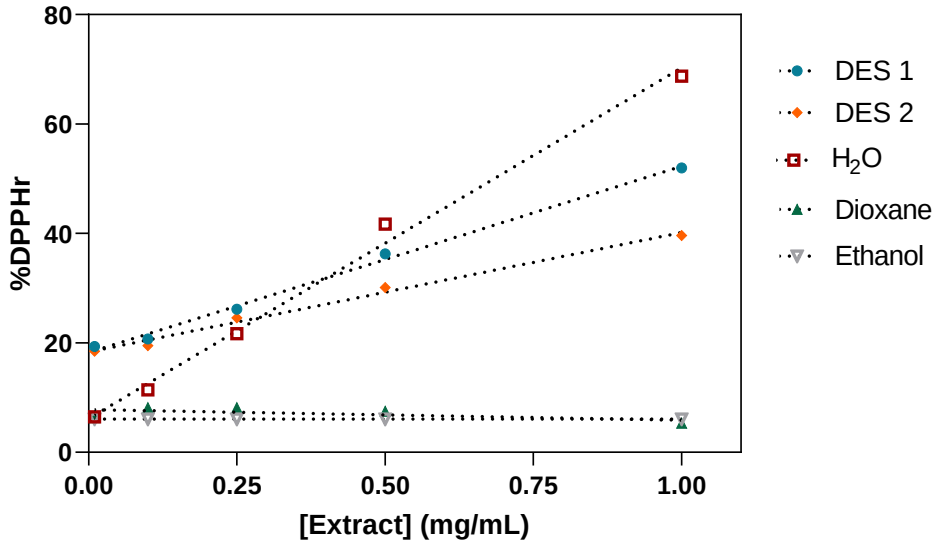

Figure 4II: Cocoa beans UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(34,0±3,9) x [Extract] (mg/ml) + (18,2±2,0)**, R^2=0,9961. The curve has a LOD of 0,62 mg/mL and a LOQ of 0,82 mg/mL. **DES 2: %DDPHr=(21,8±3,8) x [Extract] (mg/ml) + (18,3±2,0)**, R^2=0,9911. The curve has a LOD of 0,97 mg/mL and a LOQ of 1,28 mg/mL. **H_2O: %DDPHr=(64,11±9,3) x [Extract] (mg/ml) + (6,2±4,8)**, R^2=0,9938. The curve has a LOD of 0,20 mg/mL and a LOQ of 0,46 mg/mL. **Dioxane: %DDPHr=(-1,9±4,6) x [Extract] (mg/ml) + (7,8±2,4)**, R^2=0,3718.

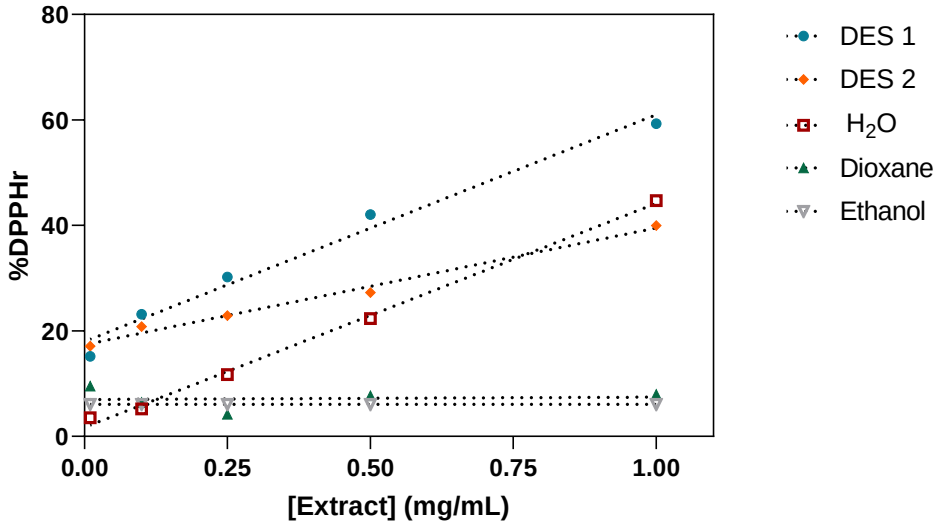

Figure 5II: Cocoa beans SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(43,0±11,1) x [Extract] (mg/ml) + (18,0±5,7)**, R^2=0,9807. The curve has a LOD of 0,61 mg/mL and a LOQ of 1,06 mg/mL. **DES 2: %DDPHr=(22,1±4,2) x [Extract] (mg/ml) + (17,4±2,2)**, R^2=0,9893. The curve has a LOD of 0,93 mg/mL and a LOQ of 1,26 mg/mL. **H_2O: %DDPHr=(42,5±4,4) x [Extract] (mg/ml) + (1,7±2,3)**, R^2=0,9969. The curve has a LOD of 0,12 mg/mL and a LOQ of 0,30 mg/mL. **Dioxane: %DDPHr=(-0,5±9,2) x [Extract] (mg/ml) + (7,0±4,7)**, R^2=0,0081.

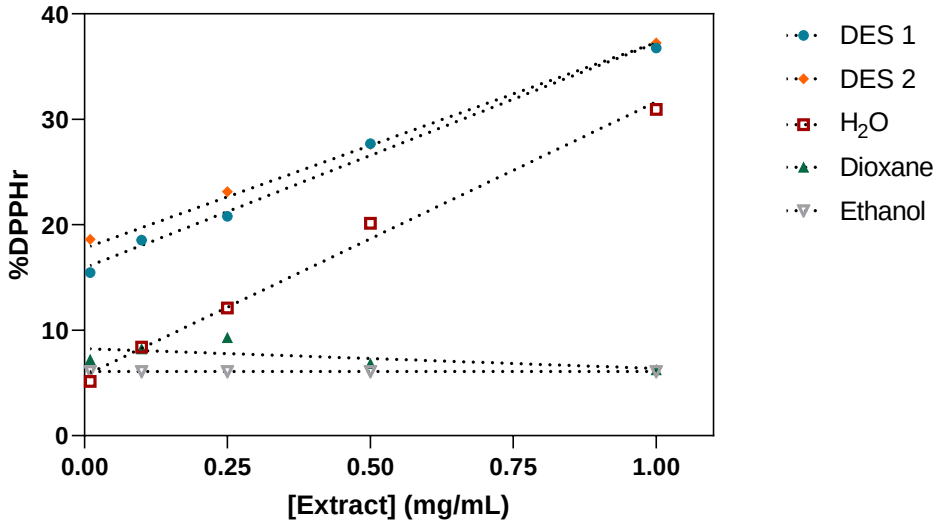

Figure 6II: Cocoa beans MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(21,3±3,6) x [Extract] (mg/ml) + (15,9±1,8)**, R^2=0,9919. The curve has a LOD of 0,87 mg/mL and a LOQ of 1,16 mg/mL. **DES 2: %DDPHr=(19,5±3,3) x [Extract] (mg/ml) + (17,8±1,7)**, R^2=0,9917. The curve has a LOD of 1,04 mg/mL and a LOQ of 1,33 mg/mL. **H_2O: %DDPHr=(26,0±4,2) x [Extract] (mg/ml) + (5,7±2,2)**, R^2=0,9923. The curve has a LOD of 0,34 mg/mL and a LOQ of 0,62 mg/mL. **Dioxane: %DDPHr=(-1,9±4,4) x [Extract] (mg/ml) + (5,3±2,2)**, R^2=0,3845.

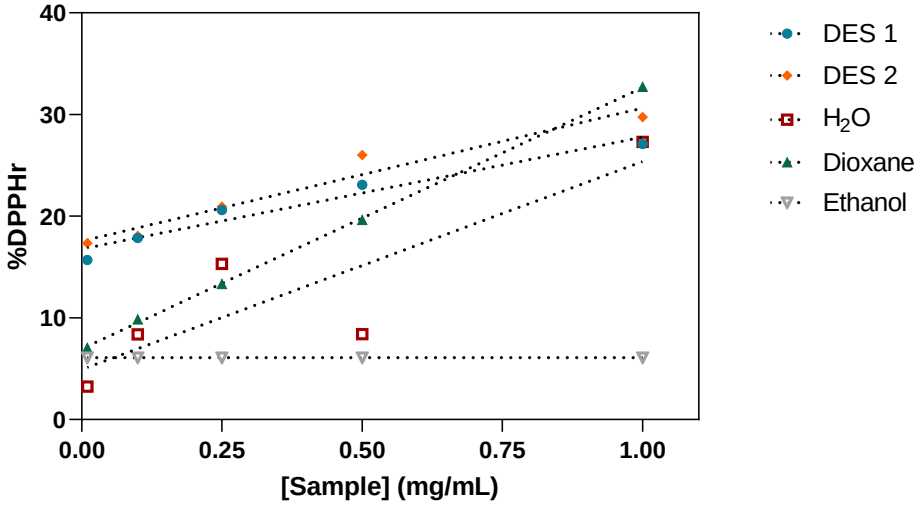

Figure 7II: Canta viril UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(11,0±4,5) x [Extract] (mg/ml) + (16,8±2,3)**, R^2=0,9537. The curve has a LOD of 1,83 mg/mL and a LOQ of 2,54 mg/mL. **DES 2: %DDPHr=(13,1±5,3) x [Extract] (mg/ml) + (16,8±2,7)**, R^2=0,9542. The curve has a LOD of 1,64 mg/mL and a LOQ of 2,34 mg/mL. **H_2O: %DDPHr=(20,5±21,0) x [Extract] (mg/ml) + (4,9±10,8)**, R^2=0,7615. The curve has a LOD of 1,01 mg/mL and a LOQ of 2,81 mg/mL. **Dioxane: %DDPHr=(25,7±0,9) x [Extract] (mg/ml) + (7,0±0,5)**, R^2=0,9996. The curve has a LOD of 0,30 mg/mL and a LOQ of 0,36 mg/mL.

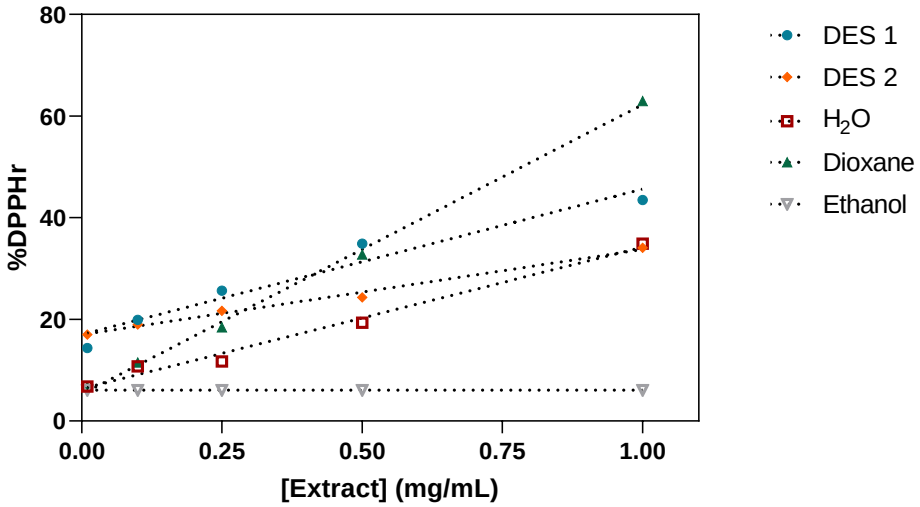

Figure 8II: Canta viril SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(28,6±12,3) x [Extract] (mg/ml) + (17,0±6,3)**, R^2=0,9482. The curve has a LOD of 0,92 mg/mL and a LOQ of 1,67 mg/mL. **DES 2: %DDPHr=(16,8±2,9) x [Extract] (mg/ml) + (17,0±1,5)**, R^2=0,9911. The curve has a LOD of 1,14 mg/mL and a limit of LOQ of 1,45 mg/mL. **H_2O: %DDPHr=(27,9±5,9) x [Extract] (mg/ml) + (6,3±3,1)**, R^2=0,9867. The curve has a LOD of 0,39 mg/mL and a LOQ of 0,76 mg/mL. **Dioxane: %DDPHr=(57,0±4,5) x [Extract] (mg/ml) + (5,3±2,3)**, R^2=0,9982. The curve has a LOD of 0,15 mg/mL and a LOQ of 0,29 mg/mL.

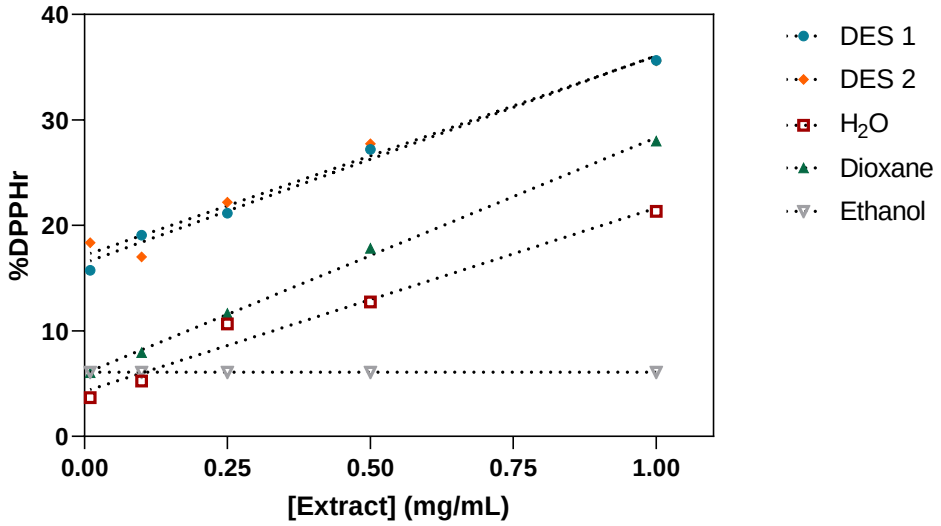

Figure 9II: Canta viril MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(19,7±3,6) x [Extract] (mg/ml) + (16,5±1,8)**, R^2=0,9904. The curve has a LOD of 0,97 mg/mL and a LOQ of 1,29 mg/mL. **DES 2: %DDPHr=(18,9±6,0) x [Extract] (mg/ml) + (17,2±3,1)**, R^2=0,9709. The curve has a LOD of 1,14 mg/mL and a LOQ of 1,70 mg/mL. **H_2O: %DDPHr=(17,4±5,5) x [Extract] (mg/ml) + (4,3±2,8)**, R^2=0,9718. The curve has a LOD of 0,48 mg/mL and a LOQ of 1,03 mg/mL. **Dioxane: %DDPHr=(22,4±1,9) x [Extract] (mg/ml) + (6,0±1,0)**, R^2=0,9979. The curve has a LOD of 0,33 mg/mL and a LOQ of 0,48 mg/mL.

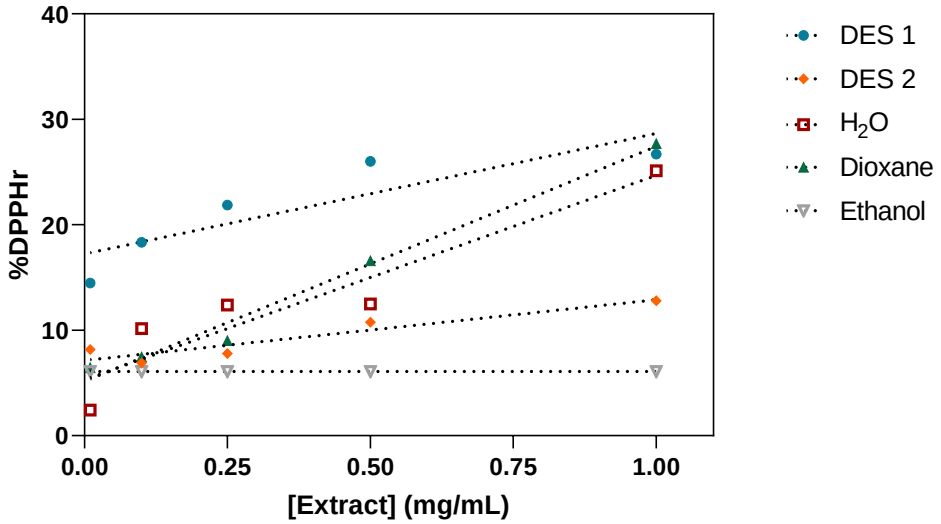

Figure 10II: Carqueja UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(11,4±11,5) x [Extract] (mg/ml) + (17,2±5,9)**, R^2=0,7694. The curve has a LOD of 2,26 mg/mL and a LOQ of 4,02 mg/mL. **DES 2: %DDPHr=(5,8±3,9) x [Extract] (mg/ml) + (7,1±2,0)**, R^2=0,8821. The curve has a LOD of 1,74 mg/mL and a LOQ of 2,92 mg/mL. **H_2O: %DDPHr=(19,4±12,6) x [Extract] (mg/ml) + (5,3±6,5)**, R^2=0,8893. The curve has a LOD of 0,76 mg/mL and a LOQ of 1,89 mg/mL. **Dioxane: %DDPHr=(22,3±4,8) x [Extract] (mg/ml) + (5,1±2,5)**, R^2=0,9866. The curve has a LOD of 0,39 mg/mL and a LOQ of 0,77 mg/mL.

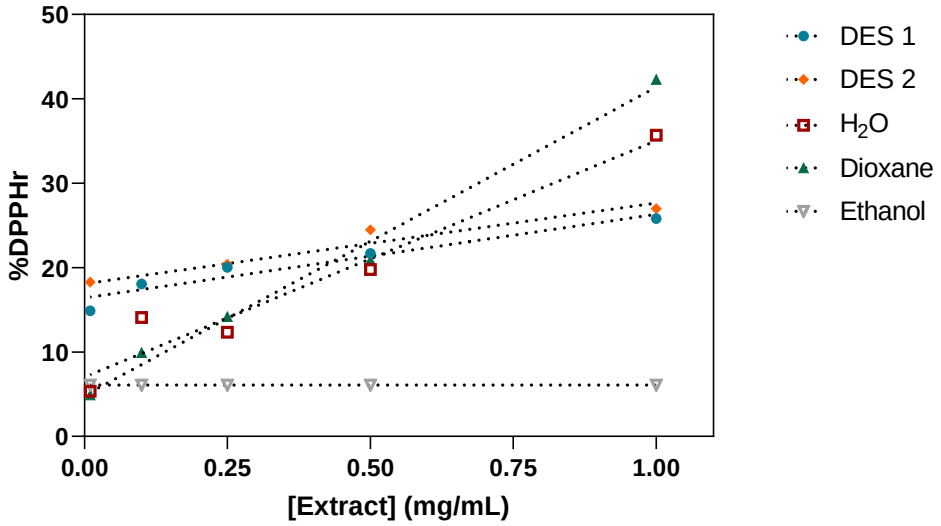

Figure 11II: Carqueja SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(9,9±5,0) x [Extract] (mg/ml) + (16,4±2,6)**, R^2=0,9295. The curve has a LOD of 2,04 mg/mL and a LOQ of 2,92 mg/mL. **DES 2: %DDPHr=(9,6±4,6) x [Extract] (mg/ml) + (18,1±2,3)**, R^2=0,9371. The curve has a LOD of 2,24 mg/mL and a LOQ of 3,07 mg/mL. **H_2O: %DDPHr=(28,0±12,0) x [Extract] (mg/ml) + (7,0±6,2)**, R^2=0,9487. The curve has a LOD of 1,57 mg/mL and a LOQ of 1,32 mg/mL. **Dioxane: %DDPHr=(36,5±6,5) x [Extract] (mg/ml) + (4,9±3,4)**, R^2=0,9906. The curve has a LOD of 0,27 mg/mL and a LOQ of 0,58 mg/mL.

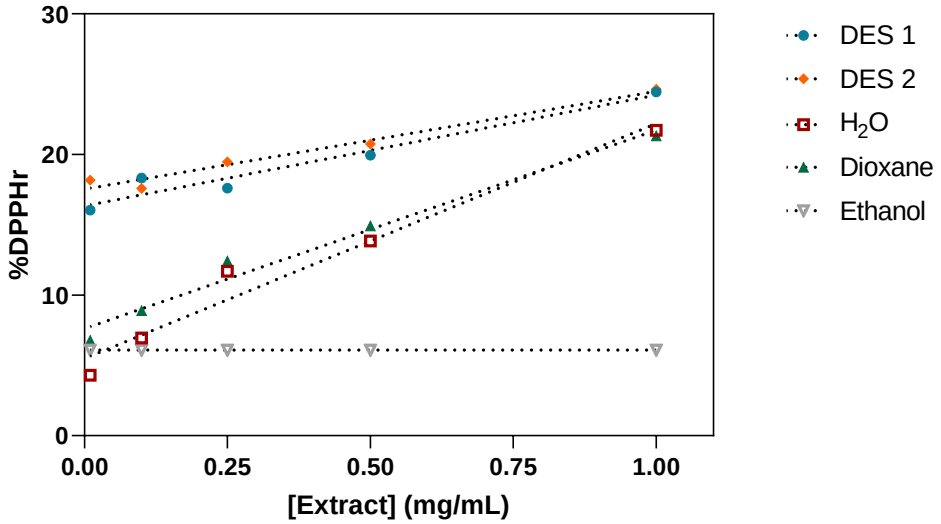

Figure 12II: Carqueja MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(7,9±3,5) x [Extract] (mg/ml) + (16,3±1,8)**, R^2=0,9455. The curve has a LOD of 2,40 mg/mL and a LOQ of 3,17 mg/mL. **DES 2: %DDPHr=(7,0±2,1) x [Extract] (mg/ml) + (17,5±1,1)**, R^2=0,9725. The curve has a LOD of 2,75 mg/mL and a LOQ of 3,29 mg/mL. **H_2O: %DDPHr=(16,7±5,8) x [Extract] (mg/ml) + (5,0±3,0)**, R^2=0,9657. The curve has a LOD of 0,59 mg/mL and a LOQ of 1,19 mg/mL. **Dioxane: %DDPHr=(14,1±3,9) x [Extract] (mg/ml) + (7,6±2,0)**, R^2=0,9780. The curve has a LOD of 0,74 mg/mL and a LOQ of 1,23 mg/mL.

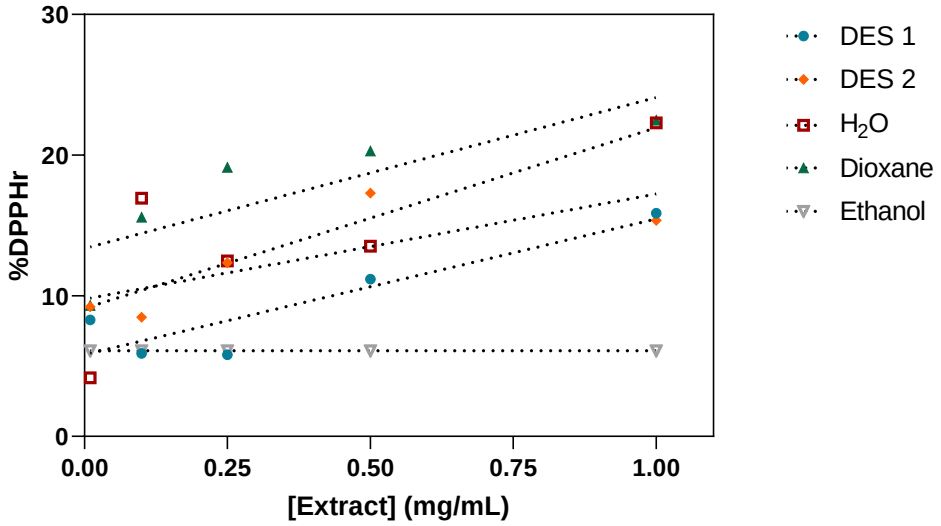

Figure 13II: Damiana UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(9,6±8,2) x [Extract] (mg/ml) + (5,8±4,2)**, R^2=0,8221. The curve has a LOD of 1,24 mg/mL and a LOQ of 2,74 mg/mL. **DES 2: %DDPHr=(7,5±11,1) x [Extract] (mg/ml) + (9,8±5,7)**, R^2=0,6049. The curve has a LOD of 2,42 mg/mL and a LOQ of 5,01 mg/mL. **H_2O: %DDPHr=(12,8±19,7) x [Extract] (mg/ml) + (9,1±10,1)**, R^2=0,5887. The curve has a LOD of 1,86 mg/mL and a LOQ of 4,54 mg/mL. **Dioxane: %DDPHr=(10,7±13,3) x [Extract] (mg/ml) + (13,4±6,8)**, R^2=0,6877. The curve has a LOD of 2,17 mg/mL and a LOQ of 4,34 mg/mL.

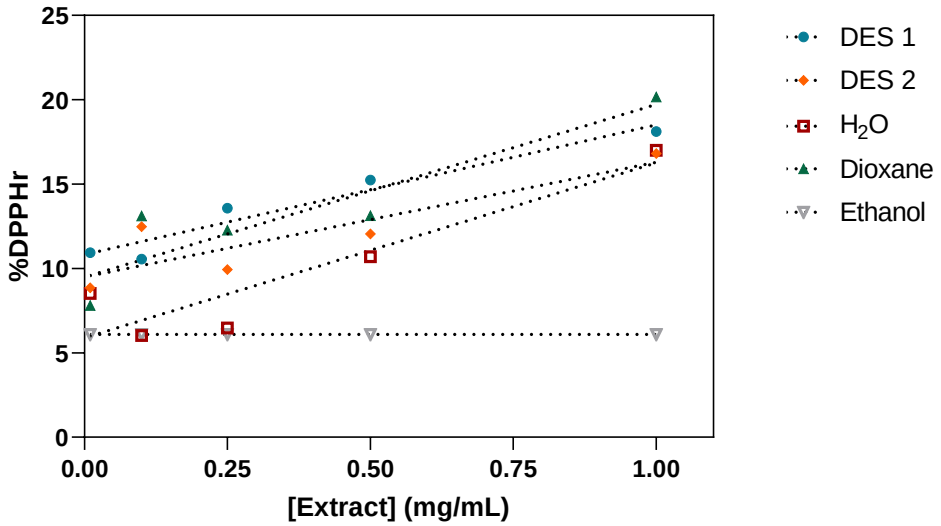

Figure 14II: Damiana SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(7,7±3,5) x [Extract] (mg/ml) + (10,8±1,8)**, R^2=0,9427. The curve has a LOD of 1,75 mg/mL and a LOQ of 2,54 mg/mL. **DES 2: %DDPHr=(6,8±6,7) x [Extract] (mg/ml) + (9,5±3,4)**, R^2=0,7776. The curve has a LOD of 2,13 mg/mL and a LOQ of 3,85 mg/mL. **H_2O: %DDPHr=(10,4±8,0) x [Extract] (mg/ml) + (5,9±4,1)**, R^2=0,8501. The curve has a LOD of 1,15 mg/mL and a LOQ of 2,49 mg/mL. **Dioxane: %DDPHr=(10,2±8,1) x [Extract] (mg/ml) + (9,5±4,2)**, R^2=0,8422. The curve has a LOD of 1,52 mg/mL and a LOQ of 2,91 mg/mL.

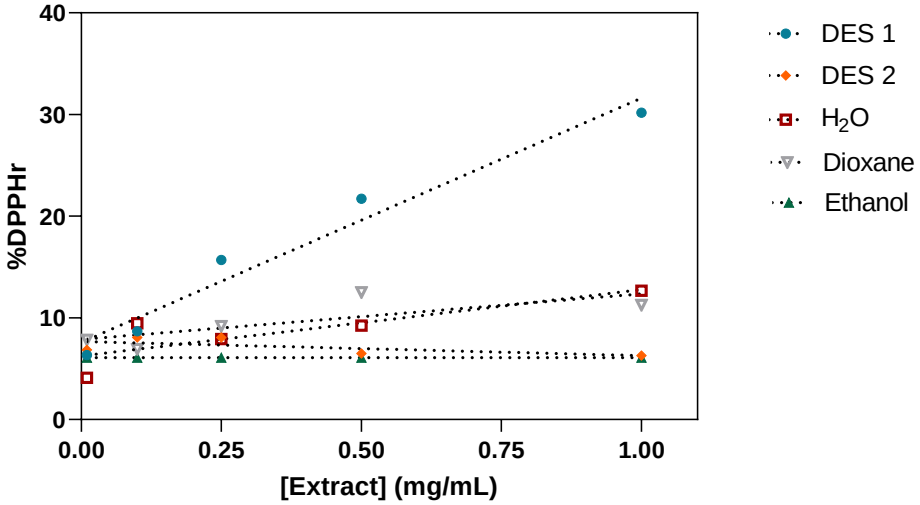

Figure 15II: Damiana MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(24,0±8,9) x [Extract] (mg/ml) + (7,6±4,6)**, R^2=0,9607. The curve has a LOD of 0,59 mg/mL and a LOQ of 1,24 mg/mL. **DES 2: %DDPHr=(1,4±3,1) x [Extract] (mg/ml) + (7,7±1,6)**, R^2=0,4080. The curve has a LOD of 7,17 mg/mL and a LOQ of 11,03 mg/mL. **H_2O: %DDPHr=(6,5±7,8) x [Extract] (mg/ml) + (6,3±4,0)**, R^2=0,7000. The curve has a LOD of 1,86 mg/mL and a LOQ of 3,96 mg/mL. **Dioxane: %DDPHr=(4,5±6,9) x [Extract] (mg/ml) + (7,9±3,6)**, R^2=0,5869. The curve has a LOD of 2,91 mg/mL and a LOQ of 5,61 mg/mL.

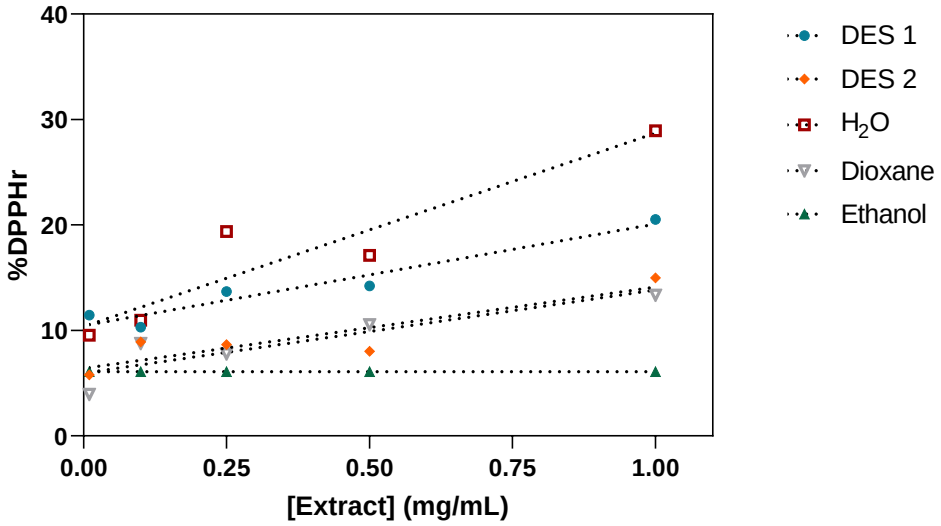

Figure 16II: Donzela UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(9,6±4,6) x [Extract] (mg/ml) + (10,5±2,4)**, R^2=0,9360. The curve has a LOD of 1,44 mg/mL and a LOQ of 2,28 mg/mL. **DES 2: %DDPHr=(7,7±7,1) x [Extract] (mg/ml) + (6,4±3,6)**, R^2=0,8016. The curve has a LOD of 1,51 mg/mL and a LOQ of 3,11 mg/mL. **H_2O: %DDPHr=(18,3±12,2) x [Extract] (mg/ml) + (10,4±6,3)**, R^2=0,8833. The curve has a LOD of 1,07 mg/mL and a LOQ of 2,23 mg/mL. **Dioxane: %DDPHr=(7,9±7,0) x [Extract] (mg/ml) + (6,0±3,6)**, R^2=0,5869. The curve has a LOD of 1,42 mg/mL and a LOQ of 2,97 mg/mL.

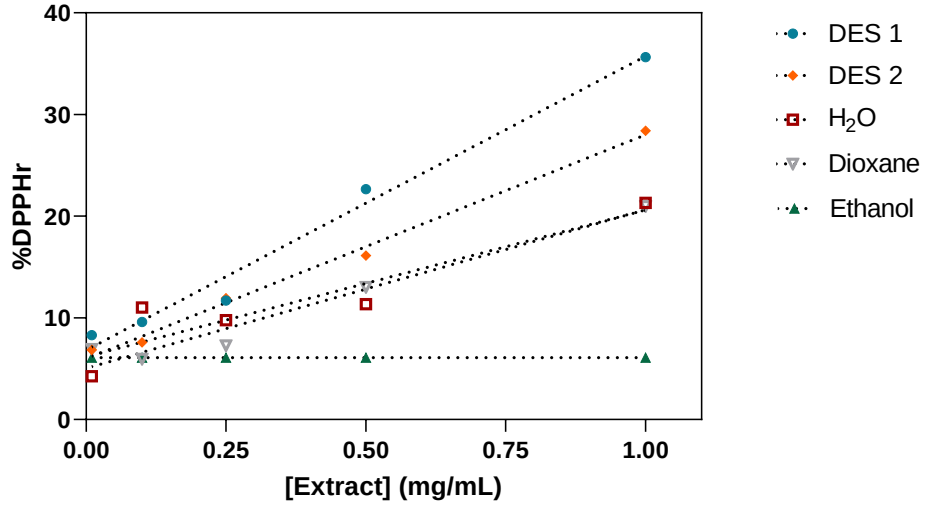

Figure 17II: Donzela SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(28,9±6,9) x [Extract] (mg/ml) + (6,8±3,5)**, R^2=0,9835. The curve has a LOD of 0,41 mg/mL and a LOQ of 0,83 mg/mL. **DES 2: %DDPHr=(22,0±3,2) x [Extract] (mg/ml) + (6,0±1,6)**, R^2=0,9939. The curve has a LOD of 0,38 mg/mL and a LOQ of 0,63 mg/mL. **H_2O: %DDPHr=(14,4±10,5) x [Extract] (mg/ml) + (6,2±5,4)**, R^2=0,8650. The curve has a LOD of 0,97 mg/mL and a LOQ of 2,24 mg/mL. **Dioxane: %DDPHr=(15,6±5,8) x [Extract] (mg/ml) + (5,0±3,0)**, R^2=0,9605. The curve has a LOD of 0,60 mg/mL and a LOQ of 1,25 mg/mL.

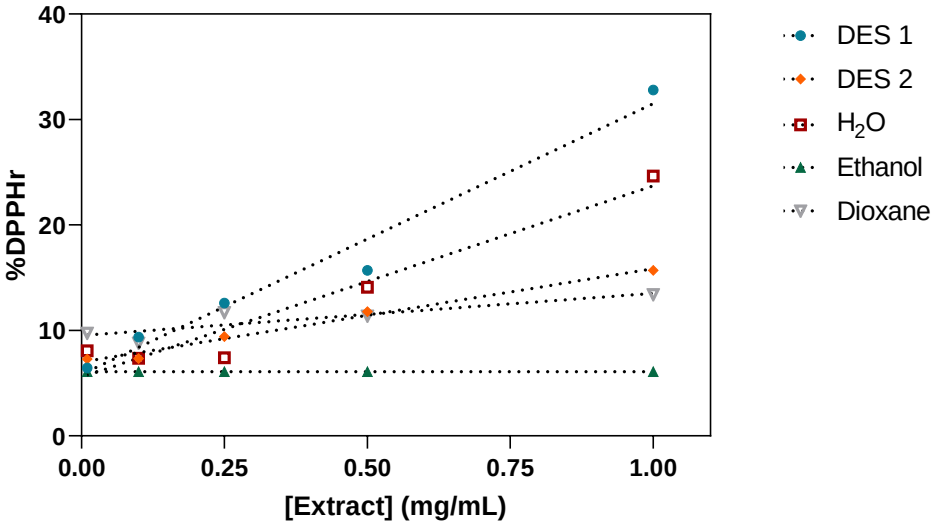

Figure 18II: Donzela MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(25,7±7,9) x [Extract] (mg/ml) + (5,8±4,1)**, R^2=0,9728. The curve has a LOD of 0,46 mg/mL and a LOQ of 0,99 mg/mL. **DES 2: %DDPHr=(8,9±1,7) x [Extract] (mg/ml) + (7,0±0,9)**, R^2=0,9897. The curve has a LOD of 0,93 mg/mL and a LOQ of 1,26 mg/mL. **H_2O: %DDPHr=(18,2±8,6) x [Extract] (mg/ml) + (5,6±4,4)**, R^2=0,9376. The curve has a LOD of 0,66 mg/mL and a LOQ of 1,49 mg/mL. **Dioxane: %DDPHr=(4,0±3,8) x [Extract] (mg/ml) + (9,5±1,9)**, R^2=0,7906. The curve has a LOD of 3,09 mg/mL and a LOQ of 4,74 mg/mL.

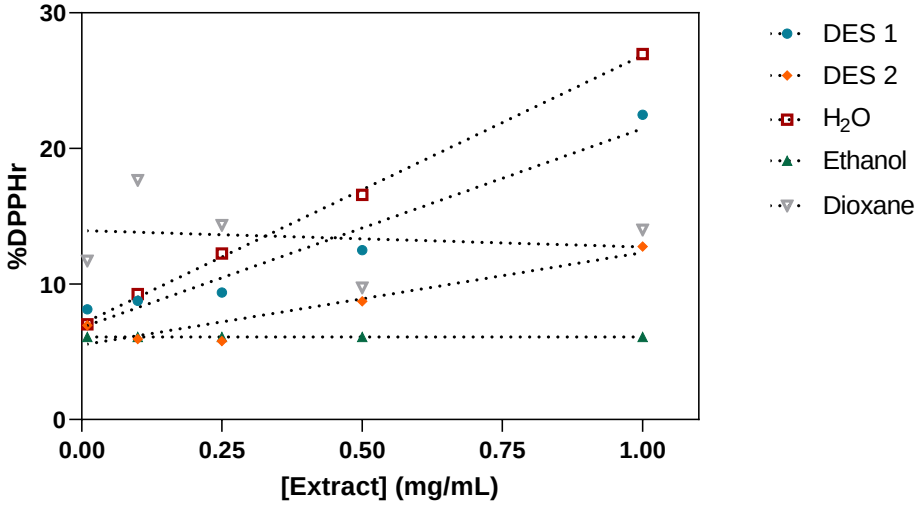

Figure 19II: African basil UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(14,7±6,0) x [Extract] (mg/ml) + (6,8±3,1)**, R^2=0,9535. The curve has a LOD of 0,77 mg/mL and a LOQ of 1,47 mg/mL. **DES 2: %DDPHr=(6,8±4,8) x [Extract] (mg/ml) + (5,5±2,4)**, R^2=0,8744. The curve has a LOD of 1,33 mg/mL and a LOQ of 2,54 mg/mL. **H_2O: %DDPHr=(19,8±1,3) x [Extract] (mg/ml) + (7,0±0,7)**, R^2=0,9987. The curve has a LOD of 0,40 mg/mL and a LOQ of 0,52 mg/mL. **Dioxane: %DDPHr=(1,2±13,6) x [Extract] (mg/ml) + (13,9±7,0)**, R^2=0,2588. The curve has a LOD of 19,98 mg/mL and a LOQ of 39,66 mg/mL.

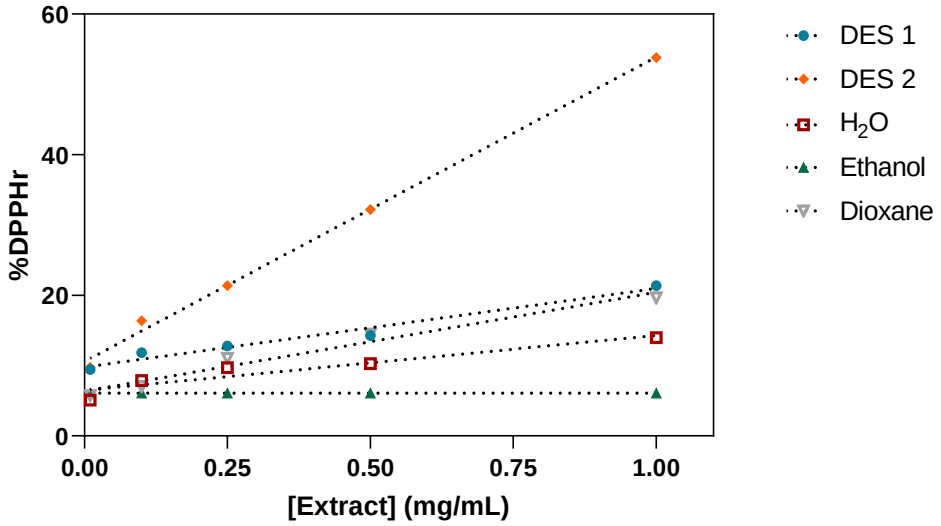

Figure 20II: African basil SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(11,2±3,7) x [Extract] (mg/ml) + (9,8±1,9)**, R^2=0,9693. The curve has a LOD of 1,12 mg/mL and a LOQ of 1,69 mg/mL. **DES 2: %DDPHr=(43,3±4,5) x [Extract] (mg/ml) + (10,6±2,3)**, R^2=0,9968. The curve has a LOD of 0,32 mg/mL and a LOQ of 0,50 mg/mL. **H_2O: %DDPHr=(7,8±4,8) x [Extract] (mg/ml) + (6,5±2,5)**, R^2=0,8993. The curve has a LOD of 1,29 mg/mL and a LOQ of 2,36 mg/mL. **Dioxane: %DDPHr=(14,1±4,8) x [Extract] (mg/ml) + (6,4±2,5)**, R^2=0,9670. The curve has a LOD of 0,71 mg/mL and a LOQ of 1,30 mg/mL.

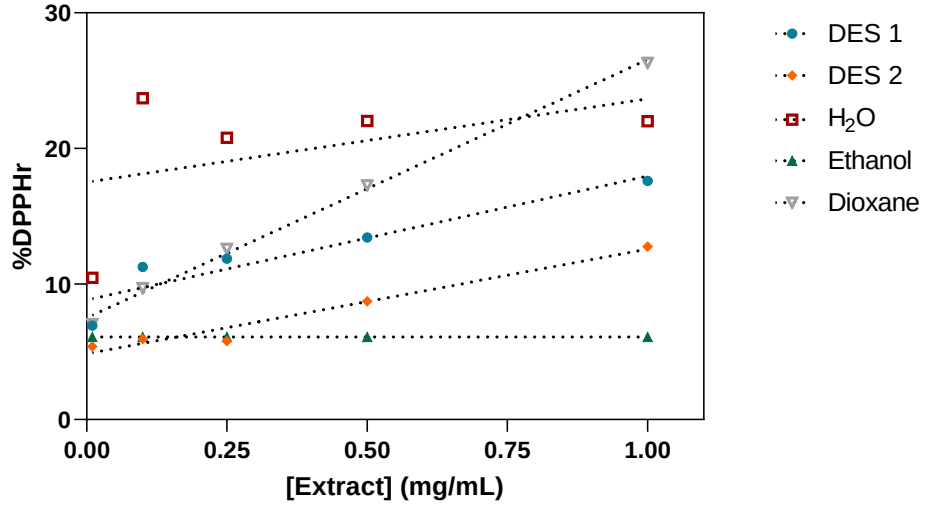

Figure 21II: Africa basil MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(9,1±6,1) x [Extract] (mg/ml) + (8,8±3,2)**, R^2=0,8826. The curve has a LOD of 1,47 mg/mL and a li LOQ of 2,64 mg/mL. **DES 2: %DDPHr=(7,7±2,7) x [Extract] (mg/ml) + (4,9±1,4)**, R^2=0,9658. The curve has a LOD of 0,89 mg/mL and a LOQ of 1,49 mg/mL. **H_2O: %DDPHr=(6,1±21,9) x [Extract] (mg/ml) + (17,5±11,3)**, R^2=0,2097. The curve has a LOD of 1,29 mg/mL and a LOQ of 3,47 mg/mL. **Dioxane: %DDPHr=(19,1±2,0) x [Extract] (mg/ml) + (7,5±1,0)**, R^2=0,9968. The curve has a LOD of 0,47 mg/mL and a LOQ of 0,65 mg/mL.

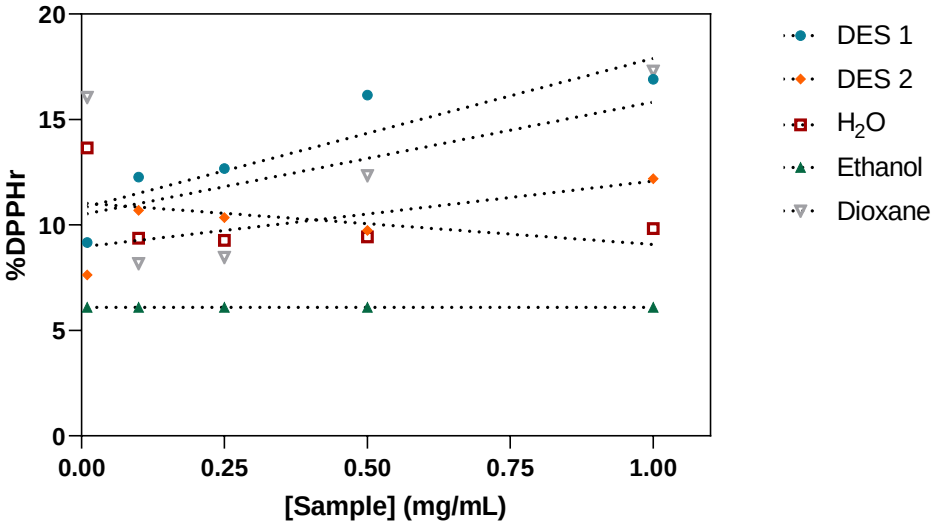

Figure 22II: Pollen UAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(7,1±6,4) x [Extract] (mg/ml) + (10,8±3,3)**, R^2=0,0388. The curve has a LOD of 2,19 mg/mL and a LOQ of 3,77 mg/mL. **DES 2: %DDPHr=(3,1±5,1) x [Extract] (mg/ml) + (9,0±2,6)**, R^2=0,5588. The curve has a LOD of 4,09 mg/mL and a LOQ of 6,94 mg/mL. **H_2O: %DDPHr=(-2,0±7,9) x [Extract] (mg/ml) + (11,0±4,1)**, R^2=0,1728. **Dioxane: %DDPHr=(5,4±16,8) x [Extract] (mg/ml) + (10,5±8,6)**, R^2=0,2560. The curve has a LOD of 4,30 mg/mL and a LOQ of 9,77 mg/mL.

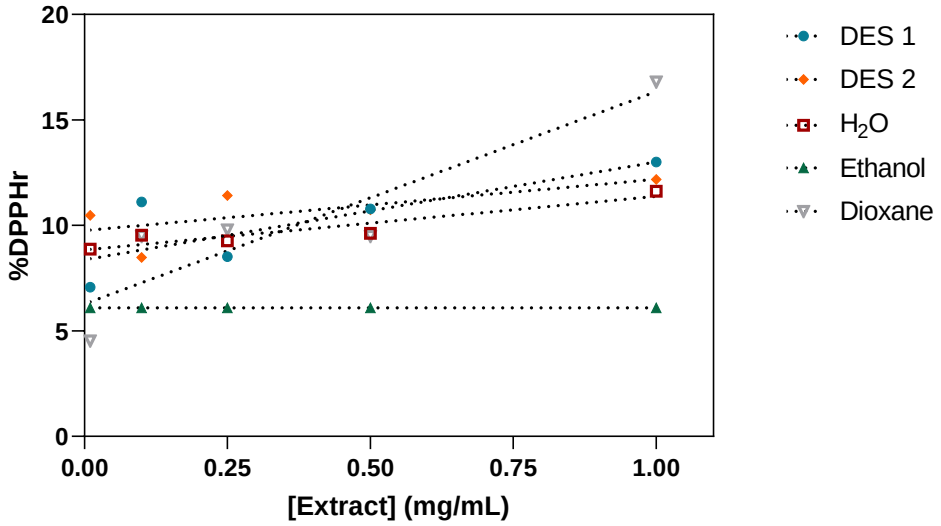

Figure 23II: Pollen SSE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(4,6±6,6) x [Extract] (mg/ml) + (8,4±3,4)**, R^2=0,6281. The curve has a LOD of 2,86 mg/mL and a LOQ of 5,33 mg/mL. **DES 2: %DDPHr=(2,4±4,6) x [Extract] (mg/ml) + (9,8±2,4)**, R^2=0,4835. The curve has a LOD of 5,44 mg/mL and a LOQ of 8,76 mg/mL. **H_2O: %DDPHr=(2,5±1,7) x [Extract] (mg/ml) + (8,8±0,9)**, R^2=0,8859. The curve has a LOD of 3,99 mg/mL and a LOQ of 5,14 mg/mL. **Dioxane: %DDPHr=(10,1±8,3) x [Extract] (mg/ml) + (6,3±4,3)**, R^2=0,8332. The curve has a LOD of 0,75 mg/mL and a LOQ of 1,04 mg/mL.

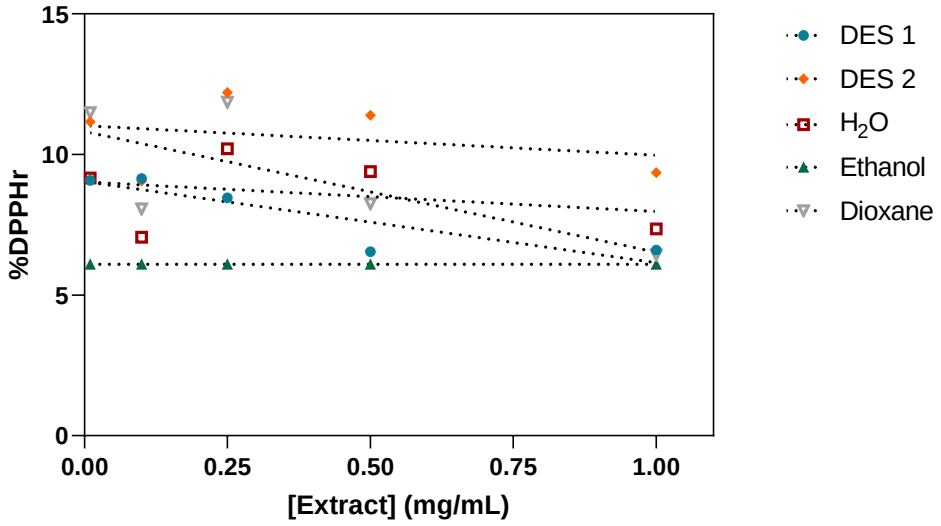

Figure 24II: Pollen MAE extracts concentration-effect linear regression curves. **DES 1: %DDPHr=(-2,9±2,8) x [Extract] (mg/ml) + (9,0±1,5)**, R^2=0,7794. **DES 2: %DDPHr=(-1,1±6,0) x [Extract] (mg/ml) + (11,0±3,1)**, R^2=0,0938. **H_2O: %DDPHr=(-1,1±7,5) x [Extract] (mg/ml) + (9,0±3,9)**, R^2=0,1011. **Dioxane: %DDPHr=(-4,3±7,5) x [Extract] (mg/ml) + (10,8±3,9)**, R^2=0,5242.

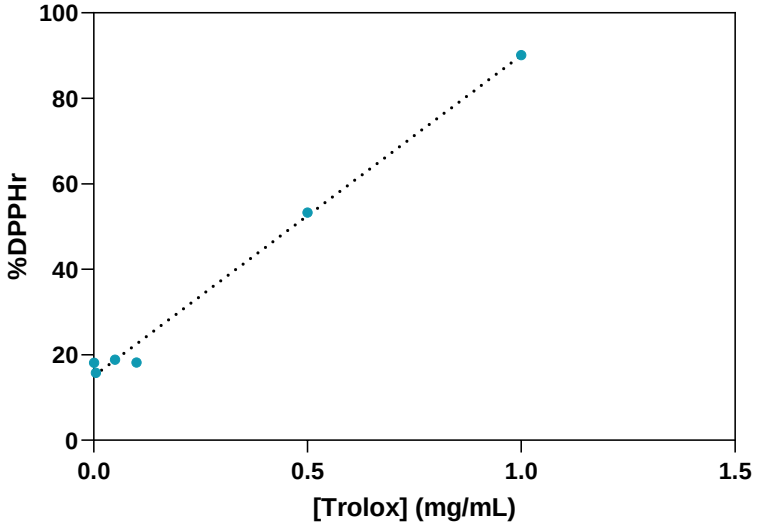

Figure 25II: Trolox concentration-effect linear regression curves, where **%DDPHr=(75,02±8,3) x [Gallic acid] (mg/ml) + (15,01±3,8)**, R^2=0,9937. The calibration curve has a limit of detection (LOD) of 0,3 mg/mL and a limit of quantitation (LOQ) of 0,6 mg/mL.

ANNEX III – Extracts chromatograms

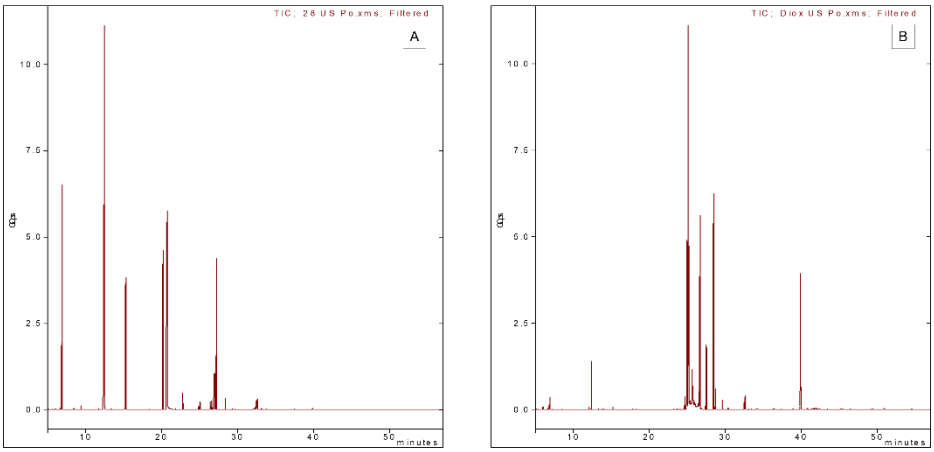

Figure 1III: Pollen extracts chromatograms. (A) DES 1 extract, and (B) Dioxane extract.

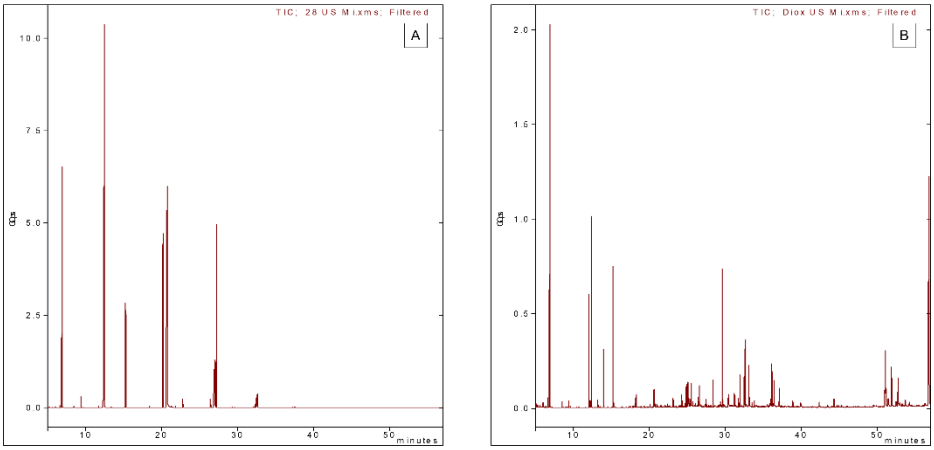

Figure 2III: African basil extracts chromatograms. (A) DES 1 extract, and (B) Dioxane extract.

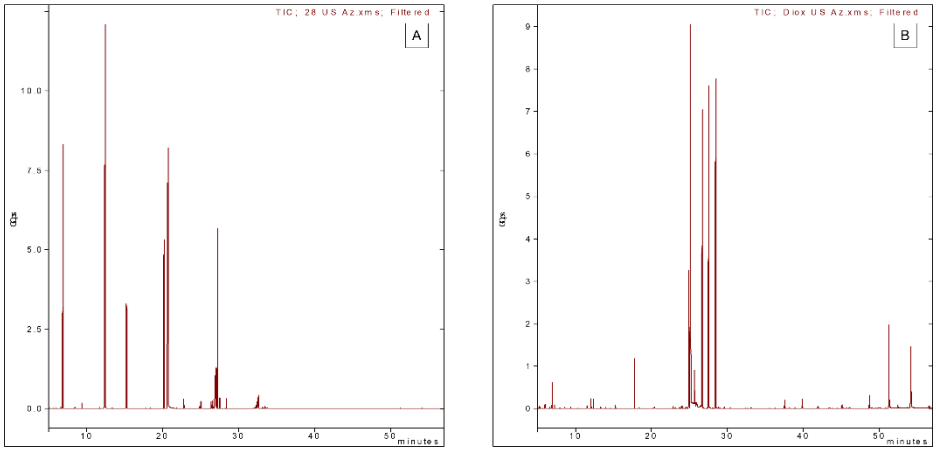

Figure 3III: Cherry bay berries extracts chromatograms. (A) DES 1 extract, and (B) Dioxane extract.

ANNEX IV – Extracts UV-Vis absorption spectra

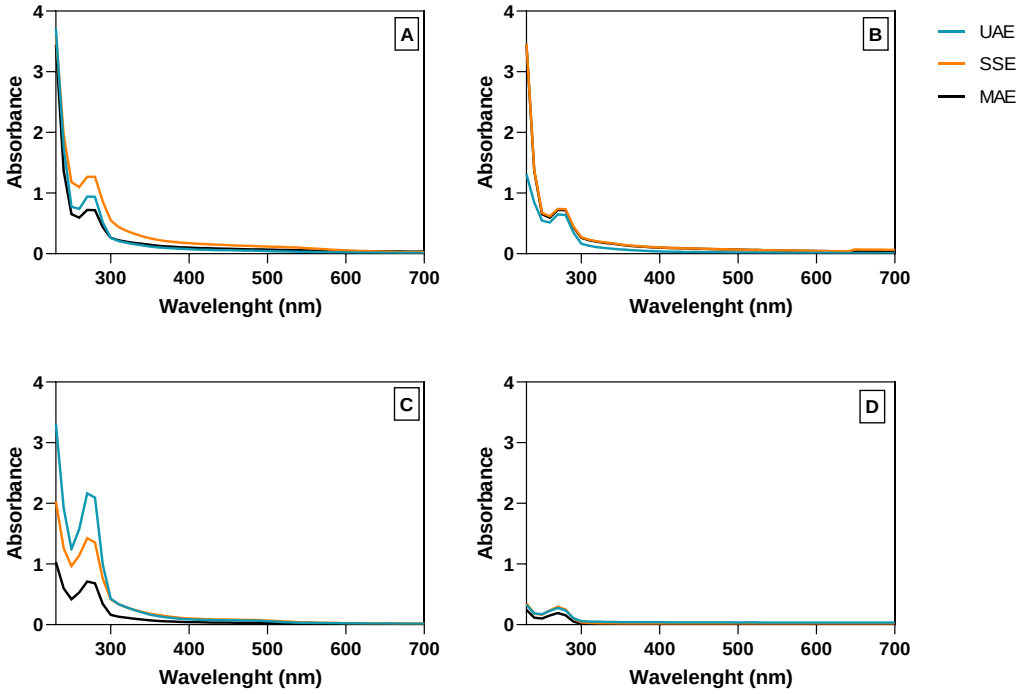

Figure 1IV: Absorption UV-Vis spectra of cocoa beans extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

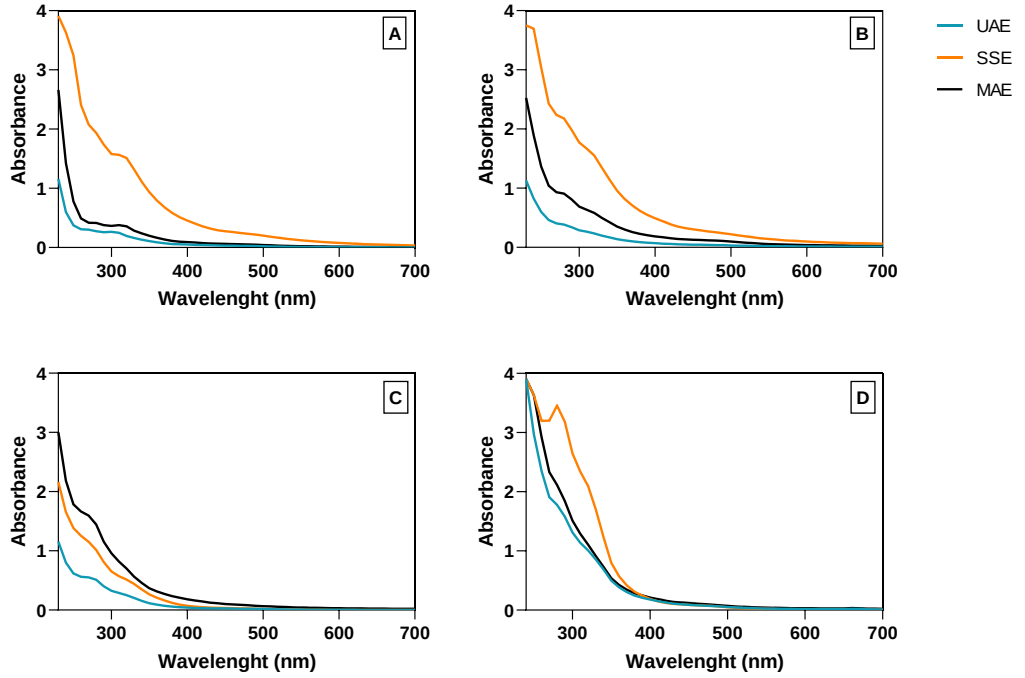

Figure 2IV: Absorption UV-Vis spectra of canta viril extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

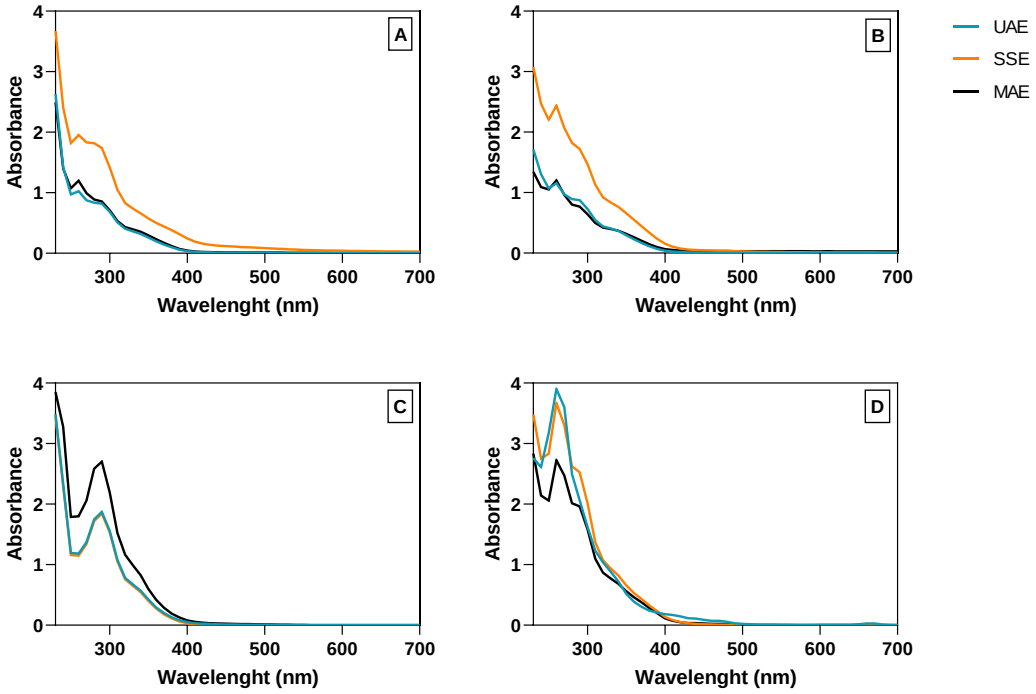

Figure 3IV: Absorption UV-Vis spectra of *P. tridentatum* extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

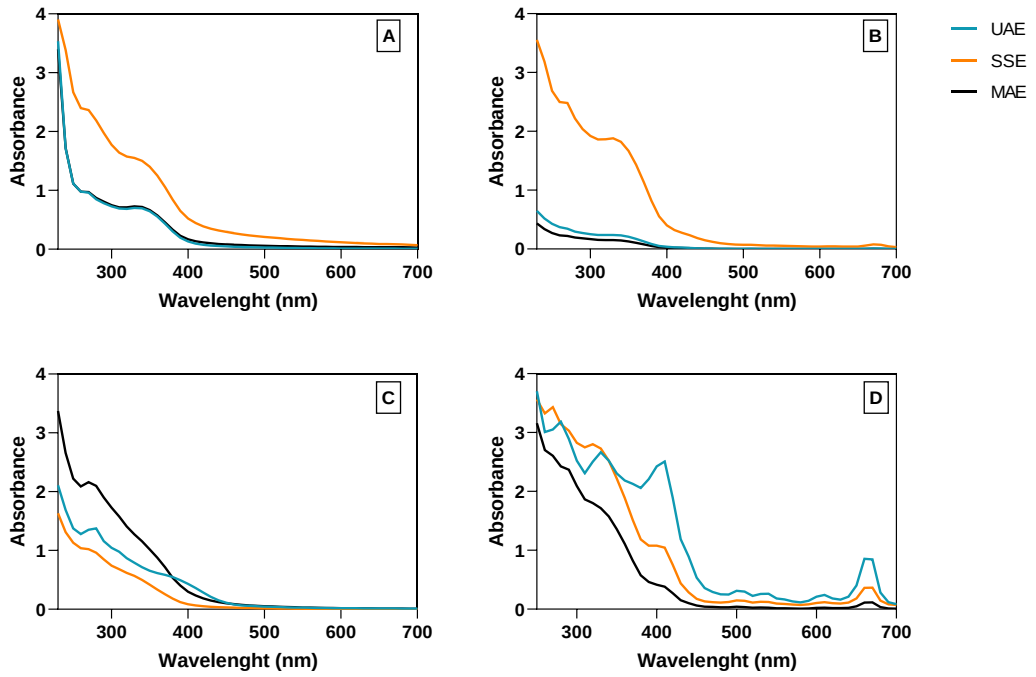

Figure 4IV: Absorption UV-Vis spectra of T. *diffusa* extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

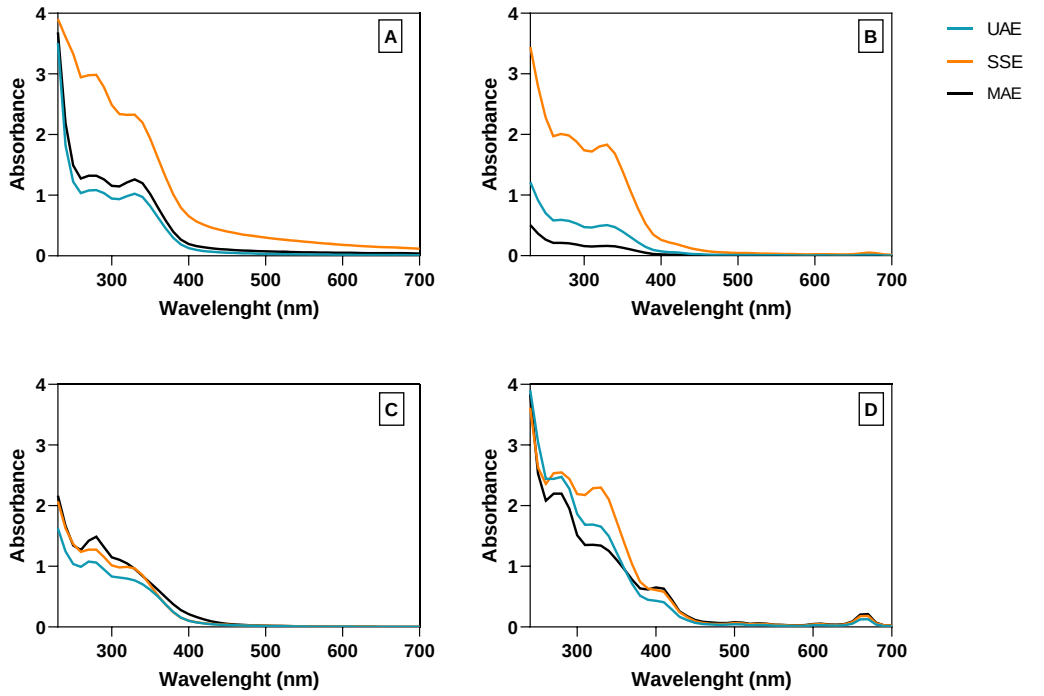

Figure 5IV: Absorption UV-Vis spectra of donzela extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

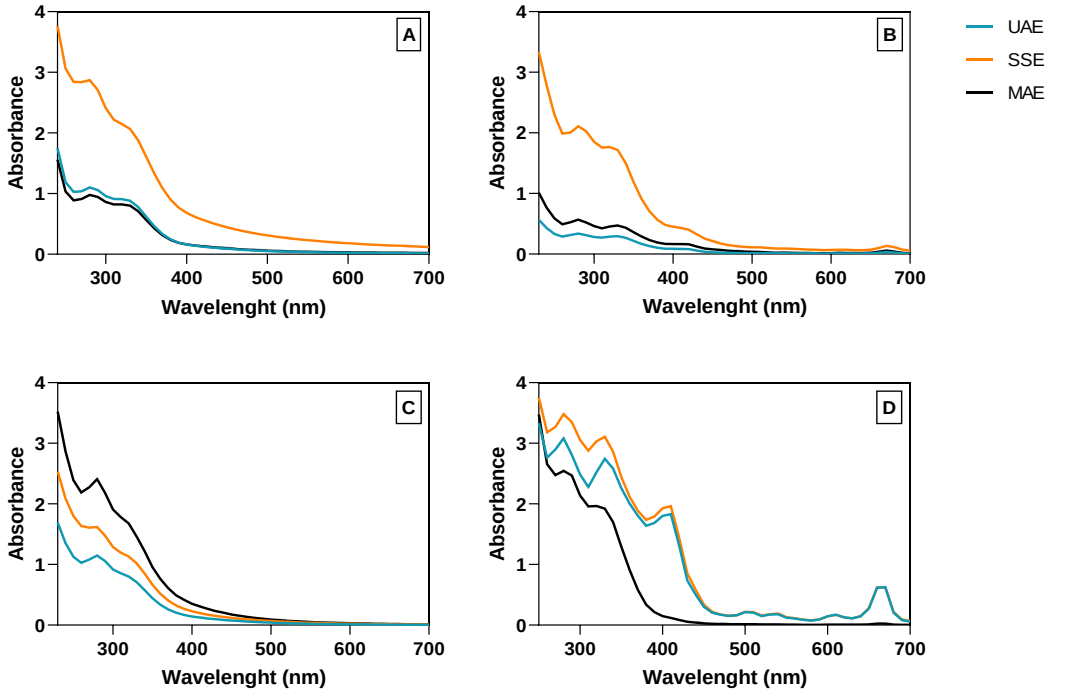

Figure 6IV: Absorption UV-Vis spectra of *O. gratissimum* extracts. (A) DES 28, (B) DES 90, (C) H_2O, and (D) Dioxane.

ANNEX V – Polyester dyeing with DES

Table V1: Polyester dyed with DES 3 + DB124 (A) and DB134 (B) - laboratory dyeing machine.

(A) DES	Sample
4	
4 – 25% H_2O	
4 – 50% H_2O	
6 – 25% H_2O	
6 – 50% H_2O	
7	
7 – 25% H_2O	
7 – 50% H_2O	

(B) DES	Sample
4	
4 – 25% H_2O	
4 – 50% H_2O	
6 – 25% H_2O	
6 – 50% H_2O	
7	
7 – 25% H_2O	
7 – 50% H_2O	

Table V2: Polyester dyed with DB124 (A) and DB134 (B) – sealed system

(A) DES	Sample
4	
4 – 25% H_2O	
4 – 50% H_2O	
5 – 25% H_2O	
5 – 50% H_2O	
6 – 25% H_2O	
6 – 50% H_2O	
7	
7 – 25% H_2O	
7 – 50% H_2O	

(B) DES	Sample
4	
4 – 25% H_2O	
4 – 50% H_2O	
6 – 25% H_2O	
6 – 50% H_2O	
7	
7 – 25% H_2O	
7 – 50% H_2O	

ANNEX VI – Calibration curves of DB124 and DB134 on DES 4

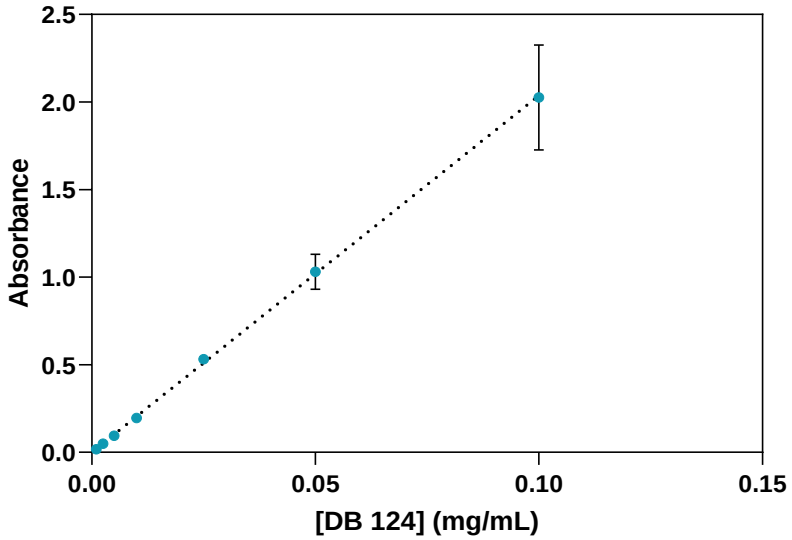

Figure 1VI: DB124 calibration curve, where **Absorbance (590nm)=(20,4±0,4) x [DB124] (mg/ml) + (0,00±0,02)**, R^2=0,9997. The calibration curve has a LOD of 0,002 mg/mL and LOQ of 0,01 mg/mL.

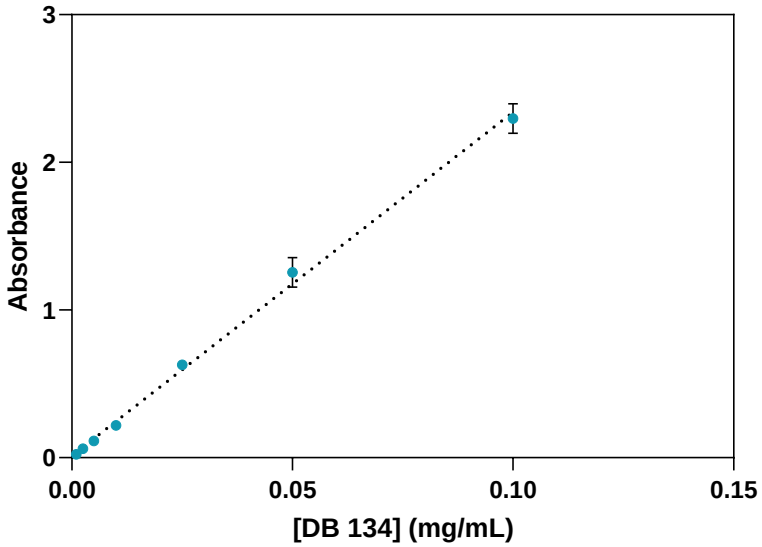

Figure 2VI: DB134 calibration curve, where **Absorbance (640nm)=(23,3±1,3) x [DB134] (mg/ml) + (0,01±0,06)**, R^2=0,9976. The calibration curve has a LOD of 0,006 mg/mL and a LOQ of 0,02 mg/mL.

ANNEX VII – Statistical analysis of DPPH scavenging activity

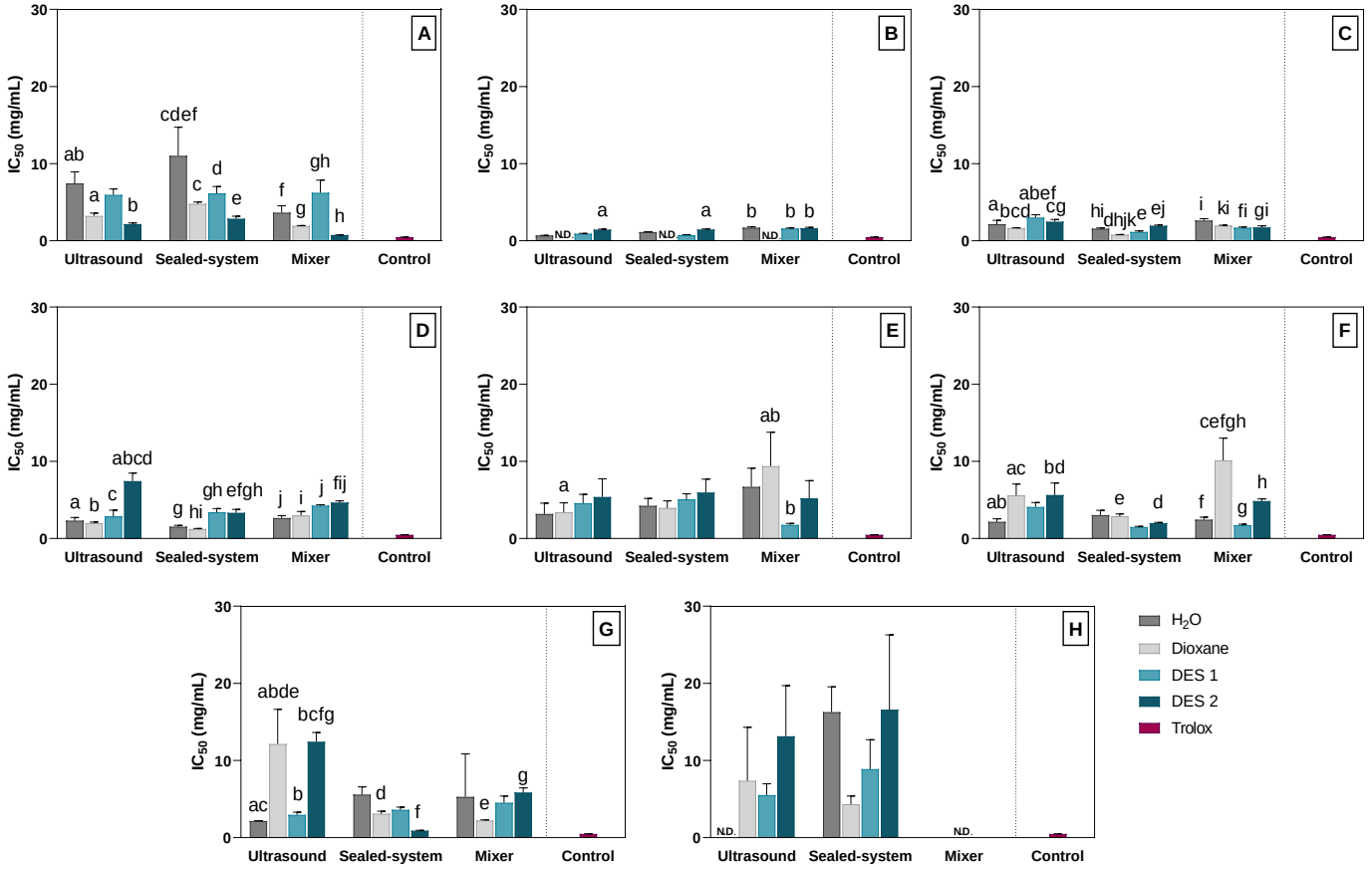

Figure 1VII: IC_{50} results of DPPH scavenging activity of plant extracts obtained with different solvents and extraction methods. (A) Cherry bay, (B) Cocoa beans, (C) Canta viril, (D) Carqueja, (E) Damiana, (F) Donzela, (G) African basil and (H) Pollen. Triplicate readings were performed for each condition, and data is expressed as mean ± SD. The results were statistically analysed by two-way ANOVA, followed by Tukey's multiple comparison test. Equal letters represent statistically significant difference ($p<0,05$), with the exception of cocoa data, where equal letter represent no statistically significant difference and pollen data in which there is no statistically significant difference.

ANNEX VIII – Information from where images were adapted

Images from Figure 2 were adapted from:

https://plants.sc.egov.usda.gov/home

https://cactusconservation.org/

https://jb.utad.pt/

http://flora-peninsula-indica.ces.iisc.ac.in/

https://www.tinroofteas.com/product/bee-pollen-organic