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Effect of solvent extraction on Tunisian esparto wax composition

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Abstract: The increase of needs for renewable and vegetable based materials will help to drive the market growth of vegetable waxes. Because of their highly variable composition and physicochemical properties, plant waxes have found numerous applications in the: food, cosmetic, candle, coating, polish etc... The aim of this project is to determine the effect of solvent extraction (petroleum ether and ethanol) on Tunisian esparto wax composition. The GC-MS was applied in order to determine the waxes compositions. Then, physicochemical parameters of these two samples of waxes: acid value, saponification value, iodine value and melting point were measured in order to deduct their properties and possible fields of uses. Results showed that esparto wax composition depended on the solvent extraction and that major components of the two samples of waxes were: alkanes, esters of fatty acids and phenols. Furthermore, esparto waxes were characterized by an antioxidant and antibacterial activities but the potential of these activities depended on the solvent of wax extraction.

Keywords: *Esparto wax, ethanol, petroleum ether, physicochemical parameters, antibacterial activity, antioxidant activity.*

I. Introduction

The surfaces of all plants are covered by a layer of cuticular waxes. The primary role of these waxes is to prevent uncontrolled water loss. The chemical composition of plant cuticular waxes can affect the resistance of plants to herbivores and herbivores behaviors. It enhances or deters insect oviposition, movement or feeding [1]. These waxes are complex mixture of both aliphatic and cyclic compounds [2][3][4], generally a various groups of long chain lipids such as hydrocarbons, wax esters(esters of long chain alcohols and fatty acids, alkyl esters), fatty acids, long chain alcohols, aldehydes and ketones. Cyclic components consist mainly of sterols and triterpenoids with various functionality (antibacterial, antioxidant…)[1]. Mangesh et al [5], for exemple, reported that the sterols and sec-butyl isothiocyanate presented in the Sugarcane wax have anti bacterial activity. The chemical composition of plant waxes depends on many factors such as: the organ or the source of wax extraction [6], age of the plant, plant species [7], environmental conditions [1] [7], technique and solvent extraction [8].

Waxes are the basis of many different applications and are important raw material for many industries due to their various compositions and properties. The main application for wax is candles which accounts for 43% of the application of waxes. The second biggest application is board sizing which holds 15% of the wax market. Impregnation of petroleum wax is typical for paper industries. Another major application for waxes is coating and this includes coating for wires, food packaging, fruits, paper, tables and wrapping as waxes are hydrophobic and can provide water proofing of the products [9,10,11].

In addition, waxes are also used in adhesives as laminator and protective coating and a number of different polishes from shoes to cars. A large number of cosmetics also have waxes in their formulations for different purposes including lip-sticks, skin cream, depilatory wax etc. Lanolin can self-emulsify into stable water in oil emulsion and has emollient properties which are ideal as a cosmetic ingredient for skin creams. Waxes can also be blended into different products for improved performance. Paraffin wax is added to rubber to increase the stiffness of the product, carnauba wax is blended into candles at a low percentage as a hardener and beeswax is incorporated into coatings to increase flexibility. Many formulation or applications involve more than one wax. In a typical lip-stick formulation, a combination of waxes is formulated into the stick for various functions. Candelilla wax is formulated in as a harder; lanolin serves as an emollient and helps the stick to remain a homogeneous mass whereas carnauba wax is added in order to increase the toughness of the stick. So the identification of the chemical compounds in the wax is important as it is directly related to its physical properties which can influence its applications. Commercial waxes are graded, characterized by a number of properties and tested according to various pharmacopeia guidelines depending on its application [11,12]. Chemical constants such as acid value, saponification value and iodine value are commonly determined for waxes as they give an overview into the chemical components.

So the aim of this work was to determine the composition and the physicochemical properties (acid value, saponification value, iodine value and melting point) of esparto wax extracted with two solvents: petroleum ether and ethanol in order to characterize identify and classify it and their possibility of uses. So, the effect of solvent extraction on esparto wax extraction was deducted. It' the first time in Tunisia that esparto wax was extracted and characterized for until uses. It was, always, eliminated to extract esparto fibers.

II. Materials and methods

Waxes were extracted from esparto leaves with soxhlet apparatus (following the AOCS method Aa [13], then stored in the freezer at 4°C. Esparto waxes were extracted with two different solvents (ethanol and petroleum ether). In order to determine the composition of these waxes, the difference between them and the effect of the solvent extraction on wax composition, gas chromatography mass spectrometry was applied. For gas chromatographic analysis, 1µg of esparto wax was dissolved (1%) in hexane. A gas chromatograph (HP5890-SERIE VI) with fused silica column HP5-MS (30m×0.25mm×0.52µm) and a HP-MSD5972A mass spectrometer. Helium was used as carrier gas at a flow rate of 1.2ml/min. The column temperature increased from 50°C to 280°C at rate of 5°C/min with two bearings for 1 minute respectively at 50°C and 280°C. The temperature of the injector, the interface and the ion source were kept respectively at 250°C, 280°C and 175°C. Nist and Wiley mass spectral data bases were used to identify the different compounds of the esparto waxes. The kovats index of each component confirmed its identification.

The chemical parameter like: acid value, iodine value and saponification value were done according to, respectively, ISO 660(1996), ISO 3961 (2009) and NF ISO 3657 (1990). The melting points were determined according to the 2nd edition of the European pharmacopoeia.

III. Results and Discussion

Gas chromatography was used for the separation of long chain fatty acids [5].The gas chromatograms of esparto waxes extracted with petroleum ether and ethanol were illustrated in the beginning paragraph.

Mass spectrums of: tributyl acetylcitrate, dibutyl sebacate, ethyl palmitate, BHT, Bis-(octylphenyl)-amine and Isodiospyrin, were represented respectively in Figure 3.

III.1. Determination of the composition of esparto wax extracted with petroleum ether

Figure 1 *represent the chromatogram of esparto wax extracted with petroleum ethe*

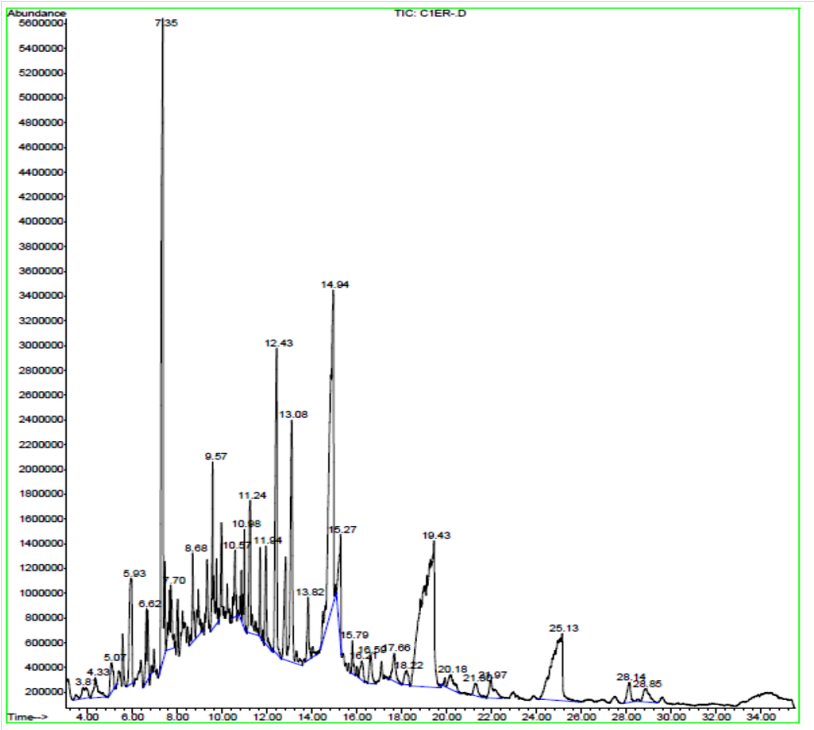

Figure1: *Chromatogram of esparto wax extracted with petroleum ether*

Table 1 shows the different compounds exiting in this esparto wax, their percents and their kovats index.

Table 1: *Classes and compounds of esparto wax extracted with petroleum ether*

Classes (%)	Major compounds	Compounds (%)	Kovats index
Alcohols (7.64%)	3-methyl-3-Cyclohexen-1-ol	2.86	1205
	2hexyldecanol	1.53	1790
	Hexadecanol	1.08	1812
Alkanes (47.15%)	Octacosane	16.59	2800
	Nonacosane	6.55	2900
	Heneicosane	2.95	2100
Alkenes (2.02%)	Tridecene	0.86	1292
Ester of fatty acids(28.13%)	Tributyl acetylcitrate	12.71	2594
	Dibutyl sebacate	5.15	2169
	Tributyl aconitate	4.63	2297
	Ethyl palmitate	2.72	1978
	Ethyl margarate	0.59	2077
Aldehydes(1.74%)	Tetradecanal	1.31	1615
Fatty acids (0.96%)	Tricosanoic acid	0.47	2664
	Pentacosanoic acid	0.5	2829
Phenols (11.83%)	BHT	11.83	1512
Total 99.47%			

According to table 1, esparto wax extracted with petroleum ether contains 7 classes of compounds namely: alkanes, alkenes, fatty acid, alcohols, esters of fatty acids, aldehydes and phenols. The major components were: alkanes (47.15%), esters of fatty acids (28.13%) and phenols (11.83%).

The alkanes presented (octacosane, nonacosane, heptacoane….) have a lubricant activity. In addition the length of these paraffins ranged from (C_{11}-C_{30}). All components presented a higher hydrocarbon chains causing the hydrophobia of this esparto wax.

The BHT was a phenolic compound having an anti oxidative activity. The majority of esters in the sample were characterized as plasticizer, emollient (Tributyl acetylcitrate, dibutyl sebacate, tributyl aconitate), lubricant products. However, the other esters presented an anti-inflammatory activity (ethyl palmitate) or were used as demulcent (ethyl margarate).

III.2. Determination of the composition of esparto wax extracted with ethanol

Figure 2 illustrates the chromatogram of esparto wax extracted with ethanol.

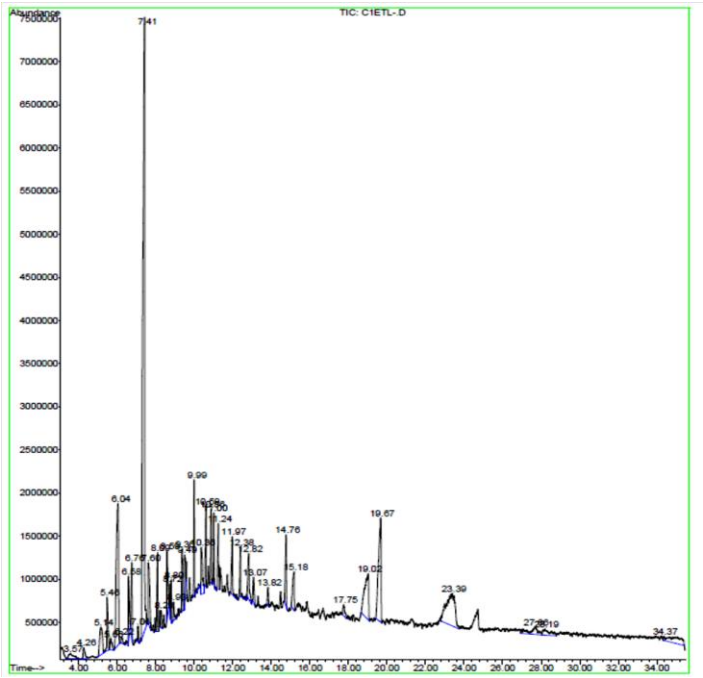

Figure 2: *Chromatogram of esparto wax extracted with ethanol*

Table 2: Different compounds of esparto wax extracted with ethanol

Classes (%)	Major compounds	Compounds (%)	Kovats index
Alcohols (9.35%)	3-methyl-3-Cyclohexen-1-ol	7.45	1205
	2hexyldecanol	0.2	1790
	Octadecanol	1.7	2084
Alkanes (27.7%)	Octacosane	4.99	2800
	Tetradecane	3.66	1400
	Tritriacontane	1.93	3300
Alkenes (7.54%)	1-dodecene	2.92	1192
Ester of fatty acids(17.6%)	Isooctylphtalate	6.1	2832
	Ethyl linoleate	2.12	2159
	Ethyl palmitate	2.1	1978
	Tributyl acetylcitrate	2.03	2594
	Methyl palmitate	1.48	1912
Aldehydes(1.03%)	Octadecanal	1.03	2017
Fatty acids (0.47%)	Tricosanoic acid	0.47	2628
Phenols (24.78%)	BHT	24.78	1512
Amines (7.36%)	Bis-(octylphenyl)-amine	7.36	2959
Aromatic hydrocarbon(1.49%)	Isodiospyrin	1.49	3656
Total 99,47%			

Table 2 showed the compound classes found in this esparto wax, their percents and their kovats index. In addition, the major compounds were indicated. According to this table, esparto wax extracted with ethanol contained 9 compound classes namely: alkanes, alkenes, alcohols, esters of fatty acids, fatty acids, aldehydes, phenols, amines and aromatic hydrocarbon. The major compound was the BHT characterized by an antioxidative activity. The major compound classes were: alkanes (27.7%), esters of fatty acids (17.6%) and phenols (24.78%). In addition, this esparto wax sample contained the isodiospyrin and the Bis-(octylphenyl)-amine having antibacterial properties. Also, the palmitate compounds (methyl palmitate and ethyl palmitate) existing at average of 3.6% had an anti inflammatory activity.

III.3. Comparison of the compositions of esparto waxes extracted with petroleum ether and ethanol

Table 3 showed a comparative account of different compounds classes for each type of esparto wax.

Table 3: Comparative table of the two esparto waxes samples

Compounds (%)	Petroleum ether wax extract	Ethanol wax extract
Alkenes	2.02	7.54
Alkanes	47.15	27.7
Fatty alcohols	7.64	9.35
Esters of fatty acids	28.13	17.6
Fatty acids	0.96	0.47
Aldehydes	1.74	1.03
Phenols	11.83	24.78
Amines		7,36
Cyclic hydrocarbons		1,49

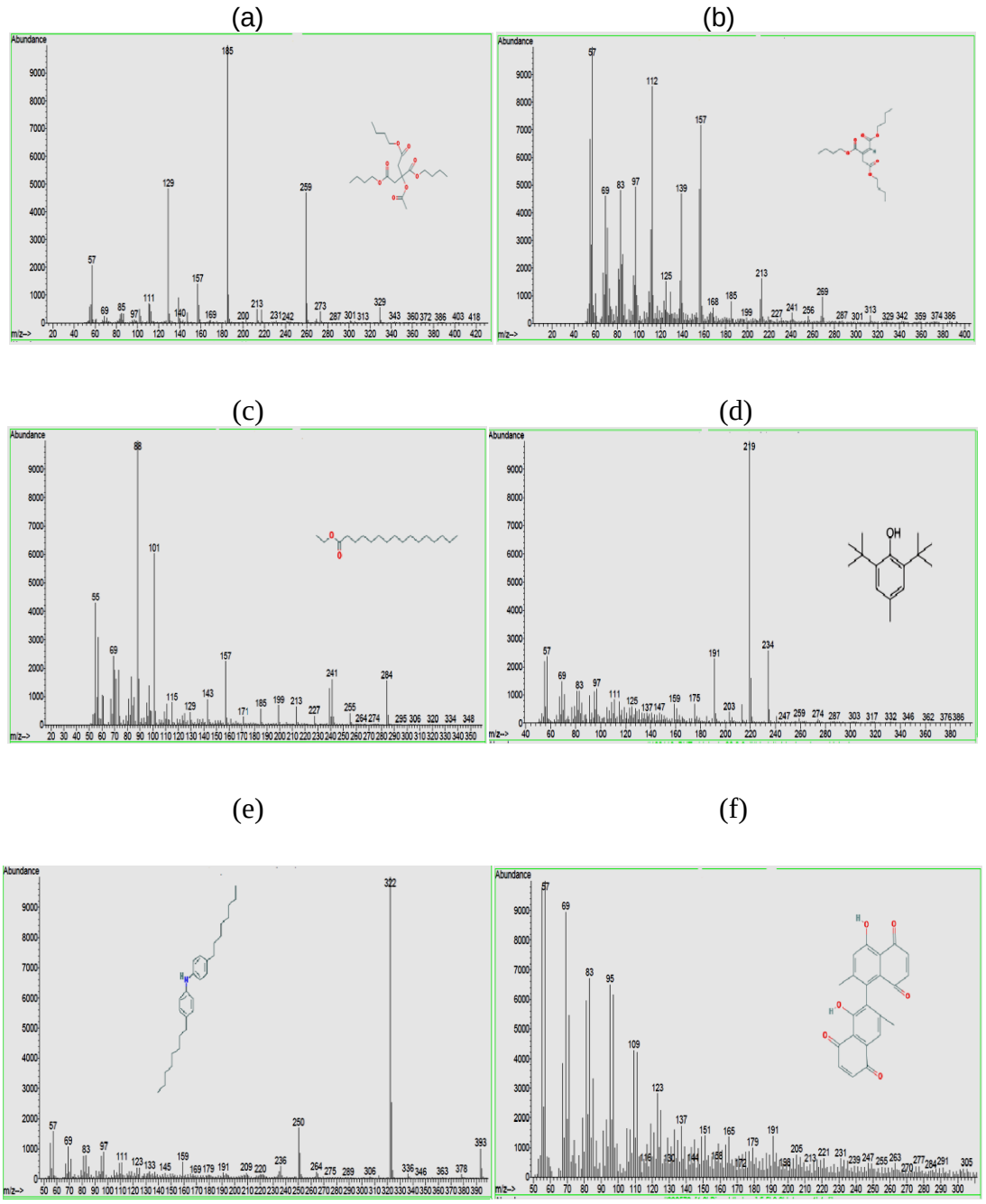

Figure 3 *: Mass spectrums of: (a)tributyl acetylcitrate,(b)dibutyl sebacate, (c)ethyl palmitate,(d)BHT, (e)bis-octylphenylamine and (f)isodiospyrin*

Table 4 showed a comparative account of physicochemical parameters of esparto waxes extracted with petroleum ether an ethanol.

Table 4: *Physicochemical parameters of esparto waxes*

Physicochemical parameters	Esparto wax extracted with petroleum ether	Esparto wax extracted with ethanol
Acide value	16.01	17.73
Iodine value	2.02	4.69
Saponification value	73.2	68.1
Melting point(°C)	64	86

According to table 3, the major compound class for the two wax samples were alkanes or hydrocarbons, which was an accord with the results found by A.P.Tulloch[14].

The quantitative analysis resulted from the GC-MS demonstrated that chemical composition of esparto wax depended on the solvent used for wax extraction. Not only, percents of the compounds changed but also a new components appeared (amines and aromatic hydrocarbon were present only in the ethanol wax extract).

The ethanol esparto wax extract presented two interesting properties: antioxidant and antibacterial derived, respectively, from the BHT and the Bis(octylphenyl)amine and the isodiospyrin. In addition, the BHT was the major antioxidant used for food and cosmetic industries etc…Also, Mei Fen Tsou et al showed that BHT presented an antibacterial affect [15]. The originality of this BHT extracted was that it was a natural product.

In referring to table 4, the acid value of ethanol wax was higher than the petroleum ether wax.

The highest percent of unsaturated content in the ethanol esparto wax extract (7.54%) justify its highest iodine value (4.69) compared to petroleum ether wax extract. This unsaturated affects the stability of this esparto wax sample for until uses. This increase in unsaturated for this sample wax was due to the increase of the temperature of extraction compared to petroleum ether (the boiling point of ethanol is 78°C whereas the boiling point of the petroleum ether is 40°C -60°C).

As result to the highest content of esters of fatty acids, the petroleum ether esparto wax extract had the highest saponification value.

In addition, the esparto wax extracted with ethanol had the highest melting point due to the presence of hydrocarbons of higher length (C_{33}). These result provided that the extracting power of ethanol was higher than the extracting power of petroleum ether. Having a high melting point, gives a high stability of this ethanol esparto wax extract for further uses.

IV. Conclusion

The physicochemical parameters: acid value, iodine value and saponification value and the melting point were related to the composition of esparto wax which depended on the solvent used for the extraction. The GC-MS analysis of esparto waxes extracted illustrated an antibacterial and antioxidant activity. Finally, the composition of esparto wax judged its properties and then its fields of uses.

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