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Properties, variations, roles, and potential applications of epicuticular wax: a review

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Abstract: The cuticular wax layer covers the aerial surface of plants and acts as a barrier between plants and the environment. The cuticle plays a key role in the protection of plants from pathogens, UV light, and transpiration. Variation in the wax quality and quantity is influenced by factors like the solvent used for extraction, species, ontogeny, and season. Compounds isolated from the cuticle layer have been studied by various methods and were found to play an important role from the ecological and physiological points of view. These compounds include esters, alcohols, ether, alkane, and aldehydes. Nonpolar compounds help reduce water loss in plants. The wax can be explored for its potential applications in developing sustainable green packaging material. This review article will facilitate biologists and nonbiologists to get comprehensive and updated knowledge about various aspects of cuticular wax including its chemical composition and variations among different species and seasons. Further studies of the wax composition will pave the way for classification of plant species and an understanding of plant protection from biotic and abiotic stresses.

Key words: Cuticle, protection, lotus effect, chemical composition

1. Introduction

The aerial plant surface is usually covered with a hydrophobic material called epicuticular wax. This waxy layer is an active interface between a plant and the environment. It is the first line of defense for the aerial plant body. Epicuticular wax protects the plant from several abiotic and biotic stresses. Epicuticular wax protects the plant from infection by insect, fungal, and bacterial pathogens and helps plants conserve water content by reducing the rate of transpiration (Taíz and Zeiger, 1991; Rhee et al., 1998; Znidarcic et al., 2008; Dutta and Laskar, 2009).

Epicuticular wax is a mixture of various classes of compounds, including n-alkanes (chain-length C21–C35), primary alcohols (C22–C40), fatty acids (C20–C24), aldehydes (C24–C36), secondary alcohols (C21–C35) with a tendency for midchain hydroxylation, ketones (C21–C35), diketones (C22–C36), and n-alkyl esters (C36–C60) in combination with long-chain primary alcohols and fatty acids (Baker, 1982; Walton, 1990).

The composition of epicuticular wax is influenced by plant genotype, the side and age of the leaf, and seasonal and climatic conditions. According to Walton (1990) the quantity of the wax is influenced by environmental factors but chemical composition remains conserved. Plant cuticular wax shows a high degree of crystallinity, low chemical reactivity, and hydrophobicity (Domínguez and Heredia, 1998). Hydrophobicity of the epicuticular wax depends upon the chemical composition of the wax. The presence of higher amounts of compounds having functional groups like –OH, -COOH, $-NO_2$, or -CO- provides hydrophilicity to the surface.

According to Barnes et al. (2009), 10% of total municipal waste consists of plastic. Plastic waste contaminates terrestrial, freshwater, and marine habitats. Plastic material and its fragments cause soil pollution (Zubris and Richards, 2005; Brinton, 2005). The need for biodegradable packaging material becomes important in the contemporary scenario.

Here, epicuticular wax offers a promising option in developing hydrophobic packaging material. Paper may be coated with epicuticular wax to obtain hydrophobic packaging material. In this aspect, recently Yadav et al. (2014) reported the use of epicuticular wax derived from *Calotropis procera* to increase hydrophobicity of paper.

In this review, various features of surface wax have been discussed in detail, like its morphology, extraction procedures, characterization, and factors affecting the composition and yield of wax along with the applications of epicuticular and cuticular wax in various sectors. Emphasis has been given on composition, yield, and application of surface wax. This paper will help readers gain knowledge

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and will aid in the goal of isolating an ecofriendly plant-derived material to develop hydrophobic material to be used in packaging and other industries.

2. Cuticle as a source of wax

The cuticle comprises the outermost layers of aerial plant parts (Jetter et al., 2006). It consists of cutin and wax. Based on the location of the wax, it can be divided into two layers: inside or outside the surface (Jeffree, 1986). In most plant species, epicuticular wax forms a smooth layer on the surface, whereas wax crystals form a rough surface in some species (Jeffree, 2006). The internal structure and design of cuticular wax can be studied easily by the extraction of the wax layer with the help of organic solvents (Jetter et al., 2006) because the solvent molecules enter the cuticle and provide a combination of epicuticular and intracuticular wax (Jetter et al., 2000). Gas chromatography and scanning electron microscopy have been used for separate analysis of both intracuticular and epicuticular wax layers (Jetter et al., 2000; Jetter and Schaffer, 2001).

Cuticle and epicuticular layers play important roles in the deposition of pesticides, growth regulators, and other agricultural chemicals (Martin and Juniper, 1970). The wax layer present on the outer surface of the plant is not only important for fruit development but also has implications on the economic aspects of viticultural commodities. It also scatters the light and gives a shiny appearance and this attracts the consumers of table grapes. The wax biosynthesis in plants is genetically governed by crosstalk, so it can also be used in the classification of plants or in establishing the relationship among them (Lemieux, 1996).

2.1. Morphology of surface wax

The cuticular wax layer consists of dendrites, filaments, plates, and tubes when viewed through a scanning electron microscope (Rashotte and Feldmann, 1998). According to Vogg et al. (2004) the cuticle is a thin, hydrophobic, and flexible membrane (0.1–10 μm). In some species, the epicuticular wax exists as a smooth layer that gives the surface a shiny appearance and sometimes it forms wax crystals, which result in the textured structure of the surface (Jeffree, 2006).

2.2. Composition

Cuticular wax is very specific in nature. It is organ- and tissue-specific in terms of composition. Plant epicuticular wax is a mixture of primarily long-chain aliphatic compounds. Primary alcohols, aldehydes, fatty acids, and alkyl esters have even-numbered chain lengths while other hydrocarbons like secondary alcohols and ketones have a majority of odd-numbered chain lengths (Walton, 1990). The composition of epicuticular wax has been investigated in several plants (Table 1).The aliphatic compounds include fatty acids, aldehydes, primary and secondary alcohols, ketones, and alkanes with chain length from C20 to C36 (Jeffree, 2006). Alkyl esters ranging from C38 to C70 are present in cuticular wax. Some cyclic compounds like triterpenoids, tocopherols, and aromatic compounds are present but their quantities vary. It is possible to extract the epicuticular and intracuticular wax separately with the help of solvents (Jetter et al., 2000; Jetter and Schaffer, 2001). The epicuticular wax of *Prunus laurocerasus* consists of aliphatic compounds and the intracuticular wax consists of high percentages of two cyclic triterpenoids (Jetter et al., 2000). Some compounds like triterpenoids are also reported in wax crystals of *Ricinus communis* (Guhling et al., 2006) and *Macaranga* spp. (Markstadter et al., 2000). There is wide diversity in the composition of cuticular wax among plant species (Vogg et al., 2004).

Hydroxy fatty acids are present as a major compound in the cuticular layer of all vascular plants (Kolattukudy, 1970, 1980; Martin and Juniper, 1970). Aliphatic compounds present in the epicuticular layer are n-alkanes, n-alkanals, n-alkanols, n-alkanoic acids, and wax esters (Kolattukudy, 1970; Simoneit and Mazurek, 1982; Simoneit, 1989).

Plant species show a very common composition of cuticular wax having very-long-chain aliphatic components, namely fatty acids, aldehydes, primary and secondary alcohols, ketones, and alkanes of chain lengths C20–C36, as well as C38–C70 alkyl esters (Bianchi, 1995; Jetter et al., 2006).

Abas and Simoneit (1998) reported hydrocarbons like carboxylic acid, ketone, and alcohol in the epicuticular wax of ten plants, namely *Calophyllum inophyllum*, *Cerbera odollarn*, *Eugenia grandis*, *Fagraea jragrans*, *Hevea brasiliensis*, *Melaleuca leucadendron*, *Mirnusops elengi*, *Mesua ferrea*, *Lagerstroemia indica*, and *Pterocarpus indicus*. *Mesua ferrea* showed the highest amount of wax (35.4 mg/g dry weight), whereas the lowest amount (1.2 mg/g dry weight) was present in *Eugenia grandis*. Analysis revealed that out of the n-alkenes present in the wax, the maximum amount was found to be of hentriacontane (C31), followed by tritriacontane (C33) and finally nonacosane (C29). A strong even-to-odd carbon number predominance is observed for both the n-alkanoic acids and alkanols. Epicuticular wax has several compounds like p-sitosterol, triterpenoids, triterpenes with α- and β-boswellic acids, α- and β-amyrin, α- and β-amyrones, friedelin, friedelanol, friedelane, olean-12-ene, taraxerene, squalene, dihydronyctanthic acid, dihydroroburic acid, and dihydrocanaric acid. *Arabis serotina* wax presumptively contains the following hydrocarbons: hexadecanoic acid, octadecanoic acid, tetracosanoic acid, hexacosanoic acid, octacosanoic acid, eitriacontanoic acid, dotriacontanoic acid, 1-docosanol, 1-tetracosanol, 1-hexacosanol, 1-octacosanol, 1-eitriacontanol, and 1-dotriacontanol (Catrow et al., 2009). Secondary alcohols like heptacosanol,

Table 1. Characterization of epicuticular wax extracted from plants in different types of solvents**.**

Plant	Solvent	Technique	Derivatized	Component	References
Arabis serotina	Hexane and chloroform	GC, MS	Yes	Alkanes, ketones, primary alcohols, secondary alcohols, or carboxylic acids	Catrow et al., 2009
Salix spp.	Chloroform	GCMS and SEM	Yes	Primary alcohols, fatty acids, aldehydes, n-alkanes, and wax esters	Szafranek et al., 2008
Ficus glomerata	Hexane	TLC, GC, SEM, FTIR, and SEM	No	n-Alkanes	Kundu and Sinhababu, 2013
Hosta spp.	Chloroform	TLC, GCMS	Yes	Primary alcohols	Jenks et al., 2002
Calophyllum inophyllum, *Cerbera odollarn*, *Eugenia grandis*, *Fagraea fragrans*, *Hevea brasiliensis*, *Melaleuca leucadendron*, *Mirnusops elengi*, *Mesua ferrea*, *Lagerstroemia indica*, and *Pterocarpus indicus*	Methylene chloride	TLC, GCMS	Yes	n-Alkanes, n-alkanoic acids, and n-alkanols	Abas and Simoneit, 1998
Pinus halepensis	Chloroform	GCMS, XRD, and differential scanning calorimetry	Yes	Secondary alcohol nonacosan-10-ol	Matas et al., 2003
Cocos nucifera	Hexane	TLC, GCMS, FTIR, NMR	No	Lupeol methyl ether, skimmiwallin, and iso-skimmiwalin	Erosa et al., 2002
Aspidospema pyrifolium, *Capparisyco*, *Maytenus rigida*, *Ziziphus joazeiro*, *Aristolochia esperanzae*, *Didymopanax vinosum*, *Strychnos pseudoquina*, and *Tocoyena formosa*	Chloroform	Column chromatography, TLC, and GCMS	No	n-Alkanes and alcoholic triterpenes, hentriacontan-16-one (a ketone) and ursolic acid (an acid triterpene)	Oliveira et al., 2003
Rosa canina	Chloroform	GCMS and SEM	Yes	Alkanes, primary alcohols, alkyl esters, triterpenoids, secondary alcohols, alkenols (unsaturated primary alcohols), and benzyl esters	Buschhaus et al., 2007a
Grape berries	Chloroform	SEM	No	-------	Rosenquist and Morrison, 1988
Sorghum bicolor	Chloroform	Colorimetric method and gravimetric	No	-------	Ebercon et al., 1977
Helicanthus elasticus	Chloroform	Column chromatography, FTIR, HPLC, and MS	No	Triterpenoid (lupeol)	Kedar and Jadhav, 2012
Ligustrum vulgare	Chloroform	GCMS and SEM	Yes	Aliphatic compound and cyclic triterpenoids	Buschhaus et al., 2007b

Table 1. (Continued).

Picea pungens	Chloroform	SEM	No	-------	Reicosky and Hanover, 1978
Clusia spp.	Hexane	GCMS	No	Alkanes and triterpenes	Medina et al., 2006
Dudleya sp.	Et_2O	TLC, FTIR	No	Long chain alkanes, wax esters, primary alcohols and carboxylic acids.	Manheim and Mulroy, 1978
Vitis vinifera	Chloroform	XRD, differential scanning calorimetry, GCMS	Yes	n-Alcohols and n-fatty acids and cyclic terpenoid oleanolic acid	Casado and Heredia, 1999
Mandevilla guanabarica and *Mandevilla moricandiana*	n-Hexane and chloroform	GC, MS, NMR	No	n-Alkanes and triterpenes	Cordeiro et al., 2011

GC: Gas chromatography, MS: mass spectrometry, NMR: nuclear magnetic resonance, TLC: thin-layer chromatography, SEM: scanning electron microscopy, XRD: X-ray diffraction; FTIR: Fourier transformation infrared spectroscopy; HPLC: high-performance liquid chromatography.

nonacosanol, hentriacontanol, tritriacontanol are also present in this wax.

The cuticular wax of *Salix* shows the presence of polar compounds like n-alcohols, free fatty acids, and n-aldehydes (Szafranek et al., 2008). Aldehyde usually varies from 0.4 to 4 g/cm^2 in cuticular wax of *Salix* (Hietala et al., 1995, 1997). However, according to Cameron et al. (2002), aldehydes are present as minor components in wax. Hydrocarbons contributed 68.82% in n-hexane extract of the epicuticular wax of *Ficus glomerata* leaves (Kundu and Sinhababu, 2013). These hydrocarbons show the presence of hexadecane (5.92%), heptadecane (6.18%) hentriacontane (5.47%), nonacosane (5.29%), heptacosane (5.11%), and docosane (2.16%). Saber et al. (2010) and Chowdhury et al. (2010) also reported that the alkanes with odd number of C chains are dominant in the epicuticular wax of plant leaves. The presence of secondary alcohol nonacosan-10-ol in cuticular wax of *Pinus halpensis* was reported by Matas et al. (2003). According to them, nonacosan-10-ol is the main component of the epicuticular wax. This study provides information about the chemical variation that occurs during leaf aging and after interaction with air pollutants in the epicuticular wax layer. Erosa et al. (2002) reported the composition of the hexane extract of leaves of *Cocos nucifera*, which consists of lupeol methyl ether, skimmiwallin, and iso-skimmiwallin. Major compositional differences were observed between the abaxial and adaxial surface layers of rose (*Rosa canina*) leaves (Buschhaus et al., 2007a). The adaxial surface shows a wax composition rich in some aliphatic compounds including secondary alcohols, whereas the inner surface of leaves had large amounts of triterpenoids. Olenolic acid, betulin, and lupeol were identified as commonly present triterpenoids in the cuticular wax of leaves of *Helicanthus elasticus* (Loranthaceae) (Kedar and Jadhav, 2012). Buschhaus et al. (2007b) reported that the outer wax layer of leaves of *Ligustrum vulgare* extracted with the help of gum arabic consisted entirely of homologous series of very-long-chain aliphatic compounds. By contrast, the inner wax layer was dominated by two cyclic triterpenoids (80%), namely ursolic and oleanolic acid. Lee et al. (2015) studied the cuticular wax of broccoli bloomed (MC91) and bloomless (MC117). The total amount of wax present in MC91 was 1.07- to 3.791-fold higher in comparison to MC117. The wax composition does not differ much, except for few compounds present in high levels in MC91, like C29 alkanes, C29 secondary alcohols, and C29 ketones. However, a high amount of C31 alkane is present in MC117. Surface wax study was carried out in *Triticum aestivum*, *Zea mays*, and *Lupinus angustifolius* (Nadiminti et al., 2015), where they reported that wax composition of *T. aestivum* had long-chain even-numbered saturated fatty acids (C16–C30), alcohols (C24–C30), and alkanes (C23–C37), except hentriacontane (C31 alkane). *L. angustifolious* and *Z. mays* followed the same trend of alkanes as observed in *T. aestivum*. Primary alcohols are the base of wax plates present in the epicuticular wax of all three species. The presence of alkyl alkanoates, terpenes, sterols, alkanals, alkanoic acids, ketones, hydrocarbons, and alkanols has been reported in leaves of *Actinidia deliciosa* (Celano et al., 2006). Batovska et al. (2009) studied the leaf wax component of 16 grapevine plants (*Vitis vinifera*) in summer and autumn. The wax components present in leaves were aldehydes, hydrocarbons, terpenes, free and esterified fatty acids, alcohols, sterols, and ketones. Hydrocarbons are mostly present in summer with a range

of 18 to 31 carbons. The leaf area that was exposed to air showed the presence of long-chain hydrocarbons, which facilitate control of the transpiration process and provide defense against microbes and chemicals. Fatty acids are only present in the summer. Tetradecanoic (myristic), octadecanoic (stearic), and hexadecanoic (palmitic) acids provide strength to cell membranes in higher plants. The absence of these compounds in autumn leads to permeability of the cell membrane, in turn leading to senescence of the plant. Decylisobutyrate, methyl tetradecanoate, and long-chain alcohols tetradecanol and hexadecanol were only present in summer, while methyl hexadecanoate, methyl tetracosanoate, and carbonyl compounds were present in winter only. Another study was carried out to understand the chemical composition of cuticular wax in 12 populations of *Plantago major* and 5 populations of *Plantago depressa* with 2.0 °C to 18.48 °C annual temperature. With the increase in temperature, the relative content of alkanes (C29, C31) decreased while C33, C35, and average chain length (ACL) total, and ACL 27–33 increased (Guo et al., 2015). ToF-SIMS was used by Jetter and Sodhi (2011) to analyze the leaves of *Kalanchoe daigremontiana*. Glutinol and friedelin were present in high concentrations on the abaxial side of the leaf. The results indicated that all the compounds were distributed evenly on the lower leaf surface, showing no apparent gradients across the outer and inner surface areas of the leaf.

2.3. Techniques used in wax analysis

To analyze the epicuticular wax in terms of its quantity and quality, several techniques have been applied. Epicuticular wax has been extracted by mechanical and chemical methods. Gum arabic has been used to peel out the epicuticular wax from several plant species, namely *Ligustrum vulgare*, *Rosa canina*, and *Prunus laurocerasus* (Jetter and Schäffer, 2001; Buschhaus et al., 2007a, 2007b). Organic solvents like benzene, chloroform, hexane, acetone, dichloromethane, methanol, and ethanol have been used on a large scale for surface wax extraction (Abas and Simoneit, 1998; Erosa et al., 2002; Szafranek et al., 2008; Yadav et al., 2014). The wax has been further quantified by the gravimetric method (Ebercon et al., 1977). The quantity of extracted wax varies (0.9 μg/cm^2 to 100 μg/cm^2) in different genera. This is evident from several reports as presented in Table 2.

Mostly chromatographic and spectroscopic techniques are in practice for characterization of surface wax. Fourier transform infrared spectroscopy has been used to reveal the information about the functional groups present in the wax (Odlyha, 1995). Separation methods such as supercritical gas chromatography (Hamilton, 1995), liquid chromatography (Asperger et al., 2001), gas chromatography-flame ionization detection (Marinach et al., 2004), or pyrolysis gas chromatography (Regert, 2005) are in practice now. Gas chromatography (GC) and mass spectrometry (MS) is the best combination of techniques to identify and quantify the compounds present in the surface wax of plants (Regert, 2005). For analysis of nonvolatile compounds of wax by GC, these compounds (fatty acids and fatty alcohols) must be derivatized (Asperger et al., 1999). However, other compounds like fatty hydrocarbons are volatile in nature so these can be analyzed directly (Grob et al., 1994). Epicuticular wax lipids from leaves of plants growing in Klang Valley, Malaysia, were extracted by dichloromethane (Abas and Simoneit, 1998). After derivatization, the wax was analyzed by GC and MS, which showed that hentriacontane was the dominant n-alkane, followed by tritriacontane and nonacosane, in most of the species (Abas and Simoneit, 1998). Catrow et al. (2009) reported the surface wax composition of *Arabis serotina* using GC. It revealed that the wax contains organic acids, alcohols, and alkanes. Buschhaus et al. (2007b) reported the composition of the outer and inner wax of leaves of *Ligustrum vulgare* using gum arabic and GC with flame ionization detection and mass spectrometry. X-ray diffraction and differential scanning calorimetry techniques have also been used to explore the structure of surface wax in *Vitis vinifera*; alcohols and fatty acids were the major compounds of the wax (Casado and Heredia, 1999).

2.4. Factors affecting yield of extracted wax

Several attempts have been made to classify plants into different groups based on the similarities or dissimilarities of their cuticular wax (Maffei, 1996; Mimura et al., 1998). The variation in wax composition may be attributed to genetic (genotype and mutation) and environmental factors (light and temperature) (Bianchi, 1995; Szafranek et al., 2008). Some of these factors are discussed in the following paragraphs.

2.4.1. Season

Jenks et al. (2002) studied the variation in the quantity of wax in three genotypes of *Hosta*, *H. plantaginea*, *H. lancifolia*, and *H.* 'Krossa Regal', during a year. The highest amount of wax was extracted in the spring season from the abaxial side of 'Krossa Regal' (17.636 μg/cm^2) soon after full leaf development. A large fall in wax amount extracted from the abaxial surface was observed from spring (17.636 μg/cm^2) to summer (7.126 μg/cm^2) in this taxon. Earlier studies also reported that the wax quantity in leaves is reduced after flowering (Freeman et al., 1979; Jenks et al., 1996).

2.4.2. Species

The compositions of epicuticular wax crystals present on leaves of *Prunus laurocerasus*, the pitcher traps of the *Nepenthes* species, and leaves of *Pisum sativum* have been

Table 2. Quantitative variation in epicuticular wax contents among various plant species.

Plant	Solvent	Leaf side/source	Quantity of wax (μg/cm^2)	References
Salix alba	Chloroform	Cuticular	98	Szafranek et al., 2008
Salix fragilis	Chloroform	Cuticular	75	
Salix × *rubens*	Chloroform	Cuticular	100	
Ligustrum vulgare	Gum Arabic	Adaxial	28	Buschhaus et al., 2007b
Hosta spp.	Chloroform	Abaxial and adaxial	17.63	Jenks et al., 2002
Clusia spp.	Hexane	Abaxial and adaxial	29.3	Medina et al., 2006
Wollemia nobilis	Chloroform	Adaxial and abaxial	35	Dragota and Riederer, 2007
Sesamum indicum	Chloroform	Cuticular wax	7.69	Kim et al., 2007
Prunus laurocerasus	Chloroform	Cuticular wax	45	Jetter et al., 2000
Prunus laurocerasus	Chloroform	Adaxial	54	Jetter and Schaffer, 2001
Arabidopsis thaliana	Chloroform	Total wax	0.9	Buschhaus and Jetter, 2012
Nicotiana glauca	Dichloromethane	Total wax	11.5	Cameron et al., 2006
Zea mays	Hexane	Adaxial and abaxial	11.09	Ristic and Jenks, 2002
Brassica oleracea	Diethyl ether	Surface wax	33·3	Denna, 1970
Pisum sativum	Chloroform	Epicuticular wax	43	Sanchez et al., 2001
Triticum spp.	Chloroform	Epicuticular wax	34.3	Uddin and Marshall, 1988

reported (Jetter et al.., 2000; Riedel et al., 2003; Gniwotta et al., 2005; Riedel et al., 2007). Wax content of leaves varies with species in *Hosta* (Jenks et al., 2002) as the maximum wax content was found to be 17.636 μg/cm^2 (abaxial side), 6.299 μg/cm^2 (adaxial side), and 7.477 μg/cm^2 (adaxial side) in 'Krossa Regal', *H. plantaginea*, and *H. lancifolia*, respectively. During summer the total wax quantity of wax from abaxial leaf surfaces decreased in *H. plantaginea*, *H. lancifolia*, and 'Krossa Regal' by 3.8-, 7.2-, and 2.5-fold, respectively. Similarly, on the adaxial side, the wax quantity decreased by 3.0-, 4.5-, and 3.3-fold in *H. plantaginea*, *H. lancifolia*, and 'Krossa Regal', respectively. Lack of precipitation immediately after the expansion of leaves during the spring season may be one of the reasons for the reduction in wax quantity (Jenks et al., 2002). Variations in epicuticular wax in two genotypes of *Salix* species (*Salix alba* and *S. fragilis*) and their hybrid (*S.* × *rubens*) were reported by Szafranek et al. (2008). The wax amount was found to be 98 μg/cm^2, 75 μg/cm^2, and 100 μg/cm^2 in *S. alba*, *S. fragilis*, and *S.* × *rubens*, respectively. All three genotypes were reported to contain a high quantity (8–18 μg/cm^2, 4–15 μg/cm^2, and 3.8 to 11.6 μg/cm^2) of polar compounds (*n*-alcohols, free fatty acids, and *n*-aldehydes, respectively). However, earlier studies of *Salix* taxa (*S. purpurea*, *S. dasyclados*, *S. eriocephala*, *S. myrsinifolia*, *S. viminalis*, *S. dasyclados* × *S. triandra*) indicated the presence of low levels of these compounds (Hietala et al., 1995, 1997; Cameron et al., 2002). Hietala et al. (1995, 1997) reported that aldehyde quantity varied from 0.4 to 4 μg/cm^2. Cameron et al. (2002) showed that quantity and composition of wax varied with species, namely *Salix* and *Populus*, under the same environmental conditions. Recently, a study was carried out on the variation of wax quantity in 35 plant species by Maiti et al. (2016) extracted from *Helietta parvifolia*, *Amyris texana*, *Leucophylum leucocephala*, *Zanthoxylum fagara*, *Karwinskia humboldtiana*, *Celtis pallida*, *Guaiacum angustifolium*, *Bernardia myricifolia*, *Forestiera angustifolia*, *Croton suaveolens*, *Eysenhardtia polystachya*, *Cordia boissieri*, *Ehretia anacua*, *Caesalpinia mexicana*, *Condalia hoockeri*, *Sargentia gregii*, *Diospyros palmeri*, *Bumelia celastrina*, *Ebenopsis ebano*, *Leucaena leucocephala*, *Celtis laevigata*, *Cercidium macrum*, *Acacia rigidula*, *Gymnosperma glutinosum*, *Acacia farnesiana*, *Lantana macropoda*, *Berberis chococo*, *Diospyros texana*, *Acacia berlandieri*, *Quercus polymorpha*, *Salix lasiolepis*, *Acacia shaffneri*, *Prosopis laevigata*, *Parkinsonia aculeata*, and *Acacia wrightii* in the month of June to examine the variations. The amount of wax varied from 11.18 μg/cm^2 (*Amyris texana*) to 702.04 μg/cm^2 (*Forestiera angustifolia*). Dragota and Riederer (2009) studied the composition of wax from the adaxial and abaxial sides of *Araucaria araucana*, *Agathis robusta*, and *Wollenia nobilis*. The main components of wax were secondary alcohols, *n*-alkanes, and alkane diols. Secondary alcohols and alkane diols were reported to be responsible for the development of

the tubular epicuticular wax crystals. Nonacosan-10-ol contributed to tubule formation. *A. robusta* and *W. nobilis* contain very small amounts of nonacosan-10-ol homologs. Epicuticular wax contains 69% of *n*-alkanes, which help in the formation of interspersed granular crystals. On the surface of *A. robusta* leaves perpendicular platelets were found, similar to the abaxial leaf side of *W. nobilis.*

Braccini et al. (2015) studied the role of cuticular waxes for oviposition acceptance by willow sawfly females in *Salix nigra* and *S. viminalis*. *S. nigra* is preferred by willow sawfly females for oviposition as it contains three times more volatile compounds in comparison to *S. viminalis*. *S. viminalis* contain 97% alkanes while *S. nigra* contains alcohols, acids, and esters.

Another study on variability in wax composition was done by Zlatković et al. (2016) on *Sedum album*, *S. micranthum*, *S. athoum*, and *S. serpentini*. The surface was covered with horizontal wax crusts, which were further divided by a prominent network of fissures. Around the stomatal apertures aggregates of wax filaments were present. Wax scales have rectangular to irregular (polygonal) shapes with crenulated edges. Many wax scales mostly covered the surface, which matched the epidermal cells. *S. album* and *S. micranthum* have low cuticular wax content of *n*-alkane C30. *S. athoum* has a high content of *n*-alkane C27 and a low content of C33, C32, and C35, whereas *S. serpentini* showed high content of *n*-alkane C32 and low content of C27 and C29.

2.4.3. Ontogeny

Jetter and Schaffer (2001) studied the seasonal development of the adaxial leaf surfaces and wax of *Prunus laurocerasus*. During epidermal cell expansion around 50 µg of alkyl acetate was present within 10 days of epidermal cell expansion and the epicuticular wax film thickness was 30 nm. After 18 days of development of leaves, alcohols started accumulating. The thickness of the epicuticular wax film also increased (approximately 60 nm after 60 days) and various other compounds also started contributing to epicuticular wax composition (fatty acids, aldehydes, and alkyl esters). The intracuticular wax showed a constant trend during the development. Variations in quantity and quality of epicuticular wax in mature and emerging leaves of oak (*Quercus robur* L.) were reported by Gülz and Boor (1992). Crystalloids increase in size and quantity on both surfaces of the leaf after a few weeks of leaf development. Observations were taken from July to November. Composition of wax components varied with season (May–August), like hydrocarbons (5%–9%), wax esters (2%–25%), fatty acids (17%–48%), aldehydes (0%–26%), and alcohols (17%–49%). Another study was done on epicuticular wax development of leaves by Prasad and Giilz (1990) on beech trees (*Fagus sylvatica*). The folded leaves in buds did not contain an aldehyde group but its presence was detected after 10 days of leaf development. The biosynthesis of lipids was fast in a few weeks (3 to 5 weeks) of leaf development and after that it remained constant, with the exception of fatty acids.

Sachse et al. (2015) identified three different periods of leaf development in evergreen tree *Quercus agrifolia*. During the first three months, *n*-alkane concentrations increased seven times and wax δ2H and ACL values were also reported to be high, which makes this period the best period for *n*-alkane formation. According to Gülz et al. (1991), epicuticular waxes of rolled leaves in buds and mature leaves in *Tilia tomentosa* have different wax compositions. Alcohols, esters, acetates, fatty acids, and α- and β-amyrin were present in young leaves. After the unfolding of leaves, synthesis of wax esters and acetates ceased. β-Amyrenyl acetate and aldehydes were only present in mature leaves. The highest wax production (hydrocarbons, aldehydes, alcohols, β-amyrin, and β-amyrenyl acetate) was observed during April to June. The quantity and quality of wax remain unchanged during July to November. Celano et al. (2006) studied the cuticular wax composition and development in *Actinidia deliciosa* leaves. The main components were found to be alkyl alkanoates (10 µg/cm^2), terpenes (3/µg cm^2), sterols (0.6 µg/cm^2), alkanals (0.7 µg/cm^2), alkanoic acids (1 µg/cm^2), ketones (1 µg/cm^2), hydrocarbons (6 µg/cm^2), and alkanols (1 µg/cm^2). After bud break at 83 days the cuticular components reached a peak (43 µg/cm^2). Before bud break, wax coverage increased at high pace from 12 µg/cm^2 to 43 µg/cm^2. After this, the wax concentration decreased, and at 169 days (after bud break) it reached a final value of 9 µg/cm^2. Takahashi et al. (2012) observed that at the early stage of growth the leaves of *Sonneratia alba* were rich in wax (21.5%–25.7%) and cutin (52.4%–63.4%) while cutan (4.3%–10.3%) and polysaccharide (2.3%–7.7%) were deposited throughout the growth period of leaves. Immature cuticular membranes (CMs) are not physically strong but are highly viscoelastic in nature. When leaf expansion and maturation occur the CMs become hard and lose their flexibility (68%–83% decrease). At the time of senescence, the strength of CMs decreases by 30%–43%. The high viscoelastic property was due to the cutin matrix, whereas wax, cutan, and polysaccharide added elasticity. Cutan and polysaccharide also contributed to rigidity. After bud burst, the accumulation of cutan, polysaccharide, wax, and cutin in CMs increased the environmental tolerance of the plant.

3. Applications of epicuticular wax

The cuticle has been assigned several important functions in plant life besides acting as a structural entity.

3.1. Protection from the environment

All higher plants have a protective layer made up of wax

covering the whole aerial body of the plant. This layer of cuticle plays very significant roles in protection from drought and UV damage (Taiz and Zeiger, 1991; Kerstiens, 1996; Rhee et al., 1998). UV protection is facilitated by some phenolic compounds like flavonoids and hydroxyl cinnamic acid derivatives (Kraus et al., 1997; Kolb et al., 2001, 2003). Besides, the cuticular wax layer acts as a barrier between the plant and the atmosphere (Schreiber et al., 1996).

3.2. Protection from pathogens

The cuticle wax layer prevents the attack of pathogenic bacteria and fungus (Taiz and Zeiger, 1991; Schreiber et al., 1996; Rhee et al., 1998). The epicuticular wax layer protects the aerial parts from insect feeding, probing, or oviposition. This property of the plant cuticle is commonly known as antiinsect (Znidarcic et al., 2008). This cuticle layer shows interaction with insect and external chemical agents (Garcia et al., 1995; Muller, 2006; Carver and Gurr, 2006), as evident from Table 3. Cutin and long-chain fatty acids present on the plant surface facilitate fungal infection (Kolattukudy et al., 1995; Ahmed et al., 2003; Dickman et al., 2003) or they may initiate defense mechanisms against pathogens. Tomato cutin-derived enantiomers of (+) DHPA (10,16-dihydroxyhexadecanoic acid) and (-) DHPA (10,16-dihydroxyhexadecanoic acid) were found to induce pathogenicity-related genes in *Colletotrichum trifolii* (Ahmed et al., 2003). Both enantiomers had different efficiencies as the (+) form had greater induction effects than the (-) form.

The thickness and three-dimensional structure of leaf wax crystalloids protect the leaves and fruits from fungal pathogens (*Uncinula necator*) in grape berries (Schwab et al., 1995). In a similar way, *Brassica oleracea* and *Pisum sativum* are protected from *Botrytis cinerea* (Ficke et al., 2004).

According to Marcell and Beattie (2002), the leaf surface wax of different mutants of *Zea mays* influences bacterial leaf colonization in different ways. The increase in epicuticular wax reduces the rate of infection of *Pantoea agglomerans* and *Clavibacter michiganensis* due to reduced availability of nutrients. Baldotto and Olivares (2008) reported that the epicuticular wax of a leaf affects the bacterial colonization on the plant surface. The amount of epicuticular wax present on a leaf of *Brassica* impedes the ability of a parasitoid (*Diaeratiella rapae*) to forage, locate, and attack its host (Gentry and Barbosa, 2006). Jones et al. (2002) reported that there is no significant correlation between total wax yield and gum moth (*Mnesempala privata*) in *Eucalyptus globulus*. However, several aliphatic phenylethyl and benzyl wax esters were found to be responsible for resistance against gum moth. Furthermore, it has been revealed that wax compounds present in the cuticular layer of *Eucalyptus globulus* provide resistance against gum moth. Kosma et al. (2010) reported that in *Triticum aestivum* the constituents and accumulation of wax can play important roles against infection of fly larvae (*Mayetiola destructor*), which causes extensive loss of the crop. According to Daoust et al. (2010), monoterpenes present in the epicuticular wax of *Picea glauca* affect the pattern of feeding of spruce budworm larvae on the host. This causes resistance against attack of spruce budworm in this genotype. According to Voigt et al. (2007), the attachment of the insect on leaf surfaces is more influenced by trichomes in comparison to wax crystals in 40 plant species. In *Gossypium hirsutum* it has been observed that cotton leaf curl virus (CLCuV)-resistant cultivar CIM-448 had higher leaf epicuticular wax than that of the susceptible cultivar (Zafar and Athar, 2013). Infection frequency of CLCuV in *Gossypium arboreum* variety 786, its wax mutant GaWM3, and *Gossypium hirsutum* MNH-93 was studied and it was reported that the wax content of leaves acts as a barrier in the transfer of virus by whitefly (Khan et al., 2011).

3.3. Role in transpiration

Transpiration takes place mainly through stomata. Besides stomatal water loss, water loss also occurs through the cuticle (Schonherr, 1982), and this is known as cuticular transpiration. The rate of cuticular transpiration is inversely proportional to the thickness of the cuticle layer (Schönherr, 1976). The role of the cuticle in decreasing transpiration was also reported by Riederer and Schreiber (2001). As the cuticle has hydrophobic compounds that repel water molecules, it does not allow water to escape. Water permeability efficiency of cuticles can be determined for intact leaves (Hall and Jones, 1961), reconstituted waxes (Grncarevic and Radler, 1967), and isolated cuticles (Schönherr and Riederer, 1989) or foliar discs (Hoad et al., 1996). However, transpiration is an unavoidable evil for plants.

The epicuticular wax layer is helpful in the foliar uptake of xenobiotics (Schreiber et al., 1996). Various environmental conditions show great impact on the surface properties and chemical compositions of plant cuticular wax. Cuticular wax provides mechanical strength and viscoelastic properties, prevents organ fusion during plant development, and protects the plant from stress factors in the environment (Catrow et al., 2009).

4. Lotus effect

Lotus leaves are able to remain clean in any muddy pond due to water contact angle of more than 160° and sliding angle lower than 5°. Whenever the lotus leaf receives any water droplet, it will convert it into a bead-like structure. This bead-like ball collects all the dust particles and debris present on the surface of the leaf and rolls down (Shirtcliffe et al., 2009). This property of self-cleanup of lotus leaves

Table 3. Interaction of pathogens with cuticular wax layer.

Insect/pathogen name	Plant name	Interaction with plant	References
Alternaria brassicae	Canola	Reduced the rate of germination and number of germ tubes	Conn and Tewari, 1989
Botrytis cinerea	*Vitis vinifera*	Susceptibility to infection decreases when wax is present	Marois et al., 1986
Erysiphe graminis	*Lolium* spp.	Abaxial surface shows resistance to disease	Carver et al., 1990
Metarhizium anisopliae	Members of Cruciferae	Germination is influenced (decreases) when in contact with wax	Inyang et al., 1999
Peltaster fructicola and *Leptodontidium elatius*	*Malus domestica*	Fungi not able to grow on epicuticular wax	Belding et al., 2000
Cotton leaf curl virus (CLCuV)	*Gossypium arboreum*	Wax acts as a barrier in transfer of virus by whitefly	Khan et al., 2011
Cotton leaf curl Burewala virus	*Gossypium* spp.	Less epicuticular wax could make plants susceptible to CLCuV	Khan et al., 2016
Pantoea agglomerans and *Clavibacter michiganensis*	*Zea mays*	Epicuticular wax reduces rate of infection	Marcell and Beattie, 2002
Pseudomonas syringae	*Arabidopsis thaliana*	*CYP86A2* may repress bacterial type III gene expression in cuticle layer	Xiao et al., 2004
Hippodamia convergens	*Pisum sativum*	Attached more strongly to reduced-wax peas	Rutledge and Eigenbrode, 2003
Phyllotreta spp., *Eurydema ventralis*, and *Thrips tabaci*	*Brassica oleracea*	Infection level is low on high epicuticular wax layer of plant	Znidarcic et al., 2008
Dicyphus errans	*Brassica oleracea*, *Plectranthus ambiguus*, and *Solanum melongena*	Insect attachment influenced by trichomes	Voigt et al., 2007
Diaeratiella rapae	*Brassica sp.*	Epicuticular wax impedes the ability of the pathogen to forage, attack, and locate.	Gentry and Barbosa, 2006
Mnesempala privata	*Eucalyptus globulus*	Wax provides genetic resistance against moths	Jones et al., 2002
Mayetiola destructor	*Triticum aestivum*	Wax provides resistance against *Mayetiola destructor*	Kosma et al., 2010
Crematogaste spp.	*Macaranga* sp.	Unable to walk on surface	Federle et al., 1997

is commonly known as the lotus effect (Marmur, 2004). Superhydrophobicity and self-cleaning of lotus leaves are due to the presence of small hydrophobic wax tubules, which are present on convex cell papillae (Barthlott and Neinhuis, 1997). This kind of surface does not absorb water droplets because the air is trapped in the cavities and they have larger water–air interface and low solid–water interface (Bhushan and Jung, 2008).

Surfaces can be broadly classified in four categories, namely superhydrophilic, hydrophilic, hydrophobic, and superhydrophobic, with contact angles of ≤10°, 11°–89°, 90°–149°, and ≥150°, respectively (Drelich et al., 2011).

Plant species having hydrophobic leaf surfaces are *Fagus sylvatica* (Paoletti et al., 1998), *Zea mays* (Beatie and Marcell, 2002), and *Fouquieria columnaris* (Neinhuis and Barthlott, 1997). Plant species with superhydrophobic leaf surface include *Nelumbo nucifera*, *Eucalyptus macrocarpa*, *Euphorbia myrsinites*, *Brassica oleracea*, *Pistia stratoides* (Neinhuis and Barthlott, 1997), *Salvinia oblongifolia* (Cerman et al., 2008), *Tropaeolum majus*, *Crambe maritima*, *Leymus arenarius* (Koch et al., 2008), and *Colocasia esculenta* (Koch and Barthlott, 2009).

5. Chemotaxonomy

Epicuticular wax has also been used for separation of one species from another. Cluster analysis of n-alkanes helps to separate the population of *Plantago major* based upon annual temperature but it is unable to separate the population at interspecies level (Guo et al., 2015).

6. Potential application of epicuticular wax as plastic/packaging material

Plastic is tough, strong, corrosion-resistant material with high thermal and electrical insulation properties. Its uses and production are increasing tremendously due to the flexibility of the polymer. It is used in industries, medical care, and day-to-day life activities (Andrady and Neal, 2009). A huge amount of plastic waste is being deposited in the environment and landfills. This plastic is ingested by many organisms and accumulation of the same results in the death of the organisms (Derraik, 2002). There is a great health risk for the human population due to the toxic chemicals used to manufacture the plastic (Talsness et al., 2009). Therefore, there is a demand for biodegradable plastic that is hydrophobic in nature. Plant-derived wax can be used in making bioplastic/hydrophobic packaging material. Cuticular wax is hydrophobic in nature, and if extracted by a nondestructive method, it can open new vistas for biodegradable plastic/packaging materials, which will be economically and ecologically beneficial.

7. Conclusion and future prospectus

Plant-derived wax may be an ecofriendly and efficient material to produce hydrophobic and superhydrophobic surfaces to be used in various sectors. The design of superhydrophobic surfaces with low surface energy is the main challenge. Currently available such surfaces are nonbiodegradable and cause accumulation of solid waste. Due to their self-cleaning and anticontamination properties, these superhydrophobic surfaces are in great demand in various sectors like paint (antibiofouling paints for boats), instruments (snow-free antenna surfaces), automobiles (windshields), textiles (self-cleaning and stain-resistant), architecture (antisoil coatings), and surgery (waterproof and contamination-free) (Pociūtė et al., 2003; Li et al., 2007). They can also be used in coating metal surfaces to protect them from corrosion. Rapid increase in the requirement for superhydrophobic surfaces has been recently reported (Latthe et al., 2014). In view of these facts, surface wax derived from plants may reduce/replace nonbiodegradable plastic and protect biodiversity. There is a need for developing nondestructive methods to extract the wax from suitable plant sources. Besides, the surface wax generally contains aliphatic and aromatic compounds along with ester and alcohols. The chemical composition and physical properties of this wax are to be studied thoroughly to ensure its nontoxic nature and the properties required for making plastic/packaging material. Use of plant wax for various prospects with nondestructive extraction methods will pave a way for the manufacturing of bioplastic in the future.

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