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# A review of Willow (*Salix* spp.) as an integrated biorefinery feedstock

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## ABSTRACT

Throughout history, the genus *Salix* (willow) has been an incredibly useful temperate plant for humans, with widespread global distribution and species indigenous to all continents except Antarctica. Estimations of the number of species range from 450-520 worldwide, and there are still more natural hybrids and multi-hybrid combinations. Several biomass willow breeding programmes have been established across the globe. All of these attempt to produce fast-growing, high-yielding stems with a straight habit and minimal side branching that are highly adaptable to different sites and are also disease and pest resistant. Short rotation coppice (SRC) cultivation involves growing willow at close spacings with a stocking rate of around 15,000 per hectare with harvests every 2-4 years. The crop is mechanically harvested, typically using a forager, and material has recently been used for bioenergy applications. Trial plots have achieved yields of up to 20 odt/ha/yr, whilst well-tended commercial crops have yielded up to 14 odt/ha/yr. Global willow breeding programmes have produced a wide variety of commercial genotypes that have suitable properties for easy planting and harvesting and have the added benefit of elevated levels of bioactive compounds, including salicin, present in the bark, which can be used in medical and veterinary applications. These high-yielding willow varieties grow well in the wetter regions of the globe, including NW Europe, and afford multiple harvests before re-planting. Salix's versatility and adaptability and the SRC cultivation process make them an ideal candidate feedstock for use in an integrated biorefinery to produce a range of biobased materials, including pharmaceuticals, and biocomposites, fuels, energy and fertiliser.

**KEYWORDS**

Integrated biorefinery, biobased products, willow, circular bioeconomy

## 1. Introduction

Throughout history, willows, the genus *Salix*, has been an incredibly useful temperate plant for humans (Stott, 1992, Kuzovkina & Quigley, 2005). They have a widespread global distribution with species indigenous to all continents except Antarctica. Estimations of species range from 450-520 worldwide, and there are still more natural hybrids and multi-hybrid combinations (Argus, 2010, Newsholme, 1992). Table 1 indicates the number of native species according to different regions.

**Table 1: Estimates of willow species according to the continent**

| **Region** | **Number of native species** | **Reference** |
|---|---|---|
| Europe, Russia and the Arctic | 135 | Skvortsov, 1999 |
| North America | 125 | Argus, 2007 |
| Central and South America | 9 | Newsholme, 1992 |
| Asia | 313 | Wang et al., 2017 |
| Africa | 12 | Newsholme, 1992 |

The distribution of *Salix* spp. is aided by the numerous tiny seeds (less than 2mm in length and a milligram in weight) that are borne in fluff and distributed by the wind over areas of up to 0.8 km (Tiebel et al., 2019). The genus includes dwarf ground-hugging shrubs, low-growing shrubs, shrubby trees with multiple stems and tall, single-stemmed trees. Willow is a pioneer species and is often one of the dominant vegetations in peripheral habitats such as fen communities, wet woodlands, tundra, mountain tree lines, shorelines, riverbanks and vacant or disturbed land (Gramlich et al., 2016). Similar types of willow occupy the same type of habitats in different parts of the world. There are, therefore, geographically isolated, exotic equivalents of many species (Stott, 1984).

The use of willow as a potential feedstock for an integrated biorefinery could be beneficial for several reasons:

- Willow is a pioneer species and grows well in the wetter regions of the world, including NW Europe, where there are high levels of annual rainfall, not as suited for the cultivation of some other biomass feedstocks (Wagner *et al* 2021; Rotherham 2022)
- Once established, a willow plantation can yield 8-9 harvests collected every three years over 25 years before re-planting might be required (Sleight *et al* 2016; Volk *et al* 2004, Karp *et al* 2008)
- Many of the current commercial willow varieties which have been cultivated over the last 20 years were developed for use in bioenergy applications and exhibit rapid growth and high yields (Mula-Yudego and González-Olabarria 2010, Lindegaard *et al* 2016)
- Cultivation of willow in flood-prone areas (e.g., next to rivers) offers the potential for flood risk mitigation because of surface flow disruption due to the high planting density, improved soil drainage and the dense network of roots, which can prevent topsoil from being washed away (Kuzovkina and Volk 2009; Borsje *et al* 2011; Evette *et al* 2009; Wilkinson 1999; van Wesenbeeck *et al* 2022)
- Use of willow as an integrated biorefinery feedstock offers considerable opportunities for diversification in the rural economy and is linked to the cultivation and processing of high-yielding varieties

There are currently no commercial examples of integrated biorefinery facilities, that the authors are aware of, that utilise willow as a feedstock to manufacture a range of biobased products. This review aims to summarise the potential value chains currently being developed from this sustainable, short-rotation crop (see figure 1), focusing on utilising the willow bark and pulp.

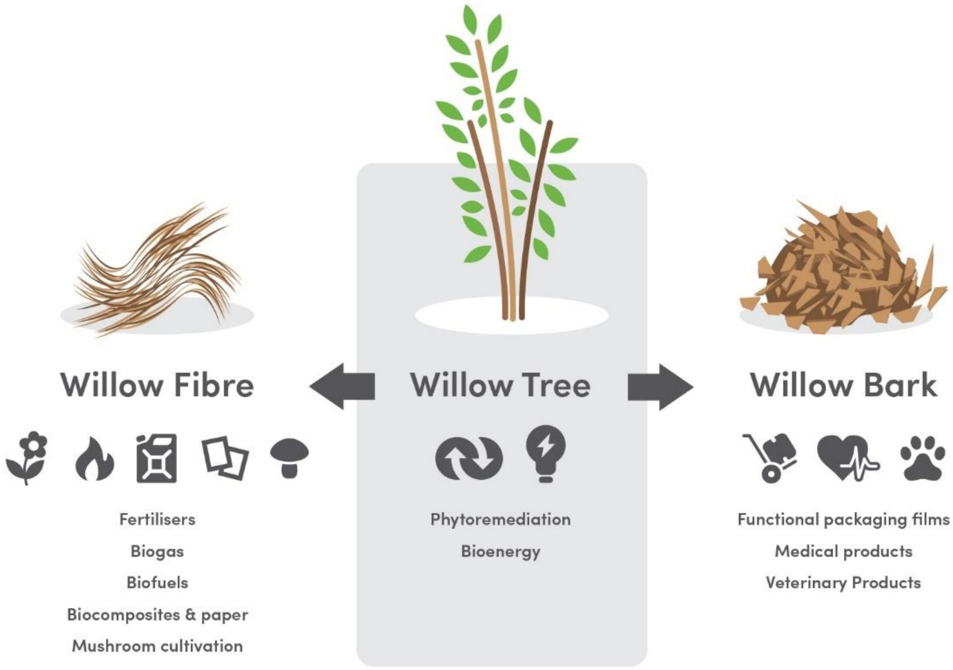

Figure 1. The potential value chain of bio-based products derived from Willow (*Salix* spp.)

## 2. Lignocellulose composition and bark bioactivity

Willow (*Salix* spp.) is an important biomass crop due to its rapid growth (Schroyen et al., 2015), with high cellulose (36-65%) and lignin contents (17-29%) reported (table 2) for a range of varieties (Schroyen et al., 2015; Yoon et al., 2015, Sassner et al., 2006, Vaher et al., 2012, Alexandropoulou et al., 2017). Some willow clones contain especially high cellulose contents ranging from 54-59% and a low lignin content ranging from 17-22% (Meng et al., 2012). Most studies have reported the fibre contents of the whole willow chips that includes the wood and bark (Alexandropulou et al., 2017; Schroyen et al., 2015; Sassner et al., 2006; Vaher et al., 2012), whereas the lignin and total polysaccharide contents were 1-2% and 5-11% lower, respectively in the central part of the willow stem, compared with the bark (Serapiglia et al., 2009; Yoon et al., 2015). The chemical structure of lignin also shows a variation where guaiacyl units are predominantly present in the bark and syringol and p-hydroxyphenyl contents were higher in the inner bark and inner wood, as determined by

NMR spectroscopy analysis (Dou et al., 2018). These results showed that the bark contained condensed lignin and high pectin and protein contents.

In an analysis of two-year-old willow shrubs from 25 willow varieties belonging to crosses of *S. viminalis* x *S. miyabeana*, *S. purpurea* x *S. miyabeana* and *S. purpurea*, the total biomass of willow bark was found to comprise between 10-18% (Serapiglia et al., 2009). The study reported that high-temperature thermogravimetric analysis directly on wood samples for fibre analysis was a rapid approach, showing a high correlation with standard methods of sugar analysis using NMR. The structure and composition of the inner bark of four willow hybrids produced from *S. myrsinofolia* and *S. schwerinii* were described (Dou et al. 2016) to help develop an approach toward complete willow biomass valorisation. The inner bark consists of highly dignified bundles of fibres and non-delignified surrounding tissue, with 25% of the dry mass composed of ash and acetone extractable substances. The lignin-to-polysaccharide ratio was similar in the inner bark and wood, but there were differences in the polysaccharide compositions. Glucose (67-70%) and xylose (28-30%) were the main monosaccharides in wood, whilst the inner bark also contained high arabinose (8%) and galactose (10%) contents. The elevated rhamnose levels in the inner bark was indicative of a higher pectin content. In addition to the potential for sugar and lignin valorisation, the high extractives content and the fibre quality were identified as additional product streams from a willow biorefinery.

Table 2. Lignocellulosic composition of different willow varieties

| Sample | Hemicellulose | Cellulose | Lgnin | reference |
|---|---|---|---|---|
| chopped 5 mm willow | | | 17 | Schroyen et al., 2015 |
| whole willow | 12.58 | 37.25 | 25.32 | Mantes et al., 2014 |
| 20 mesh size ground goat willow - woody core | | | 17.3 | Yoon et al., 2015 |
| 20 mesh size ground goat willow - bark | | | 22.3 | Yoon et al., 2015 |
| 2-10 mm wood chips, from 5-year-old stems Tora (*Salix schwerinii* × *Salix viminalis*) | 14.9 ± 0.1 | 43.0 ± 0.5 | 24.2 ± 0.9 | Sassner et al., 2005 |
| air-dried 10-20 mm willow chips | 11.1 | 36.8 | 28.5 | Vaher et al., 2012 |
| milled, sieved and air-dried willow | 21.50 ± 0.89 | 35.59 ± 0.91 | 28.71 ± 0.23 | Alexandropoulou et al., 2017 |
| *Salix purpurea* S95042 | | 64.4 | 16.8 | Meng et al., 2012 |
| *Salix dasyclados* SV1 | | 57.3 | 17.4 | Meng et al., 2012 |
| *Salix erio* x *S. erio* 95316 | | 57.4 | 18.1 | Meng et al., 2012 |
| *Salix discolor* S625 | | 57.7 | 18.1 | Meng et al., 2012 |

| | | | | |
|---|---|---|---|---|
| *Salix purpurea* 94009 (B 193) | | 56.9 | 18.5 | Meng et al., 2012 |
| *Salix erio* S287 | | 55.4 | 18.6 | Meng et al., 2012 |
| Salix alba SA2 | | 55.4 | 18.8 | Meng et al., 2012 |
| *Salix eriocephala* S25 | | 58.9 | 19.4 | Meng et al., 2012 |
| *Salix erio* x *S. erio* 95018 | | 53.9 | 20.1 | Meng et al., 2012 |
| *Salix purpurea* 94005 (FC 189) | | 57.6 | 20.3 | Meng et al., 2012 |
| *Salix purpurea* 94006 (FC 190) | | 54.2 | 21.7 | Meng et al., 2012 |
| 99202-004 *S. viminalis* x *S. miyabeana* | 28.61 | 41.49 | 20.25 | Serapiglia et al., 2009 |
| *Salix dasyclados* Wimm. SV1 | 26.96 | 41.73 | 20.36 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99202-043* | 28.7 | 40.68 | 20.51 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99202-011* | 28.85 | 40.04 | 21.19 | Serapiglia et al., 2009 |
| *S. miyabeana SX67* | 24.45 | 41.85 | 21.53 | Serapiglia et al., 2009 |
| *S. purpurea 99113-010* | 27.09 | 39.71 | 21.57 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99208-038* | 25.21 | 42.28 | 21.66 | Serapiglia et al., 2009 |
| *S. sachalinensis* x *S. miyabeana 9970-036* | 26.22 | 35.73 | 21.83 | Serapiglia et al., 2009 |
| *S. purpurea* x *S. miyabeana 99217-023* | 27.32 | 39.5 | 21.86 | Serapiglia et al., 2009 |
| *S. miyabean*a Seemen SX64 | 25.77 | 41.76 | 21.97 | Serapiglia et al., 2009 |
| *S. purpurea 99239-028* | 24.21 | 41.17 | 22.01 | Serapiglia et al., 2009 |
| *S. purpurea* x *S. miyabeana 99217-015* | 27.06 | 38.81 | 22.15 | Serapiglia et al., 2009 |
| *S. purpurea 99239-020* | 24.02 | 40.6 | 22.49 | Serapiglia et al., 2009 |
| *S. purpurea* x *S. miyabeana 9980-005* | 27.71 | 37.52 | 22.58 | Serapiglia et al., 2009 |
| *S. viminalis* x *S.miyabeana 99207-018* | 27.12 | 41.99 | 23.01 | Serapiglia et al., 2009 |
| *S. purpurea 99239-015* | 25.35 | 38.92 | 23.04 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99207-003* | 28.05 | 39.68 | 23.17 | Serapiglia et al., 2009 |
| *S. sachalinensis* x *S. miyabeana 9970-014* | 24.67 | 39.81 | 23.32 | Serapiglia et al., 2009 |
| *S. purpurea* L. x *S. miyabeana 9979-036* | 27.76 | 36.33 | 23.43 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99207-020* | 26.76 | 38.04 | 24.27 | Serapiglia et al., 2009 |
| *S. sachalinensis* F. Schmidt SX61 | 26 | 36.92 | 24.4 | Serapiglia et al., 2009 |
| *S. purpurea 99113-012* | 24.92 | 40.3 | 24.42 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99201-001* | 27.21 | 38.65 | 25.24 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99201-002* | 27.25 | 39.27 | 25.38 | Serapiglia et al., 2009 |
| *S. viminalis* x *S. miyabeana 99201-007* | 25.95 | 39.32 | 26.25 | Serapiglia et al., 2009 |

For millennia, willow bark has been used in traditional medicine because of its excellent medicinal and pain-relieving qualities. Some of the earliest medical documents recognise the merits of using willow. For instance, the Papyrus Ebers, written in Egypt circa 1500 BC, describes a concoction involving willow, beer, figs and frankincense as a cure for indigestion (Aikmann, 1977). Throughout history and wherever willows were found, they were used for their healing and recuperative properties (Gensler, 1981, Moerman, 2009, Mahdi, 2010) and, more recently, within dietary supplements, where the bioactive compounds present in willow may contribute to easing musculoskeletal pain. Phenolic glucosides, also known as salicylic glucosides, are now known to be responsible for this effect (Vlachojannis et al., 2009). A comparison of these supplements with typical doses of synthetically produced acetylsalicin (aspirin) revealed that while the quantity of salicin present in bark extracts was considerably lower, it nevertheless had an analgesic effect, which was partially attributed to polyphenols and flavonoids present in the extracts (Vlachojannis et al., 2011).

Following the approval of willow bark extract for phytopharmaceutical applications by the European Medicines Agency, an application was submitted for use as a medication in 2017 to reduce the effects of fever, headache, and minor articular pain (Oketch-Rabah et al., 2019). Although the initial application was rejected, the potential pharmaceutical applications of the compounds in the willow bark are still undergoing investigation to evaluate the effectiveness of each of the compounds present and to develop methods for their recovery and purification. While the pharmaceutical, cosmetic, chemical, food and fodder industries have increased their use of willow varieties for the manufacturing of their products (Oleszek et al., 2019), industrial applications are dominated by five varieties: *S. purpurea*, *S. daphnoides*, *S. fragilis, S. alba* and S. *nigra* (European Medicines Agency, 2017). Following the extraction and isolation of these high-value constituents, the remaining willow biomass can be used as raw material to produce biofuels and heat (Parajuli et al., 2015).

## 3. Willow breeding

Several international willow breeding programmes were established in Sweden (1987), the United Kingdom (1996) and the USA in 1998 (Larsson, 1998; Lindegaard and Barker, 1997; Karp et al., 2011; Smart et al., 2008). All were attempting to produce fast-growing, high yielding stems with a straight habit and minimal side branching that are highly adaptable to different sites and disease and pest resistant.

Many species have been used in hybridisations, but the most successful parental species (based on commercialised varieties) are outlined in Table 3.

Table 3: The most important *Salix* species used in SRC willow breeding programmes are based on representation in the parentage of commercialised varieties (Lindegaard (2012), Karp et al., 2011, Macalpine et al., 2011, Cornell University (2022).

| **Species** | **Number of released varieties in which it figures as a parent or grandparent** | | |
|---|---|---|---|
| | European breeding programmes | US Breeding programme | Total |
| *S. viminalis* | 35 | 3 | 38 |
| *S. schwerinii* | 25 | 0 | 25 |
| *S. dasyclados* | 10 | 0 | 10 |
| *S. miyabeana* | 2 | 7 | 9 |
| *S. triandra* | 5 | 0 | 5 |
| *S. eriocephala* | 3 | 0 | 3 |
| *S. purpurea* | 0 | 3 | 3 |
| *S. aegyptiaca* | 2 | 0 | 2 |
| *S. rehderiana* | 2 | 0 | 2 |
| *S. sachalenensis* | 0 | 2 | 2 |
| *S. petiolaris* | 1 | 0 | 1 |

Most of these willows are shrubs that are classified in just a few sections of the different sub-genera of *Salix*. Many successful crosses resemble natural hybrids that occur in the wild but utilise exotic equivalents of each other (Stott, 1984). In some circumstances, the phenomenon of heterosis has been demonstrated where the performance of progeny (in height, biomass yield and other characteristics) is far superior to the parents (Lindegaard, 2001).

The breeding process involves choosing the suitable male and female parents. *Salix* sp. are generally diecious, meaning that different individuals are single-sexed. Many crosses are made each year, and seeds are harvested from successful crosses and grown in nursery beds. A 5-10% selection is made from many thousands of seedlings reared. The best individuals are then made into cuttings and are planted in a series of observation trials. Over a period of years, further selections are made based on the breeding objectives. After around ten years of successful trial results, a new genotype may be selected for multiplication and commercial release (Lindegaard, 2001).

The process of willow breeding is expensive, involving sizeable up-front investment in rearing, screening, multiplying, IP protection (Plant Breeders' Rights) and commercialisation. As a result of the slow, embryonic development of the industry and the long payback period to recoup costs from variety development, the anticipated conveyor belt of varieties has not yet materialised. Many of the current varieties were bred in the mid-1990s. In addition, crosses during the primary years often utilised already established varieties. This means that many of the existing varieties have a narrow genetic base. However, many near-market genotypes could be commercialised once the industry gains momentum (Lindegaard, 2012).

The cultivation techniques used for basketry willows have also devised many new environmental applications. The concept of Short Rotation Coppice (SRC) for biomass production was developed in Sweden and Northern Ireland in the early 1970s (Dawson, 1991). Both countries were exposed to the OPEC oil crisis and were looking for ways to use vacant land for energy production (Lindegaard, 2015). SRC cultivation involves growing willow at close spacings with a stocking rate of around 15,000 per hectare with harvests every 2-4 years (Karet et al., 2011). The crop is mechanically harvested (normally by a forager producing woodchips,) and the biomass is used in power stations or biomass boilers. Trial plots have achieved yields of up to 20 oven-dried tonnes per hectare per year, whilst well-tended commercial crops have yielded up to 14 odt/ha/yr (Lindegaard et al., 2001, 2011).

The versatility and adaptability of *Salix* and the SRC cultivation process make them an ideal candidate feedstock for use in an integrated biorefinery. Global willow breeding programmes have produced many varieties and near-market genotypes with ideal properties for easy planting and harvesting. So there is considerable potential to use these as a sustainable source of high-value pharmaceuticals, biocomposite materials, fuels and energy.

## 4. Integrated biorefinery models utilising willow

In recent years, the concept of an integrated biorefinery approach to generating a range of biobased products from different biomass feedstocks has been a focus for many research groups and companies worldwide (Singh et al., 2022; Volk et al., 2016). The concept involves utilising a range of mechanical, thermal, chemical and biological pre-treatment methods to deconstruct and partially degrade the cellulose, hemicellulose and lignin fractions that compose the biomass matrix (Kumar et al., 2008). In theory, this facilitates the downstream conversion of the deconstructed biomass using chemical, thermochemical and fermentation processes into a range of products, including fuels, energy, speciality and platform chemicals and materials, including biocomposites and packaging materials, whilst minimising waste. Whilst this approach is theoretically possible the authors are unaware of any examples of currently operating, commercial biorefineries using willow, although there are some state-subsidized commercial willow plantations with an emphasis on producing bioenergy (Volk *et al* 2016, Rickerby, 2022).

In terms of upstream processing, there are a number of technical challenges, including the growth, harvesting, drying, transport and supply of biomass at commercially viable prices, along with the development of robust and scalable pre-treatment processes to deconstruct a wide range of lignocellulosic feedstocks. Many of the technologies required to convert the pre-treated biomass into the broad range of products required are available. However, these still need to be fully integrated into commercial multi-product biorefineries. At the downstream (commercial end-user) part of the supply chain, generating markets for new biobased products that are cost competitive with existing fossil fuel-derived products, whilst retaining the same or ideally better levels of functionality compared to existing products demanded by industry, is another key challenge. Despite the potential opportunities for using willow as an integrated biorefinery feedstock, there have been relatively few reports in the literature that have investigated approaches to producing a range of biobased materials from this plant.

A research article published by a Polish research group actively involved in willow research outlined the results of studies into the use of willow as an integrated biorefinery feedstock for the recovery of hemicellulose, cellulose and lignin, each with different applications. Krzyzaniak et al. (2014) reported the compositional analysis of seven varieties and clones of *S. viminalis,* which grows well in Europe, revealing seasonal variation in cellulose and consequently, cultivars were selected that would yield high biomass. In addition, the cultivars studied exhibited good thermophysical compositions, with only trace amounts of undesirable components present, including ash, sulphur or chlorine, but which contained high levels of cellulose (up to 47%) and hemicellulose (up to 31.8%). However, it was noted that the high moisture content (~50%) in the fresh biomass could present an issue from a processing perspective, and for a commercial biorefinery operating in Poland, the minimum volume of material required annually would be 37,000 tonnes.

Sas et al. (2021) reported the potential to use willow plantations for treating primary municipal wastewater, noting that the impact of wastewater irrigation on the willow biorefinery potential had not yet been assessed. The study reported details of a range of approaches to assess the potential impact, including biomass compositional analysis, ionic liquid pre-treatment and enzymatic saccharification, along with the abundance of extractable phytochemicals. It was concluded that substantially increased biomass yields of field-grown willow were achieved with primary effluent municipal wastewater, replacing synthetic agricultural inputs, and without any observed adverse impacts on biomass quality and bioenergy potential. Untargeted metabolite abundance analysis indicated the presence of potentially high-value phytochemicals, including flavanols, lignans and flavonolignans. The glucan content significantly increased by 8% in wastewater-grown trees, while arabinose and galactose significantly decreased by 8 and 29%, respectively, and finally, xylose, mannose and lignin content remained unaltered. It was noted that both ionic liquid pre-treatment and enzymatic saccharification efficiencies did not vary significantly, releasing >95% of the cell wall glucose and recovering 35% of the lignin. It was concluded that there is potential for an integrated willow biorefinery to generate bioenergy, lignin, platform chemicals and high-value specialised phytochemicals with reduced or even reversed feedstock cultivation costs using wastewater irrigation.

## 5. Pre-treatment approaches to deconstruct willow

One of the key technical challenges linked to the utilisation of lignocellulosic feedstocks, including willow and as part of an integrated biorefinery, is pre-treatment, and many forestry residues can be particularly recalcitrant and difficult to degrade because of the high levels of lignin present. Any potentially scalable pre-treatment technologies also need to ensure that the lignin fraction can still be valorised as well following deconstruction. In relation to the pre-treatment of willow to access the main components - cellulose, hemicellulose, lignin and bioactive compounds, a range of approaches has been reported.

The use of pressurised organosolv systems consisting of mixed aqueous and organic solvents have been utilised as one pre-treatment approach for willow and to extract lignin (Wang et al., 2022). Lignin fractions samples were extracted (2 MPa, 200°C, 2 hrs) from an unspecified, powdered willow source, using three typical organosolv-water co-solvents systems {ethyl acetate (EAC), tetrahydrofuran (THF), and γ-butyrolactone (GBL)}, and their properties and subsequent pyrolysis were investigated. It was determined that lignin extracted using an EAC-water mixture contained more oxygen-functional groups, whilst the GBL-water system afforded higher molecule weight fragments. Subsequent pyrolysis of the GBL-water extracted lignin resulted in the formation of hydrogen, methane and carbon monoxide. The other two solvent systems yielded carbon dioxide as an additional gaseous product.

Steam explosion is another process that has been evaluated as a pre-treatment approach for willow, which resulted in the solubilisation of elements from material cultivated on soils contaminated with zinc and manganese as part of a bioethanol production process (Ziegler et al., 2019). Batch steam explosion (180-220 °C preceded by 2% sulfuric acid soaking) of milled *S. viminalis* enabled up to 80% of the metal contamination to be removed from the willow and extracted from the aqueous effluent generated. Enzymatic hydrolysis of the pulp following incubation (75 h) afforded a high yield of glucose (80%) and subsequent successful conversion to ethanol using *Saccharomyces cerevisiae* (~65% yield) which was uninhibited by the presence of metal contaminants.

Lignin condensation following a high-pressure steam explosion is an issue that can lead to increased recalcitrance and reduced digestibility/ sugar release (saccharification). The use of radical scavengers (1- and 2-naphthol, formaldehyde) to reduce lignin condensation and improve downstream sugar release as part of a hydrothermal pre-treatments process for forestry feedstocks (pine, birch, and willow wood) has been reported (Borrega et al., 2021). In birch and willow wood, almost quantitative (> 90%) saccharification could be achieved even after relatively mild pre-treatment severities. So the addition of chemical additives had little effect on improving enzymatic hydrolysis. The presence of willow bark inhibited saccharification in this feedstock, but the addition of 2-naphthol or formaldehyde increased it from 60–70% to about 90% at the highest pre-treatment severity. Willow was deemed a suitable feedstock as a source of fermentable sugars, but the need for improved commercial debarking methods to improve enzyme access and downstream hydrolysis was highlighted.

An alternative pre-treatment approach for willow involving the use of milder experimental conditions to separate the fibre bundles has been reported (Dou et al., 2021 i). Harvested two-year-old willow (hybrid Klara) stems were subjected to hot water extraction, followed by a mild alkaline (sodium hydrogen carbonate) treatment. Hot water extraction was applied first to remove soluble materials from the willow bark (80 °C for 20 min), and the second stage of alkaline treatment (liquid-to-bark ratio 8:1, 20 wt % dose of $NaHCO_3$) was conducted in a preconditioned water bath (100 °C, 60 min). The separated fibre bundles were washed with water, ethanol and acetone and finally collected. The fibres were subsequently converted into spun yarns, which provided excellent protection from ultraviolet radiation (UPF $\geq$ 140) and also exhibited antibacterial activity (A $\geq$ 8) against the Gram-positive pathogen *Staphylococcus aureus*, resulting in the complete eradication of viable bacteria after 24 -h incubation with the material.

This hot water extraction (HWE) approach was further developed, and willow bark separated from samples of four-year-old willow hybrid “Karin” (SalixEnergi Europa AB) were converted into lignin-containing cellulose nanofibril films for food packaging applications (Dou et al., 2021 ii). The HWE extracted bark required further processing using a disc refiner to obtain coarse fibre bundles prior to treatment with aqueous p-toluenesulfonic acid to liberate the fibrous cellulosic solid residue. It was reported that moisture and oxygen permeability (3 $cm3 \cdot \mu m/m^2 \cdot kPa \cdot day$ at 50% relative humidity) of the HWE willow bark films were among the lowest achieved for single bio-based materials and comparable to commercially available synthetic barrier films.

## 6. Medical properties of willow Bark

Willow (*Salix*) is widely used in industry, mainly to produce kitchen items, containers, construction materials and sports equipment. For example, the variety *Salix alba* var. Caerulea is informally called ‘cricket bat willow’ due to its wide use for manufacturing cricket bats. However, the industrial use of willow is based on the treatment of its pulp. In contrast, in recent years, the interest in the presence of several non-structural wood components with antioxidant properties, which can be extracted and isolated from willow bark, has increased (Houston Durrant et al., 2016). In fact, willow bark contains several metabolites which have been studied for their bioactivity, with phenolic compounds present in relatively high concentrations (Sulima and Przyborowski, 2019). Bioactive phenolics generally refer to a range of compounds, including phenolic glucosides, phenolic acids, flavonoids, lignans, stilbenes and tannins (Augusti et al., 2021). One of the most important bioactive compounds, which is present in willow bark, is salicin, which acts within the metabolic pathway in response to pathogens and environmental stresses to limit the negative effects of these external factors (Tyśkiewicz et al., 2019).

## 6.1.Bioactive Compounds and Medical Applications

Willow bark is a source of salicin and phenolic glycosides. However, in addition to salicylic compounds, willow bark contains several valuable non-salicylic compounds, such as flavonoids, lignans and tannins (Brereton et al., 2017). High levels of phenolic glycosides are present in *Salix daphnoides*, *Salix purpurea* and *Salix pentandra*; and the main compounds identified in those varieties can be divided into two main classes: salicylates (salicin, salicortin, acetylsalicin, acetylsalicortin, tremulacin); and phenolic glycosides (syrengin, picein, catechin, ampelopsin, vimalin, purpurein, naringenin) (Förster et al., 2008). In addition, *Salix viminalis* and *Salix purpurea* contain commercially relevant concentrations of phenolic acids, including hydroxybenzoic, chlorogenic, vanillic, ferulic, cinnamic, protocatechuic and p-coumaric acids (Tyśkiewicz et al., 2019).

These compounds possess several important pharmaceutical properties and exhibit antipyretic, analgesic, anti-inflammatory and anti-cancer effects (Fan et al., 2020). In addition, other bioactives, such as flavonoids, usually found in higher concentrations in flowers and fruits of most plants (Zielinska et al., 2018), are present in willow bark and foliage, which possess antioxidant and antimicrobial properties (Hage and Morlock, 2017).

Salicin formulations, including zinc salicylate, form non-steroidal anti-inflammatory inhibitors, inhibiting enzyme cyclooxygenase, which is required to convert arachidonic acid into thromboxane, prostaglandins, and prostacyclin (Gurpinar, Grizzle and Piazza, 2013).

### *6.1.1* Phenolic glycosides

The main bioactive molecules used in pharmaceutical and cosmetic products isolated from willow bark are phenolic glycosides. However the phenolic glycoside profiles of *Salix* barks are affected by a range of factors, including the willow variety, the time of harvest during the growing season and the local soil composition (Förster et al., 2021).

Salicin (Figure 2) is a key chemotaxonomic chemical found in the leaves of all species of the genus *Salix* sp. (willow) (Binns et al., 1968). It is also found as salicin and as well as derivatives in the bark and leaves of other plants within Salicaceae (Dickmann and Kuzovkina, 2014). One study quantified salicin, its derivatives and other phenolic glycosides in the bark of six *Salix* spp.  (Poblocka-Olech et al., 2007), reporting free salicin levels, ranging from 6.9 to 15.3 mg per g dry plant material and a total salicin content, including bound salicin in an esterified form, ranging from 16.8 to 96.3 mg per g dry bark . The salicin content measured in 91 different genotypes of *Salix purpurea* ranged from 3-11% of the total dry weight of the bark, and the authors suggested that it would be possible to breed willows with higher proportions of salicin (Sulima et al., 2017). Salicylates, especially salicin and salicortin, account for 72% of the phenolics in willow bark stems, with no difference in content between female and male genders of the willow tree and a slight decrease with age (Nissinen et al., 2018). Other phenolic acids amounted to 19% of the total phenolics present.

Ten different genotypes were grown in a field trial, revealing that the highest biomass yield was obtained with *S. purpurea* × *S. daphnoides.* But higher quantities of bark were obtained with another genotype, *S. americana* UWM (Warmiński et al., 2021).  However, *S. purpurea* × *S. daphnoides* contained the highest salicin content at 29 mg/g dry weight and the highest yield per hectare at 92 kg/ha.  In addition to salicin, a novel sulphated derivative, salicin-7-sulphate (Figure 2), was found at the highest concentration of 0.3% dry weight in the bark in *S. koriyanagi,* which is concerning due to potential gastrointestinal problems and toxicity that could be associated with this compound and therefore requiring further investigation (Noleto-Dias et al., 2018).  The same study also reported significant variation of this compound in the stem tissue of 86 willow varieties ranging from 0.3% per g dry wood in *S. maccaliana* to 5.8% per g dry wood in *S. acutifolia*.

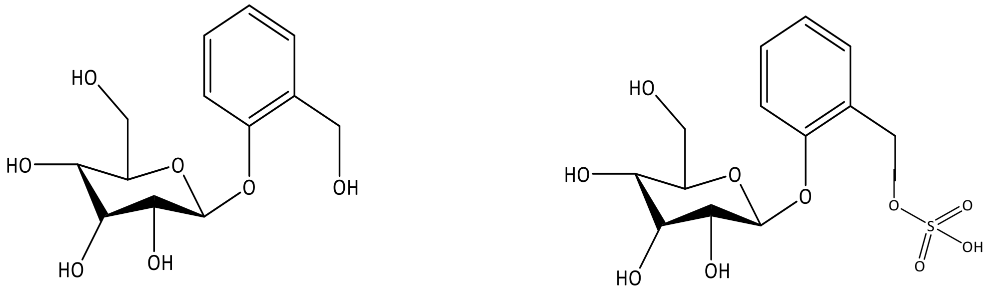

Figure 2. Chemical structure of salicin (left) and salicin-7-sulphate (right)

### *6.1.2* Flavonoids and Phenolic Acids

Salicin has been generally used as the bioactivity marker for willow bark, which has resulted in less attention being paid to the action of polyphenols (tannins, phenolic acids, and flavonoids) also present in the bark; up to 20% of the dry biomass weight. These compounds include naringenin, eriodictyol, flavan-3-ols catechin, epicatechin, gallocatechin, and procyanidand ins B1 and B3 (Nahrstedt et al., 2007).

Flavonoids constitute ~9% of the total phenolic compounds in willow bark (Nissinen et al., 2018), comprising different compounds within at least two groups, such as flavanols and procyanidins (Piątczak et al., 2020). A wide variety of compounds have been isolated from different willow species. In one study, 14 different phenolics were isolated from the bark of *Salix acutifolia Willd*. Including 7 different flavonoids (Zapesochnaya et al., 2002). Some of the flavonoids were sugar derivatives of parent molecules, where the glucose moiety could be removed using β-glucosidase. Two highly similar acylated flavan-3-ols were extracted from fresh bark of *S. sieboldiana* using acetone, along with two procyanidins and one trimeric procyanidin (Hsu et al., 1985). Acidification of one of the flavan-3-ols resulted in the formation of (+)-catechin and catechol. The study speculated that one of the flavan-3-ols (1-hydroxy-6-oxo-2-cyclohexene carboxylic acid) was a precursor in the biosynthetic pathway to the production of salicin, indicating that many of these different flavanols may be derivatives of each other.

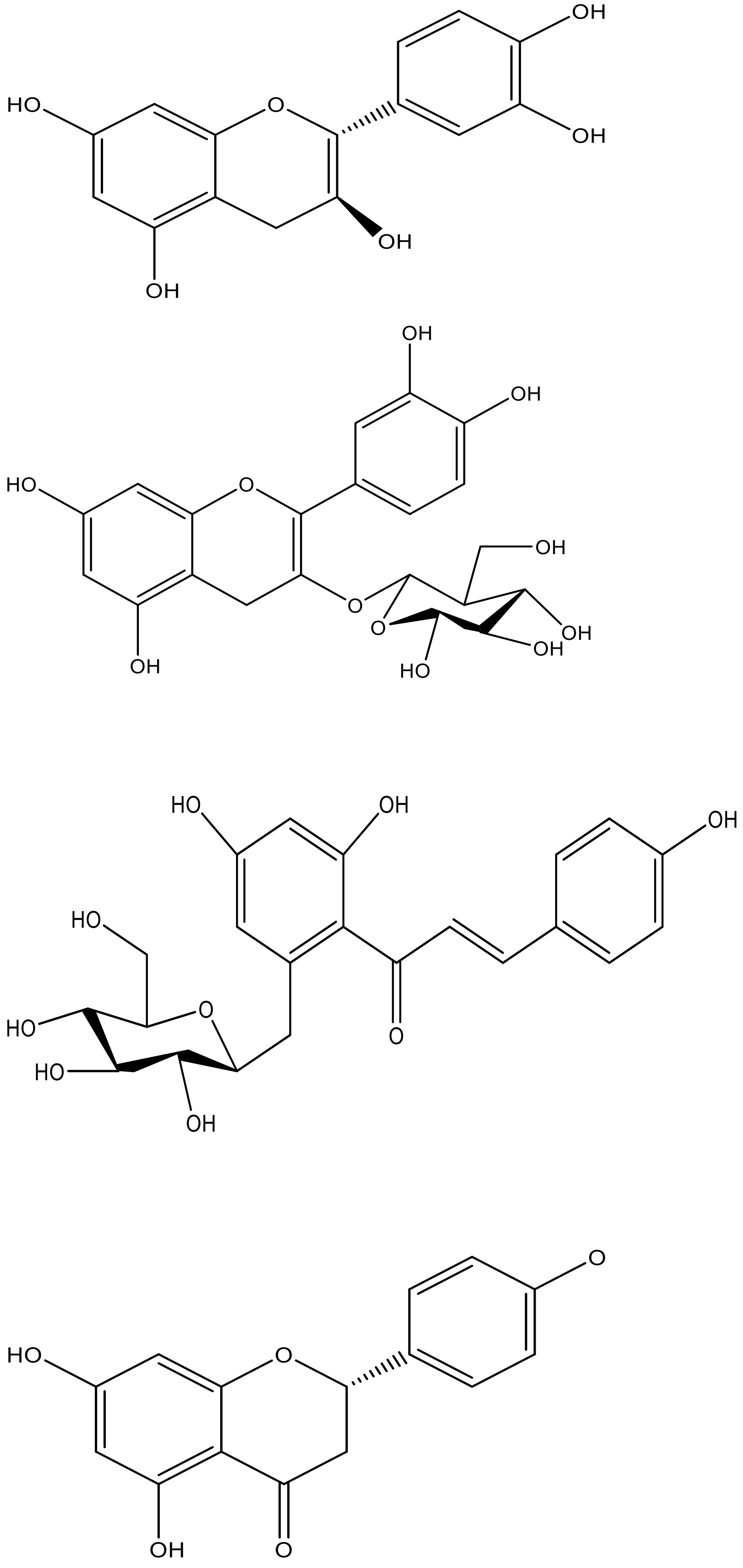

Figure 3. Chemical structures from top to bottom of (+) catechin, cyanidin-3-glucoside (note that the aglycone, in this instance, is (+) catechin), isoalipurposide and naringenin

Highly coloured flavonoids belonging to anthrocyanins were recovered using acidified methanol from the bark of 29 cultivars of *S. purpurea* and other *Salix* species. They were identified as cyanidin 3-glucoside (Figure 3) and delphinidin 3-glucoside as a minor component (Bridle et al., 1973). Another anthrocyanin was also found as a minor component buwasre recovered only in some varieties of *S. purpurea*, possessing leaves at the smallest range within willow. In a hybrid species of willow, *Salix* x *alberti*, the progeny of *S. integra* and *S. suchowensis*, the presence of four sulphated flavonoids have been reported (Notelo-Dias et al., 2020). Following extraction in a water-methanol mixture (4:1) at 50°C, the structures of dihydroflavonols (flavanonols), taxifolin-7-sulfate and dihydrokaempferol-7-sulfate, and flavanones, eridictyol-7-sulfate, and naringenin-7-sulfat were characterised using NMR spectroscopy and high-resolution mass spectrometry. Interestingly, this was the first time these compounds had been reported in plants, although the two flavanones have been partially characterised previously and respectively as dietary and fungal metabolites.

Factors affecting changes in phenolic composition were investigated in a study on 43 different phenolic compounds in *S. myrsinifolia*, and it was determined that wetter growing seasons resulted in willow infections by rust fungi, which in turn caused increasing phenolic content because the phenolics were acting as plant defence compounds (Nissinen et al., 2018). The effects of drought showed no overall changes in phenolic contents, but the production of specific flavonoids showed varying responses that differed from the norm in contrast to salicins that were unaffected by drought conditions (Köhler et al., 2020). Factors such as tree ageing and gender resulted in only minor variations of six phenolic compounds, including salicin. Such changes were described in a previous study revealing isosalipurposide to be present in young twigs of *S. purpurea* contained and naringenin to be present in older, where the structures are shown in Figure 2 (Jarrett and Williams, 1967).

The predominant flavonoids extracted as pure compounds from *S. alba* were eriodictyol (120 mg/g), 5,7-dihydroxychromen-4-one (29.5 mg/g), and naringenin (50 mg/g), respectively (Du et al., 2004). Eriodictyol and naringenin have been found in other *Salix* species (Freischmidt et al., 2015) that are commonly associated with therapeutic willow barks (Piątczak et a., 2020). Eriodictyol has reported antioxidant and anti-inflammatory properties (Islam et al., 2020), while naringenin has anti-inflammatory properties (Zeng et al., 2018). These studies seem to confirm the effectiveness of willow extracts despite the lower-than-expected salicin content contributing to efficacy (Vlachojannis et al., 2011).

## 6.2. Medical applications

### 6..2.1. Antioxidant properties

Several compounds are reported to possess antioxidant effects in willow bark. In one study, five compounds were identified as derivatives of phenolic acids, 26 between flavanols and procyanidins and three flavonols. The extracts exhibited strong antioxidant potential, determined by the assessment of their radical scavenging activity compared to 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,20 -azinobis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), common assays to determine the antioxidant potential. The results of the experiments confirmed that willow bark extract has stronger antioxidant properties even than the ascorbic acid used as a standard to assess the antioxidant activity of the extract (Piątczak et al., 2020).

The antioxidant activities of willow bark extracts from *S. purpurea* and *S. myrsinifolia* were shown to have a similar level of activity to the antioxidant activity in coffee, which contains a range of polyphenolics including such as caffeine, trigoelline and chlorogenic, nicotinic, quinolic, tannic and pyrogallic acids (Durak et al., 2015). One study reported high antioxidant activity associated with the bark extract of *S. aegyptiaca* which was a two-fold increase compared with leaf extract, and this activity was attributed to the presence of a polyphenolic compounds, quercetin, while salicin exhibited no activity (Enayat and Banerjee, 2009). While flavonoids may possess antioxidant properties, the highest levels of antioxidant activity are likely associated with polyphenolics (Durak et al., 2015). This would appear to be confirmed with higher levels of 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity associated with samples predominantly composed of polyphenolics compared with those comprising flavonoids (Enayat and Bannerjee, 2009).

The bark particle size was reported to have a significant effect on antioxidant activities in one study examining the granulometric classes ranging from <20 µm to >500 µm of milled bark from *Salix alba* (Zaiter et al., 2016). Extractions performed with 70% methanol resulted in the highest antioxidant activities using the DPPH and ABTS (2,2′-azino-bis (3-ethyl benzothiazoline-6-sulphonic acid) assays for extracts from the 50-100 µm fraction. Further downstream, three different protocols were examined to improve the extraction of antioxidants from the bark of *S. eleagnos* after grinding particles to <0.35 mm, followed by the determination of antioxidant activities using two assays, DPPH (2,2-diphenyl-1-pycrylhydrazil) and hydroxyl radical scavenging (Gligoric et al., 2020). The highest recovery yield of 22% was obtained with microwave extraction compared with a 19% yield using ultrasound and up to 17% yields using aqueous ethanol extraction.

These comparatively high antioxidant activities could offer potential treatment for chronic diseases, including arthritis. This potential was investigated using a methanolic bark extract obtained from *S. nigra,* which was reported to have high scavenging activity against superoxide, hypochlorous acid and hydrogen peroxide radicals, along with the suppression of lipid peroxidation in the rat model of collagen-induced arthritis (Sharma et al., 2011). These activities led to significant inhibition of paw swelling in female wistar rats. They showed maximum collagen-induced arthritis inhibition of 93.7%, with improvements to reduced infiltration of polymorphonuclear cells, smooth synovial linings, osteophyte formation, soft tissue swelling and bone resorption.

### 6.2.2. Analgesic effect

The consumption of willow bark extract has been used as a therapeutic practice for centuries to relieve pain. A study was conducted that evaluated effect of oral administration of willow bark extract and reported that >80% of salicin was absorbed (Steinegger and Hövel, 1972). In later studies, both salicin and another predominant salicylate, salicortin, were both shown to be metabolised to saligenin in the gut, absorbed into the bloodstream where saligenin was metabolised by the liver to salicylic acid (Fötsch et al., 1989; Julkunen-Titto et al., 1992).

Drying willow bark to produce homeopathic drugs at elevated temperatures (70°C) has been shown to reduce flavonoid concentrations, whereas concentrations of condensed tannins increased (Harbourne et al., 2009). It was postulated that flavonoids were polymerised at increased temperatures to form condensed tannins, causing the material to show increased redness. Consequently, the therapeutic phenolic glycoside content found in commercial extracts of willow barks may show differences depending on the species or type of extraction method used to recover the phenolics (Agnolet et al., 2012).

One clinical trial was performed using tablets containing a dosage of 240 mg per day of a bark extract of *Salix purpurea* x *daphnoides* and reported a moderate analgesic effect on 78 patients suffering from osteoarthritis over two weeks, compared with the group consuming a placebo (Schmid et al., 2001). In contrast, another clinical trial using tablets with the same dosage levels of a bark extract of *Salix daphnoides* revealed no analgesic effect on 178 patients suffering from osteoarthritis and rheumatoid arthritis (Biegert et al., 2004). However, the reduction in pain in both studies was similar, between 14-15%. Yet, the perception of the placebo group drifted with an increase in pain of 2% after two weeks of participation, compared with a decrease in pain of 2% after six weeks of participation. Consequently, only one set of results was significantly different. A comparison of the composition of the tablets from both bark extracts revealed only minor differences, although an additional phenolic, picein, was present in the extract that showed no efficacy (Kammerer et al., 2005).

### 6.2.3. Anti-inflammatory effect

Willow bark contains salicylic acid, which is in the form of acetylsalicylic acid (ASA), which is one of the most widely used anti-inflammatory drugs (aspirin). However, an interesting early study by Mayer and Mayer (1949) concluded that the overall anti-inflammatory effect of willow bark extract is produced by the presence of other compounds rather than salicylic acid. In addition, more recent experiments examined the medicinal properties of willow bark extract by assessing the contribution of salicin and other compounds to its anti-inflammatory effect. It was found that the beneficial effect of the whole extract was due to the presence of polyphenols such as flavonoids, phenolic acids and proanthocyanidins. Furthermore, willow bark extract proved to be as strong as ASA in terms of the rapid anti-inflammatory response of the body (Nahrstedt et al., 2007),

In a similar study, the anti-inflammatory effect of willow bark extract was compared with comparable doses of acetylsalicylic acid (non-selective COX inhibitor) and celecoxib (selective COX-2 inhibitor). The extract from willow bark produced a very similar anti-inflammatory response to ASA in terms of reducing inflammation zones and cytokines production. In addition, the extract proved to be more effective than ASA in suppressing the production of leukotrienes. In a subsequent report, polyphenols were found to play a major role in improving the free radical scavenging properties of the extracts (Khayyal et al., 2011).

In recent years, several researchers have supported the application of willow bark extracts for the treatment of chronic inflammatory diseases, such as osteoarthritis (Schmid *et al* 2001, Chrubasik, *et al* 2007, DeSilva *et al* 2011). In addition, these extracts are also widely advertised in sports, where they are used to improve the performance of athletes and help them with weight loss. The reason for the ability of willow bark extract to reduce inflammation is due to the suppression of the two main inflammatory response mediators: tumour necrosis factor-α and nuclear factor-kappa B. Nevertheless, the recurrence of adverse side effects of willow bark extract is much lower than non-steroidal drugs such as aspirin. However, the high incidence of subjects allergic to salycilates might be considered a downside to using untreated willow bark extract over common aspirin (Shara and Stohs, 2015).

Finally, in another study, 100 patients with chronic joint pain and inflammation were treated with a commercial product containing 250mg of willow extract and other herb extracts or a placebo. The results demonstrated that the group consuming the commercial product containing the willow bark extract benefited from a decrease in joint inflammation, pain and stiffness compared to the group that consumed the placebo. (Nieman et al., 2013).

### 6.2.4. Anti-microbial activity

Salicylic acid is a plant phytohormone regulating many aspects of plant growth and development. Salicylic acid is associated with pathological processes, binding with the glycolytic enzyme GAPDH, suppressing the protein's ability to enhance hepatitis C virus replication (Choi et al., 2015). The disruptive cell wall germicidal activity of zinc salicylate and the metabolites of zinc salicylate ensures that micro-organisms cannot develop resistance to their effect, unlike that of antibiotics (Gupta et al., 2014).

## 7. Veterinary applications for willow

Growing willows have found use in New Zealand as fodder for sheep (National Poplar and Willow Users Group, 2007) and at Chester Zoo, UK, where 25 hectares of Short Rotation Coppice (SRC) willow are grown as a feed for their large animals (Lindegaard, pers comm.). Willow foliage has high levels of Vitamin E and is favoured by black rhinoceros (Ricketts et al., 2020) and high levels of condensed tannins, enabling enhanced iron absorption in herbivores (Lavin et al., 2015). Several recent studies have focussed on producing winter silage for giraffes and gorillas using ensiled finely chopped whole willow and stripped willow leaves (Sauer, 2013; Sauer et al., 2015; Papies, 2019; Depauw, 2020). Willow silage was consistently preferred by one giraffe in the small-scale study compared with the standard hay silage, while other giraffes preferred other silage types.

The anthelmintic (de-worming) properties in weaned male lambs infected with *Teladorsagia* spp. and other organisms, including *Trichostrongylus*, *Cooperia* and *Haemonchus*, and subsequently fed a diet of willow have been reported (Mupeyo et al., 2011). A number of parameters were measured, including voluntary feed intake, apparent digestibility, blood composition, faecal nematode egg counts, and the production of eggs and hatched larvae.

Following slaughter, it was determined that the adjusted total daily egg production was lower in willow-fed sheep than lucerne-fed sheep due to reductions for *Haemonchus* and *Teladorsagia* spp.

A number of other studies have reported that willow feeds can also provide additional sources of micronutrients such as zinc and cobalt (Kendall et al., 2019) and can also reduce the greenhouse gas emissions from the digestive systems of ruminants (Ortuño et al., 2021).

The efficacy of a willow bark extract in treating canine osteoarthritis has also been reported (Shakribaei et al., 2012). This involved excising primary canine articular chondrocyte samples, which are the cells responsible for producing cartilage, from the joints of client-owned dogs undergoing orthopaedic surgery as a treatment for osteoarthritis. The research was based on evaluation of a chloroform extract produced from *Salix alba,* and following a series of *in vitro* experiments, it was determined that the bark extract did have an impact on the anti-inflammatory response, thus preventing the immune system from attacking the chronodrocytes.

A potential willow based treatment for heat stress, which is a major problem in commercially bred broiler chickens exposed to temperatures above 30 °C, has been investigated. There is increasing commercial interest in the use the of natural feed additives, including willow, in broiler diets to help combat this issue. A recent review revealed a dose-dependent effect of willow bark extract as a replacement for acetylsalicyclic acid, where lower concentrations had no effect on this condition (Saracila et al., 2021). A reduction in pathogenic bacteria populations in broiler caecum, including Enterobacteriaceae (−6.36%), E. coli (−2.03%) and staphylococci (−8.22%), was reported as part of this study. In addition an increase in immunological response in chickens fed with this willow bark extract, along with a lowering of both cholesterol and glucose levels, and an increase in weight gain by 10% were all reported as part of this study.

## 8. Biofuels and energy production

The production of bioethanol from willow and its use as a bioenergy crop are established areas of research. One of the challenges linked to bioethanol production, as with many lignocellulosic feedstocks is that cellulose, hemicellulose and lignin are interconnected, which can limit access by cellulases and hemicellulases during enzymatic hydrolysis. In order to optimise the yields of fermentable sugars for downstream fermentation following hydrolysis, strategies are required to deconstruct the biomass matrix and improve enzyme accessibility. The application of a thermal pre-treatment at 190°C for 20 min to crushed willow branches measuring 5 cm in diameter, followed by hydrolysis with xylanase and glucosidase at 50°C, has been investigated (Vahar et al., 2012). The heat treatment only reduced hemicellulose content, whereas the cellulose fraction remained unaffected, and the proportion of lignin associated with the insoluble fibre material increased. The use of enzymes released about 28% monomeric sugars during 24 h incubation, predominantly glucose and xylose. However, most of the sugars contained within the process effluent were comprised of xylose due to the breakdown of hemicellulose during the pre- treatment.

Pre-treatment of willow to assist with downstream processing and fermentation can be achieved using either acidic (Kraft), alkaline (sulphite), or organic solvent (organosolv) processing and each of these approaches have been investigated. When a chemical approach using either sulphur dioxide or sulphuric acid treatment on willow chips obtained from five-year-old willow shrubs (*S. schwerinii* × *S. viminalis*) was combined with steam pre-treatment, 60% of sugars were released from xylans compared with only 30% of sugars released from xylans using only heat treatment (Sassner et al., 2006). Therefore, both the combined and heat treatments resulted in the deconstruction of fibres with some loss of hemicellulose to form sugars, although the cellulose and lignin fractions remained mostly intact. Another study using an alkaline approach identified the optimum conditions for the process to be immersion of willow chips in four volumes of liquor containing sodium oxide (22%) and heating to 160°C (Pinto et al., 2015).

Organosolv treatment of 280 g of two-year-old *S. schwerinii* in 35% ethanol at an elevated temperature of 185°C reduced the lignin content (MacFarlane et al., 2008). Increasing concentrations of ethanol, 35% compared with 70%, resulted in higher lignin concentrations remaining because slightly more acidic conditions were associated with the lower ethanol concentrations that assisted lignin removal. A later study confirmed the role of low acidity in the organosolv process when comparing three different catalysts, sulphuric acid, hydrochloric acid and magnesium chloride, in treating willow chips (Huijgen et al., 2011). Combining both acid and organic solvent with sulphuric acid in an ethanol-water ratio of 55% at 190°C for 180 minutes resulted in the release of 87% of available sugars after enzymatic hydrolysis with Accelerase 1500 for 72 h at 50°C.

An analysis of the willow chips of 40 different genotypes in the UK revealed one genotype, a shrubby willow (*S. miyabeana*), yielded the highest glucose liquor concentrations, through acid hydrolysis and the highest glucose concentrations released through enzymatic hydrolysis with Celluclast 1.5L and Novozyme 188. Yet, this genotype possessed neither the highest glucan nor the lowest lignin content (Brereton, 2011). An analysis of the same study of focusing on 35 different genotypes of the original 40 revealed no correlation between sugar release and glucan or lignin contents for the different genotypes (Ray et al., 2012). A higher level of monomeric sugar release was achieved when an acid pre-treatment was incorporated prior to enzymatic hydrolysis. Following enzymatic hydrolysis, it was reported that the lignin contents had increased from the ranges of 23.8-28.0% to 36.1-40.7%, demonstrating the effect on composition between the different willow genotypes. It was evident that another genotype, Endurance, was found to yield the highest biomass per hectare, which was also amenable to high enzymatic hydrolysis to release glucose for bioethanol production (Brereton, 2011; Ray et al., 2012).

It is possible that the presence of phenolic compounds could act as fermentation inhibitors during downstream conversion (fermentation). Phenolic inhibitors were reduced by the treatment of hydrolysates of *Salix caprea* formed by steam treatment at 205°C for 6 min with 2 µM laccase, allowing ethanolic formation by the yeast *Saccharomyces cerevisiae* to proceed (JoÈnsson et al., 1998). Another later study showed a reduction of phenolics by 33% in willow with laccase activity prior to bioethanol production (Dhiman et al., 2015). This study showed that a saccharification yield of 41% could be achieved using optimal enzymatic concentrations, which compared favourably with 65% using alkali-treated willow. Finally, the sugar to the ethanol conversion efficiency of 72% was achieved, although no comparisons were made without using laccase.

The generation of biogas was compared with the direct combustion of willow chips which revealed that anaerobic digestion was preferential in terms of global warming potential, resulting in carbon capture of 310 tonnes in the remaining solids from 921 tonnes of wet willow (Ericsson et al., 2012). However, the quantities of biogas produced were low compared with manure. Instead, the pyrolysis process was recommended that would also result in a carbon captured solid as biochar. In another anaerobic digestion study of 94 different types of willow representing 71 genotypes, it was found that most types of guaiacyl lignin had a negative impact on biogas production along with phenolic compounds present in the bark (Ohlsson et al., 2019). The study was performed using the longest shoots of two-year-old willows, which were ground into sawdust and treated over 94 days. Therefore, the factors that affected anaerobic digestion were similar to those involved in the enzymatic release of sugars for bioethanol production, where crop biomass yields was important along with the ratio of syringyl/guaiacyl ratio with the additional factor of phenolic inhibitors. However, fungal pretreatment with *Abortiporus biennis* of willow sawdust for 28 days increased methane production by 48%, which was unaffected by different types and concentrations of nitrogen input during the fermentation process (Alexandropoulou et al., 2017). The increase was attributed to delignification which was determined to be 24% for most of the experiments. The use of fungal fermentation along with alkaline hydrolysis was also examined which revealed only a 7% increase in methane production compared to the control without fungi. Methane production levels using alkaline hydrolysis resulted in a 100% increase in methane production compared with the original, untreated willow samples.

## 9. Willow pulping

Wood fibre length and fibre aspect ratios are important parameters for pulping and subsequent paper production, and these were investigated as part of a study based on prediction modelling using different willows (Monteoliva et al., 2008). In contrast within the same study, lignin content showed a negative correlation with pulp properties. It was determined, following the production of test paper handsheets by cooking at temperatures between 70-80 °C for 30-90 minutes of two-year old pulped willow trees, without debarking (Ai and Tschirner, 2010). It was found that the fibre length (0.34 mm) was 50% shorter and the pulp yield was 7% lower compared to material obtained from aspen, which is traditionally used in papermaking, and this resulted in lower tensile paper properties. However, the fibre lengths of willow were similar to aspen when the woods were left untreated and contained lower ash contents compared with the other lignocellulosic substrates examined, including poplar, switchgrass and alfafa. In another study, which investigated the fibre lengths of pulped wood chips obtained from 13-year-old willow trees, following alkaline pre-treatment and atmospheric refining, a range of fibre lengths from 812-1167 µm was generated using six different *Salix* clones (Monteoliva et al., 2007). It would appear longer fibre lengths were obtained during the atmospheric refining of 13-year-old willows (Monteoliva et al., 2007) compared with the cooking two-year-old old willows (Ai and Tschirner, 2010). However, another study revealed no distinct trends in fibre lengths and widths of two different clones of *Salix excelsa* during an analysis of earlywood and latewood over three years using blending (Elmas et al., 2018). A longer duration study over 13 years revealed a significant increase in fibre lengths from two years to seven years and a minor increase thereafter at 12 years (Monteoliva and Marlats, 2006). The growth site was found to be important for some clones besides age and while there were minor differences in fibre lengths and widths between clones, major differences were evident based on wood density which is an important factor in paper production. Furthermore, the predicted paper properties based on fibre measurements in terms of elasticity and rigidity revealed both clones to be highly similar.

An evaluation of different drying processes, including supercritical carbon dioxide extraction, freeze drying and vacuum evaporation after immersion in acetone on willow pulp formed using the Kraft process (moisture content of willow pulp at the start was 79%) indicated that the type of drying process used had a significant effect on the available surface area of cellulose and xylan (Saito et al., 2018). Larger surface areas would indicate less adhesion between xylan and cellulose to cellulose surfaces that could acetylated lead to biocomposite board with improved properties such as increased hydrophobicity.

The use of cellulose and willow lignin blends as a potential source of sustainable carbon fibres has also been reported, following extraction of the lignin using trialkyl ammonium salts (Vincent *et al* 2018). Lignin-cellulose fibres were subsequently produced using a dry-jet, wet spinning technique and in relation to the mechanical properties of these fibres, resulting in increases in tensile strength and Young’s modulus when the cellulose weight fraction in the fibres was increased. Values of 3.04 ± 0.48 GPa to 8.31 ± 0.61 GPa for Young’s modulus and 29.91 ± 4.48 MPa to 152.45 ± 6.02 MPa for tensile strength were reported. The lignin/cellulose fibres produced exhibited better mechanical properties than previously reported pure lignin fibres or those combined with non-renewable synthetic polymers. Blending was also achieved with 20% hot water extracted and alkaline treated willow bark from one-year-old trees with 80% polylactic acid to produce a composite board revealed an increase (Dou et al., 2019). The tensile and elastic properties of the blended composite board increased compared with using a board prepared with polylactic acid only.

## 10. Fungal delignification of willow and mushroom cultivation

The utilisation of white rot fungi as a biological approach to deconstructing a range of lignocellulosic feedstocks, including willow, has been investigated over a number of years. White rot fungi have been studied because they contain a range of lignin-degrading enzymes which assist with this deconstruction process (Baker et al., 2017). These fungi would perform a dual process in obtaining mushrooms and generate remaining biomass that has lower lignin content and might assist in improving the tensile properties of packaging material.

Long term storage of willow logs after harvesting revealed that the white rot fungi, *Schizophyllum commune, Phanerochaete flavido-alba* and *Trametes versicolor,* were the primary colonizers after 17 weeks, which was followed by an increasing diversity of fungi after 34 weeks (Eslyn and Lombard, 1984). Presumably, the initial colonizers had altered the composition of the willow, allowing other white rot fungi to subsequently grow on this substrate. Fungal degradation of willow wood by *Trametes trogii* revealed that almost 30% of the wood block was degraded, showing equal degradation of cellulose and lignin (Levin and Castro, 1998). Most of the degradation occurred within two months and fine fibres were produced after six months of degradation. It was found that 30% of the lignin was lost when *Leiotrametes meizeisii* was inoculated into 6 g willow sawdust and incubated over 30 days, although higher losses of hemicellulose and cellulose were determined compared with fungal degradation by *Abortiporus biennis* (Alexandropoulou et al., 2017a). The addition of nitrogenous compounds such as yeast extract, urea or ammonium nitrate resulted in higher cellulose degradation leading to higher fungal biomass of *A. biennis* but reduced delignification (Alexandropoulou et al., 2017b). A much higher level of delignification of 46% was achieved after 120 days when a plug of *Echinodontium taxodii* was inoculated into 5 g of willow dust (Yu et al., 2009).

Willow chips are used for the commercial cultivation of mushrooms and in one study the biological efficiency was evaluated for 14 different *Pleurotus* strains belonging to five different species (Lechner and Albertó, 2011). The biological efficiency is the percentage of fresh weight of mushrooms per weight of material used for mushroom growth and the results revealed a significant variation in this parameter, ranging from 14-171%. In a more recent study, the growth of three different fungi: *Pleurotus ostreatus*, *Pleurotus eryngii* and

*Pleurotus geesteranus* on three different types of wood chips (*Pennisetum sinese*, willow and pine) that were AFEX (ammonia fibre expansion) pre-treated and were compared with untreated wood chips (Hu et al., 2021). Higher hemicellulose, cellulose and lignin decomposition rates were observed with the AFEX treated wood chips compared with the untreated wood chips, which was generally accompanied by higher lignin-degrading enzymes activities. Consequently, the formation of fruiting bodies on AFEX treated samples occurred within a shorter timeframe and resulted in almost two-fold higher quantities. Furthermore, of the fungal species examined, higher yields were produced on certain wood species, regardless of AFEX treatment, although none showed higher yields on *Salix* chips. Similarly, the laccase activities of *Pleurotus ostreatus* and *Ganoderma lingzhi* were comparatively low on *Salix babyloncia* when the study was evaluating the production of laccase on different lignocellulose substrates (An et al., 2021).

## 11. Phytoremediation applications

The fast growth and high yield of SRC willow have also been combined with their ability to tolerate inundation and contaminated sites, take up large quantities of water and remove chemicals from the soil (Kuzovkina & Quigley, 2005). In Sweden and Northern Ireland, there are examples of using SRC for biofiltration of municipal wastewater (McCracken, Johnston, 2015, Werner & McCracken 2008) and livestock farming dirty water (Forbes et al., 2017). Furthermore, applying phytoremediation to landfills associated with leachate treatment has several potential advantages including providing a water and nutrient source for enhanced plant performance and crop yield. The application of SRC willows with the explicit intention of managing landfill leachate in Sweden has been reported (Dimitriou & Aronsson 2010).

For many reasons, the use of willow and poplar for energy and environmental purposes is still a very immature part of the farming and land use sector (Adams and Lindegaard 2016, Lindegaard et al, 2016). However, the climate emergency and net zero targets are encouraging policymakers to find ways to encourage farmers to make this change (BEIS. 2021). There is a growing body of modelled research indicating that the integration of willows for water quality protection incorporated with livestock agriculture, can lead to the reduction of overall GHG and pollution emissions and thus reduce the overall strain on the food-energy-water nexus (Livingstone et al., 2021; Livingstone et al 2022). These findings would seem to support current government aspirations, such as the UK’s planting of 700,000 hectares of biomass crops which could incorporate approximately 3.8% of UK agricultural land by 2050 (CCC, 2020).

## 12. Biocomposite materials

The use of willow fibres, flour, bark and residual biomass in biocomposite materials for potential applications in construction, food packaging and battery technology, is another important sector of area of interest in creating a value chain of higher value products from this feedstock.

The anatomy of the willow fibres has been investigated using optical and electron microscopy (Okatenee et al., 2017) in order to determine their broader application in wood polymer composite (WPC) applications. Thermogravimetric analysis of these fibres indicated that the major mass loss occurred at 257 °C and their density was measured with a gas pycnometer (1.19 ± 0.2 g/cm$^3$). A preliminary assessment of the mechanical properties of single willow bast fibres indicated that the tensile strength (307.6 ± 130.1 MPa) and Young's modulus (16.9 ± 8.4 GPa) were comparable to some commonly used natural fibres, indicating that this material has the required mechanical properties and thermal stability for potential use in natural fibre composites.

The production of willow-fibre reinforced polylactic acid (PLA) based composites and their foam processability has been reported (Zafar et al., 2016), using microcellular materials prepared by foam injection moulding and nitrogen as the blowing agent. The impact of including the willow fibre (30% loading) on a range of properties, including morphology, mechanical properties, thermal stability, crystallisation, and heat deflection temperature, was reported. The inclusion of the willow resulted in improved tensile and flexural properties along with increased crystallinity in the foamed composites, confirmed by differential scanning calorimetry.

The use of willow flour obtained chipped material as an alternative for standard hardwood (e.g., beech, oak) or softwood (e.g., pine, spruce) flours used in the production of wood plastic composites (WPCs)  made from polyethylene has been reported (Barton-Pudlick, 2018). The structural and functional properties were investigated, and higher hemicellulose and lower lignin levels in the resultant WPCs resulted in better impact strength values of those materials. WPCs with conventional hardwood fillers (30–50 wt % fibre loading) typically have tensile and impact strengths of 20–30 MPa and 3–5 $kJ/m^2$, respectively, and the reported materials produced using the willow flour had slightly better mechanical properties. The willow flour, therefore, has the potential for use as a more cost-effective alternative to hardwood fillers.

Kumar et al (2019) described the use of short-rotation wood particles from both willow and aspen in injection moulded composite samples produced using PLA and the effect on the physical/ mechanical properties and microstructure of these biocomposite materials. Willow stems were sawn into small blocks, following the removal of bark, branches, and larger knots then chipped and dried with warm air to stabilise the moisture content. The processed willow was mixed with a natural binder in 10%, 20%, 30%, and 40% amounts and based on the dry weight percentage of PLA prior to injection moulding. Testing of the injection moulded samples indicated that the tensile and bending strength initially decreased with 10% weight percentage of wood particles when compared to pure PLA, but showed an increasing trend with higher wood particle contents. Microstructure analysis indicated the presence of good interfacial bonding between wood particles and biopolymer, but also variations in the homogeneity with different weight percentages of wood particles. Despite this, it was concluded that willow has excellent potential for use in the production of biocomposite materials.

Willow bark has also been used to produce biobased barrier films for packaging applications as an alternative to fossil fuel-derived materials (Dou et al., 2021). Lignin-containing cellulose nanofibril films were produced from willow bark using a hot water extraction method, which resulted in materials with good moisture and oxygen barrier properties. The hot water extracted films achieved an oxygen permeability of 3 $cm^3 \cdot \mu m/m^2 \cdot kPa \cdot day$ at 50% relative humidity, which is among the lowest achieved for single bio-based materials and comparable to commercially available synthetic barrier films. These films also exhibited complete blocking of UV light transmission within the wavelength range of 290–400 nm, highlighting their potential to limit issues associated with UV radiation and oxygen permeation in food packaging applications.

In a further development of this work, combining biopolymers and natural bioactive phenolic components as protective coating layers in packaging was investigated (Lohtander et al., 2021). Polyphenols are naturally occurring bioactive molecules that protect organisms from physical and chemical threats such as UV irradiation and oxidative stress. A fully wood-based and crosslinked film material, incorporating polyphenolic compounds obtained from willow bark, embedded in nanocellulose was produced to impart greater resistance to UV and oxidative degradation was produced. Crosslinking was achieved using both UV irradiation and enzymatic methods. The material produced displayed enhanced rheological properties that could be cast into optically active films exhibiting good antioxidant and tunable oxygen barrier properties for potential applications in the packaging, medical, pharmaceutical, food, and feed sectors.

## 13. Life Cycle Assessment aspects of willow valorisation

The success of an integrated willow biorefinery model will depend not just on its economic viability but also on its environmental credentials. Life cycle assessment (LCA) is a methodology that can be used to model the environmental performance of a willow biorefinery system, providing feedback on potential hotspots within the process and overall results on a product-by-product basis. However, modelling the complex multi-product systems typical of refinery operations (whether petrochemical or bio-based) is typically complex and involves important methodological choices regarding the apportioning of process burdens across different product streams, as well as other key attributes of the system modelling (Ahlgren et al., 2013). Such choices significantly affect result outcomes (Luo et al., 2009; Ardente & Cellura, 2011), so close adherence to and understanding of LCA standards, such as ISO-14040 and ISO-14044 (International Organization for Standardization, 2006) are key to transparency and inter-comparability of results. Issues too, relating to the accounting of biogenic carbon, both within biomass and soil at the start of the process and as stored in downstream products, such as bio-composites materials, need clarifying to ensure harmonisation of approach when modelling LCA in biorefinery systems (Ahlgren et al., 2013).

With respect to biomass production, where crops are grown for the primary purpose of providing feedstock for onward processing, burdens associated with their cultivation sit within the lifecycle boundary. Such burdens in relation to climate change can be significant, especially if significant nitrogen fertilisers (e.g., Krzyzaniak et al., 2016; González-García et al., 2013) or land use change, whether direct (e.g., Lark et al., 2022) or indirect (e.g. Searchinger et al., 2008) are involved.

Modelling of willow biomass produced on land converted from cropland and pasture or grassland has been reported (Yang et al., 2020), including assessing impacts up to receipt of chipped willow by the processor. When planted on cropland or pasture, GHG emissions associated with the chips were negative (−0.053 to 0.18 kg $CO_2e$ / tonne), whereas they were slightly positive when grown on converted grasslands. The difference was due to changes in soil organic carbon associated with direct land use change. Willow yield and transport distances were also identified as key determinants in the carbon balance of this system.

Fertiliser manufacture and application, followed by harvesting, accounted for the majority of the primary energy usage (approximately 75% of 98.3 GJ $ha^{-1}$) over 7 rotations in a willow biomass crop production system (Heller et al., 2003). The fertiliser type and application technique were also highlighted as environmental hotspots in a short rotation coppice (SRC) willow feedstock system for small-scale electricity generation (Goglio and Ownede, 2009). Other important inputs are the drying technique utilised and the type of biomass-to-energy conversion plant chosen. The scale of cultivation is also an important factor, with smaller willow plantations having larger environmental footprints (Kowalczyka & Kwaśniewski (2019).

Life cycle inventory (LCI) data for willow stem cutting production (for planting) is available through the Ecoinvent v3 database (Wernet et al., 2016), based on a German study (Hölscher et al., 2007). This suggests a global warming potential (GWP) of 1.19 kg $CO_2e$ / kg cut stems (moisture content 48%) after electricity for cold storage post-harvest (which would not be required in a willow biorefinery system) is removed. This is based on modelling all inputs associated with growing the willow, including the establishment of the plantation, input of mineral fertilisers and pesticides, machine operations and transport of stems from field to farm (2km). Planting density was assumed to be 13,500 stems per ha and direct field emissions were included. The same study also provided data for air-dried willow wood chips and particles produced from SRC. These have a GWP of 0.0651 kg $CO_2e$ / kg dry matter (moisture content 23%), based on a plantation with 20 years lifetime, harvested after 4 years and every 3 years thereafter, yielding an average 36,333 kg/ha dry matter each harvest.

Krzyzaniak *et al.* (2014) examined the potential to use specific cultivars of willow to optimise yield and chemical composition for use as a biorefinery feedstock. Certain clones of *Salix viminalis L.*, for example, were identified as being highly useful for an integrated multi-product biorefinery due to their content and yield of cellulose and hemicelluloses. Optimising such factors would reduce the lifecycle impacts associated with willow processing. The environmental impact of growing willow specifically for bioenergy or biorefinery systems has been assessed, and a carbon footprint of 0.1 kg $CO_2$e / kg dry matter feedstock was reported (Parajuli et al., (2017). This was lower than the value obtained using straw obtained from spring barley as a feedstock and marginally higher than that for alfalfa (0.084 kg $CO_2$e / kg dry matter). Eutrophication potential, which can significantly impact agricultural systems, was considerably lower for willow biomass than for these two other crops. The lower carbon footprint reported for willow was primarily due to higher soil carbon sequestration and lower $N_2O$ emissions.

The post-harvest transportation distance to the processing plant is an important factor, and it has been reported that the movement of willow chips from up to 38 km did not have a significant impact on net energy production, or carbon emissions, in a willow-based small-scale electricity generation model (Goglio and Ownende 2009). However, over greater distances, transportation burdens played a greater role in the system's overall efficiency (energetic and carbon). Similarly, Caputo et al. (2014) highlighted impacts associated with diesel use during transportation of willow to processors site as being a key determinant in overall environmental impacts, suggesting that decentralised biorefineries should be promoted as a means of improving system-wide performance.

The lifecycle impacts associated with bioethanol production from SRC willow, pre-treated using sulphur dioxide and heat (205°C), followed by saccharification and co-fermentation have been assessed (Budsberg et al., 2012). Bioethanol produced this way was slightly carbon negative due to the displacement of fossil-based electricity with renewable electricity generated during the bioconversion process. However, it required significantly more water to produce than gasoline. This was predominantly due to water consumption during the conversion process, combined with that required to produce chemicals and enzymes used in the process. Willow performed slightly better in terms of climate change, water consumption and non-renewable energy, when compared to a similar system using SRC poplar as the feedstock. The study concluded that willow-based bioethanol was a highly viable alternative to gasoline, but that attention was needed when designing and operating plantations and biorefineries to minimise unintended negative consequences. Issues associated with freshwater toxicity also need to be considered when planning willow wood chip production systems (Krzyzaniak et al., 2016).

The production of bioethanol from sugars obtained using a hot water extraction of willow grown on grasslands has also been reported (Therasme et al., 2021). In relation to the environmental impact of this process, it was determined that ethanol produced from the fermentation of the sugars released during this extraction step can sequester 0.012±0.003 kg CO2eq $MJ^{-1}$ for a supply system incorporating summer harvest and storage. However, the proportion of organic soil carbon does decrease using this approach. It was, however concluded that the production of willow and conversion into biofuels, while using a portion of this biomass as the energy source for the operation, can generate a transportation fuel with a negative carbon footprint.

Maximising the energetic efficiency of biorefinery operations is essential to their economic and environmental balance. The Borregaard biorefinery facility, located in Norway, reported that approximately half the GHG emissions associated with its wood-derived range of bio-based products were generated from the combustion of oil and waste biomass, used for operational heat and steam generation. These inputs outstripped emissions from feedstock and chemical production, transport and electricity generation combined (Modahl & Vold, 2011). Drying, in particular, can be an energetically expensive step in biomass processing and use of biomass burners and combined heat and power operations are essential to minimizing carbon emissions associated with these steps. Therasme et al. (2021) reported a negative carbon balance for bioethanol (0.012 ± 0.003 kg $CO_2$e / MJ) produced from the fermentation of sugars from hot water extraction of willow grown on cropland, but only when electricity and heat required for the conversion process came from renewable sources. Accounting for sequestration of carbon in the belowground portion of the plant was also essential for this negative footprint result.

Ultimately, the environmental burdens of each integrated biorefinery proposition will depend on the specific mix of products and co-products being manufactured, as well as the feedstock(s) used and the approaches to their cultivation, harvesting and downstream processing. Maximising the environmental efficiency of such operations will therefore be determined by the extent to which all component fractions can be valorised and upgraded and, by implication, the degree to which the generation of low-value waste streams is minimised. A comprehensive lifecycle assessment of an optimised willow biorefinery model would be of great interest and would add to the growing knowledge base of biorefinery lifecycle understanding and SRC willow-based energy and biofuels production.

## 14. Conclusions

The term circular bioeconomy is now being used to describe the transition for the production of commodity and speciality materials, away from non-renewable, fossil fuels, to using more sustainable biomass feedstocks and residues, as alternative raw materials for the manufacturing sector. This can be achieved by growth of high yielding industrial SRC crops, including willow, on land unsuitable for food production as a lignocellulosic feedstock to supply integrated biorefineries that will support this emerging sector. The ultimate aim in utilising lignocellulosic residues is to improve resource-efficiency and optimise the inherit economic value of biomass, through production of a spectrum of chemicals, materials and energy. One of the key technical challenges in this sector is the development of robust, scalable pre-treatment technologies to facilitate the downstream separation of key components including polysaccharides, lignin and bioactive molecules.

This review has focused on the potential value chains of products that could be generated from high yielding willow varieties that are cultivated commercially using a SRC approach. There is potential to valorise the willow bark, pulp and whole plant to produce medical and veterinary products, energy (biogas, bioenergy and biofuels) and biocomposites materials (packaging and wood plastic composites).

In addition to the commercial potential of willow as a source of biobased products, there are environmental benefits associated with this plan which have been alluded to in this review, including its use in phytoremediation and flood mitigation strategies. The impact of global warming and more extreme weather patterns mean that these environmental benefits could increase the future economic value of this crop. This could encourage commercial cultivation by greater numbers of farmers, creating additional opportunities for diversification in the rural economy, which is a key component in any biobased product supply chain.

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