

INTEGRATION OF “WILLOW ENERGY” WITH EXTRACTION TECHNOLOGIES

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Abstract

Biomass is the plant material derived from the reaction between CO₂ in the air, water and sunlight, via photosynthesis, to produce carbohydrates that form the building blocks of biomass. Biomass contains varying amounts of cellulose, hemicellulose, lignin and a small amount of other extractives. Woody plant species are typically characterized by slow growth. The attention paid to particular woody plant species varies around the world, taking account of the soil and climatic factors that affect growth. In the context of northern Europe, much attention has been focused on the woody species, especially willow and poplar. These wood plants grow 6-10-fold faster in comparison to other trees. One hectare of willow (25% moisture) produces approximately 13 tonne of willow every year with an energy content of 13.2 Giga Joules (GJ) per ton. Therefore one hectare produces 172 GJ's of energy per year. Our investigation deals with extractives which content about 15% of whole biomass. The extracts obtained from willow and poplar bark and foliage, and purified phytochemicals arising from these extracts, can find various applications, e.g., as biopharmaceuticals, food additives and nutraceuticals, biopesticides, and in cosmetic products. Considering the increased progress in the development of innovative biorefinery technologies, the renewable biofuel market will continue to provide substantial opportunities for potential investors in this area. We found more than 80 lipophilic constituents from raw materials of willow and poplar bark and foliage.

Key words: willow energy, extraction technologies, biomass

The conversion of biomass to energy (also called bioenergy) encompasses a wide range of different types and sources of biomass, conversion options, end-use applications and infrastructure requirements. Biomass can be derived from the cultivation of dedicated energy crops, such as short rotation coppice (SRC), perennial grasses, etc.; by harvesting forestry and other plant residues (forest thinnings, straw, etc.); and from biomass wastes such as sludge from organic industrial waste and organic domestic waste or the wastes themselves. In each case the biomass feedstock has to be harvested/collected, transported and possibly stored, before being processed into a form suitable for the chosen energy conversion technology.

The use of biomass to produce energy is only one form of renewable energy that can be utilized to reduce the impact of energy production and use on the global environment. As with any energy resource there are limitations on the use and applicability of biomass and it must compete not only with fossil fuels but with other renewable energy sources such as wind, solar and wave power. Literature data (McKendry, 2002, Stokes, 2006, Volk, 2004) show an overview of the sources and main types of biomass and typical plant characteristics was presented. First-generation biorefineries use feedstocks such as corn starch or sugar cane that are renewable, but that also have feed/food uses. As production levels have increased, along with human populations, concerns about competition with food needs have arisen (Field, 2007, Kamm, 2007). Nevertheless, over the past 30 years these first-generation feedstocks have paved the way for production of biofuels via a more sustainable system without negative impacts on the environment or food supplies. Second-generation biorefineries are based on biomass feedstocks that are more widely available and that are not directly used as food, although some are used as livestock. Technologies are under development to efficiently convert biomass into ethanol as well as valuable co-products. These are leading the way to sustainably meeting energy needs while also supplying materials for chemical and manufacturing industries. Biomass has the unique advantage among renewable energy sources that it can be easily stored until needed and

provides a liquid transportation fuel alternative for the near term. However, cellulosic ethanol can displace only the 40% of a barrel of crude oil that is used to produce light-duty gasoline. Research, development, and demonstration on a range of technologies are needed to replace the remaining 60%, which is primarily converted to diesel and jet fuel. About 15% of our current crude oil consumption is used to produce solvents, plastics, cleaners, and adhesives. Thus, cost-effective technologies are needed to produce biofuels that are suitable for drop-in use in cars, trucks, and jet planes. These advanced biofuels can be sustainably produced from cellulosic and algal feedstocks. Biomass conversion technologies are also needed to produce chemical intermediates and high-value chemicals. Compatibility with the existing infrastructure will aid in process integration and increase profitability of biorefineries. The feedstocks evaluated were those that are currently produced from agriculture and forestry sources, including grain crops (mainly corn for ethanol, sorghum, and barley), sugarcane, sugar beets, oil crops (primarily soybeans for diesel), canola, sunflower, rapeseed, municipal solid wastes, fuel wood, mill residues, pulping liquors, and urban wood wastes, as well as potential forest and agricultural biomass and waste resources. Under conservative assumptions, the combined resources from forests and agricultural lands, assuming feedstock prices of \$40-\$60 per dry ton, will increase from 138-258 million dry tons in 2012 to 243-767 million dry tons by 2030. Total energy crops, including perennial grasses such as switchgrass and miscanthus, woody crops such as poplar, willow, southern pine, and eucalyptus, and annual energy crops such as high-yield sorghum, are projected to contribute significantly to this increase, going from less than 4 million dry tons in 2012 to 34-400 million dry tons in 2030. Energy crops have the potential advantages of being produced on marginal lands not used for growing food, requiring essentially no fertilizers or irrigation, and, especially if perennial, requiring little or no tilling. Advanced biofuels may include any of the following: 1) ethanol derived from cellulose, hemicellulose, or lignin; 2) ethanol derived from sugar or starch (other than corn starch); 3) ethanol derived from waste material, including crop residue, other vegetative waste material, animal waste, food waste, and yard waste; 4) biomass-based diesel; 5) biogas (including landfill gas and sewage waste treatment gas) produced through the conversion of organic matter from renewable biomass; 6) butanol or other alcohols produced through the conversion of organic matter from renewable biomass; 7) other fuel derived from cellulosic biomass (Taylor, 2008, Lynd, 2008, Kokossis, 2010). The obtaining of energy from biomass includes main pathways. Combustion is the production of heat through burning the biomass directly. Gasification is incomplete combustion of the biomass producing a syngas. The syngas can be combusted to generate heat and power. Hydrogen gas can be isolated from the syngas for fuel cells. The syngas can also be converted to liquid fuels through the Fischer-Tropsch method. Pyrolysis is thermochemical breakdown of biomass into liquid oils and gases. Liquifaction is thermochemical breakdown of biomass in the presence of hydrogen to produce bio-crude oil. Biochemical conversion is the production of ethanol and other alcohols, such as butanol, via fermentation of the biomass (Farrell, 2006, Saha, 2003).

Reinvention of the forest industry is vital for its continuous and sustainable growth, and this can be achieved by implementing innovative and environmental-friendly approaches. The goal is to produce more and more value from a given volume of harvested trees, and this can best be achieved by producing a greater range of diversified product lines. This makes companies more resilient towards market demand or price changes for any one commodity. A new business model or strategy is required to invigorate the wood and paper/pulp industry through sustainable resource exploitation. The identification of new products to replace existing commodity products offers one avenue for transforming the forestry industry. Bioenergy, cellulosic bioethanol, bio/chemicals, biomaterials and tree bark and foliage utilization are examples that are more competitive and more resilient (Devappa et al, 2015; Towers et al., 2007). The integrated biorefinery approach provides an innovative pathway to develop new value-added products, which have the potential to increase revenues through better utilization of existing on-site plant biomass, as well as the development of new, non-traditional biomass resources in plantations. Nonetheless, the combustion approach for producing bioenergy is not part of the principles behind sustainable development, as this leads to the production of greenhouse gases (CO₂ and other gaseous products). Besides, the valuable chemicals in wood go up in smoke and are lost forever. Presently, cellulosic bioethanol and thermochemical production of bio-oils are still considered expensive. One approach to improve economic viability is by utilizing maximum by-

product/co-products to produce high-valued products (bio/chemicals and bio-based materials) that will offset production costs. One such example is the extraction of natural products or phytochemicals from forest residues can be recovered (in the form of pure or as mixtures/extracts) as value-added products. However, this will initially require basic research that includes systematic identification of bioactive chemicals from forest residues that are present in significant amounts to be considered as biopharmaceuticals. A similar approach has been reported to obtain phytochemicals (e.g. terpenoids and proteins/ peptides) as value-added products from the *Jatropha* seed oil-based biodiesel production chain (Devappa et al, 2015). Pharmaceutical and biotechnology companies are constantly on the lookout for natural biochemicals that command high value in medical and bulk/fine chemical segments. Hence there is considerable potential to critically analyze chemicals present in plant species. This emphasizes the need for further research requiring a synergistic collaborative framework between industry and academia. New product development will allow industry players to access new markets. Recent advances in analytical, metabolomics and metabolite profiling/ fingerprinting tools have made it possible to explore the natural products and phytochemicals as biopharmaceuticals, oleochemicals, fragrance chemicals, food additives and nutraceuticals, among others. The potential of natural chemical products or phytochemicals from poplar (*Populus*) and willow tree species that can generate additional revenue to the forest products industry. Some reviews (Devappa et al, 2015) intend to focus and attract the interest of the natural products' researchers throughout the world to address the unexplored potential of the poplar tree species. This genus needs to be investigated systematically so that potential species can be exploited as sources of therapeutic agents.

There are around 35 species of poplar trees and more than 100 different clones are available. The genus *Populus* includes species such as balsam poplar (*Populus balsamifera*), black cottonwood (*P. trichocarpa*), trembling aspen (*P. tremuloides*), eastern cottonwood (*Populus deltoides*) and bigtooth aspen (*Populus grandidentata*), among others. Poplars possess several attributes that make them potential candidates to be used in the production of solid wood products, pulp and paper, bioenergy including biofuels, and bio-based products. For example, poplar trees have a short life span, fast growth rate, ease of propagation and high wood/fiber quality, among others. In intensive farming with appropriate soil conditions, poplar trees can yield up to 20 tonne d.w./hectare/year. The most commonly found poplar species in Canada are *P. tremuloides* (67–68%), *P. grandidentata* (0.25%), *P. deltoides* (0.0006%), *P. balsamifera* (4.41%), *P. trichocarpa* (0.05%), and other species (28.1%) (Devappa et al, 2015). The figure 1 shows the ratio of constituents in poplar and willow biomass. The sector “other” presents extractive substances. The amount of them depends on part of tree biomass. The content of extractives in poplar and willow bark and leaves measure up 30%.

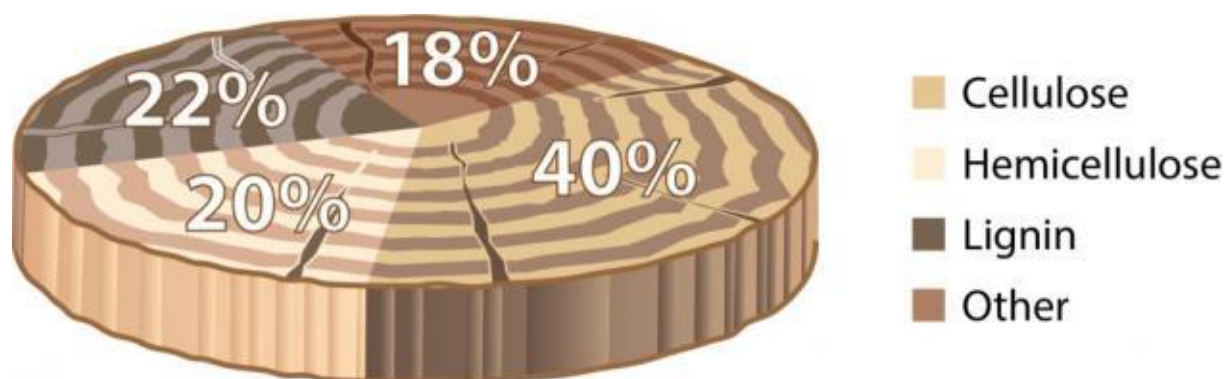


Fig. 1. The ratio of constituents in poplar and willow biomass.

Figure 2 (Devappa et al, 2015) demonstrates the scantiness of data about poplar lipophylic constituents. The data concerning Russian poplar species such as *Populus tremula*, *P. nigra* and *P. alba* are too poor. The information about willows constituents is also insufficient (Azimova, et al,

2012). Thereby, our aim was the investigation of poplar and willow lipophylic constituents by GC-MS and NMR-spectroscopy.

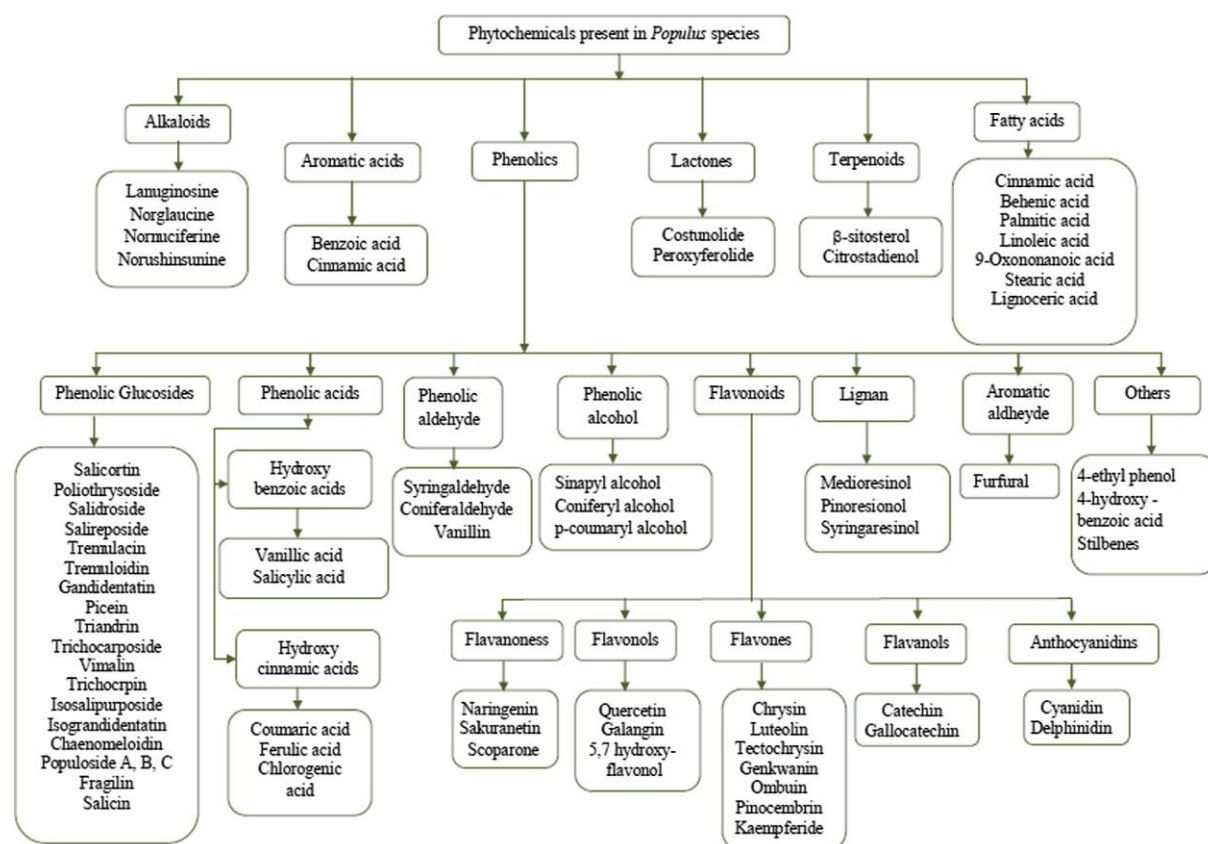


Fig. 2. The scantiness of data about poplar lipophylic constituents.

2. MATERIAL AND METHODS

GC-MS spectra were recorded on a Hewlett–Packard G1800A instrument consisting of an HP 5890 Series II gas chromatograph and an HP 5971 mass-selective detector using an HP-5MS column (30 m × 0.25 mm × 0.25 μm; diphenyl, 5%; dimethylsiloxane, 95%) and He carrier gas (1 mL/min). The conditions for recording spectra and identifying constituents were analogous to those published earlier (Kukina, 2014).

Raw Material Characteristics. Raw material was collected in the Ogurtsovo-village (Novosibirsk, Russia) on July- August, 2014-2016.

Raw Material Extraction. Air-dried raw material was ground in an electric grinder and passed through a 2-mm sieve. A weighed portion of raw material was loaded into a Soxlet-apparatus, drawn with MTBE, and extracted for 1–1.5 days. The extracts were drained through the lower tap. The solvent was removed in vacuo. The yield of extracted substances was determined.

Isolation of Unsaponified Substances (USs). Extracts were saponified with a mixture of KOH (15% w/w), distilled H₂O (10%), and EtOH (75%), calculated to be a 10-fold excess of the saponifying mixture relative to the sample weight. The mixture was refluxed in a flask equipped with a water-cooled condenser with vigorous stirring on a magnetic stirrer/heater for 1.5 h. When the reaction was finished (TLC monitoring of the disappearance of the ester fraction), the mixture was diluted with four volumes of H₂O. Unsaponified compounds were extracted with four portion of MTBE. The combined ether extracts were washed with distilled H₂O until neutral, dried over anhydrous Na₂SO₄, and evaporated in vacuo.

Isolation of Acids. The pH of the combined aqueous phases, containing sodium salts of the acidic constituents was adjusted to 2 with 10% aqueous HCl. Acids were extracted by MTBE (3×50 mL). The combined ether extracts were washed in a separatory funnel with distilled H₂O until the water phases were neutral (pH 7) according to universal indicator, dried over anhydrous Na₂SO₄, and evaporated to dryness in vacuo. The samples of acids were methylated with diazomethane, and analyzed by GC-MS as methyl ethers.

Chromatographic Fractionation of USs. A weighed portion of USs (1.5 g) from leaf (needle) extract was loaded on to a silica-gel (30 g) column as a hexane solution. The column was eluted with a linear gradient from 0 to 50% diethyl ether in hexane. The resulting fractions were evaporated to dryness in a vacuo. The fractions were analyzed by GC-MS.

3. RESULTS AND DISCUSSION

We carried out previously a GC-MS analysis of and identified more than 50 aliphatic and triterpene compounds. The unsaponified constituents of the extract of the plant raw material were the focus of the present investigation because of their important contribution to the biological activity of the lipophilic extracts. Lipophilic compounds were extracted from raw material by MTBE in 3.2–6.4 yields. Acidic constituents made up from 0.8% to 2.6%; unsaponified residue (USs), from 0.3% to 2.2% of the raw material mass. Considering the share of vegetative and generative plant organs in the total mass of the aerial part of *C. angustifolium*, it could be concluded that neutral constituents of leaves contributed substantially to the activity of the lipophilic extract of the plant aerial part. The USs of extract was separated into individual fractions and analyzed by GC-MS. According to GC-MS analysis, nonpolar fraction 1 contained mainly aliphatic hydrocarbons; fraction 2 consists of aldehydes; fractions 3 and 4 contained mainly aliphatic alcohols; fraction 5 and 6 contained mainly triterpene alcohols and sterols.

Table 1 presents the qualitative and quantitative compositions of aliphatic constituents of USs that were obtained from GC-MS analyses taking into account the masses of fractions obtained from the chromatographic separation.

Constituent/Sample	<i>Populus nigra</i> bark	<i>P. nigra</i> phloem	<i>P. alba</i> bark	<i>P. tremula</i> bark	<i>Salix alba</i> bark
Heneicosane	0.7			1.8	
Tricosane	1.6	1.0	3.0	11.8	4.2
Pentacosane	3.2	1.5	135.1	44.3	18.9
Heptacosane	2.7	1.6	527.4	134.0	189.5
Nonacosane	1.2	0.8	44.6	76.6	229.9
Hentriacontane	ND	ND	ND	13.2	24.2
Squalene	26.8	43.2	3.5	6.0	ND
Phytol	8.0	4.8	8.2	2.1	ND
Eicosanol	6.7	ND	ND	ND	ND
Docosanol	10.4	ND	0.8	12.4	ND
Tetracosanol	42.3	0.7	4.4	5.5	ND
Pentacosanol	0.9	ND	ND	ND	ND
Hexacosanol	190.4	4.1	5.0	36.7	ND

Heptacosanol	0.3	ND	ND	ND	ND
Octacosanol	15.7	ND	1.1	90.9	ND
Nonacosanol	0.6	ND	ND	ND	ND

Table 1. The qualitative and quantitative compositions of neutral aliphatic constituents from poplar and willow bark (mg/100 g of raw material dry weight)

Table 2 shows the qualitative and quantitative compositions of triterpene alcohols and sterols.

Constituent/Sample	<i>Populus nigra</i> bark	<i>P. nigra</i> phloem	<i>P. alba</i> bark	<i>P. tremula</i> bark	<i>Salix alba</i> bark
Campesterol	1.4	8.1	2.2	2.5	2.8
β -Sitosterol	165.8	432.2	116.9	164.8	189.7
β -Amyrin	11.0	56.5	8.2	15.3	16.2
α -Amyrin+lupeol	60.3	229.4	2.4	28.2	40.8
Cycloartenol	1.6	2.4	Trace	Trace	3.5
24-Methylenecycloartanol	2.5	14.3	Trace	Trace	1.5
Euphol	9.2	15.9	2.5	Trace	ND
Butirospermol	60.5	265.4	7.1	65.5	ND
Moretenol	2.3	ND	ND	ND	ND
Citrostadienol	1.1	13.2	1.1	2.2	0.4

Table 2. The qualitative and quantitative compositions of triterpene alcohols and sterols from poplar and willow bark (mg/100 g of raw material dry weight)

The comparison of data from tables 1 and 2 indicates differences of poplar and willow composition and features of phloem constituents. One of main constituents of poplar phloem is butirospermol. This compound was identified earlier in *Populus tremula* foliage (Roshchin, 1986) and *P. tremuloid* wood (Abramovitch, 1963). The NMR spectra of purified compound confirmed right of identification. This data may serve for chemotaxonomic aims because constituted in other poplars and absent in willow extracts.

Table 3 presents the qualitative and quantitative compositions of aliphatic and triterpenoic acidic constituents of extracts that were obtained from GC-MS analyses of methyl ethers of acids.

Constituent/Sample	<i>Populus nigra</i> bark	<i>P. nigra</i> phloem	<i>P. alba</i> bark	<i>P. tremula</i> bark	<i>Salix alba</i> bark
Lauric	12.7	2.9	2.0	1.6	4.3
Miristic	9.7	5.8	6.0	3.7	11.3
Pentadecanoic	7.2	14.2	5.9	2.4	13.4
Palmetic	197.7	641.8	242.2	75.0	340.3
Palmitoleic	ND	2.9	ND	ND	

Margaric	7.3	15.9	4.0	2.8	19.1
Stearic	36.7	70.1	35.1	15.4	69.7
Oleic	21.5	Trace	Trace	Trace	14.1
Linoleic	191.7	3244.1	1425.3	45.0	215.4
Linolenic	92.5	499.7	477.0	23.4	57.5
Hexadecandioic	69.1	ND	ND	ND	24.7
Arachinoic	61.8	25.6	6.6	26.2	34.2
Octadecandioic	10.6	ND	ND	ND	15.9
Heneicosanoic	ND	3.7	ND	9.2	10.5
Behenic	422.5	35.4	26.3	95.0	152.0
Tricosanoic	21.6	13.3	ND	32.9	29.5
Lignoceric	467.5	17.5	30.2	172.9	131.7
Pentacosanoic	18.6	3.0	ND	35.4	19.5
Cerotic	241.1	18.2	9.2	290.6	123.4
Montanic	28.1	8.2	ND	413.9	83.7
Melissic	2.3	ND	ND	25.5	91.3
Ursolic	ND	1.7	ND	ND	ND
Oleanolic	ND	9.0	ND	ND	ND
Betulonic	ND	6.2	ND	ND	ND

Table 3. The qualitative and quantitative compositions of aliphatic and triterpenoic acidic constituents from poplar and willow bark (mg/100 g of raw material dry weight)

Table 3 shows the presence of little amount of triterpenoic acids in *P. nigra* phloem. The main constituents all of samples are palmetic, linoleic, linolenic, behenic, lignoceric acids. *P. tremula* bark contains a significant amount of cerotic and montanic acids. Some phenylpropanoids and benzoic derivates such as benzoic, anis, veratric, caffeic, cumaric, and ferulic were also found in these samples.

4. CONCLUSIONS

The obtained research data enable us to conclude that:

1. more than 50 aliphatic and triterpene compounds were identified in poplar and willow bark by a GC-MS analysis at first time;
2. investigated plants may be a source of triterpene alcohols and sterols with biological activity;
3. the content of polyisoprenoids depends on place of growth and phase of vegetation.

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