



# Potential of three years old plantation willow bark as a source of proanthocyanidins

Matiss Pals<sup>1,2\*</sup>, Sarmite Janceva<sup>1</sup>, Liga Lauberte<sup>1</sup>, Galina Telysheva<sup>1</sup>

<sup>1</sup>Laboratory of Lignin chemistry, Latvian State Institute of Wood Chemistry, Riga, Latvia

<sup>2</sup>Faculty of Chemistry, University of Latvia, matiss232@gmail.com

## INTRODUCTION

Proanthocyanidins, known also as condensed tannins, are complex class of polyphenols that mainly consist of epicatechin and gallic acid units. The proanthocyanidins obtained in significant amounts from different Latvian wood resources (pine, oak, goat willow) are confirmed as a potential ingredient in antioxidant additives for food, cosmetic and health industries<sup>1</sup>, as a component of adhesives<sup>2</sup> and in many other areas of industrial application. Willows - fast growing, easily cultivable trees from the genus *Salix* are widespread in cold and moderate temperate regions of the Northern Hemisphere. Willows are known as a source of such important compounds as phenolic glycosides, namely, salicin, and their esters (tremulacin or salicortin), as well as proanthocyanidins. The aim of the present work was detection and characterization of proanthocyanidins in the bark of plantation willow (exemplified by *Salix Klara* sp.) and comparison of data obtained with well-known rich-in proanthocyanidins pine (*Pinus Sylvestris*) bark.

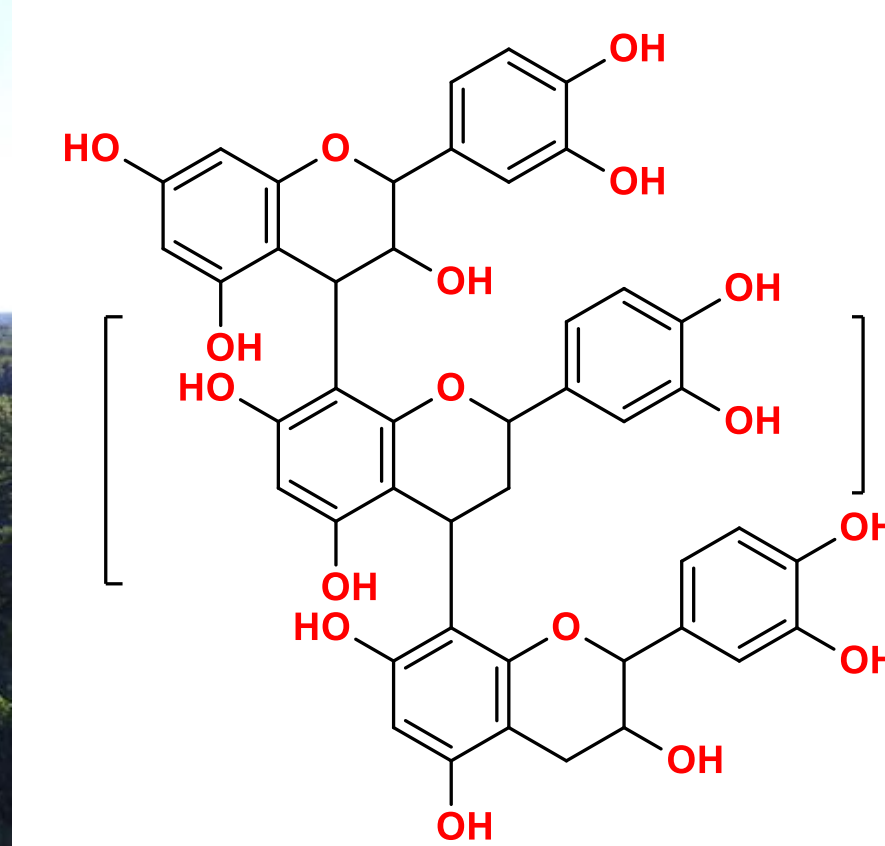


Fig. 1. *Salix* plantation 3 years after planting<sup>3</sup> and chemical structure of proanthocyanidins



Fig. 2. Obtained salix bark extracts

## EXPERIMENTAL METHODS

*Salix "Klara"* bark was collected from plantation in Skriveri municipality in Latvia, from 3 year old trees. *Pinus Sylvestris* bark was collected from Ogres municipality in Latvia, from 76 years old trees. Bark samples were dried on open air, milled using Retch ( $\leq 2$ mm). Soxhlet extraction with acetone during 8 hours<sup>3</sup> and accelerated solvent extraction (ASE) using 60% ethanol water extraction after removal of lipophilic compounds with hexane were performed. Total proanthocyanidins content in the extracts was determined using acid – butanol assay<sup>1</sup>.

Individual compounds in the extracts were identified using UHPLC-qToF-MS/MS (Waters) system with UPLC CSH C18 column (100 x 2,1 mm, 1,  $\mu$ m). Mobile phases were ultra-pure water with 0,1 % formic acid (A) and acetonitrile (B).

## RESULTS AND DISCUSSION

The yields of proanthocyanidins extracts obtained from plantation *Salix "Klara"* bark were 22.3 % and 17.8 % for Soxhlet and ASE, correspondingly (Fig.3.). Meanwhile the yield of extractives from *Pinus Sylvestris* bark was slightly lower: 18.1 % for ASE and 14.2 % for Soxhlet extraction. Results from acid – butanol assay show that in these extracts proanthocyanidins content varies from 17.6 % in pine bark to 28.7 % in *Salix "Klara"* bark. Respectively, the total yield of proanthocyanidins from investigated *Salix* bark is higher than obtained from *Pinus Sylvestris* bark (Fig. 4.).

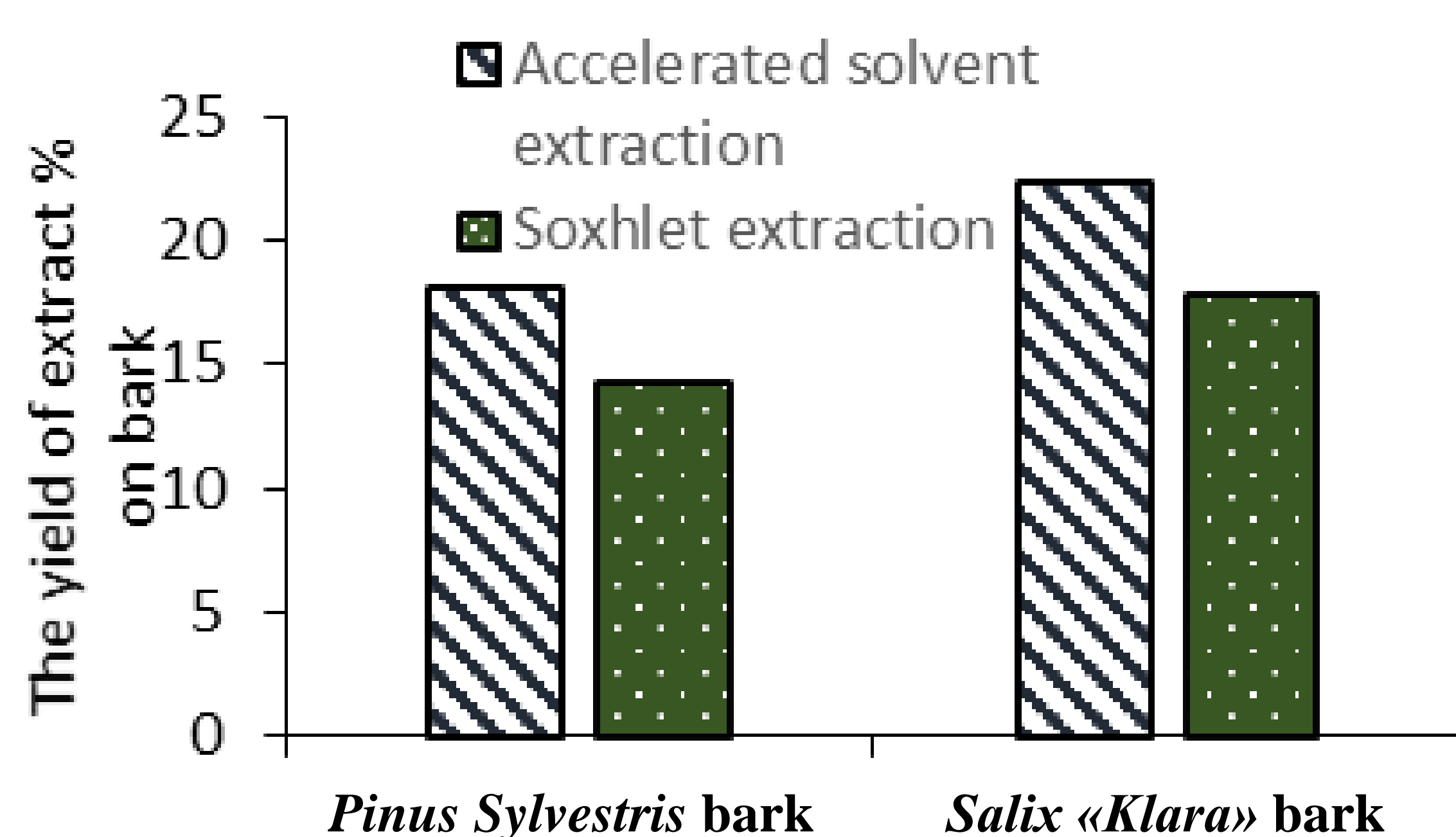


Fig. 3. The yield of extractives obtained by Soxhlet extraction and ASE from bark of *Salix «Klara»* and *Pinus Sylvestris*

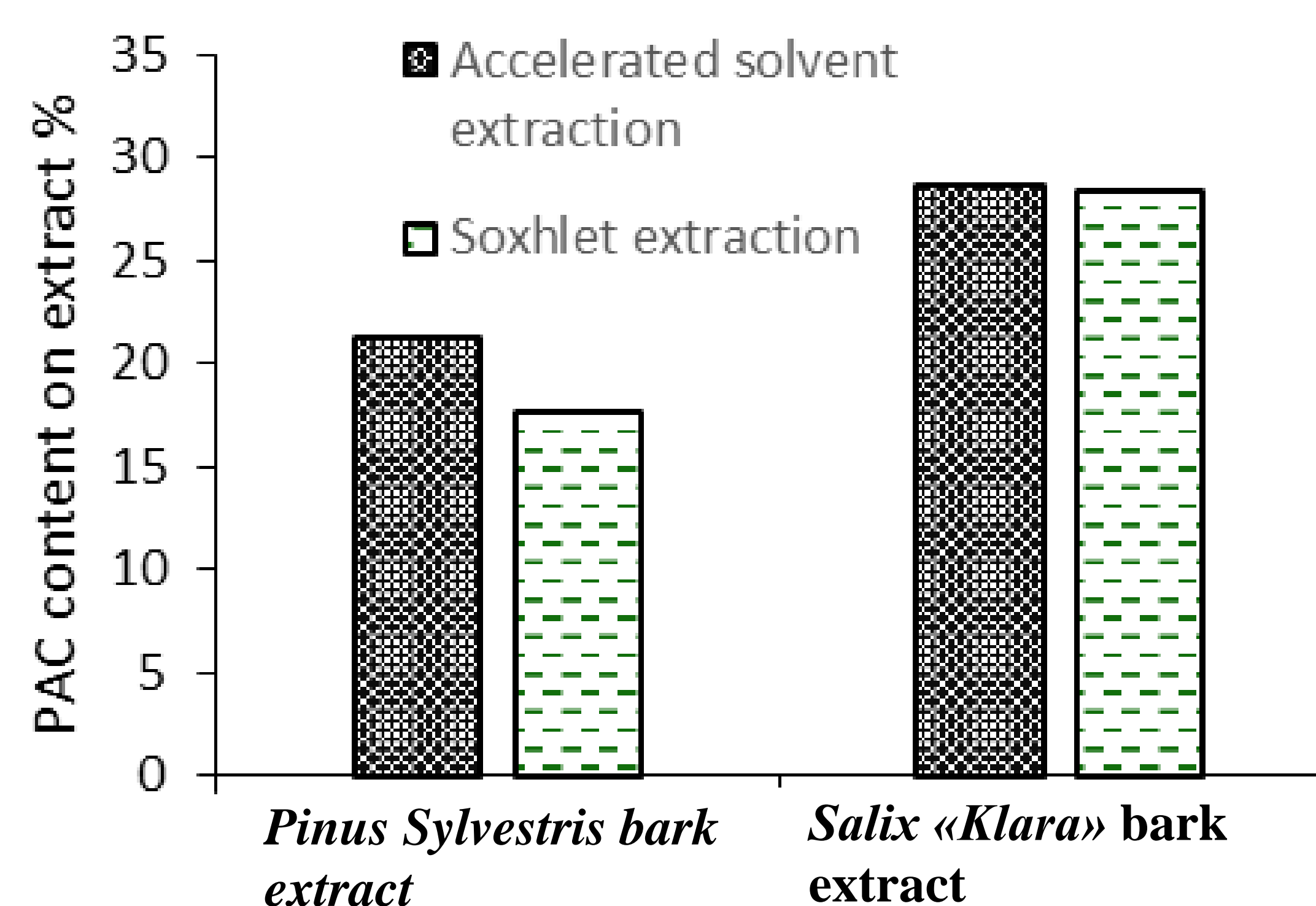


Fig.4. The total content of proanthocyanidins (PACs) in the extracts of *Salix «Klara»* and *Pinus Sylvestris* bark

Qualitative composition of willow bark extracts determined using UPLC-MS/MS showed the presence of the following PAC monomers – epicatechin and gallic acid, as well as dimers: ((E)C-GC and (E)C-(E)C), trimers: ((E)C-(E)C-(E)C and (E)C-(E)C-GC) as shown in Fig 5 (2-6), in addition tetramers: ((E)C-(E)C-(E)C-(E)C, (E)C-(E)C-(E)C-GC and ((E)C-(E)C-GC-GC) were also identified. Both A type and B type linkages between monomeric units were estimated. In addition to proanthocyanidins salicin derivatives were also identified in extract as shown in Fig 5 (7-9). All of those compounds were identified in extracts obtained with soxhlet extraction as well as in extract obtained with ASE. Such wide variety in composition opens different opportunities of application for these extracts.

## CONCLUSIONS

From acquired results, it's possible to conclude that *Salix "Klara"* bark could be considered as a valuable potential source of proanthocyanidins rich extracts. Further studies are necessary to characterize other individual phenolic compounds, quantify them and describe possible practical applications of these compounds.

## REFERENCES

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## ACKNOWLEDGMENTS

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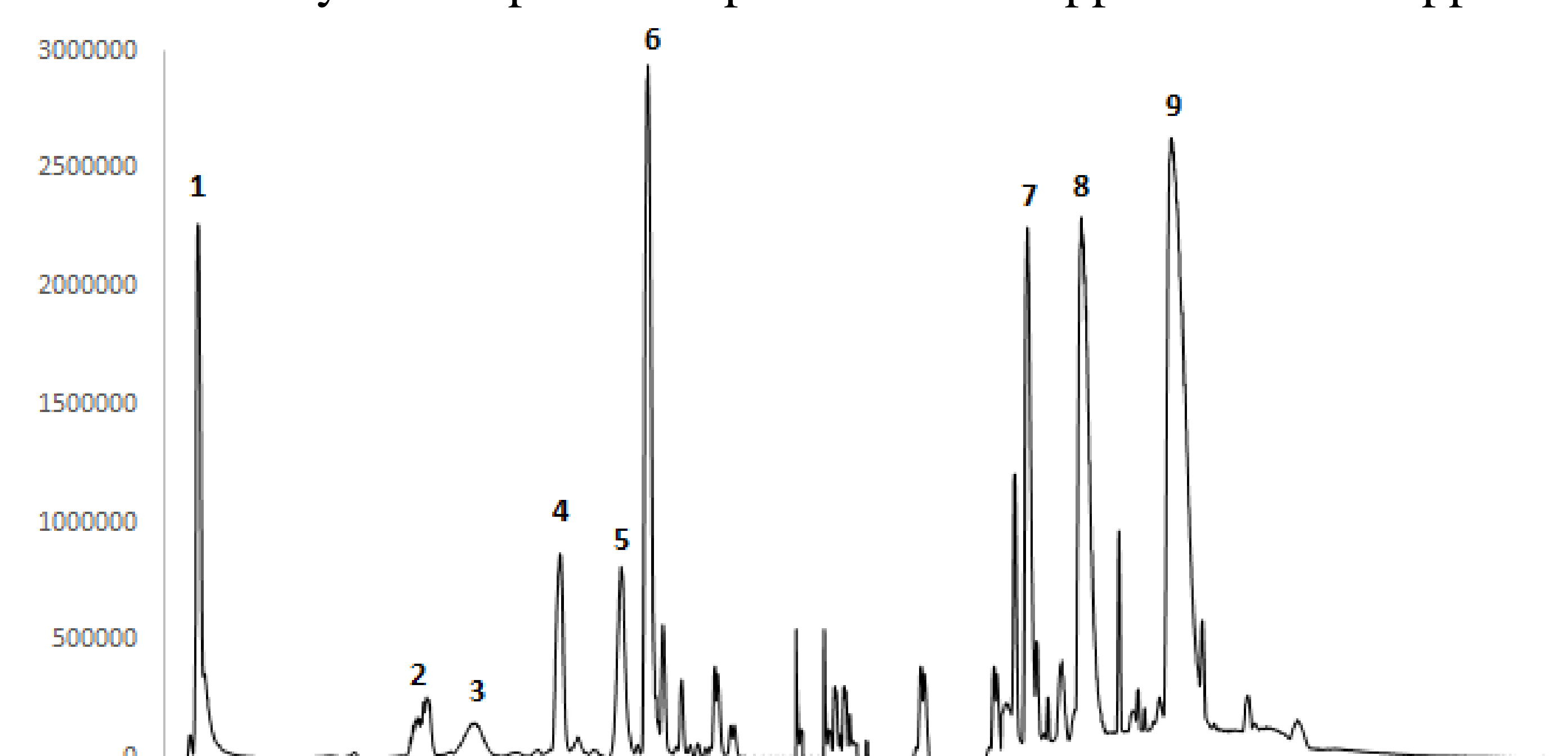


Fig. 5. UHPLC-MS chromatogram of *Salix «Klara»* acetone extract obtained using soxhlet extraction