PP8A-13
Isolation and preliminary characterization of a serine protease inhibitor from a South African medicinal plant, *Acacia Schweinfurthii var Schweinfurthii*

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Introduction: While serine proteases perform a wide range of functions essential to life, they can also be dangerous and must be controlled. One of the many control mechanisms is their specific inhibition by protein protease inhibitors. Protease inhibitors in plants, also present in their seeds, participate in defense mechanisms and their production is induced by herbivory or wounding. For the first time we isolated a canonical serine protease inhibitor from *Acacia schweinfurthii* seeds.
Methods: The purification includes 0.15 M NaCl extraction of the seeds, fractionation of the extract by 80% (v/v) acetone, followed by ion exchange-and affinity chromatograpy and then by reverse phase HPLC.
Results: Both HPLC analysis and SDS-PAGE indicated that inhibitor with a molecular mass of approximately 21 kDa and reducing conditions revealed, however, that the native inhibitor is composed of two polypeptide chains with molecular masses of approximately 14 and 8 kD, respectively. Our inhibitor was shown to inhibit both trypsin and chymotrypsin, with an inhibitory constant (K_i) estimated to be in the range of $1–6 \times 10^{-10}$ M.
Conclusions: The specificity of inhibition together with the two-chain composition of the inhibitor protein and results of N-terminal sequencing of the two constituent polypeptide chains showed that this new inhibitor is homologous to a serine protease inhibitor previously isolated from *Acacia confusa*. Based on this homology the new serine protease inhibitor from *Acacia schweinfurthii* appears to be a Kunitz-type canonical inhibitor.

PP8A-14
Biochemical characterization of GSTs in *Pinus brutia,* Ten (Kizilcam)

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Glutathione *S*-transferases (GST, EC. 2.5.1.18) are a family of multifunctional, dimeric enzymes that play important roles in detoxification metabolism and stress telorance in plants. Today, searches on GSTs in plants have mainly focused on agricultural crop species. However, there are few studies considering the isolation, characterization and purification of GSTs from forest trees, no information were available about the *Pinus brutia*, Ten (Kizilcam) in the literature. In this study, the GST activity for the conjugation of xenobiotic substance (CDNB) was isolated from needles of *Pinus brutia,* Ten. trees from a nursery in Yalıncak area/METU (Ankara-Turkey). Trees that exhibited healthy appereance were selected from the same altitude profile. GSTs activities in the cytosolic fractions prepared from *Pinus brutia* needles were determined spectrophotometrically by using 1-chloro-2,4-dinitrobenzene (CDNB), 2,3-dichloro-4-(2-methylene butyryl)-phenoxy acetic acid (Ethacrynic acid; EA), 1,2-Dichloro-4-nitrobenzene (DCNB), 1,2-Epoxy-3-(*p*-nitrophenoxy) propane (EPNP), and p-Nitrobenzyl chloride (p-NBC) as substrates at 25°C. There was at about 68% decrease in the GST activities after 25°C. Only CDNB (160 ± 10 nmoles/min/mg) and DCNB (2.30 ± 0.38 nmoles/min/mg) activities were detected and the rest were found as negligible. Therefore, CDNB was used as the substrate during purification of GSTs from needles of *P.brutia*. The sequential application of cytosol to gel filtration column chromatography on Sephadex G-25, anion exchange DEAE cellulose column chromatography, and *S*-hexylglutathione agarose affinity chromatography were performed for purification of GSTs. After the final step of purification procedure, CDNB conjugating activity of *P.brutia* cytosolic GSTs was purified about 15.45 fold with 1.95% yield. SDS-PAGE results showed that distinctly purified GST isozyme had an Mr of 24 kDa. In this study, we report for the first time the GST isozymes in a gymnosperm, *P. brutia*.

PP8A-15
Sequence analysis of Tau class glutathione S-transferase from *Pinus brutia (Pinaceae)*

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Ubiquitously distributed multifunctional superfamily of Glutathione *S*-transferases (GST, EC.2.5.1.18) generally constitute a dimeric enzymes and catalyze the conjugation of the thiol group of the glutathione (GSH) to diverse electrophilic centres on lipophilic molecules with the formation of rather less active end products.
Plant GSTs had an attention because of their roles in herbicide detoxification. Therefore, GSTs have been identified and characterized with a differential and overlapping substrate specifities mainly from the agricultural crop species. However, there is almost no information about molecular characterization of this superfamily in gymnosperms, except for a very recent study from *Pinus tabulaeformis* and our previous studies by using *Pinus brutia*, which is the climax vegetation of the Mediterranean Region in Turkey. As conifers have wide distributions and have to cope with several environmental stresses, the definitions of detoxification enzymes like GSTs in conifers is very important for their adaptations. Due to this purpose, it was decided to examine the molecular cloning and expression of GST-Tau isoenzymes in *E. coli*.
In this study, the total RNA was isolated from *Pinus brutia* needles and subsequently cDNA was prepared with RT-PCR strategy. *Pb*GST-Tau gene was amplified by gene specific primers designed to open reading frame of the sequence of *Pt*GST-Tau gene and then cloned into pGEM-Teasy vector. DNA sequencing was performed to confirm the sequence in pGEM-Teasy vector. Automated sequence analysis of the *Pb*GST-Tau gene and sequence comparisons of *Pb*GST-Tau gene to other plant GST genes were performed. *Pb*GST-Tau was subcloned into expression vector pET21a(+). IPTG induced expression conditions of *Pb*GST-Tau were optimized in *E. coli* (BL21 Codon Plus).

PP8A-16
Bacterial species isolated from untreated olive mill wastewaters reduce its toxic load and produce high added value antioxidants

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Introduction: Olive mill wastewater (OMW), a byproduct of olive oil extraction, constitutes a significant agroindustrial waste and an important environmental pollutant due to its high organic load, low pH and presence of biotoxic substances (polyphenols) that are difficult to degrade. A specific group of the OMW phenolic profile called secoiridoids includes oleuropein, tyrosol and hydroxytyrosol, which have antimicrobial activity and act as antioxidants, thus having a health-beneficial role.
Methods: A group of bacterial strains that grew with ease on untreated OMW were isolated based on their ability to bioremediate and decrease total organic carbon (Folin-Ciocalteau method) and phenolic concentration (e.g. vanillic, π-coumaric feroulic and syringic acids; GC spectrophotometer) in diluted OMW. Furthermore, they were found to produce significant amounts of hydroxytyrosol and tyrosol (Reverse-phase HPLC). The selected strains were characterised based on their 16S rRNA gene sequence and the microbial flora of OMW was investigated by means of 16s rDNA array probing.
Results: OMW is a substrate for a number of bacterial phyla, with emphasis to Cyanobacteria and γ-Proteobacteria - mostly Enterobacteria. The microbial load varies depending on OMW treatment/geographical area. An uncultured isolate of Enterobacteria and a Gram+ species were able to decrease the total organic load, in some cases as much as 69% in 24 hours and significantly decreased phenolic concentration. In addition, a 7 hour incubation in diluted OMW yielded an almost 100% increase in its tyrosol/hydroxytyrosol content. Based on remediation treatments and the phylogenetic status of these bacteria we provide alternative tools for OMW management.