UNIVERSITY OF PLOVDIV
"PAISII HILENDARSKI"
FACULTY OF BIOLOGY

DEPARTMENT "BOTANY AND BIOLOGICAL EDUCATION"

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# PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON THE INVASIVE FOR THE BULGARIAN FLORA PLANT SPECIES *AILANTHUS ALTISSIMA* (MILL) SWINGLE AND *KOELREUTERIA PANICULATA* LAXM.

# ABSTRACT

of a dissertation for obtaining the educational and scientific degree "PhD"

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The thesis consists of 148 pages, 21 tables, 25 figures, 8 applications The reference list includes 247 sources (1 in Cyrillic and 246 in Latin).

The experimental studies were carried out in the scientific laboratories of the Department of Botany and Biological Education of the Faculty of Biology at Paisii Hilendarski University, the Chemical Technology Department of the Faculty of Chemistry at Paisii Hilendarski University, the Department of Plant Physiology and Molecular Biology of Faculty of Biology at "Paisii Hilendarski" University, "Laboratory of Applied Biotechnology" – "Stefan Angelov" Institute of Microbiology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, (IEMPAM-BAS), department "Technology of fats, essential oils, perfumery and cosmetics" at University of Food Technology – Plovdiv, "AgroBioInstitute" – Sofia, (ABI).

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The defense materials are made available for free access, to those interested, in the library of University of Plovdiv "Paisii Hilendarski".

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## LIST OF ABBREVIATIONS:

| | |
|---|---|
| ABTS | 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid |
| AMA | antimicrobial activity |
| AOA | antioxidant activity |
| CUPRAC | copper-reducing antioxidant activity |
| DMSO | dimethyl sulfoxide |
| DPPH | 2,2 diphenyl-1-picrylhydrazyl |
| DW | dry weight |
| Eur Pharm | European Pharmacopoeia |
| EO | essential oil |
| FRAP | ferric-reducing antioxidant activity |
| GC/MS | gas chromatography, mass spectrometry |
| GAE | gallic acid equivalents |
| HPLC | high-performance liquid chromatography |
| HT-29 | human colorectal adenocarcinoma cell line |
| IFS | invasive foreign species |
| MTT | (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) |
| PC3 | cell line from adenocarcinoma of the prostate |
| RE | rutin equivalents |
| ROS | reactive oxygen species |
| SD | standard deviation |
| SPM | spectrophotometry |
| TE | trolox equivalent |
| ГФР | State Pharmacopoeia of Russia |

## INTRODUCTION

In search of new sources of bioactive compounds for the prevention and treatment of various diseases, in recent years there has been a growing interest in plant extracts, essential oils, pure substances, etc., with the aim of investing in natural phytoproducts. The in-depth study of the chemical composition of plant species is an important step in the discovery of new chemical compounds that are sufficiently effective and at the same time more easily absorbed and with fewer side effects, given their natural origin. There is increasing scientific interest in widespread species that are invasive to a number of countries due to the fact that they suppress and displace native species and pose a threat to biodiversity. However, at the same time, they can be a valuable and accessible natural resource of bioactive compounds exhibiting a number of pharmacological effects. Extensive studies on the chemical composition of such species, as well as on manifested biological effects of plant extracts from them, enriches the set of plant species, sources of medicinal raw materials. On the other hand, the more serious exploitation of such invasive species with medicinal properties would help to solve the problem of their uncontrolled spread.

In Bulgaria, the natural medicinal flora is rich and the subject of many scientific studies. As for foreign and especially invasive species, the research is mainly ecologically oriented. In the present work, the interest is directed to two tree species, widespread, foreign to the territory of the country and Europe, namely *Ailanthus altissima* (Mill.) Swingle (ailant) and *Koelreuteria paniculata* Laxm. (Chinese goldenrain tree). Their origin is associated with Southeast Asia and more than a century ago they were imported for decorative purposes, but they went wild and spread quickly, especially the ailant. The invasion risk assessment carried out in different countries defines the ailant as highly invasive and the Chinese gorse as potentially invasive, with the possibility of being highly invasive (Petrova et al., 2013; Ljubojević, et al., 2021). They represent a threat to local plant species, the negative effect of which is more pronounced in protected areas with a rich plant diversity and less so in urban areas.

*K. paniculata* (family Sapindaceae) is a tree species reaching up to 10 m in height, with an ovoid crown, compound odd-pinnate leaves and grey-brown to black, furrowed and fissured bark. Anemochoria, zoochoria and hydrochoria are typical for the species. A highly adaptable, fast-growing, drought- and wind-resistent plant species. It also grows on poor, calcareous soils that can tolerate heavy metal contamination. In Bulgaria, it is

widespread (the Rhodope mountains, Black Sea coast, Northeast Bulgaria, Pre-Balkan, etc.) up to 1000 m above sea level. (Petrova et al., 2013).

***K. paniculata*** ***A. altissima***

*A. altissima* (family Simaroubaceae) is a deciduous tree species reaching 18-30 m in height. Its bark is gray-brown, longitudinally fissured (in older tree species). Its leaves are large (0.6 - 1 m), consecutive, unpinnate. It reproduces by seeds and root shoots. In Bulgaria, it is found everywhere up to 1800 m above sea level, because it is undemanding to environmental conditions - it tolerates hot and cold climates, it withstands droughts, as well as excess moisture in the soil, polluted air; it grows on poor, calcareous soils, it is easy to reproduce and distribute (Petrova et al., 2013).

At the same time for *A. altissima and K. paniculata* data on the presence of some valuable secondary metabolites and the manifestation of various biological activities have been reported (Kožuharova et al., 2014; Sladonja et al., 2015; Caramelo et al., 2021), with studies on the ailant are predominant. The review of the scientific literature showed that until 2019 in Bulgaria there were no studies on chemical composition and biological activities for the two species, with the exception of one study on a carotenoid fraction isolated from the flowers of Chinese goldenrain tree (Zhelev et al., 2016). All this directed our attention to these two plant species and determined the purpose and tasks of the present dissertation work.

## THE PURPOSE AND OBJECTIVES OF THE DISSERTATION

**The purpose** of the dissertation is to study the phytochemical structure and some biological activities of the plant species that are foreign to the Bulgarian flora *Koelreuteria paniculata* and *Ailanthus altissima*.

To achieve the purpose, the following **objectives** are set:

1. Monitoring the seasonal dynamics of the accumulation of the main classes of phenolic compounds in aboveground plant substances of the two species, in three consecutive growing seasons (2019-2021).
2. HPLC analysis of aerial plant substances for the content of phenolic acids and flavonoids.
3. Gas chromatographic and mass spectrometric determination of the chemical composition of ethanol extracts and essential oils from the aerial parts of the two species.
4. Finding the physicochemical characteristics and chemical composition of fatty oils from the seeds of the species through chemical and chromatographic analyses.
5. *In vitro* studies on some biological activities (antimicrobial, antioxidant, and antitumor) of different extracts of the species.
6. Determining the DNA protective potential of extracts from *K. paniculata* and *A. altissima*.
7. Analysis of powder of plant substances from *K. paniculata* and *A. altissima* to determine basic microscopic diagnostic features.

## MATERIAL AND METHODS

**The plant material** from *K. paniculata* and *A. altissima* was collected during the period April – September (2019 – 2021) in the region of the city of Plovdiv. Herbal specimens from *K. paniculata* Laxm. (№ 060436) and *A. altissima* (Miller) Swingle (№063263) are deposited in the Herbarium of the University of Agriculture (SOA), Plovdiv, Bulgaria. Leaves, stem barks, flower parts (flower buds, blossoms), and seeds of both species were examined phytochemically, biologically, and microscopically. In *A. altissima* the fruits were also studied (for essential oil composition).

To carry out the analyses, reagents with the highest analytical frequency were used, purchased from Sigma-Aldrich, Merck, Karl Roth, Germany and Duchefa, The Netherlands. The bacterial strains were supplied by the National Bank for Industrial Microorganisms. Cell lines antibiotics and cell culture media were obtained from American Type

Cultures Collection (ATCC, Manassas, Virginia, САЩ) and Orange Scientific, Braine-l'Alleud, Belgium. A trinocular light microscope Magnum T CETI (Medline Scientific, Oxfordshire, UK) was used for the microscopic analysis of the substances. The microphotographs were taken with a digital camera (Si 5000 5 Mpx) attached to the microscope.

Several types of **plant extracts** were subjected to analysis:

- Extracts (70% ethanol) with **triple extraction** (under reflux) of dry substances for determination of phenolic profile, antioxidant activity;
- Extracts (70% ethanol and water) with a **single extraction** (under reflux) from dry substances for the study on antioxidant activity, SPM;
- Dry extracts (under vacuum) of **fresh substances**, for GC/MS analysis, DNA protective potential, antimicrobial, antitumor and antioxidant activities;
- Isolation of **essential oils** (EO) from fresh substances by hydrodistillation with a Clevinger apparatus;
- Isolation of **fatty oil** from seeds. Well-ripened seeds were ground and then subjected to extraction with hexane in a Soxhlet apparatus

The following methods have been applied:

- ✓ Quantitative spectrophotometric methods (SPM) for the determination of total water-soluble polyphenols, tannins, total phenolic acids and flavonoids (Eur. Pharm. 10, 2019a, b; ГФР XI, 1990);
- ✓ HPLC analysis to determine the content of flavonoids and phenolic acids (Krasteva, 2022);
- ✓ Gas chromatographic/mass spectrometric analysis (GC/MS) of ethanol extracts and essential oils from aerial plant parts;
- ✓ Chemical, physicochemical and chromatographic methods for the analysis of the chemical composition of glyceride oils (ISO 659:2014; ISO 12228-1:2014; ISO 9936:2016; ISO 10540-1:2014, etc.);
- ✓ Methods for *in vitro* studies of biological activities of extracts – antimicrobial (agar-diffusion method); antitumor (MTT test, Mosmann, 1983) against PC3 and HT-29 cell lines; antioxidant – DPPH, ABTS, CUPRAC and FRAP analyzes (Kivrak et al.,

2009; Ivanov et al., 2014; Thaipong et al. 2006; Apak et al., 2006; Benzie & Strain, 1999); DNA protection potential – supercoiled plasmid DNA (pUC 19) test according to Rajiv et al. (2021); light-microscopic analysis of powdered plant substances (Eur. Pharm. 10, 2019c).

## RESULTS AND DISCUSSION

- **Seasonal dynamics of the accumulation of the main classes of phenolic compounds, tracked in three vegetational seasons (2019-2021)**

In ***K. paniculata,*** the results of the quantitative SPM determination show that the **total water-soluble polyphenols** in the investigated plant parts have the highest values in flower buds (12.64%) and flowers (8.55%), followed by leaves and bark. The total polyphenols in the leaves are the highest in the months of May - June (7.57 - 9.36%) and in the three seasons, while in the bark their variation during the months is weakly expressed (2.12 - 3.75%). The tendency in the accumulation of **tannins** is similar. The **flavonoid** content is in the following descending order: leaves, flower buds, flowers and barks. The leaves, in addition to containing the highest amounts of flavonoids, show a clear maximum of the reported values at the beginning of the season (April 3.68 – 4.98%). In the flower parts, the content is 2-6 times lower, compared to the leaves. In the stem barks, flavonoids are about 0.1%, with a peak in the month of August and in all three seasons. **Total phenolic acids** accumulate in the highest amounts in the stem barks, with a characteristic maximum at the end of the vegetation season (4.06 – 4.76%), followed by leaves and flower parts. A well-defined peak for the month of May is observed in the leaves (2.76 - 3.14%). The considered group of phenols is the least represented in the flower parts (about 2% in flower buds and 1.6% in flowers).

There are no studies in the scientific literature regarding the seasonal dynamics of the monitored groups of phenols in *K. paniculata*, to make a comparison with.

In ***A. altissima,*** the quantitative data of the monthly determined **total polyphenols** in the leaf substances show maximum values in the months of May - July (4.12 - 5.08% for 2020; 4.87-5.71% for 2019 and 5.39 -6.72% for 2021), in the period of the most active photosynthesis. A well-defined peak can be observed at the end of the growing season in barks, but in them the

measured amounts are significantly lower - 1.19 - 1.78%. Of the three analyzed generative parts, the richest in polyphenols are flower buds (over 5%), followed by flowers and fruits. The **tannins** determined in the plant substances from the island clearly follow the following descending order: leaves, flower buds, flowers, fruits and barks. Tannins in the leaves show a gradual increase during the growing season and reach maximum values in the month of September (between 3.38% in 2020 and 4.16% in 2021), which are almost twice as high compared to the beginning of the season. Regarding the flower parts, tannins have the highest content at the beginning of flowering, in the flower buds (3.74 - 4.42%), after which they decrease in the flowers and are the least in the fruits (about 2.6%). Ailant barks accumulate small amounts of tannins (below 1%), with the highest amount reported in all three years towards the end of the growing season, in the month of September. **Total flavonoids** are best represented in the leaves (with the maximum of 4.04% for 2021) of all the investigated substances. The dynamics of the accumulation of this group of compounds shows higher amounts at the beginning, during leafing and in the phase of young leaves, while at the end of the vegetation period, they decrease almost by half (3.48 – 1.85 in 2019; 2.7– 1.74 in 2020 and 4.04 – 2.43% in 2021, respectively). In the generative substances, their content is lower than in the leaves (about 1.7% flavonoids in well-developed flowers to 1.1% in fruits). They have the weakest presence in the barks of ailant (maximum of 0.17% in April, 2019). **Total phenolic acids** are best represented in leaf substances (1.4 – 2.5%), with higher quantitative data recorded in the summer months (July and August). In flower buds, flowers and wing phenolic acids are about 1%, and their accumulation is significantly weaker in the stem barks of the ailant.

Literature sources report measured amounts of total polyphenols and flavonoids from ailant, but data on the dynamics of their accumulation by season are lacking. Marinas et al. (2017) found for ethanolic fractions (leaves and flowers) of *A. altissima* total polyphenols of the order of 24.66 mg GAE/g dw for leaves and 17.94 mg GAE/g dw for flowers, and flavonoids – 1.82 mg QE/g dw (in leaves) and 1.11 mg QE/g dw (in flowers).

- **Phenolic profile of ethanolic extracts of aerial plant substances of *K. paniculata and A. altissima***

HPLC analysis was performed to prove the presence of some significant bioactive phenolic compounds in ethanol extracts of the species studied.

In the **extracts from the aerial substances** of ***K. paniculata*** 14 phenolic compounds were identified (10 flavonoids, 9 phenolic acids

(Table 1). Data on the content of **flavonoids** in *K. paniculata* show that the leaves have the highest concentration of rutin, followed by hesperidin and quercetin. Flower buds contain a high concentration of (-)-epicatechin, which decreases 4.5 times in well-developed flowers. In stem barks (-)-epicatechin is the best represented flavonoid, in comparison with the other identified (+)-catechin, quercetin and rutin. Hesperidin was not detected in the bark, and kaempferol - in none of the analyzed extracts. **9 phenolic acids** were found in leaf extracts, 8 in flowers and flower buds and 7 in stem barks. The leaves have the highest content of rosmarinic acid, followed by gallic and vanillic acids. *p*-coumaric, rosmarinic, salicylic and protocatechuic acids predominate in flowers and flower buds. Their presence is the weakest in the barks. Ferulic acid in bark has not been established. Chlorogenic acid was not detected in any of the plant samples, and gallic acid was identified only in the leaves. The phenolic acids that showed the highest content (over 1.0 mg/g dw) can be arranged in the following descending order: rosmarinic > *p*-coumaric > salicylic > vanillic > gallic.

In ethanol extracts of the aerial substances of A. altissima, 16 phenolic compounds were identified: 6 flavonoids and 10 phenolic acids (Table 2). The **leaves** have the highest content of hesperidin, rutin and (+)-catechin. Quercetin and (-)-epicatechin are in significantly lower amounts. In the **flowers**, the concentration of rutin is the highest, followed by (+)-catechin. The other identified flavonoids in the flowers are in significantly lower amounts. In **stem barks**, catechins are best represented: (+)-catechin and (-)-epicatechin, and rutin and hesperidin are missing. Kaempferol has been proven only in the bark of *A. altissima.* 9 **phenolic acids** were found in the leaf extracts of A. altissima, among which rosmarinic acid is in the highest content, followed by salicylic acid. The same two acids are best represented in the flower parts, but in several times lower concentrations than those in the leaves. Protocatechuic acid is absent in the leaves. 7 phenolic acids were identified in the bark extracts, of which vanillic, chlorogenic and rosmarinic acids are best represented. Protocatechins, caffeic and *p*-coumaric are absent in stem barks of *A. altissima.*

**Table 1.** Content of flavonoids and phenolic acids in ethanolic extracts of aerial substances of *K. paniculata* (mg/g dw).

| No. | Compounds | Leaves | Stem Bark | Flowers | Flower Buds |
|---|---|---|---|---|---|
| | **Flavonoids** | | | | |
| 1 | Rutin | 4.23 ± 0.96 $^{a}$ | 0.03 ± 0.01 $^{b,c}$ | 0.34 ± 0.08 $^{b}$ | 0.24 ± 0.09 $^{b,c}$ |
| 2 | Hesperidin | 2.97 ± 0.42 $^{a}$ | n.d. | 0.37 ± 0.07 $^{b}$ | 0.19 ± 0.06 $^{b}$ |
| 3 | Quercetin | 2.66 ± 0.54 $^{a}$ | 0.04 ± 0.01 $^{b,c}$ | 0.42 ± 0.09 $^{b}$ | 0.24 ± 0.04 $^{b,c}$ |
| 4 | (+)-Catechin | n.d. | 0.09 ± 0.02 | n.d. | n.d. |
| 5 | (−)-Epicatechin | 0.38 ± 0.06 $^{b,c}$ | 0.80 ± 0.14 $^{b}$ | 0.59 ± 0.05 $^{b,c}$ | 2.69 ± 0.82 $^{a}$ |
| | **Phenolic acids** | | | | |
| 6 | Gallic | 1.02 ± 0.22 | n.d. | n.d. | n.d. |
| 7 | Protocatehuic | 0.30 ± 0.10 $^{c}$ | traces | 0.75 ± 0.10 $^{a}$ | 0.53 ± 0.06 $^{b}$ |
| 8 | Vanillic | 1.04 ± 0.08 $^{a}$ | 0.19 ± 0.04 $^{b,c}$ | 0.24 ± 0.04 $^{b}$ | 0.14 ± 0.05 $^{b,c}$ |
| 9 | Caffeic | 0.06 ± 0.02 $^{n.s.}$ | 0.11 ± 0.03 $^{n.s.}$ | 0.10 ± 0.02 $^{n.s.}$ | 0.14 ± 0.08 $^{n.s.}$ |
| 10 | Syringic | 0.13 ± 0.07 $^{a,b,c}$ | 0.07 ± 0.02 $^{c}$ | 0.23 ± 0.08 $^{a,b}$ | 0.24 ± 0.06 $^{a}$ |
| 11 | *p*-Coumaric | 0.26 ± 0.06 $^{c}$ | 0.05 ± 0.01 $^{c}$ | 6.97 ±1.04 $^{a}$ | 4.97 ± 0.97 $^{a,b}$ |
| 12 | Ferulic | 0.07 ± 0.02 $^{b}$ | n.d. | 0.13 ± 0.04 $^{b}$ | 0.94 ± 0.2 $^{a}$ |
| 13 | Salicylic | 0.39 ± 0.04 $^{b,c}$ | 0.10 ± 0.03 $^{b,c}$ | 0.77 ± 0.17 $^{a,b}$ | 1.64 ± 0.65 $^{a}$ |
| 14 | Rosmarinic | 10.34 ± 1.80 $^{a}$ | 0.22 ± 0.08 $^{c}$ | 3.00 ± 0.38 $^{b}$ | 2.62 ± 0.93 $^{b,c}$ |

n.d.—not detected; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$). $^{n.s.}$—not significant.

**Table 2.** Content of flavonoids and phenolic acids in ethanolic extracts of aerial substances of *A. altissima* (mg/g dw).

| № | Compounds | Leaves | Stem Bark | Flowers |
|---|---|---|---|---|
| | **Flavonoids** | | | |
| 1 | Rutin | 1,02±0,026$^{**}$ | n.d | 5,68±0,142$^{**}$ |
| 2 | Hesperidin | 2,67±0,067$^{**}$ | n.d | 0,72±0,018$^{**}$ |
| 3 | Quercetin | 0,19±0,005$^{b}$ | 0,01±0,001$^{c}$ | 0,33±0,008$^{a}$ |
| 4 | Kaempferol | n.d. | 0,11±0,003 | n.d. |
| 5 | (+)-Catechin | 0,95±0,024$^{c}$ | 2,15±0,054$^{a}$ | 1,55±0,039$^{b}$ |
| 6 | (−)-Epicatechin | 0,23±0,006$^{b}$ | 0,54±0,014$^{a}$ | 0,13±0,003$^{c}$ |
| | **Phenolic acids** | | | |
| 7 | Gallic | 0,30±0,008$^{a}$ | 0,01±0,001$^{c}$ | 0,16±0,004$^{b}$ |
| 8 | Protocatehuic | n.d. | n.d | 0,15±0,004 |
| 9 | Chlorogenic | 0,97±0,024$^{b}$ | 0,27±0,007$^{c}$ | 1,40±0,035$^{a}$ |
| 10 | Vanillic | 0,09±0,002$^{c}$ | 0,73±0,018$^{a}$ | 0,16±0,004$^{b}$ |
| 11 | Caffeic | 0,13±0,003$^{**}$ | n.d | 0,23±0,006$^{**}$ |
| 12 | Syringic | 0,20±0,005$^{b}$ | 0,08±0,002$^{c}$ | 0,39±0,010$^{a}$ |
| 13 | *p*-Coumaric | 0,24±0,006$^{**}$ | n.d | 1,35±0,034$^{**}$ |
| 14 | Ferulic | 0,11±0,003$^{b}$ | 0,07±0,002$^{b}$ | 1,31±0,033$^{a}$ |
| 15 | Salicylic | 6,19±0,155$^{a}$ | 0,07±0,002$^{c}$ | 3,81±0,095$^{b}$ |
| 16 | Rosmarinic | 10,32±0,258$^{a}$ | 0,13±0,003$^{c}$ | 2,01±0,050$^{b}$ |

n.d. – not detected; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$). $^{n.s.}$ – not significant.

- **Chemical composition of ethanolic dry extracts of *K. paniculata* and *A. altissima***

The obtained dry ethanolic extracts from the flowers, leaves and stem barks of ***K. paniculata*** are viscous liquids with a dark brown color and characteristic smell. 56 compounds were identified (40 in flowers, 50 each in leaves and bark), with predominant compounds: pyrogallol

(flowers), α-terpinyl acetate (leaves), neryl acetate (barks). The distribution of the components by chemical groups is presented in Fig. 1a. Oxygenated monoterpenes predominate in the three extracts (flowers 32.49%, leaves 35.21% and stem bark 29.84%), followed by oxygenated aliphatic derivatives and phenylpropanoids. The other groups are less represented, and diterpenes are only found in the barks (1.39%).

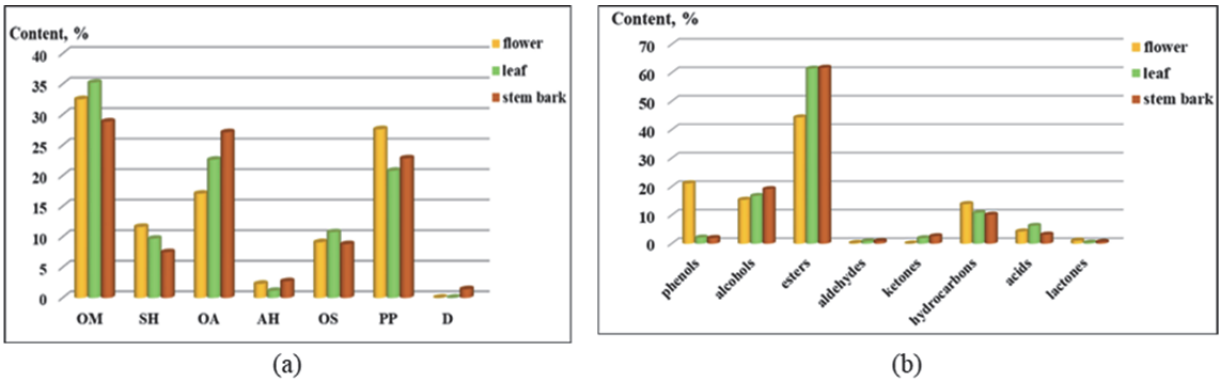



**Figure 1**. Chemical composition of ethanol extracts from *K. paniculata* in chemical (a) and functional (b) groups, in (%): AH - Aliphatic hydrocarbons; OA - Oxygenated aliphatics; OM - Oxygenated monoterpenes; SH - Sesquiterpene hydrocarbons; OS - oxygenated sesquiterpenes; PP - Phenyl propanoids; D - Diterpenes

The distribution of the functional groups (in %) is presented in Figure 1b. The group of esters had the highest percentage (44.29–61.74%) in all of the three extracts studied, followed by alcohols and hydrocarbons. The groups of acids, phenols, ketones, lactones, and aldehydes were very poorly represented in the extracts, an exception is the high content of phenols in the flowers (21.13%).

**Plant extracts of *A. altissima*** are viscous liquids with a dark brown and dark green color and a specific smell. A total of 47 components were identified in the three tested extracts (31 each in flowers and leaves, 42 in barks), with a predominance of hexenyl hexanoate (flowers and leaves), α-terpinylacetate, oleic acid (barks). The distribution of the identified components by chemical groups shows that in the three extracts aliphatic oxygenated hydrocarbons predominate (55.44 – 33.19%), followed by oxygenated monoterpenes (25.42% in the bark and 15.87% in the leaves), diterpenes (in the flowers - 14.22%), sesquiterpene hydrocarbons (in the bark) and other groups that are less represented (Fig. 2a).

The distribution of the components by functional groups in the ethanol extracts shows the highest content of esters (61.33% in leaves; 51.72% in flowers and 33.83% in barks), followed by alcohols (24.89% in flowers; 15.25 in leaves; 14.88% in bark), acids and hydrocarbons (Fig.

2b). Aldehydes and ketones are poorly represented, lactones are present only in bark extracts, and phenols have not been identified.

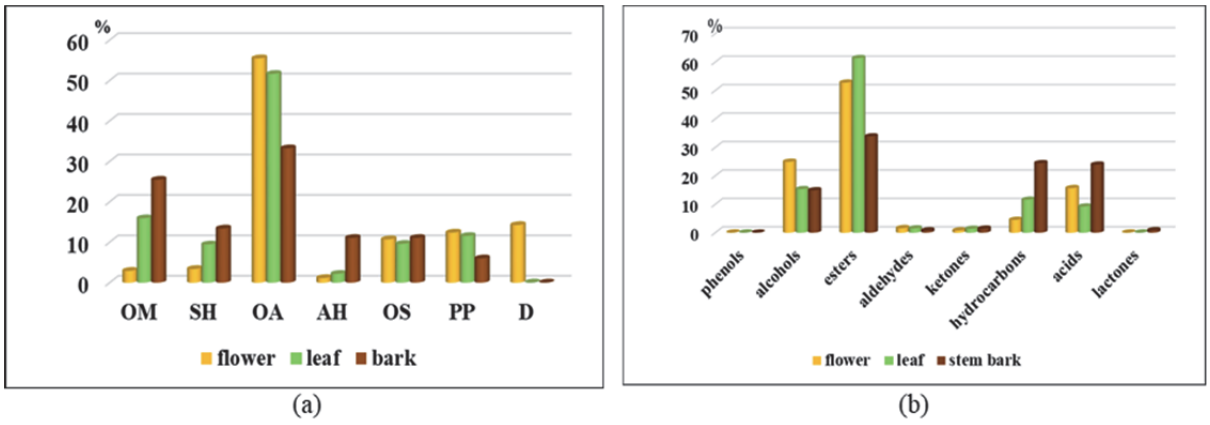



**Figure 2.** Chemical composition of ethanol extracts from *A. altissima* in chemical (a) and functional (б) groups, in (%): AH - Aliphatic hydrocarbons; OA - Oxygenated aliphatics; OM - Oxygenated monoterpenes; SH - Sesquiterpene hydrocarbons; OS - oxygenated sesquiterpenes; PP - Phenyl propanoids; D – Diterpenes

- **Chemical composition of essential oils of *K. paniculata* and *A. Altissima***

Four essential oils (EO) obtained by water distillation from stem bark, leaf, flower and flower buds were analyzed. Isolated EOs are transparent, slightly yellowish, oily liquids. Fifty-four volatile compounds were identified (49 in leaves, 44 in flower buds, 38 in flowers, and 36 in bark), with 32 common to the four EOs. Certain differences, also, were observed in relation to their chemical composition. In flower buds, 11 main compounds were found (linoleic, palmitic, linolenic acids, etc.), in flowers - 12 major ones, among which (Z, Z)-farnesyl acetone is the best represented, in barks - 9 major ones (drimenol, pentacosane etc.), and 6 main compounds in the leaves (α-farnesene, *α*-copaene, *β*-farnesene, isophytol, etc. When the components were distributed by chemical groups (Fig. 3), oxygenated aliphatic hydrocarbons predominate, followed by sesquiterpenes. Diterpenes, triterpenes and aromatics were represented poorly.

There is a lack of data in the literature regarding the composition of the *K. paniculata* essential oil, with which to make a comparison.

Flowers, unripe fruits, leaves and stem barks of ***A. altissima*** were analyzed to determine their essential oil content. EM from ailant are oily liquids with a pale yellow color. A total of 75 compounds have been identified from the various plant parts of *A. altissima.* Forty-four are the volatile components found in flowers and fruits, while in leaves and stem

barks there are 41. The total compounds in the four plant substances are 14. With a similar EO composition are fruits, leaves and barks, having 39 common components. Aliphatic oxidized hydrocarbons predominated in the four EOs, followed by sesquiterpene and aliphatic hydrocarbons. The remaining groups of compounds are less represented (Fig. 4)

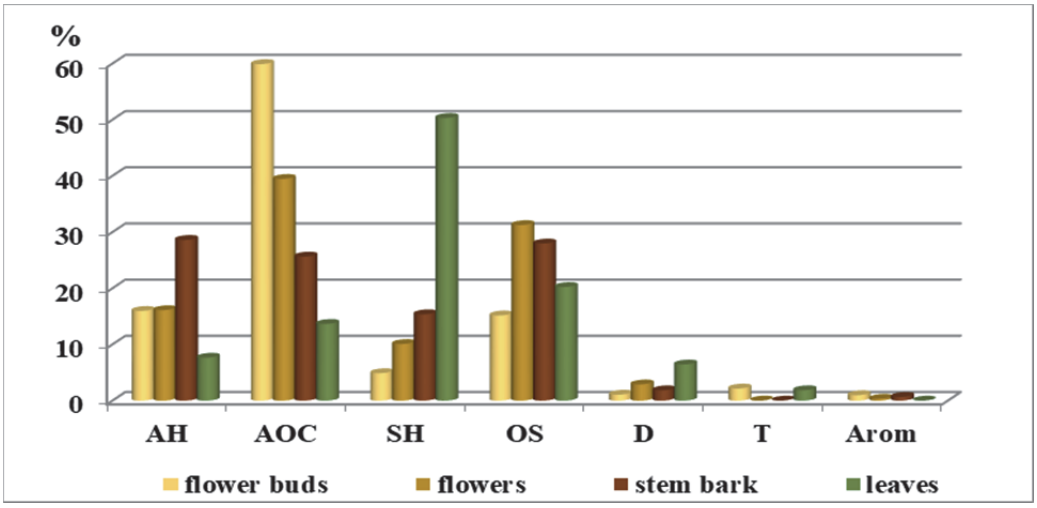



**Figure 3.** Distribution of the compounds by chemical groups in the essential oils of *K. paniculata* (%): AH- Aliphatic hydrocarbons; AOC- Aliphatic oxygenated compounds; SH- Sesquiterpene hydrocarbons; OS- Oxygenated sesquiterpenes; D- Diterpenes; T- Triterpenes; Arom- Aromatic compounds

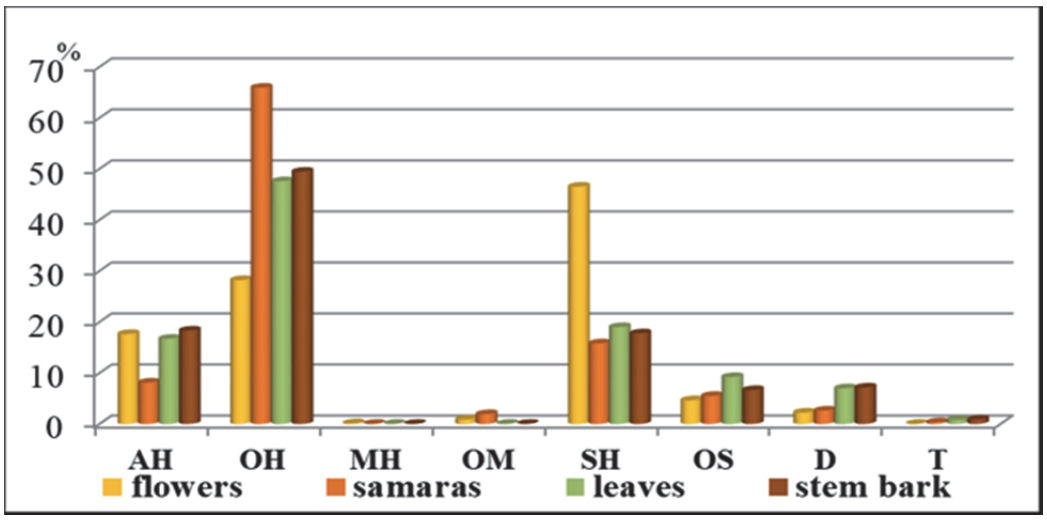



**Figure 4.** Distribution of the compounds by chemical groups in the essential oils of *Ailanthus altissima*: AH- Aliphatic hydrocarbons; OH- Oxygenated hydrocarbons; MH- Monoterpene hydrocarbons; OM-Oxygenated monoterpenes; SH - Sesquiterpene hydrocarbons; OS- Oxygenated sesquiterpenes; D – Diterpenes; T – Triterpenes

- **Chemical composition and physicochemical characteristics of fatty seed oils of *K. paniculata* and *A. altissima***

The data from the chemical composition and physicochemical parameters of glyceride oils are presented in Tables 3 and 4. The seeds from the two plant species are distinguished by a relatively high content of glyceride oil and carbohydrates. Reducing sugars and invert sugar are relatively low in both types of seeds. The established amounts of fiber can characterize the seeds of *A. altissima* and *K. paniculata* as a good source of dietary fiber.

**Table 3.** Chemical composition of seeds

| Content | A. altissima | K. paniculata |
|---|---|---|
| Oil content, % | 30.7±0.2 | 20.4±0.3 |
| Proteins, % | 18.7 ± 0.1 | 15.1 ± 0.2 |
| Carbohydrates, % | 38.9 ± 0.6 | 54.5 ± 0.7 |
| » starch, % | 4.5 ± 0.1 | 14.2 ± 0.1 |
| » reducing sugars, % | 1.31 ± 0.11 | 0.48 ± 0.04 |
| » invert sugar, % | 1.67 ± 0.10 | 2.66 ± 0.16 |
| Fiber, % | 29.6 ± 0.3 | 17.2 ± 0.2 |
| Ash, % | 5.7 ± 0.2 | 3.3 ± 0.1 |
| Moisture, % | 6.0 ± 0.1 | 6.7 ± 0.1 |

The oil from *K. paniculata* shows high oxidative stability. The peroxide number is an indicator by which the degree of oxidation of vegetable oils is calculated, such as the value for seed oil of *A. altissima* is significantly higher (9 times), compared to that of *K. paniculata.* The iodine number of *A. altissima* seed oil characterizes it as a semi-drying oil, while that of *K. paniculata* seed oil as a non-drying oil.

**Table 4.** Physicochemical parameters of glyceride oils

| Content | *A. altissima* | *K. paniculata* |
|---|---|---|
| Peroxide value, meq $O_2$/kg | 90.2 ± 0.6 | 10.0 ± 0.1 |
| Acid value, mg KOH/g | 4.3 ± 0.1 | 0.8 ± 0.0 |
| Iodine value, g$I_2$/100g | 125 ± 0.3 | 44 ± 0.2 |
| Saponification value, mg KOH/g | 210 ± 2 | 209 ± 1 |
| Relative density | 0.8891 ± 0.0002 | 0.8788 ± 0.0003 |
| Refractive index | 1.4736 ± 0.0001 | 1.4661 ± 0.0002 |
| Oxidative stability, h | 5.0 ± 0.0 | Over 50 |

The key component in the sterol composition of both oils is *β*-sitosterol, followed by stigmasterol and campesterol. The cholesterol is only identified in the seed oils of *A. altissima*, and campesterol – only in the seed oils of *K. paniculata*. Of the reaserched tocopherols, a greater percentage

was found for γ-tocopherol in ailant (74.6%) and β-tocopherol (56.6%) in the Chinese gorse. The individual phospholipid composition of the seeds of *A. altissima* and *K. paniculata* was determined for the first time in the present study. Four phospholipid classes were identified in the seeds of *A. altissima*, and six in the seeds of *K. paniculata*. Phosphatidylinositol (29.5%) is in the highest amount in ailant, and phosphatidylcholine (29.1%) prevails in the seeds of Chinese gorse. Regarding the studied content of fatty acids in the oils, it was found that the unsaturated ones predominate in both studied oils, as their content corresponds to 95.3% in the oil from the seeds of *A. altissima* and 92.2% - in that from the pulp. The content of monounsaturated fatty acids is predominant (88.7%) in burdock seed oil. Main fatty acids in *A. altissima* are oleic and linoleic acids, and in *K. paniculata* eicosenoic and oleic acids.

- **Biological activities of plant extracts from the species studied**

- ***Antimicrobial activity (AMA)***

AMA was tested on extracts of ailant and Chinese tree against 9 strains of pathogenic bacteria reported as the causes of infections, toxic infections and toxicoses: Gram-positive bacteria *Listeria monocytogenes, Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*; and Gram-negative bacteria – *Escherichia coli*, *Salmonella abony*, *Pseudomonas aeruginosa* ATCC 6027*, Proteus vulgaris* and *Klebsiella* (clinical isolate).

The extract from the bark of ***K. paniculata*** showed the greatest efficiency against *B. subtilis*, *B. cereus, P. aeruginosa*, and *Proteus vulgaris* at the higher concentration tested (150 μL). The size of the sterile zone in flower extract is quite similar against *P. vulgaris* (10 mm), *B. subtilis* (14 mm) and *B. cereus* (14 mm). The leaf extract did not inhibit the test cultures except *E. coli* (6 – 9 mm) (Fig. 5).

The data from the research conducted on extracts from ***A. altissima*** show that the leaf extracts have the most significant inhibitory effect against *B. subtilis* ATCC 6633 and *Klebsiella* (clinical isolate) (Fig 6). The size of the sterile zone in relation to *B. subtilis* is smaller and close to that of the positive control (39 mm), and against *Klebsiella*, even larger than the control sample (21 mm) with chlorhexidine. Bark extracts are most active against *L. monocytogenes and B. subtilis* (11 – 14 mm, at 150 μL extract, respectively). Flowers indicated no activity. The proven antimicrobial activity in the barks could be connected to the higher values of catechins found in this substance, in the HPLC analysis.

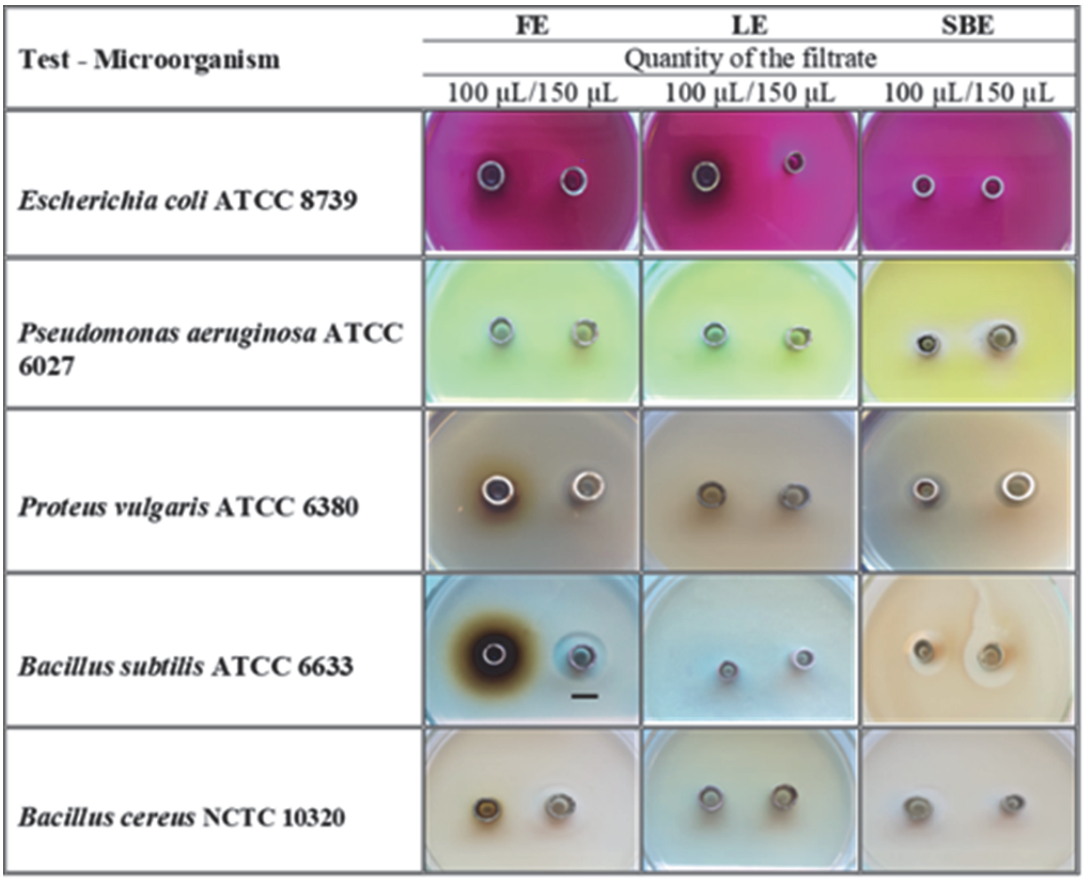



**Figure 5.** Zones with inhibited bacterial growth (above 6 mm); (indicator bar shows 10 mm)

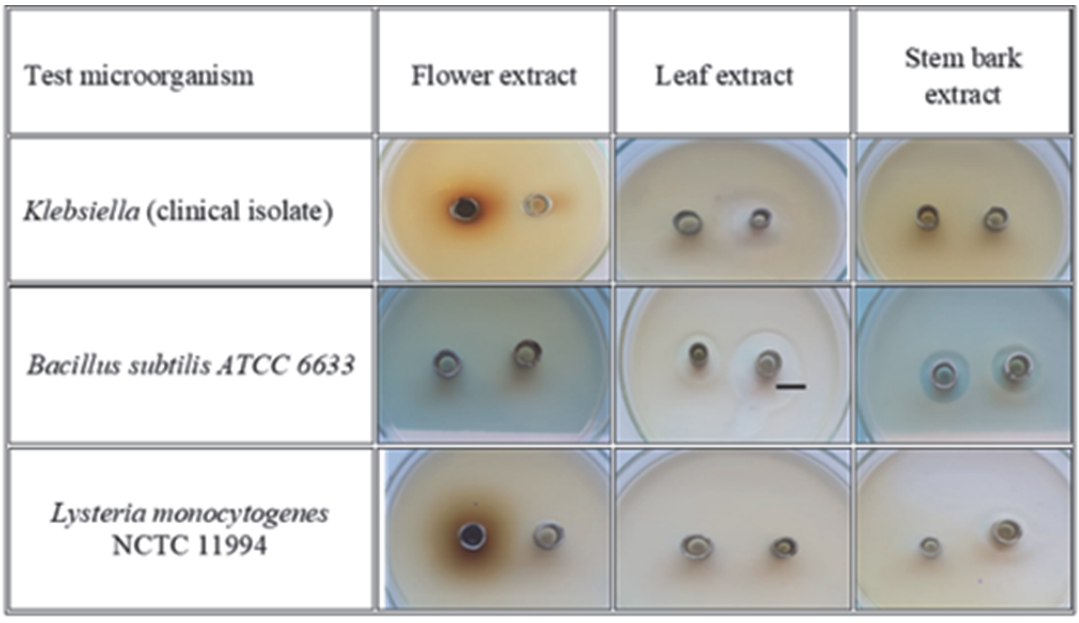



**Figure 6.** Zones with inhibited bacterial growth (above 10 mm). The indicator bar shows 20 mm.

➢ ***Antitumor activity***

The antiproliferative activity of ethanolic extracts of Chinese gorse and ailant was investigated on two tumor cell lines: HT-29 (colorectal adenocarcinoma) and PC3 (prostate carcinoma). The received results (Fig. 7) for ***K. paniculata*** show the best activity for the flower extract on both (IC50 – 21.44 µg/mL for HT-29; IC50 – 58.76 µg/mL for PC3) cell lines. The leaf extract had almost the same activity as the flower extract on the HT-29 cell line (IC50 - 23.63 µg/mL), while PC3 cancer cells were less sensitive to this extract (IC50 – 80.56 µg/mL ). The bark extract shows a weak (dose-dependent) effect on cell lines.

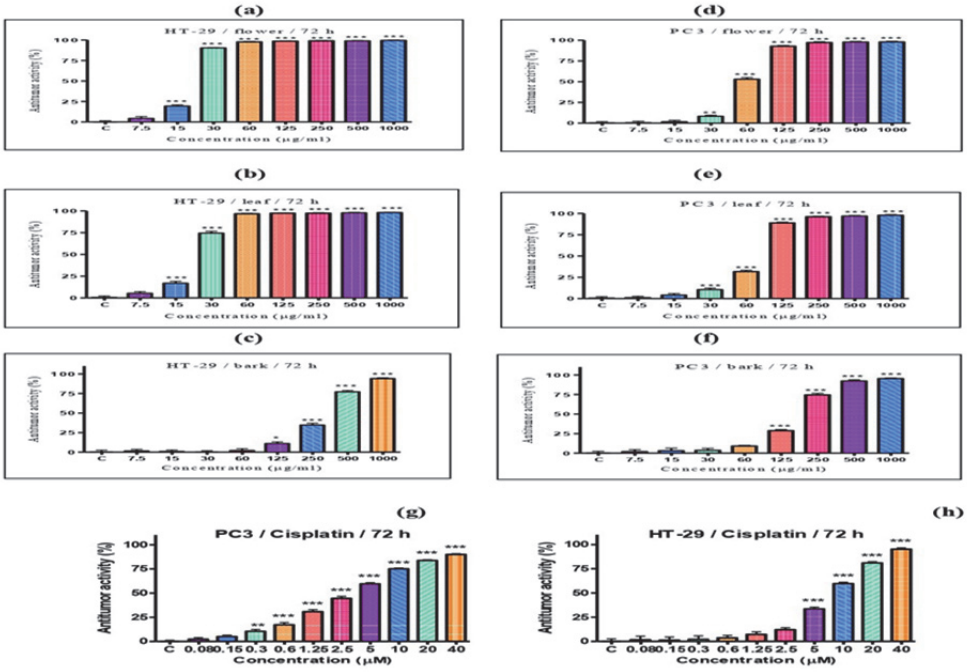



**Figure 7.** Antitumor activity of ethanolic extracts of K. paniculata on cell line HT-29 (human colorectal adenocarcinoma), respectively from: flower, leaf, bark (a, b, c) and PC3 (human prostate carcinoma) from: flower, leaf, bark (d, e, f) and standards for HT-29 (g) and PC3 (h).

The data from the antitumor activity in ***A. altissima*** (Fig. 8) found the best effect against the HT-29 cell line in the flower extract (IC50 - 64.85 µg/mL) and a weaker (more than four times) expressed effect of the other two extracts to the same cell line (IC50 - 275.9 µg/mL and 278.4 µg/mL, leaves and bark, respectively). The bark extract showed the strongest suppressive ability on the growth of PC-3 (71.28 µg/mL) cell line. Of the two lines, RS-3 is more sensitive to the components in the ethanol extracts of *A. altissima*. The antiproliferative activity of the bark and leaf extracts was dose-dependent in both cell lines and also resembled the antiproliferative effect of cisplatin (study control).

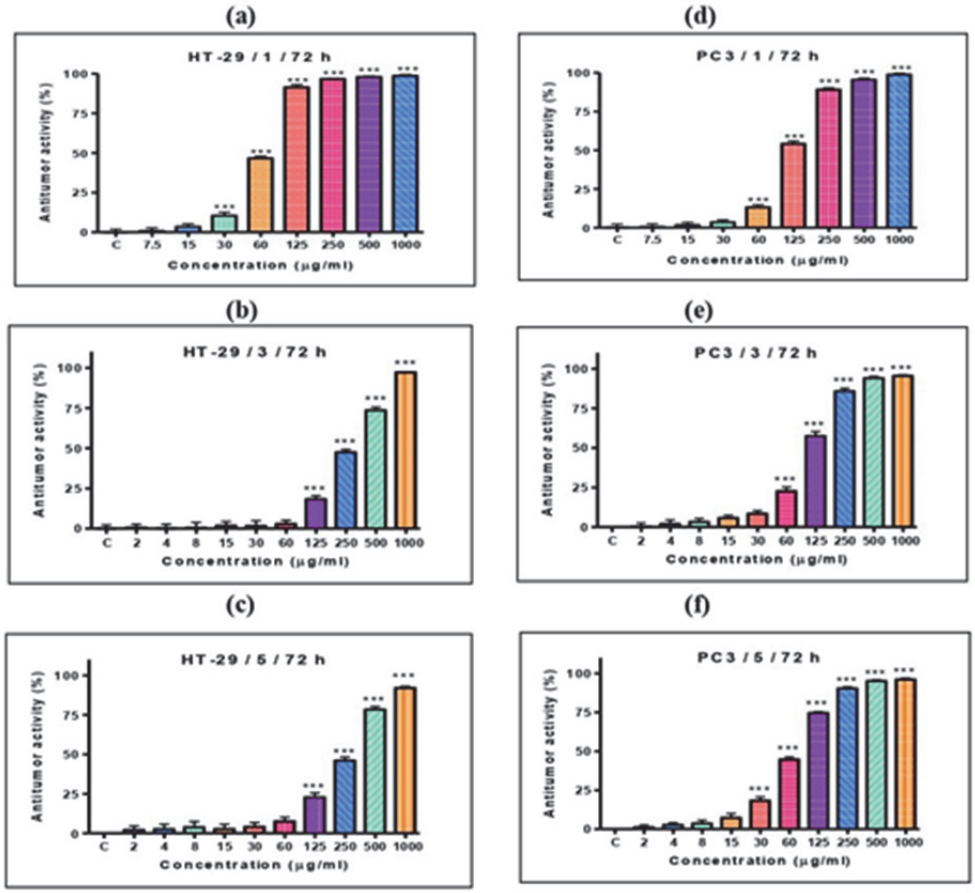



**Figure 8.** Antitumor activity of ethanolic extracts of *A. altissima* on cell line HT-29 (human colorectal adenocarcinoma), respectively from: flower, leaf, bark (a, b, c) and PC3 (human prostate carcinoma) from: flower, leaf, bark (d, e, f).

- ***Antioxidant activity (AOA)***

AOA of **ethanol extracts** was determined**,** with **triple extraction** from dry substances, which had been ground, – leaves, stem barks, flowers, and flower buds by the means of different methods (DPPH, ABTS, CUPRAC and FRAP) (Table. 5). Extracts from the flower of *K. paniculata* showed the best antioxidant potential followed by the flower buds, leaves and bark. The arrangement is different only when using the CUPRAC test, where the quantitative values from the bark extracts follow those of the flowers. To more fully clarify how the way of extraction affect the AOA of the substances investigated, analysis of water extracts and ethanol extracts with a single extraction were done, as well as ethanol dry (under vacuum). The results obtained of the ABTS and DPPH analyses tested on them showed the following trend: the extracts from flowers showed the highest radical-scavenging capacity, followed by the leaves and barks (Table 6). The most expressed AOA effect was shown by the ethanol vacuum extracts for fresh leaves and flowers. In barks, however,

this type of extracts showed lower values than the extracts from dry substances with temperature treatment.

**Table 5.** *In vitro* antioxidant activity of ethanol extracts of dry substances from *K. paniculata*.

| Plant extract | DPPH-Assay, mmol TE/g DW [1] | ABTS-Assay, mmol TE/g DW | FRAP-Assay, mmol TE/g DW | CUPRAC-Assay, mmol TE/g DW |
|---|---|---|---|---|
| leaf | 751.27 ± 1.27 [c] | 645.88 ± 1.83 [c] | 1838.92 ± 2.42 [c] | 576.68 ± 2.58 [d] |
| stem bark | 278.39 ± 1.44 [d] | 342.55 ± 0.98 [d] | 637.62 ± 3.16 [d] | 846.16 ± 2.17 [b] |
| flower extract | 1133.47 ± 1.97 [a] | 1437.49 ± 0.76 [a] | 4308.02 ± 2.84 [a] | 1748.50 ± 2.69 [a] |
| flower buds | 904.12 ± 1.75 [b] | 686.68 ± 1.45 [b] | 2464.10 ± 2.93 [b] | 731.81 ± 1.88 [c] |

[1] mmolTE/g dw—mmol Trolox equivalent (Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of dry weight; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$).

**Table 6.** *In vitro* antioxidant activity of different extracts from *K. paniculata.*

| Plant extract | ABTS- Assay, mmol TE/g dw[1] | DPPH- Assay mmol TE/g dw |
|---|---|---|
| **aqueous** | | |
| leaf | 1529,17±3,00[c] | 1306,20±1,51[c] |
| stem bark | 554,15±4,00[d] | 399,16±2,68[d] |
| flower | 2810,88±2,51[b] | 2565,57±4,10[b] |
| flower buds | 4082,02±3,05[a] | 5282,16±3,52[a] |
| **70% ethanol** | | |
| leaf | 1626,52±4,01[c] | 1434,96±1,52[c] |
| stem bark | 686,69±1,67[d] | 664,26±1,51[d] |
| flower | 2976,90±4,01[b] | 2647,49±2,02[b] |
| flower buds | 4135,17±3,25[a] | 5285,02±3,00[a] |
| **ethanol dry (under vacuum)** | | |
| leaf | 6142,05±3,05[b] | 3722,73±2,50[b] |
| stem bark | 418,12±1,52[c] | 524,21±1,56[c] |
| flower parts | 8191,37±1,94[a] | 5337,21±3,08[a] |

[1] mmolTE/g dw—mmol Trolox equivalent (Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of dry weight; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$).

All of the plant samples from ***A. altissima*** showed AOA summarized in Tables 7, 8. The activity (DPPH, ABTS, CUPRAC, FRAP methods) of the ethanol extracts, with a triple extraction, decreases in the following sequence: extracts from blossoms > leaves > stem barks. By comparing the three ways of obtaining the extracts, the most fully extraction of the antioxidant components from flower buds and stem barks was obtained in vacuum extracts, while for the leaves in the extracts obtained by a single extraction

(water and 70% ethanolic). It is obvious that the thermal treatment helps better extraction of biologically active substances from the leaves.

**Table 7.** *In vitro* antioxidant activity of the ethanol extracts of dry substances from *A. altissima*.

| Plant extract | ABTS-Assay mmol TE/g dw[1] | DPPH- Assay, mmol TE/g dw | FRAP- Assay, mmol TE/g dw | CUPRAC- Assay, mmol TE/g dw |
|---|---|---|---|---|
| leaf | 299,54±4,29[b] | 225,62±3,36[b] | 906,01±1,53[a] | 548,07±1,54[b] |
| flower | 893,14±1,54[a] | 729,72±2,04[a] | 661,48±1,50[b] | 789,54±2,19[a] |
| stem bark | 31,24±1,01[c] | 24,96±1,52[c] | 16,65±1,02[c] | 10,22±0,53[c] |

[1]mmolTE/g dw—mmol Trolox equivalent (Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of dry weight; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$).

**Table 8.** *In vitro* antioxidant activity of different extracts from *A. altissima*.

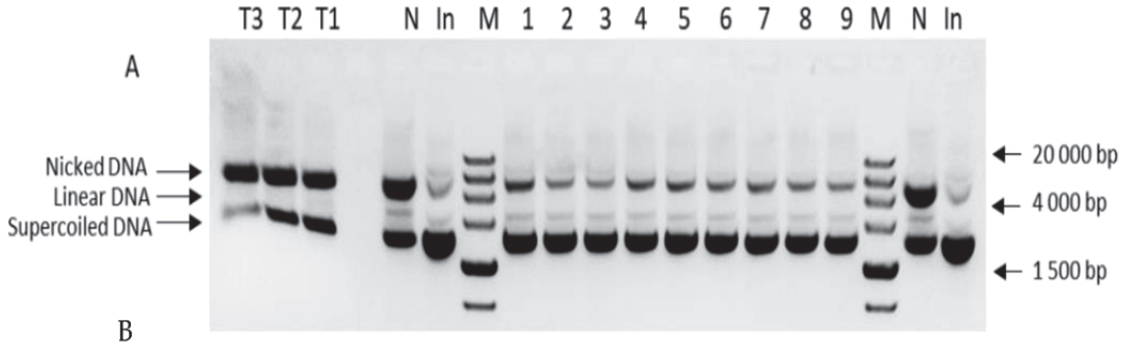
| Plant extract | ABTS-Assay, mmol TE/g dw[1] | DPPH-Assay, mmol TE/g dw |
|---|---|---|
| **aqueous** | | |
| leaf | 399,59±4,00[c] | 361,92±1,13[c] |
| stem bark | 81,78±1,51[d] | 52,19±2,51[d] |
| flower | 803,76±2,24[b] | 721,61±1,50[b] |
| flower buds | 1548,76±3,17[a] | 932,11±1,54[a] |
| **70% ethanol** | | |
| leaf | 504,75±1,66b | 404,72±1,66[c] |
| stem bark | 80,26±2,01[c] | 52,54±1,60[d] |
| flower | 1022,62±2,03[a] | 961,51±1,50[a] |
| flower buds | 1018,62±2,21[a] | 953,16±2,53[b] |
| **ethanol dry (under vacuum)** | | |
| leaf | 392,01±1,53[c] | 52,96±1,00[b] |
| stem bark | 411,61±2,53[b] | 53,19±2,05[b] |
| flower parts | 3272,28±2,13[a] | 2125,67±1,07[a] |

[1] mmolTE/g dw—mmol Trolox equivalent (Trolox: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of dry weight; The samples were analyzed in triplicate, and results were expressed in mean ± standard deviation (SD). Different superscript letters indicate significant differences according to Tukey's test ($p < 0.01$).

- ***DNA protective potential***

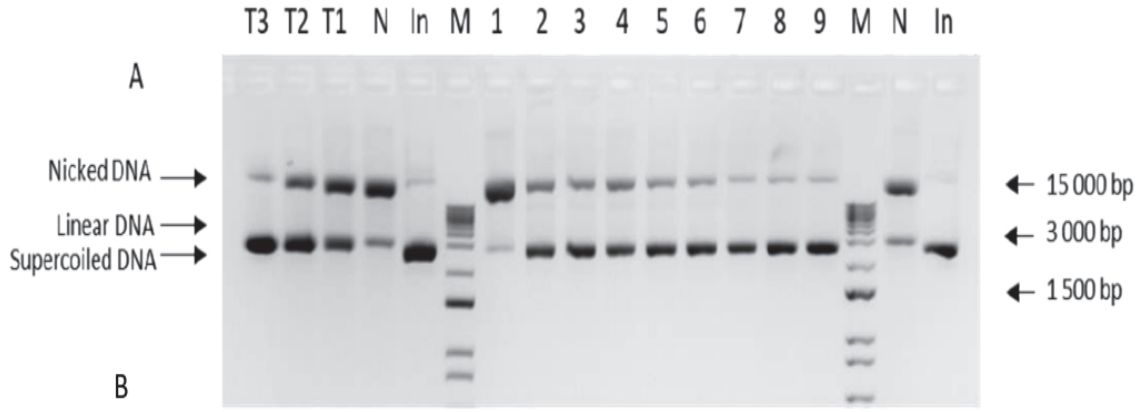
The experiment performed demonstrates the ability of antioxidants contained in the plant extracts of *A. altissima* and *K. paniculata* to neutralize the oxidative DNA damages caused by reactive oxygen species (ROS). A complete DNA protection from damages was found in the higher concentrations tested in leaf, flower and bark extracts (5.25 to 10 μg/ml) obtained from both plant species. The results obtained for ***K.***

***paniculata*** in the lower concentrations (0.6 – 2.5 µg/ml) showed the best protective effect when using bark extracts, followed by flower and leaf extracts (Fig. 9). A clearly expressed gradation of the protective effect was observed for barks and flowers, and less in leaf extracts.




| | Stem bark extract | | | Leaf extract | | | Flower extract | | |
|---|---|---|---|---|---|---|---|---|---|
| Plant extract concentration µg/ml | 0.6 | 1.25 | 2.5 | 0.6 | 1.25 | 2.5 | 0.6 | 1.25 | 2.5 |
| Nicked DNA concentration (ng) | 61 | 12 | 7 | 41 | 36 | 24 | 35 | 20 | 11 |

**Figure 1.** DNA nicking protection assay with (**A**) 1.5% agarose gel electrophoresis, and (**B**) relative concentration of necked plasmid DNA. 1–3—*K. paniculata* stem bark extract at concentrations of 0.6, 1.25, and 2.5 µg/ml; 4–6—*K. paniculata* leaf extract at concentrations of 0.6, 1.25, and 2.5 µg/mL; 7–9—*K. paniculata* flower extract at concentrations of 0.6, 1.25, and 2.5 µg/mL. T1—Trolox 100 µg/mL; T2—Trolox 50 µg/mL; T3—Trolox 25 µg/mL; N—negative control; In-pUC19 input; M-Zip Ruler 2 (Thermo Scientific, SM1373).




| | Stem bark extract | | | Leaf extract | | | Flower extract | | |
|---|---|---|---|---|---|---|---|---|---|
| Plant extract concentration µg/ml | 0.6 | 1.25 | 2.5 | 0.6 | 1.25 | 2.5 | 0.6 | 1.25 | 2.5 |
| Nicked DNA concentration (ng) | 360 | 140 | 88 | 122 | 86 | 63 | 48 | 27 | 23 |

**Figure 10.** DNA nicking protection assay with (**A**) 1.5% agarose gel electrophoresis, and (**B**) relative concentration of necked plasmid DNA. 1–3—*A. altissima* stem bark extract at concentrations of 0.6, 1.25, and 2.5 µg/ml; 4–6—*A. altissima* leaf extract at concentrations of 0.6, 1.25, and 2.5 µg/mL; 7–9— *A. altissima* flower extract at concentrations of 0.6, 1.25, and 2.5 µg/mL. T3—Trolox 100 µg/mL; T2—Trolox 50 µg/mL; T1—Trolox 25 µg/mL; N—negative control; In-pUC19 input; M-Zip Ruler 2 (Thermo Scientific, SM1373).

In the extractions of ***A. altissima*** t is also noticeable that as their concentration decreases, the degree of DNA damage increases. As can be seen from Fig. 10, the extracts of flowers (lines 7,8,9), followed by leaves (lines 4,5,6) and barks (lines 1,2,3) show the highest protective potential. For flowers and barks, a gradation of the protective effect is again observed, while for leaves this effect is less pronounced.

- ***Determination of basic diagnostic microscopic features of plant substance studies***

The identification of substances from medicinal plants is the first step in conducting pharmacognostic analysis, in which microscopic examination plays an important role. The objects of the microscopic analysis are powdered stem barks, leaves, flower parts (flowers and flower buds) of the two tree species (when using a chloral hydrate solution).

Some more important microscopic features observed in powder from leaves, barks and flower parts of ***K. paniculata*** are presented in Figures 11, 12, 13. Powdered plant substances have different colors (grass green, light brown and yellowish, respectively).

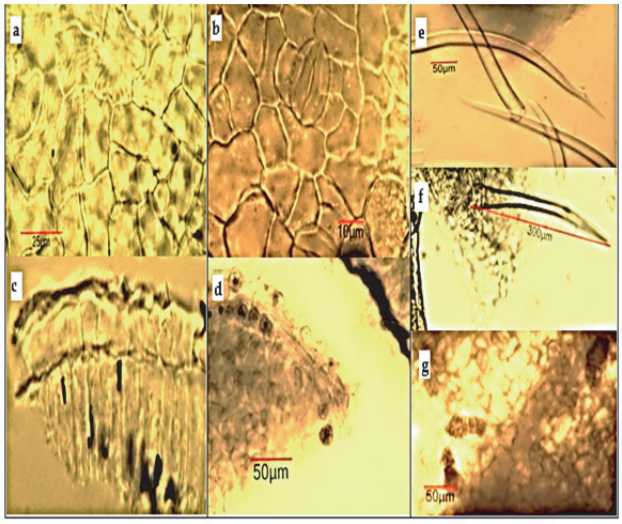



**Figure 11.** Microscopic photographs of powdered leaf samples of *K. paniculata*: (a) upper leaf epidermis in anfas; (b) lower leaf epidermis in anfas with anomocytic type of stomata; (c) fragments of leaf plate in cross section with upper epidermis and palisade parenchyma; (d) oxalate drusen around the vascular bundle; (e) isolated covering trichomes; (f) unicellular covering trichome with part of epidermis; (g) glandular trichomes of the lower leaf epidermis.

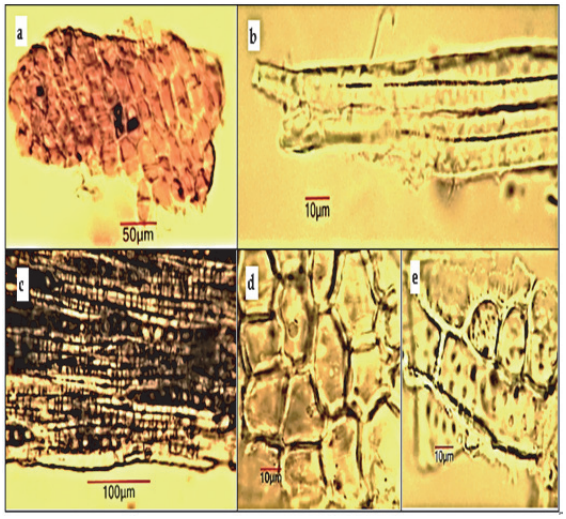



**Figure 12.** Microscopic pictures of powdered samples from the bark of the stem of *K. paniculata*: (a) cork in anfas; (b) isolated bundle of phloem fibers; (c) fiber with crystalline druses of calcium oxalate; (d) parenchymal cells in anfas; (e) sclereids.

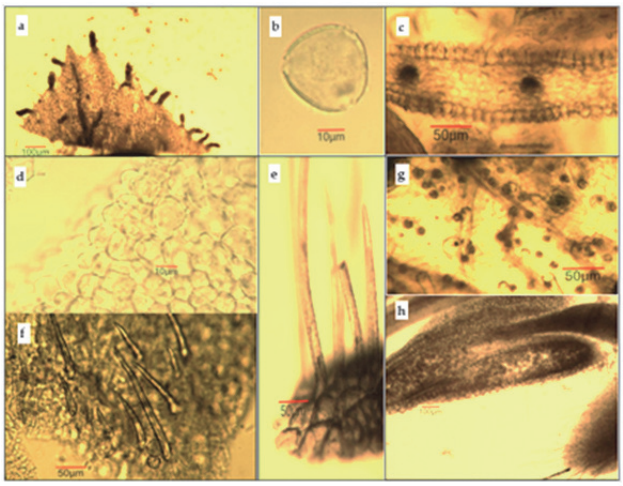


**Figure 13.** Microscopic photographs of powdered samples of flower parts of *K. paniculata:* (a) petal fragment and pollen grains; (b) pollen grain; (c) petal fragment in cross section; (d) papillose epidermal cells of the petal in surface view; (e) fragment of calyx with unicellular covering trichomes with surface incrustations; (f) fragment of sepal with covering trichomes; (g) fragment of sepal with oxalate drusen; (h) fragment of flower buds with anther and parts of perianthium.

The main microscopic features observed in powder from the plant substances from ***A. altissima*** are presented in Figures 14, 15, 16. The leaf powder samples have a grassy green color, the stem barks - light gray and flower parts - yellow green color, respectively.

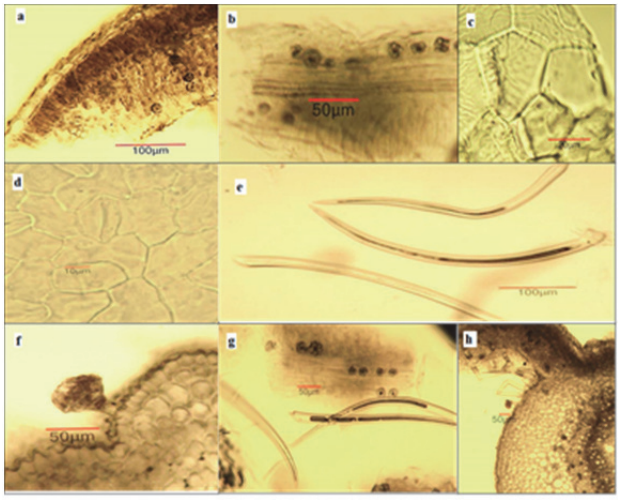


**Figure 14.** Microscopic images of powdered leaf samples *Ailanthus altissima*: (a) fragment of a leaf plate in cross section, with upper and lower epidermis, with palisade parenchyma and spongy parenchyma with crystalline druses of calcium oxalate; (b) oxalate drusen around the vascular bundle; (c) upper leaf epidermis in anfas; (d) lower leaf epidermis in surface view, with anomocytic type of stomata; (e) isolated unicellular curved covering trichomes; (f) glandular head trichome along vein; (g) unicellular linear covering trichomes (isolated long curved and straight and with part of epidermis short curved), parts of spongy mesophyll with crystalline druses; (h) fragment of a central vein with a leaf blade in cross section and trichomes (covering and glandular) on the epidermis.

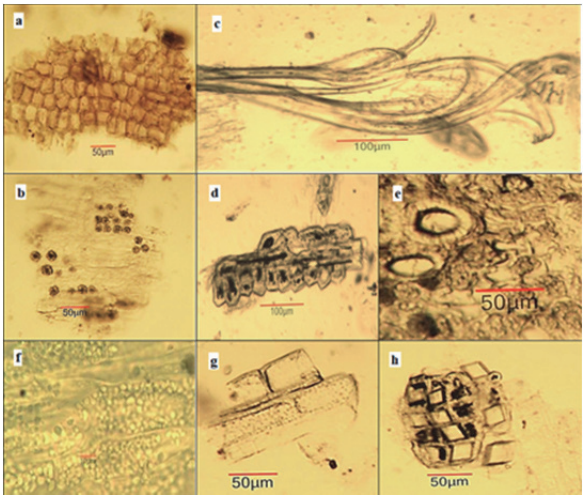


**Figure 15.** Microscopic images of powdered samples of the stem bark of *Ailanthus altissima*: (a) Cork in anfas; (b) phloem parenchyma with crystalline drusen; (c) bundle of phloem fibers; (d) group of sclereids around a bundle of phloem fibers; (e) schizogenous oil gland in the phloem; (f) phelloderm fragment with chloroplasts; (g) fragment of face tubes; (h) oxalate crystal cubes around a group of sclereids.

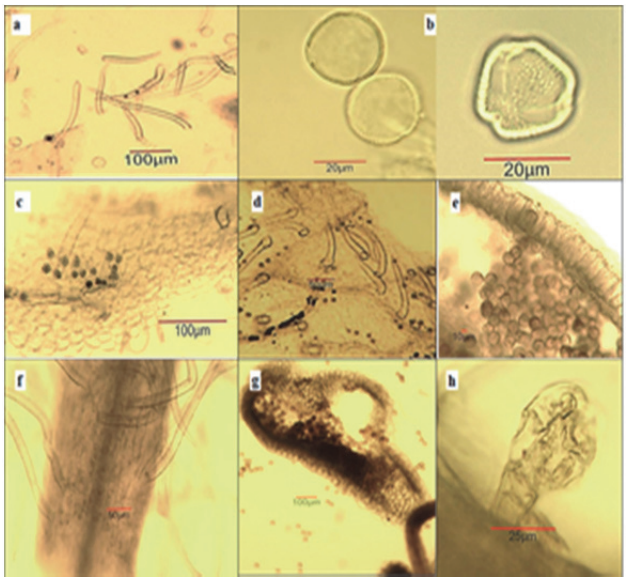



**Figure 16**. Microscopic images of powdered samples of flowere parts of *Ailanthus altissima*: (a) scattered pollen grains and covering trichomes; (b) pollen grains; (c) petal fragment with papillose epidermis and crystal drusen, (d) sepal fragment with unicellular covering trichomes on the epidermis and crystal drusen mainly around the veins; (e) fragment of anther with exothecium, endothecium and pollen grains; (f) fragment of funiculus with covering trichomes; (g) fragments of anther and ovary and numerous pollen grains; (h) glandular trichome on flower stalk.

## CONCLUSIONS

### A) Regarding the plant species *K. paniculata*

1. SPM analysis of plant substances from *K. paniculata* showed that the total water-soluble polyphenols and related tannins accumulate the most in flower buds and flowers, followed by leaves and the least in bark, for total flavonoids the leaves occupy the first position, and for phenolic acids – barks. The monitored seasonal dynamics of accumulation showed that the most suitable period for collection for the leaves is the phase of leafing and young leaves, for the barks – the end of the growing season, and for the inflorescences – the beginning of flowering.

2. HPLC analysis of the phenolic profile of ethanol extracts of *K. paniculata* showed the presence of five flavonoids and nine phenolic acids. Rutin, hesperidin and quercetin in the leaves and (-)-epicatechin in the flower buds are in the highest concentrations (over 2.6 mg/g dw). Phenolic acids, which showed the highest content (from 1.0 to 10 mg/g dw), arranged in the following ascending order, are: gallic < vanillic < salicylic < p-coumaric < rosmarinic.

3. GC/MS analysis of the ethanol extracts of *K. paniculata* shows a wide range of a total of 56 compounds, among which oxigenated monoterpene derivatives predominate. Among the identified components with the highest concentrations (over 10%) are: pyrogallol (in the flowers) and α-terpinyl acetate (in the leaves and flowers), nerylacetate (in the bark) and α-terpinyl isobutanoate (in the flowers).

4. 54 compounds were identified (GC/MS) in EO from substances (stem barks, leaves, flowers and flower buds) of *K. paniculata.* In EOs some differences in the chemical composition have been established. Oxygenated aliphatic compounds predominate in flower buds and flower EOs, in leaf EO - sesquiterpenes, and in bark EO - aliphatic hydrocarbons, oxigenated aliphatic and oxigenated sesquiterpenes. Main compounds in EOs, above 10%, are: linoleic and palmitic acids (flower buds); farnesylacetone (flowers); farnesene (leaves) and drymenol (bark).

5. The oil from the seeds of the species shows a high oxidative stability and it could be a good source of the unsaturated oleic and eicosene acids, as well as of β-sitosterol, vit. E and phospholipids.

6. Activity has been demonstrated against the following bacterial strains: *Bacillus subtilis ATCC 6633, Bacillus cereus NCTC 10320, Escherichia coli ATCC 8739, Pseudomonas aeruginosa ATCC 6027, and Proteus vulgaris ATCC 6380*, as the bark and leaf extracts show better antimicrobial potential.

7. All tested extracts show *in vitro* antioxidant activity, determined by DPPH, ABTS, FRAP and CUPRAC analyses, the highest being recorded in the flower parts, followed by the leaves and the weakest – in the bark. The type of plant substances (fresh or dry) and the method of extraction have an influence on AOA.

8. Significant antiproliferative activity against the HT-29 cell line (human colon adenocarcinoma) was established for the flower and leaf extracts of *K. paniculata*.

9. The extracts of the leaves, flowers and bark of *K. paniculata* in concentrations from 5.25 to 10 μg/ml show complete protection against oxidative DNA damage. At lower concentrations of the plant extracts (0.6-2.5 μg/ml) the DNA protective potential is dose-dependent and best in barks.

10. The main pharmacognostic microscopic features of powdered substances from the leaves, bark and flower parts of *K. paniculata*, data that are currently missing.

**B) Regarding the plant species *A. altissima***

1. Leaves are the plant substances that accumulate the largest amounts of the four studied groups of phenolic compounds, and a suitable period for their collection is the leafing phase (for flavonoids), as well as for well-developed phenolic and phenolic acids (in leaves). Flower buds and flowers are next in terms of content of the considered BAB, and in the barks they are the least represented.

2. The phenolic profile of the extracts from *A. altissima* showed 16 compounds, of which 6 flavonoids, the best represented being rutin (5.68 mg/g dw in flowers), hesperidin (2.67 mg/g dw in leaves) and (+)-catechin (2.15 mg/g dw in bark). Rosmarinic and salicylic acids are dominant (2-10 mg/g dw) among the ten established phenolic acids (in leaves and flowers).

3. In the ethanol extracts of flowers, leaves and bark, 47 components were identified in the conducted gas chromatographic study, among which aliphatic oxygen derivatives predominate. The main component in the flower and leaf extracts is (3Z)-hexenyl hexanoate (28.59%, 12.61%), and the peels - α-terpinylacetate (15.55%).

4. 75 compounds have been identified in EOs from various above-ground substances of *A. altissima.* Aliphatic oxygen derivatives (mainly fatty acids, their esters) predominate in EOs from fruits, leaves and bark, while in flowers - sesquiterpene hydrocarbons. The main compounds in EOs (over 10%) are: *β*-caryophyllene and germacrene D (in flower EO); oleic acid (in bark, leaf and fruit EOs), palmitic acid (in fruit EO).

5. *A. altissima* seed oil contains fatty acids (mainly oleic and linoleic), *β*-sitosterol, *γ*-tocopherol, as well as phospholipids.

6. There has been proved antibacterial activity of extracts from the leaves and bark of *A. altissima* against 6 of the tested pathogenic strains, the strongest effect against *Bacillus subtilis* ATCC 6633 and *Klebsiella*, clinical isolate.

7. Antioxidant potential is shown by all tested extracts, which is best expressed in flower buds and flowers. The different types of extracts tested show that the highest antioxidant effect for flowers and peels is reported for the vacuum extracts obtained from fresh material, while for the leaves – for the ethanol extracts obtained from dry substances, with temperature treatment.

8. Antiproliferative activity against two cell lines was demonstrated by the extracts from the flowers of the island (namely HT-29) and those from the stem barks (against RS-3 – human adenocarcinoma of the prostate).

9. Extracts of bark, leaves and flowers from *A. altissima* show complete protection of DNA from oxidative damage at a concentration above 5.25 μg/ml. At lower concentrations (0.6-2.5 μg/ml) the DNA protective effect is strongest in flowers.

10. The pharmacognostic microscopic analysis of powdered flower parts of *A. altissima* so far it has not been published, and the obtained results for barks and leaves enrich the available information.

## CONTRIBUTIONS

### Original scientific contributions:

1. For the first time, main diagnostic microscopic features of powdered plant substances from flowers, leaves and stem barks from *K. paniculata,* and flowers from *A. altissima*
2. Seasonal dynamics in the accumulation of total water-soluble polyphenols, tannins, flavonoids and phenolic acids in plant substances from *K. paniculata* and *A. altissima* for a period of 3 years has been determined for the first time.
3. Volatile components were isolated by water distillation and identified by GC/MS analysis, from aerial parts of *K. paniculata,* for the first time.
4. For the first time, the phenolic profile (flavonoids and phenolic acids) of ethanolic extracts of dry substances (flower buds, blossoms, leaves and stem barks) of *K. paniculata,* through HPLC analysis, has been studied.
5. For the first time, the phytochemical composition (GC/MS) and various biological activities (antimicrobial, antioxidant, antiproliferative) of ethanolic extracts of fresh plant substances of *K. paniculata* and *A. altissima,* has been studied.
6. Phospholipid profile of fatty oils from the seeds of *A. altissima* and *K. paniculata* was reported for the first time.
7. DNA-protective potential of ethanolic extracts of flowers, leaves and stem barks of *K. paniculata* as well as flowers and leaves of *K. paniculata* was demonstrated for the first time, as well as from flowers and leaves of *A. altissima*.

### Applied scientific contributions:

1. The chemical composition found of the isolated essential oils from *K. paniculata* shows that they could be a potential natural source for input into food, cosmetic and medicinal products.
2. The studied composition of the fatty seed oils of both species showed that they are rich in beneficial omega fatty acids, phospholipids, sterols and vitamin E, making them a valuable source of these components with possible future applications.
3. Due to the presence of valuable biological active components in extracts of aerial substances from *K. paniculata* and *A. altissima* (especially flowers and leaves) they would be a good source of natural antioxidants.

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## SCIENTIFIC PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC FORUMS ON THE TOPIC OF THE DISSERTATION

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## PARTICIPATION IN SCIENTIFIC FORUMS

**International Conference on Technics, Technologies an Education ICCTE 2020, 4-6 November**. Ts G Andonova, I Zh Dimitrova-Dyulgerova, I Zh Slavov, I N Dincheva, A S Stoyanova**.** Volatile compounds in flowers, samaras, leaves and stem bark of *Ailanthus altissima* (Mill.) Swingle, growing in Bulgaria.

**International Conference on Technics, Technologies an Education ICCTE 2021**, **3-5 November.** Ts G Andonova, H N Fidan, I Zh Slavov and I Zh Dimitrova-Dyulgerova. „Phytochemical composition and antimicrobial activity of *Ailanthus altissima* (Mill.) Swingle extracts from Bulgaria “.