



**PROCEEDINGS OF
IV. INTERNATIONAL
AGRICULTURAL, BIOLOGICAL
& LIFE SCIENCE CONFERENCE
AGBIOL 2022**

29-31 AUGUST, 2022

EDIRNE, TURKEY



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**Organized by
Trakya University**

**ISBN #:
978-605-73041-3-1**

WELCOME NOTES

You are welcome to our IV. AGBIOL Conference that is organized by Trakya University. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal was to bring three communities, namely science, research and private investment together in a friendly environment of Edirne, Turkey in order to share their interests and ideas and to get benefit from the interaction with each other.

In September 2018, we organized the first AGBIOL Conference with more than 700 scientists and researchers from all over the world with over 800 scientific papers. Due to COVID-19 situation, II. AGBIOL 2020 has organized fully on-line event which was one of the biggest online conferences in recent years in the world with 499 papers and 1133 authors with 333 oral and 166 e-poster presentations from 55 countries. Due to COVID-19 situation, AGBIOL 2021 was organized on-line again. There is a worldwide participation from 44 countries with 422 papers by contributing 1066 authors with 288 oral, 134 e-poster presentations.

The AGBIOL 2022 is organized with normal participation in controlled conditions as well as with online participation in Trakya University Balkan Congress Center in Edirne, Turkey on August 28-31, 2022. There is a worldwide participation from 44 countries with 522 papers by contributing over 1300 authors.

The participants with paid conference fee will be able to access all the normal and virtual presentation talks in each session, as well as to visit the virtual poster hall via preliminary provided participant ID and codes. The selected ABSTRACTs will be published in the Conference ABSTRACT and Proceedings Book. Participants might send us their full papers, which based on their preferences will be published either in our Conference ABSTRACT and Proceedings Book or in selected International Indexed Scientific Journals.

Conference Topics:

Agriculture, Forestry, Life Sciences, Agricultural Engineering, Aquaculture and Biosystems, Animal Science, Biomedical science, Biochemistry and Molecular Biology, Biology, Bioengineering, Biomaterials, Biomechanics, Biophysics, Bioscience, Biotechnology, Botany, Chemistry, Chemical Engineering, Earth Sciences, Environmental Science, Food Science, Genetics and Human Genetics, Medical Science, Machinery, Pharmaceutical Sciences, Physics, Soil Science.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

Prof Dr Yalcin KAYA
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DETERMINATION OF BIOLOGICAL POTENTIAL OF *TILIA CORDATA* FLOWER EXTRACTS

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ABSTRACT

Extraction is a very important stage in the isolation, as well as the identification of different bioactive compounds in the plants. The aim of this research was to produce aqueous and ethanolic extracts from *Tilia cordata* flowers, as well as to determine its antioxidant and antimicrobial potential. Ethanolic extract was characterized with higher ($p < 0.05$) ability to capture free DPPH radicals compared to the aqueous extract. From the point of ability to chelate iron ions can be proved that ethanolic extract was characterized with slightly higher ($p < 0.05$) values compared to the aqueous one, whereas at the highest tested concentration both of the extracts (51.57%, i.e. 49.03%, respectively) had higher ($p < 0.05$) antioxidant potential compared to the citric acid (12.66%). These values were followed by IC₅₀ values. Furthermore, ethanolic linden extract had higher ($p < 0.05$) antimicrobial potential against most of the tested strains compared to the aqueous linden extract. Even that ethanolic extract showed the highest activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Proteus vulgaris* and *Pseudomonas aeruginosa* only in one case, against *Listeria monocytogenes* (15.9 mm), showed higher ($p < 0.05$) activity compared to the tetracycline. According to that, ethanolic linden flower extract showed good antioxidant and antimicrobial potential while it can be used in the food industry for producing functional food with increased biological value.

Keywords: *Tilia cordata*, extracts, antioxidant potential, antimicrobial potential.

INTRODUCTION

Plants, their extracts or pure components isolated from them are used in various industries, such as pharmaceutical, cosmetic, food, etc. Consumption of the plants and its products have been constantly growing. Studies showed that several classes of compounds present in these plants are responsible for biological activity (Mitic et al., 2021).

Among the four species of the genus *Tilia* that grow naturally in Europe, the small-leaved lime (*T. cordata* Mill.) is the most widespread in temperate woodlands. Although it is a relatively rare and scattered species, it was very abundant in the past. Indeed, its relatively good shade-tolerance and its mid- to late-successional character in forest dynamics made it originally a co-dominant species of temperate primeval woodlands of central and Eastern Europe (Jaegere et al., 2016).

Tilia cordata is a minor, broadleaved species with wide, but scattered distribution in Europe, characterized as a species with wide ecological tolerance and numerous ecosystem services (Jaegere et al. 2016). Besides its natural distribution, *T. cordata* is a common species in parks, or other urban green areas in the Eastern Balkan region (Zorić et al., 2020). As flowers of *Tilia* species are widely used in the traditional medicine as herbal tea for cough treatment or restlessness, most of the previous research have put its focus on the organic composition of the dried inflorescence (Zorić et al., 2020). These plants contain a number of derivatives such as hydrocarbons, esters, terpenoids, quercetin, kampferol, phenolic compounds, condensed tannins and scopoletin (Wissam et al., 2017). Linden flowers have been used to treat several illnesses like bacterial infections as well as their effects in reducing tension. Alcoholic extracts have antibacterial properties while flower infusion is used to treat diseases of respiratory tract (Özbucak et al., 2013). These effects could be attributed to the presence of flavonoids and phenolic compounds.

Extraction is a very important stage in the isolation, as well as the identification, of phenolic compounds. However, the compositions of natural sources of phenolic compounds and the structure and physicochemical properties make a universal extraction protocol not conceivable. A definite extraction procedure must be designed and optimized for each phenolic source, compounds that are correlated to the antioxidant activity of the extracts (Mitic et al., 2021).

The aim of this research was to produce aqueous and ethanolic extracts from *Tilia cordata* flowers, as well as to determine its antioxidant and antimicrobial potential.

MATERIAL AND METHODS

Collection, preparation and drying of linden flowers

As a work material *Tilia cordata* flowers were used, that are located in Ohrid, the southwestern part of North Macedonia, on the shore of Lake Ohrid, at an elevation of 695 m above sea level.

In order to remove dust particles and other impurities, fresh flowers were washed with distilled water. After that, the fresh linden flowers were dried in a laboratory dryer (60 °C, 4 to 5h) to a constant mass (Stojanova, 2019). After drying linden flowers were grounded to a fine powder and were stored in a refrigerator until the analysis.

Preparation of aqueous extract

Aqueous extract was prepared according to Sławińska et al. (2013) and Ribeiro et al. (2015) method. 10g of dried and powdered linden flowers was poured with 200 mL of distilled water, and after that was extracted on a boiling water bath for 1h. The extract was strained through filter paper, then rinsed once more with boiling water and the sample was filtered again. The resulting supernatant was combined and evaporated on a vacuum evaporator. For each sample, the extraction procedure was done in triplicates.

Preparation of ethanolic extract

Ethanolic extract was prepared according to Vidović et al. (2011) method. 10 g of dried and finely powdered flower samples was poured with 100 mL of 70% ethanol and extract was covered for 40 minutes on an ultrasonic bath at 45°C. The sample was filtered through filter paper. The resulting supernatant extract was evaporated at 60°C to constant mass. For each sample, the extraction procedure was done in triplicates.

Determination of antioxidant potential of linden extracts

Ability to capture DPPH radicals

The ability to capture DPPH radicals was determined by Brand-Williams et al. (1995) method.

$$I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100\%$$

The radical scavenging capacity of the samples was calculated as IC₅₀ values (inhibitory concentration of extract reducing the absorbance of DPPH solution by 50%) by regression analysis:

$$IC_{50} \text{ (mg/mL)} = (50 - b)/a^* \text{ (*a - slope; b - intercept)}$$

BHT was used as positive control.

The results are expressed as the mean of the three measurements.

Ability to chelate iron ions

The chelating ability of iron was determined by Dinis et al. (1994) method. The chelating ability of iron ions is calculated by the formula:

$$\text{Ability to chelate iron \%} = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100\%$$

The ability to chelate iron ions of the samples was calculated as IC₅₀ values by regression analysis:

$$IC_{50} \text{ (mg/mL)} = (50 - b)/a^* \text{ (*a - slope; b - intercept)}$$

Citric acid was used as positive control. The results are expressed as the mean of the three measurements

Determination of antimicrobial potential of linden extracts

Antimicrobial potential was determined by disk-diffusion method. 9 pathogenic bacteria were used: *Staphylococcus aureus* ATCC 25923; *Bacillus cereus* ATCC 10876; *Listeria monocytogenes* ATCC 19115; *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 11230; *Yersinia enterocolitica* ATCC 27729; *Shigella sonnei* ATCC 29930; *Proteus vulgaris* ATCC 8427; *Pseudomonas aeruginosa* ATCC 35554. The tested bacteria were stored on suitable oblique agar at +4 °C.

Disc-diffusion method

Disc diffusion analysis was performed by Klaus et al. (2015) method. Tested microorganisms were prepared in the appropriate broth, sieved 2 times for 24 h, whereby the concentrations were about 1·10⁶ to 1·10⁸ CFU/mL. Then the suspension of each culture of microorganisms (100 µL) was seeded on appropriate agar. Three sterile filter discs (6 mm) were placed on the agar surface and then soaked with 50 µL of suspension of each of the extracts. After standing for 2 hours 25 °C, petri dishes were incubated for 24 h at 37 °C. After incubation, the zone of inhibition (mm) was measured.

Statistical analysis

The obtained results were statistically processed using the software package SPSS 20. To determine the statistical significant differences of the obtained values the Independent Sample T-test ($p = 0.05$) as well as ANOVA post hoc Tukey's test ($p = 0.05$) was performed.

RESULTS AND DISCUSSION

The healing properties of different plant species are numerous, and many plants are used in folk medicine as a good source of different biologically active compounds (Stojanova et al., 2022). The importance of antioxidant components of natural origin has been increasing lately, since some of the frequently used synthetic antioxidants, especially in the food industry (butylated hydroxyanisole [BHA] and butylated hydroxytoluene [BHT]), have been found to possess certain toxic properties (Vidović et al., 2011). Resistance to available antibiotics in pathogenic bacteria is currently a global challenge since the number of strains that are resistant to multiple types of antibiotics has increased dramatically each year, and the strains have spread worldwide (Stojanova et al., 2022).

Antioxidant potential of linden extracts

From the data presented in Figure 1, can be seen that ethanolic extract was characterized with higher ($p < 0.05$) ability to capture free DPPH radicals compared to the aqueous extract at all of the tested concentrations. At the highest tested concentration (10 mg/mL) can be highlighted that aqueous (55.39%) as well as ethanolic (57.66%) extracts were competitive with the BHT as a positive control (58.10%).

From the point of ability to chelate iron ions (Figure 2), once again can be proved that ethanolic extract was characterized with slightly higher ($p < 0.05$) values compared to the aqueous one, whereas at the highest tested concentration (5 mg/mL) both of the extracts (51.57%, i.e. 49.03%, respectively) had much higher ($p < 0.05$) antioxidant potential compared to the citric acid (12.66%).

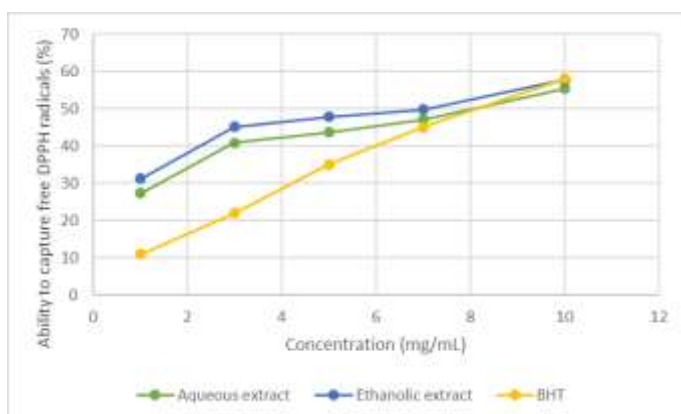


Figure 1: Ability of linden extracts to capture DPPH radicals

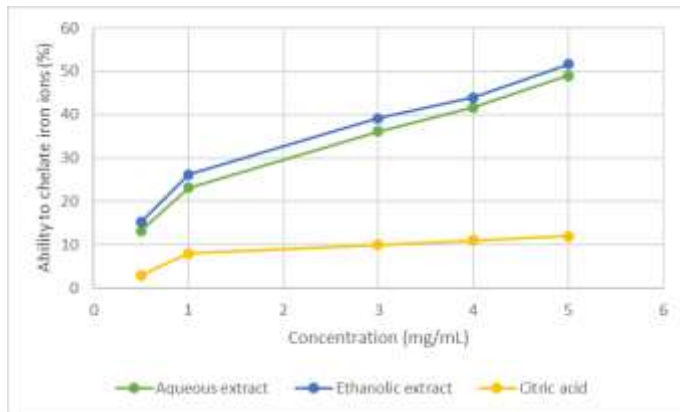


Figure 2: Ability of linden extracts to chelate iron ions

Table 1: IC₅₀ values of tested linden extracts

| Linden flower extract | n | IC ₅₀ (mg/mL) | |
|--------------------------|---|---------------------------|---------------------------------|
| | | DPPH | Chelating Fe ³⁺ ions |
| | | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ |
| Aqueous extract | 3 | 3.12 ± 0.12 ^{aA} | 2.65 ± 0.16 ^{aB} |
| Ethanolic extract | 3 | 2.01 ± 0.05 ^{bA} | 1.37 ± 0.10 ^{bB} |

a, b - values of the different extract and the same test marked with different letters, have a statistically significant difference ($p < 0.05$), T-test.

A, B - values of same extract and the different test, marked with different letters, have a statistically significant difference ($p < 0.05$), T-test.

These values were followed by those for the IC₅₀ values (Table 1). In this case, significantly lower ($p < 0.05$) values were determined for the ethanolic extract, compared to the aqueous extract for both antioxidant tests. Furthermore, can be seen that both of the extracts had higher ($p < 0.05$) ability to chelate iron ions, compared to its ability for capturing free DPPH radicals.

Antioxidants, on interaction with DPPH, transfer electron or hydrogen atoms to DPPH, and thus neutralize its free-radical character (Naik et al., 2003). Meanwhile, Akyuz et al. (2014) pointed that the DPPH scavenging activity of linden extracts and fractions, expressed in the term of SC₅₀, was in the range of 0.106–0.231 mg/mL, with the strongest antioxidant potency for phenolic extracts of tilia leaves.

According to Wissam et al. (2017) the ethanolic extracts of *Tilia cordata* leaves is a rich source of polyphenols and exhibit high antioxidant activity (IC₅₀=0.3303±0.0896 mg/ml calculated as DPPH scavenging activity).

Antimicrobial potential of linden extracts

According to data presented in Table 2, can be seen that ethanolic linden extract was characterized with higher ($p < 0.05$) antimicrobial potential against most of the tested strains compared to the aqueous linden extract. Even that ethanolic extract showed the highest activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Proteus vulgaris* and *Pseudomonas aeruginosa* only in one case, against *Listeria monocytogenes* (15.9 mm), showed higher ($p < 0.05$) activity compared to the

tetracycline. Aqueous linden extract had better ($p<0.05$) activity only against *Enterococcus faecalis* and *Shigella sonnei* compared to the ethanolic one.

In accordance, Pavlovic et al. (2020) in their study for antimicrobial activity of linden extracts found that yeast strains of *C. albicans* and Gram-negative pathogens such as *K. pneumoniae*, *E. coli*, *R. nepotum*, *P. aeruginosa*, *P. syringe* pv. *tomato*, and *E. persicina* have been shown highest resistance to the tested linden extracts. Slightly higher susceptibility has been observed against *C. glabrata*, *E. coli* and *P. aeruginosa*. Authors found that the most sensitive strains, were Gram-positive isolates of *S. mutans*, *S. pyogenes*, *E. faecalis* and *S. aureus* with clear zones of inhibition in range from 12 to 15 mm. On the other hand, Yıldırım et al. (2000) and El-Farmawi et al. (2014) have reported the absence of antimicrobial activity of linden tea extracts against *C. albicans*, *P. aeruginosa* and *K. pneumoniae*.

Table 2: Antimicrobial activity of tested extracts (mm)

| Microorganism | n | Linden flower extract | | Tetracycline 30 µg/disc | Chloramphenicol 30 µg/disc |
|--|---|-----------------------------|-----------------------------|-----------------------------|-------------------------------|
| | | Aq* | EtOH** | | |
| | | $\bar{x} \pm SD$ | $\bar{x} \pm SD$ | | |
| <i>Staphylococcus aureus</i> ATCC 25923 | 3 | 10.5 ± 0,01 ^a | 17.1 ± 0,02 ^b | 29,0 ± 0,01 ^c | 21,2 ± 0,03 ^d |
| <i>Bacillus cereus</i> ATCC 10876 | 3 | 1.0 ± 0,07 ^a | 5.3 ± 0,05 ^b | 11,5 ± 0,02 ^c | 19,3 ± 0,02 ^d |
| <i>Listeria monocytogenes</i> ATCC 19115 | 3 | 13.7 ± 0,05 ^a | 15.9 ± 0,02 ^b | 15,3 ± 0,03 ^c | 14,5 ± 0,04 ^d |
| <i>Enterococcus faecalis</i> ATCC 29212 | 3 | 10.9 ± 0,03 ^a | 7.2 ± 0,06 ^b | 15.8 ± 0,01 ^c | 17,6 ± 0,03 ^d |
| <i>Escherichia coli</i> ATCC 11230 | 3 | 6.6 ± 0,03 ^a | 9.5 ± 0,04 ^b | 11,2 ± 0,02 ^c | 12,1 ± 0,02 ^d |
| <i>Yersinia enterocolitica</i> ATCC 27729 | 3 | 18.1 ± 0,01 ^a | 21.4 ± 0,07 ^b | 27,0 ± 0,02 ^c | 26,3 ± 0,01 ^d |
| <i>Shigella sonnei</i> ATCC 29930 | 3 | 9.7 ± 0,03 ^a | 6.8 ± 0,02 ^b | 11,5 ± 0,01 ^c | 13,7± 0,02 ^d |
| <i>Proteus vulgaris</i> ATCC 8427 | 3 | 14.5 ± 0,09 ^a | 15.0 ± 0,05 ^a | 18,2 ± 0,01 ^b | 16,6 ± 0,01 ^c |
| <i>Pseudomonas aeruginosa</i> ATCC 35554 | 3 | 11.2 ± 0,02 ^a | 13.7 ± 0,03 ^b | 15.4 ± 0,01 ^c | 13.9 ± 0,01 ^b |

a, b, c, d – values marked with different letters have statistically significant difference ($p<0.05$), ANOVA, post hoc Tukey’s test.

* Aqueous extract

** Ethanolic extract

CONCLUSION

Based on the results, it can be concluded that water and ethyl alcohol are suitable for producing linden flower extracts. Ethanolic extract was characterized with higher ($p < 0.05$) ability to capture free DPPH radicals as well as ability to chelate iron ions, compared to the aqueous one, that is proved by the appropriate IC_{50} values. Moreover, both can be competitive with BHT and citric acid at the tested concentrations. However, ethanolic extract showed higher ($p < 0.05$) activity against *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Escherichia coli*, *Yersinia enterocolitica*, *Proteus vulgaris* and *Pseudomonas aeruginosa* compared to the aqueous linden extract.

According to that, ethanolic linden flower extract showed good antioxidant and antimicrobial potential while it can be used in the food industry for producing functional food with increased biological value.

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