**Proceeding Book of ISESER 2023**

# P 3. DETERMINATION OF PHYTOCHEMICAL COMPONENTS OF ACHILLEA MILLEFOLIUM IN DIFFERENT AREAS OF ALBANIA

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**ABSTRACT:** Medicinal plants are increasingly being used as an alternative to synthetic products, finding use not only in traditional medicine and pharmaceutical industry but also in the food and cosmetic industries due to their nutritional properties and bioactivity. Based on their economic, ecological and scientific value, they constitute one of the national natural assets of great importance. One of the widely spread plants in Albania is Achillea millefolium, a plant of the Asteraceae family with therapeutic properties. The health benefits of this plant's extracts result from a variety of secondary metabolites that include flavonoids, phenolic acids, terpenes, guaianolides, phytosterols, fatty acids, and organic acids. This study is focused on the evaluation of chemical components of Achillea millefolium essential oil using the hydro distillation method with gas chromatography, using flame ionization gas chromatograph. The plant material was collected in May-July 2023 in 4 different areas of Albania (Kukës, Tirana, Librazhd, Përmet). Obtained results showed that the essential oil of Achillea millefolium has a high content of secondary metabolites. The most abundant components are: alpha pinene (1.9-4.13%), beta-Pinene (6.8 – 12.1%), Cineole (7.68 -24,33 %), Camphor (2.64-4,23%), Borneol (19,62-20.72%), Azulenes (3.19–17,83%), Cineol (17.6– 24.33 %).

***Keywords****: Achillea Millefolium, Essential Oil, Chemical Components*

## 1. INTRODUCTION

Medicinal plants are increasingly being used as an alternative to synthetic products. These plants are used not only in traditional medicine and the pharmaceutical industry, but also in the food and cosmetic industries due to their nutritional properties and bioactivity. Due to their economic, ecological and scientific value, they constitute one of the national natural resources of great importance. One of the most widespread plants in Albania is *Achillea millefolium*, a plant of the Asteraceae family with therapeutic properties (Wolfgang et. al., 2003). *Achillea millefolium* was named after *Achilles*, the Greek mythological figure who used it to stop the bleeding wounds of his soldiers (Kowalchik C & Hylton WH, 1998). *Achillea millefolium* is found throughout Albania, from the northernmost to southernmost regions. This herbaceous plant has an unbranched stem that grows up to 80 cm tall and is characterized by its white, umbrella-shaped flowers and green to grey leaves. It is lightly aromatic with a bitter taste and blooms from June to November. *Achillea millefolium* is prevalent in meadows and mountainous areas, sometimes even in forests. It is found at elevations starting from 500-600m above sea level and can reach up to 2400 m above sea level in mountain peaks. The health benefits of this plant's extracts are due to a range of secondary metabolites, including flavonoids. Numerous studies have extensively documented flavones and 3-hydroxyflavones, which are frequently isolated compounds, along with sesquiterpene lactones. *Luteolin, apigenin and quercetin* are among the flavonoids. Phenolic acids, which are distinguished by the presence of derivatives such as *benzoic, caffeic, chlorogenic,* and *caffeoylquinic acid.* Additionally, *terpenes, guaianolides, phytosterols, fatty acids, and organic acids* are also present (Kowalchik & Hylton, 1987). The aerial parts of the plant are believed to aid in the treatment of gastrointestinal disorders by promoting proper bile flow. They can also stimulate blood circulation and may be used to alleviate high blood pressure. Additionally, a decoction of the whole plant is used to treat bleeding piles and kidney disorders. The plant has a mild stimulant effect and can be used to treat various allergic mucus problems such as hay fever. Fresh leaves may be chewed to

alleviate acute toothache or used as a mouthwash to promote the healing of cuts in the mouth and for tooth cleaning purposes (Noureddini et. al., 2008). In folk medicine, *Achillea millefolium* is ingested as an herbal tea to treat gastrointestinal disorders, headaches, hepato-biliary disorders, and as an appetite enhancer. Additionally, it is topically applied as a lotion or ointment for skin inflammations, wounds, cuts and abrasions.

*Achillea millefolium* extracts exhibit promising antimicrobial and antioxidant properties, which make them valuable ingredients in pharmaceutical and cosmetic products. The exploration of the use of flavonoids found in *Achillea millefolium* L. (seed oil) for formulations like sunscreens is prevalent. Additionally, traditional utilization of plants for medicinal purposes or for enhancing beauty has become a foundation for countless research studies resulting in novel developments in cosmetics (Strzepek et. al., 2023). In recent years, numerous studies have been conducted in the field of nanotechnology utilising Achillea millefolium as a component of nanoparticles. The results have shown promising effects of *Achillea millefolium* formulations as a practical distribution system. The aerial parts of the plant are utilized for treating gastrointestinal disorders by supporting proper bile flow, stimulating blood circulation to alleviate high blood pressure, and treating kidney disorders and bleeding piles with decoctions. Additionally, the plant has a mild stimulant effect and can be used to treat various allergic mucus disorders like hay fever (Noureddini et. al., 2008).

For acute toothache, fresh leaves can be chewed or used as a mouthwash to promote the healing of cuts in the mouth and for dental hygiene. In traditional medicine, *Achillea millefolium* is ingested as an herbal infusion for the treatment of digestive problems, headaches, and liver and

bile disorders, as well as for stimulating the appetite (Kumar et. al., 2021). As a topical agent, it is utilized in the form of a lotion or ointment to alleviate skin inflammations, wounds, cuts, and abrasions.

*Achillea millefolium* extracts additionally exhibit potential antioxidant and antimicrobial properties, making them useful in various pharmaceutical and cosmetic formulations. The exploration of the potential use of flavonoids in *Achillea millefolium* L. (seed oil) for formulation into sunscreens is ongoing. Furthermore, the traditional application of plants for medicinal or cosmetic purposes has formed the foundation for extensive research studies and innovative developments in the cosmetic industry (Strzepek et. al., 2023). These green synthesised nanoparticles show potential for pharmaceutical and biomedical applications (Yousaf et; al., 2020). They exhibit considerable boosting of antibacterial and antioxidant properties whilst significantly enhancing therapeutic performance (Yousaf et; al., 2020). This study aims to evaluate the chemical composition of *Achillea millefolium* essential oil using the hydro distillation method and gas chromatography with a flame ionization detector. The plant material was collected from four different areas in Albania (Kukësi, Tirana, Librazhdi, and Përmeti) between May and July 2023.

## 2. MATERIAL AND METHODS

- **Collection and prepare of plant material**

The plant was collected between May and July 2023 in four distinct regions across Albania: Kukësi, Tirana, Librazhdi and Përmeti (Fig. 1).

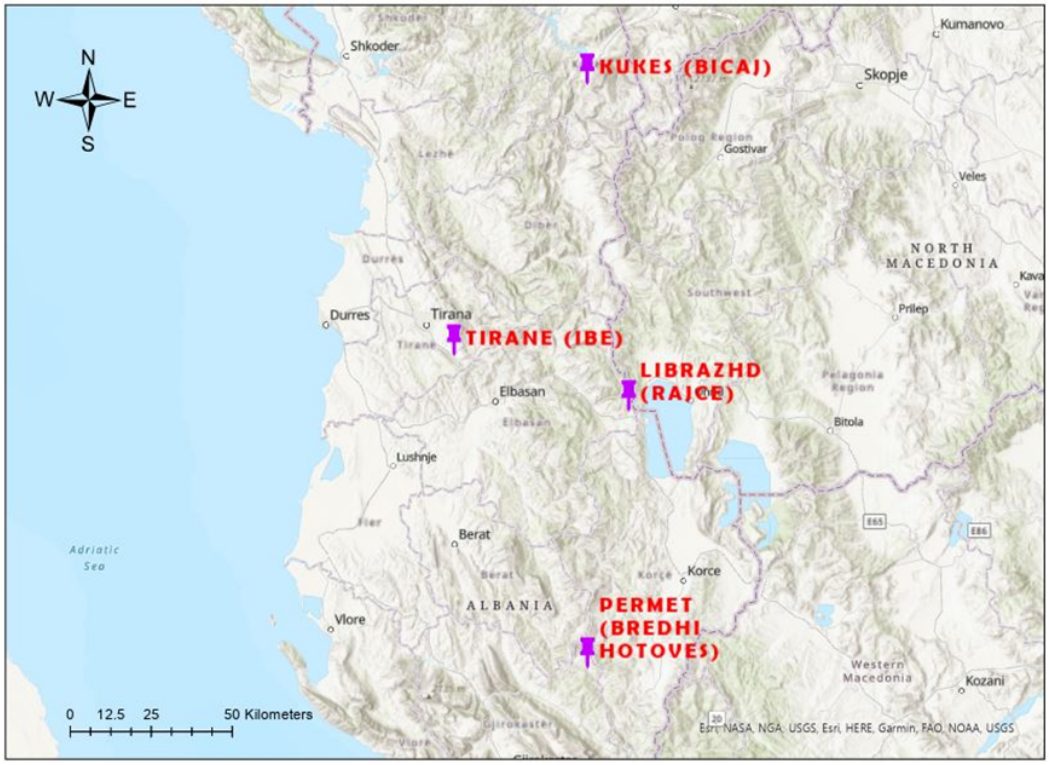


**Figure 1:** Map of sampling sites of *Achillea millefolium*

The sites are indicated in figure 1. To maintain the morphological and chemical properties, the *Achillea millefolium* specimens were air-dried. This herbaceous perennial plant has an upright growth habit and generates one to numerous stems reaching a height of 0.2–1 metre (8–40 inches), with a rhizomatous growth pattern. The leaves are evenly spread out throughout the stem, with the largest ones situated near the middle and bottom of it (Fig. 2).

The plant material was subsequently cut into small pieces. 50 g of the dried Achillea millefolium specimens were placed in a 250 ml flask. 150 ml of distilled water and 2 ml of toluene were then added to the flask to extract the essential oils from the *Achillea millefolium* specimens.

**Figure 2:** *Achillea millefolium*

- **Isolation of essential oil from Achillea millefolium**

Technical abbreviations have been explained upon their first use. Plant material from 50 g of *Achillea millefolium* was distilled for 3 hours using a Clevenger-type apparatus, as recommended by the European Pharmacopoeia for 6 hours, resulting in the production of the essential oil. The oil, which was made into a 2 ml Toluene solution, was then dried using anhydrous sodium-sulphate and kept sealed in a dark glass vial at +4°C until injection into GC/FID. Gas chromatographic analyses were conducted using a Varian 450 GC instrument, which featured a flame ionisation detector and PTV detector. The PTV injector was maintained at a temperature of 260°C. A sample of *Achillea millefolium* essential oil, which had been diluted in Toluene, was injected in spitless mode at a volume of 1 µl. An FID temperature of 280°C was maintained. Nitrogen served as the carrier (flow rate: 1 ml/min), while the make-up gas flowed at a rate of 25 ml/min. The flame detector gases comprised hydrogen (30 ml/min) and air (300 ml/min). A VF-1ms capillary column (30 m x 0.33 mm x 0.25 µm) was employed to separate the compounds of Achillea millefolium essential oil. The oven temperature was programmed in the following way: an initial temperature of 40°C (maintained for two minutes) was raised to 150ºC (with a gradient rate of 4°C/min), further raised to 280°C with a gradient rate of 10°C/min and kept at this temperature for two minutes.

## 3. RESULTS AND DISCUSSIONS

During the analysis of *Achillea millefolium* essential oil, it was observed that their chromatograms contained 40-50 compounds. After analysis, 18 main constituents were identified, (Tab. 1) which ranged in concentration from 89.57% (Tirana) to 96.1% (Kukësi).

**Table 1:** Displays the percentages of the 18 main constituents of *Achillea millefolium.*

| **Components of essential oil** | **Kukësi** | **Përmeti** | **Tirana** | **Librazhdi** |
|---|---|---|---|---|
| *Alpha-Pinene* | 2.53 | 4.13 | 2.73 | 1.9 |
| *Camphene* | 3.16 | 1.48 | 4.67 | 6.8 |
| *Beta-Pinene* | 2.96 | 4.98 | 2.26 | 1.1 |
| *Limonene* | 1.17 | 3.25 | 1.46 | 1.58 |
| *Para-Cimen* | 5.63 | 7.65 | 3.96 | 4.25 |
| *Cineole* | 17.68 | 16.32 | 24.33 | 23.46 |
| *Gama-Terpinene* | 5.56 | 6.53 | 3.69 | 2.24 |
| *Alfa-Thujone* | 1.75 | 5.28 | 0.81 | 0.3 |
| *Beta-Thujone* | 1.59 | 4.63 | 0.55 | 0.11 |
| *Camphor* | 3.73 | 4.23 | 2.64 | 1.87 |
| *Borneol* | 20.32 | 19.62 | 20.72 | 20.9 |
| *Terpylen-4-ol* | 0.99 | 0.56 | 1.52 | 1.81 |
| *Alpha-Terpineol* | 5.77 | 5.11 | 3.89 | 4.65 |
| *Acetate-Terpinol* | 0.62 | 0.18 | 1.89 | 1.65 |
| *Acetate Bornil* | 1.42 | 1.96 | 1.58 | 1.41 |
| *Caryophyllene* | 1.34 | 0.63 | 1.05 | 0.44 |
| *Cadinene* | 1.05 | 0.43 | 2.12 | 0.85 |
| *Azulenes* | 18.83 | 3.19 | 9.7 | 17.45 |
| **Total** | 96.1 | 90.16 | 89.57 | 92.77 |
| *Total monoterpenes* | 74.88 | 85.91 | 76.7 | 74.03 |

| | | | | |
|---|---|---|---|---|
| *Bicyclic monoterpenes* | 5.49 | 9.11 | 4.99 | 3 |
| *Monocyclic monoterpenes* | 6.73 | 9.78 | 5.15 | 3.82 |
| *Aliphatic monoterpenes* | 3.16 | 1.48 | 4.67 | 6.8 |
| *Oxygenated monoterpenes* | 53.87 | 57.89 | 57.93 | 56.16 |
| *Aromatic monoterpenes* | 5.63 | 7.65 | 3.96 | 4.25 |
| *Sesquiterpenes* | 21.22 | 4.25 | 12.87 | 18.74 |

The chemical constituents of the sample were analysed, (Fig. 3) determining *Borneol* as the most prominent compound, ranging from 19.62% to 20.72%. *Cineole* came next, with percentages ranging from 16.32% to 24.33%, followed by *Azulenes*, with values ranging from 3.19% to 18.83%. It is worth noting that technical term abbreviations used have been previously explained. Other compounds present but in lower percentages were *Para-Cimen, Camphene, Gama-terpinene, Camphor, alpha pinene, beta pinene, and alpha terpineol*, with percentages ranging from 1.1% to 6.8%.

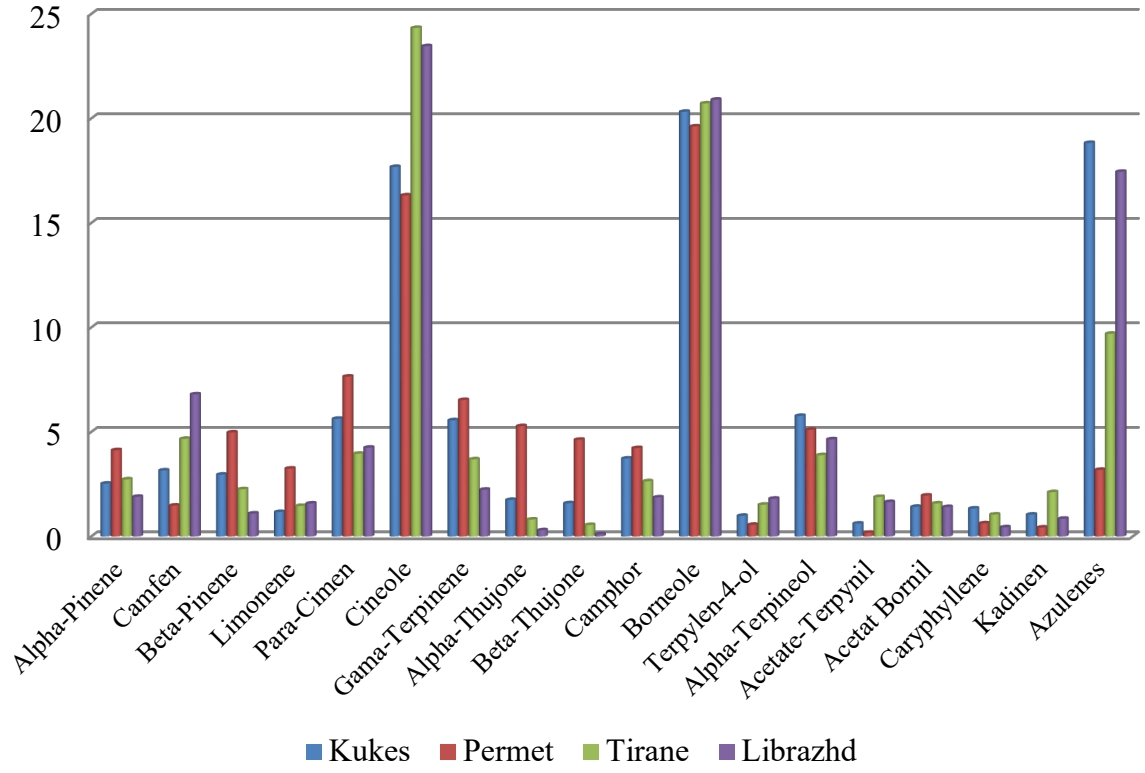


**Figure 3:** Chemical components of *Achillea millefolium* in four Albania sites.

Figure 4, demonstrates that monoterpenes comprise the largest group of terpenes (74.03%-85.91%). Within the group of monoterpenes, oxygenated terpenoids were the most abundant (*Cineole, alpha-Thujone, beta-Thujone, Camphor, Borneol, Terpylen-4-ol, alpha-Terpineol, Terpinol Acetate, Bornyl Acetate*), accounting for 53.87%-57.93% of the total. Sesquiterpenes, including *Caryophyllene*, *Azulenes,* and *Cadinene,* were found to range from 4.25% to 21.22% in the oil extracted from *Achillea millefolium.* The composition of the oil is influenced by multiple factors, such as the time of plant harvesting, geographical location, and sample composition.

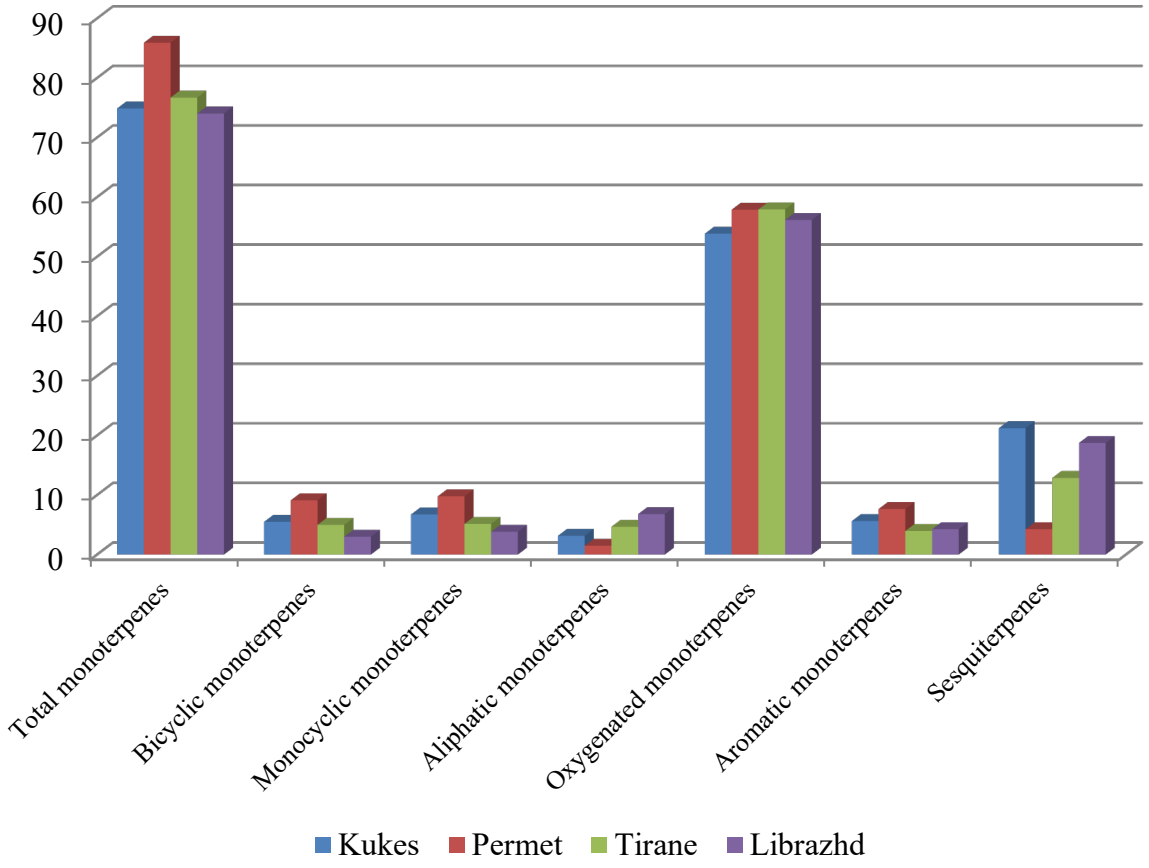


**Figure 4:** Terpenes content in *Achillea millefolium* in four Albanian sites.

## 4. CONCLUSION

- The chromatograms of the essential oil from *Achillea millefolium* contained 40-50 components upon observation.
- The sample's chemical constituents were analysed, revealing borneol as the most noteworthy compound, with a percentage range of 19.62% to 20.72%. Cineole follows, with values ranging from 16.32% to 24.33%, succeeded by Azulenes with values ranging from 3.19% to 18.83%. Notably, technical term abbreviations have been defined beforehand.
- Additional compounds found in smaller amounts include para-cymene, camphene, gamma-terpinene, camphor, alpha-pinene, beta-pinene, and alpha-terpineol, with percentages ranging from 1.1% to 6.8%.

## LITERATURE

Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat HA., 2021, Antioxidant and antimicrobial activity of the Çiğdem Aydın Acar. /Turkish Journal of Health Science and Life, 4(1), 40-45.

Evans WC.11., 2002, Trease and Evans pharmacognosy, 15th ed. Edinburgh, WB Saunders.

Kowalchik C & Hylton WH, 1998, Rodale's Illustrated Encyclopedia of Herbs, Eds, P.293, 367, 518. ISBN 978-0-87596-964-0.

Kumar P, Shruthi R, Bindu I, Raghavendra P., 2021, Pharmacognosy, phytochemistry, and molecular studies of an important medicinal herb Achillea millefolium L. Ayu., 42(2): 93-102.

Lakshmi T, Geetha RV, Roy A, Kumar SA., 2011, Yarrow (*Achillea millefolium* L.), A herbal medicinal plant with broad therapeutic use –A review. Int J Pharm Sci Rev Res., 9:136–41.

Mohammad Hosseini, Majid, Satyajit D. Sarker, and Abolfazl Akbarzadeh., 2017, "Chemical composition of the essential oils and extracts of Achillea species and their biological activities: A review." Journal of ethnopharmacology, 199, 257-315.

Noureddini M., Rasta V.-R., 2008, Analgesic Effect of aqueous extract of Achillea millefolium L. on rat's formalin test Pharmacology online, 3 (659-664).

Saeidnia S, Gohari A, Mokhber-Dezfuli N, Kiuchi F., 2011, A review on phytochemistry and medicinal properties of the genus Achillea. Daru., 19(3):173-86.

Si XT, Zhang ML, Shi QW, Kiyota H., 2006, Chemical Constituents of the Plants in the Genus *Achillea*. Chem Biodiversity, 3:1163–1180.

Strzępek-Gomółka M, Gaweł-Bęben K, Kukula-Koch W. 2021, Achillea Species as Sources of Active Phytochemicals for Dermatological and Cosmetic Applications. Oxid Med Cell Longev., doi: 10.1155/2021/6643827. PMID: 33833853; PMCID: PMC8018854.

Wolfgang Kathe, Susanne Honnef & Andreas Heym, 2003, Medicinal and Aromatic Plants in Albania, Bosnia-Herzegovina, Bulgaria, Croatia and Romania. A study of the collection of and trade in medicinal and aromatic plants (MAPs), relevant legislation and the potential of MAP use for financing nature conservation and protected areas" WWF Deutschland / TRAFFIC Europe-Germany. BfN, 2003, 200.

Yousaf H, Mehmood A, Ahmad KS, Raffi M. Green synthesis of silver nanoparticles and their applications as an alternative antibacterial and antioxidant agent. Mater Sci Eng C Mater Biol Appl., 2020 Jul; 112:110901. doi: 10.1016/j.msec.2020.110901; PMID: 32409057.