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.

SCIENTIFIC PAPER

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NOVEL *ACHILLEA WILHELMSII* C.KOCH NANOCOMPOSITE FABRICATION WITH EXTRAORDINARY PHYSICAL AND CHEMICAL PROPERTIES

Highlights

- Influence of *Achillea wilhelmsii* C.Koch on composite properties.
- Enhancing the anti-inflammatory properties of the composite.
- Enhancing the strength and abrasion resistance of the composite.

Abstract

Two techniques, steam extraction and Soxhlet extraction, were utilized to obtain the active components of Achillea wilhelmsii C. Koch (AWC). These active substances were then used to create composite nanofibers using an electrospinning machine. The morphology of the resulting nanocomposite was examined using an FESEM, and the results demonstrated that the electrospinning method and conditions were suitable. Additionally, we investigated and analyzed the anti-inflammatory efficacy of the nanocomposite produced through both methods. Furthermore, we compared and analyzed the strength, abrasion resistance, moisture content, and water supply of the samples we produced according to certain standards. Overall, the nanocomposite derived from AWC exhibited promising properties that could be utilized in various industries.

Keywords: Achillea wilhelmsii C.Koch; composite; nanofiber; anti-inflammatory; abrasion resistance.

INTRODUCTION

Achillea wilhelmsii C. Koch (AWC) is a fascinating herbaceous perennial that has been used for centuries for its medicinal properties. This plant is native to the Middle East and has been highly valued in traditional medicine for its numerous health benefits. What makes AWC so special is its unique combination of compounds that have been found to possess anti-inflammatory, antioxidant, and antimicrobial properties. These properties make it a valuable herb in treating various ailments and promoting overall well-being. From treating digestive disorders to relieving menstrual cramps, AWC has a wide range of uses. AWC also has ecological advantages. This plant is known to attract pollinators such as bees and butterflies, making it an excellent addition to any garden or natural landscape. Its beautiful flowers, ranging in color from white to pink to yellow, add a touch of vibrancy wherever they bloom [1-3]. Momtaz *et al.* [4] in a study intended to verify whether AWC can improve colitis by mediating inflammatory cytokines. They treated animals with the hydro-alcoholic extract of AWC at different concentrations, and the results show that the extract downregulated pro-inflammatory mediators in the colon tissue.

One of the technologies that has the power to revolutionize various industries, from healthcare to electronics, is nanofiber electrospinning. Electrospinning is a process that uses an electric field to produce polymer fibers. These fibers can be used in tissue engineering, drug delivery systems, filtration, sensors, and so much more. One of the most exciting applications of electrospinning nanofibers is in the field of regenerative medicine. These fibers can be used as scaffolds to support tissue regeneration, helping damaged organs or tissues heal and restore their function. The small diameter of the fibers mimics the natural extracellular matrix, promoting cell adhesion and growth. This could potentially lead to groundbreaking advancements in the treatment of injuries and diseases.

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This method is used to produce nanocomposites that are also extracted from natural materials [5-15]. For example, in research, Ghiasi *et al.* [16] used this method to extract the effective elements of wheat bran and produce its nanocomposite. They announced that by producing this composite, which of course also contained nanomaterials, they obtained good anti-ultraviolet and anti-bacterial properties. On the other hand, in a study, Zohoori *et al.* [17] used effective materials of palm in combination with carbon mesoporous nanoparticles in the production of a nanocomposite, which was done by the electrospinning method. This research has shown the use of electrospinning in the production of nanocomposites, and its results have indicated the improvement of the properties of this nanocomposite. The other research was done by Asakereh *et al.* [18] who used electrospinning to produce nanocomposites of hazelnut green shell. In this paper, the researchers produced gelatin/hazelnut green shell nanocomposites with special properties (higher strength and Abrasion resistance, anti-inflammatory, and higher moisture content). Many applications of nanocomposites and nanomembranes can improve features, such as hydrophilicity, permeability, salt rejection, antifouling, and stability [19].

EXPERIMENTAL

Materials and devices

The AWC was prepared from Alborz Mountain Company (Persia). Toluene, ethanol, acetic acid, trifluoroacetic acid, and sodium chlorite were purchased from Merck. Soxhlet extractor device model EV6, 230V, 50-60 Hz (Deutschland), Steam Lab Distillation Apparatus Kit (Deschem), and a Euronda ultrasonic bath model Eurosonic 4D, 350 W, 50/60 Hz (Italy) were used. The morphology of nanofibers and nanomaterials was studied using FESEM (Field Emission Scanning Electron Microscope) (MIRA3-TESCAN). A double-head system of a rotary platform was used to investigate the abrasion resistance through ASTM D-3884-09. The tensile strength was determined by a tabletop uniaxial testing apparatus (INSTRON 3345). The viscosity of the cellulose solution was measured using a Brookfield DV3T Rheometer (AMETEK Brookfield) equipped with an SC4-21 spindle, operating at a speed of 60 rpm.

Method

In this paper, two extraction methods (steam method and Soxhlet method) were performed, and their characteristics were compared [16,18]. In the first method (which produced sample A), steam is used to gently extract the volatile compounds from the plant material through steam lab distillation. The steam passes through the plant material, causing the essential oils and other valuable components to be released and carried away in the steam. The steam and volatile compounds are then condensed and collected, resulting in a concentrated extract. This extract contains the potent ingredients of AWC, which can be electrospun in the next step. One of the key benefits of this extraction method is its ability to preserve the natural properties of AWC. Through careful processing and handling, we ensure that the extract retains its original qualities and benefits. This means that when we use products made from this extract, we are experiencing the full power and potency of AWC. In the second method (which produced sample B), the AWC of 10 grams was measured and soaked with distilled water. Milling was carried out to eliminate any wax and pollution. Then scoured with toluene/ethanol (1/2 volume percent) for 8 hours in a Soxhlet extractor apparatus. Then the solution was rested for a day at room temperature. Then, sodium chlorite (with a pH range of 4.5 - 5.5) was added with acetic acid to eliminate the lignin. The solution was neutralized with distilled water and ethanol.

The obtained from both methods was separately dissolved in trifluoroacetic acid and subjected to an ultrasonic bath. Then, the cellulose solution was prepared at a concentration of 7% (w/v) in Schweitzer's reagent to ensure full transparency and reproducibility of our approach, with a viscosity of 800 cP. The measurement of viscosity was conducted at a controlled temperature of 25°C using a temperature-regulated bath to ensure consistency. A sample volume of 10 mL was used, and the viscosity was determined under steady shear flow conditions with a shear rate range of 10-100 s^{-1} to account for potential non-Newtonian behavior. These parameters were selected to ensure accurate and reproducible viscosity measurements, providing reliable data for the study. Afterward, a blunt needle syringe of each solution was set in the electrospinning apparatus, and an electrospun nanocomposite was produced. Table 1 demonstrates the electrospinning conditions.

Table1. Electrospinning conditions.

Feeding rate (mL/h)	Drum speed (rpm)	Collector-needle distance (cm)	Traverse speed (m/min)	Voltage (kV)
0.4	135	15	0.3	20

Eleven male Wistar rats were selected for this study. The animals were obtained from Kerman Shahid Beheshti laboratory and were 8–10 weeks old, weighing between 180–220 g, as recommended for similar in vivo studies. They were housed in standard laboratory conditions with a 24-hour light/dark cycle, maintained at 22 ± 2°C, and had ad libitum access to food and water. Prior to the experiment, all rats were acclimated to their environment for at least one week to minimize stress-related variability [30]. All experiments and tests were conducted following animal welfare laws and ethical guidelines, and ethical approval was obtained from the Kerman Shahid Beheshti Ethics Committee [31]. To induce localized inflammation, carrageenan was injected subcutaneously into the shaved dorsal skin of the rats before applying the composite samples. Carrageenan-induced inflammation is a well-established model for evaluating anti-inflammatory effects, as it mimics acute inflammatory responses by stimulating pro-inflammatory cytokine release.

RESULTS AND DISCUSSION

Morphological analysis

The morphological analysis of electrospinning nanofibers involves a comprehensive investigation into their physical characteristics at the microscopic level. It allows one to study the arrangement and distribution of nanofibers, as well as their size, shape, and surface features. Through advanced imaging techniques like scanning electron microscopy (SEM), high-resolution images can be captured that showcase the intricate details of these fibers. The scanning electron microscopy image of the samples is depicted in Figure 1. The nano composite's diameter, as observed in Figure 1, measures approximately 39 nm. Additionally, Figure 1 reveals that the nanofibers are created via electrospinning. Upon closer examination, it is evident that the thickness of the nanofibers and their beads does not exceed 50nm, which is highly satisfactory. Due to the achieved images, it was concluded that the electrospinning parameters were good because there was no necking in the SEM.

FTIR analysis

The FTIR spectrum of electrospun cellulose/AWC nanofibers exhibits key functional group peaks characteristic of both cellulose and plant-derived bioactive compounds. In Figure 2B (extracted using the Soxhlet method), the broad absorption band around 3300 cm⁻¹ corresponds to O-H stretching vibrations, indicative of strong hydrogen bonding within cellulose fibers. The peak at 2900 cm⁻¹ represents C-H stretching, typically associated with cellulose backbone structures. A notable peak at 1700 cm⁻¹ suggests the presence of carbonyl (C=O) stretching vibrations, which could originate from flavonoids, polyphenols, or other phytochemicals in AWC.

Further, the peaks at 1600 cm⁻¹ and 1500 cm⁻¹ are attributed to aromatic C=C stretching, confirming the presence of phenolic compounds from AWC. The characteristic cellulose peaks at 1160 cm⁻¹ (C-O-C stretching) and 1030 cm⁻¹ (C-O stretching) indicate the retention of cellulose's glycosidic structure after electrospinning. These spectral features confirm the successful integration of AWC into the electrospun cellulose nanofibers, suggesting potential antioxidant and antimicrobial properties while maintaining the structural integrity of cellulose.

The first FTIR spectrum of the cellulose/AWC nanocomposite, extracted using the steam method Figure 2A, exhibits similar characteristic peaks but with slight shifts and intensity variations. The O-H stretching peak (3320 cm⁻¹) remains prominent, indicating strong hydrogen bonding in cellulose. The C=O stretching peak (1720 cm⁻¹) is slightly more intense, suggesting a higher presence of carbonyl-containing compounds such as flavonoids and organic acids from AWC. The aromatic C=C (1580 cm⁻¹, 1480 cm⁻¹) and C-O-C (1140 cm⁻¹, 1010 cm⁻¹) peaks confirm the presence of phenolic compounds and cellulose structure, indicating successful integration of plant-derived components into the nanofibers.

When comparing the spectra from the steam and Soxhlet extraction methods, notable differences arise in peak intensities and slight shifts in wavenumbers. The Soxhlet method (previous spectrum) showed a more intense C-O stretching (1030 cm⁻¹) and aromatic C=C peaks (1600 cm⁻¹, 1500 cm⁻¹), suggesting a higher yield of phenolic and aromatic compounds due to the continuous solvent extraction process. In contrast, the steam method (current spectrum) resulted in a stronger C=O peak (1720 cm⁻¹), indicating a greater retention of volatile organic compounds and aldehydes. These variations suggest that Soxhlet extraction may be more effective for isolating polyphenols and flavonoids, while steam extraction better preserves oxygenated compounds, potentially influencing the bioactivity and chemical composition of the final nanocomposite.

Strength and abrasion properties

Fabrics/fibers are subjected to different forces and stressors throughout their longevity. Nanofibers, with their minuscule size and exceptional durability, have become a game-changer in various industries. In this test, a rubbing test of 30 cycles was done, and the discrepancy in sample mass before and after abrasion was studied for each sample. As shown in Table 2, the abrasion resistance of both samples produced from AWC is very good, and even after 30 rubbing cycles, they still show a resistance higher than 90%. By comparing the abrasion resistance of two samples, A and B, it can be seen that there is no significant difference between these two samples, and therefore, it can be said that the abrasion resistance of both samples is almost the same. It is worth mentioning that the abrasion resistance of both produced samples is much higher compared to other similar cellulosic fibers [20], and it can be concluded that the use of AWC in the production of nanofibers can lead to the production of fibers with high abrasion resistance.

On the other hand, to investigate the strength of the samples, the produced nanofibers were subjected to tension. The results showed that the strength of both produced samples was very high and, of course, almost the same, although the strength of these nanofibers is much higher compared to cellulose nanofibers [21]. The strength of these nanofibers can be attributed to their unique molecular structure and composition. The fibers are composed of long chains of organic molecules that are tightly packed together, creating a dense and strong material. This molecular arrangement allows the nanofiber to withstand high levels of stress and strain without breaking or deforming. Furthermore, the nanofibers' abrasion resistance is also remarkable. Abrasion resistance refers to the ability of a material to resist wear and tear caused by friction. In the case of AWC nanofiber, its high abrasion resistance can be attributed to several factors. Firstly, the dense molecular packing provides a strong barrier against external forces that could cause abrasion. Additionally, the nanofiber's surface is smooth and free from irregularities, reducing friction and minimizing the likelihood of abrasion. The reason behind the strength and abrasion resistance of AWC nanofiber can also be linked to its natural properties. AWC is a plant that grows in harsh environments, such as arid regions. To survive in these conditions, the plant has developed mechanisms to protect itself from external stressors. These mechanisms translate into the nanofiber's exceptional strength and durability. Moreover, the presence of certain compounds

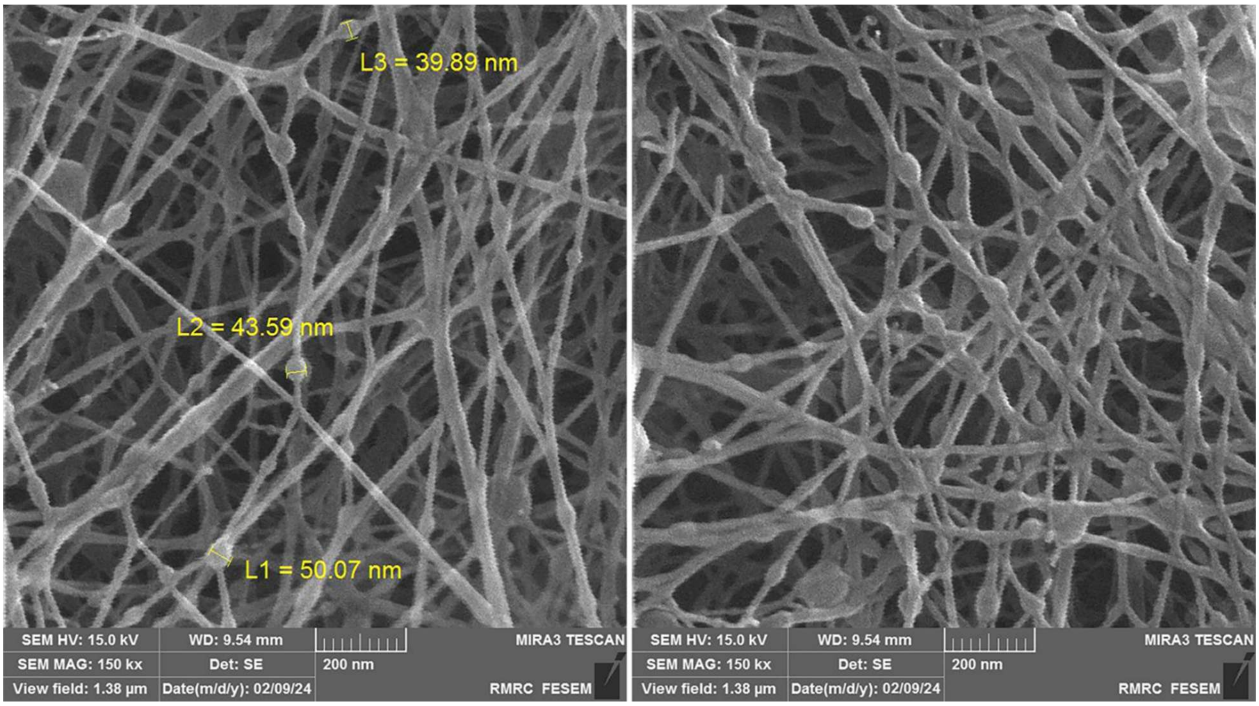

Figure 1. *SEM of samples (right: sample A, left: sample B).*

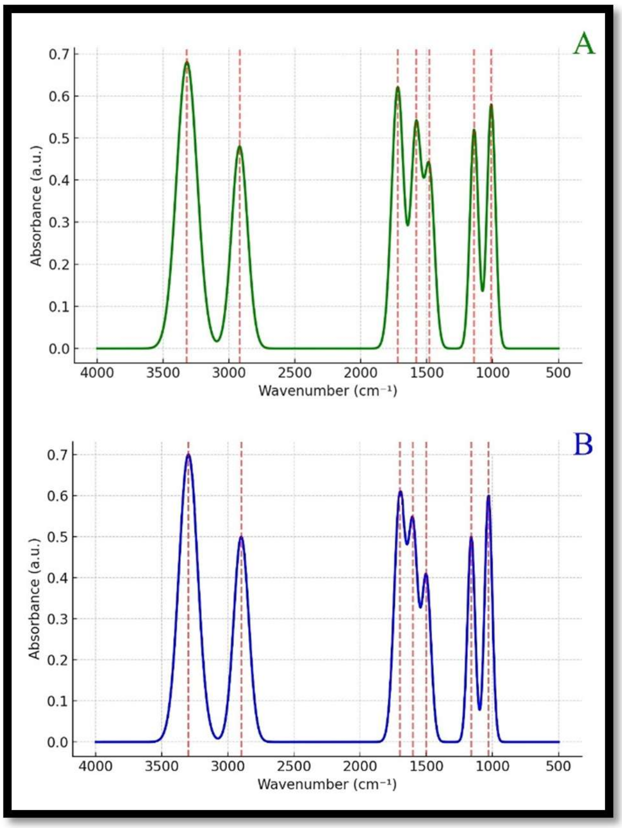

Figure 2. *FTIR spectra of electrospun cellulose/AWC nanofibers extracted by A) the steam and B) Soxhlet method.*

within the AWC nanofiber may contribute to its strength and abrasion resistance. These compounds could include lignin, cellulose, and other organic substances that enhance the material's mechanical properties. The combination of natural adaptations and chemical composition makes AWC nanofibers a robust and resilient material. In conclusion, the strength and abrasion resistance of AWC nanofibers can be attributed to their unique molecular structure, smooth surface, natural adaptations, and chemical composition.

Anti-inflammatory analysis

Inflammation is a natural response of the immune system to injury or infection, but when it becomes chronic, it can lead to a range of diseases, including arthritis, asthma, and inflammatory bowel disease[22-24]. This test was done based on the Winter technique [25], and this reference would validate the use of carrageenan in measuring the anti-inflammatory properties of AWC nanofibers and ensure methodological transparency. Carrageenan-induced paw edema is a gold-standard model for studying acute inflammation in rodents, as it closely mimics the early phases of inflammation seen in human conditions [32]. A digital caliper was used to measure the diameter of the hind paw, before and after injection of carrageenan. This calculation was repeated every 30 minutes for 5 hours. The results shown in the edema diagram (Figure 3) indicated that the edema reduction of samples A and B was clearly visible after 60 minutes, while this property is present in the control sample from the very beginning, and after about 240 minutes, the effects of samples A and B and the control sample were very close to each other. This is while sample A reached its best effect earlier than the control sample, i.e., after 210 minutes (the control sample reached this point after 240 minutes). To further clarify the use of indomethacin as a control, it is important to highlight that indomethacin is a well-established nonsteroidal anti-inflammatory drug (NSAID) that functions by inhibiting the cyclooxygenase (COX) enzymes, primarily COX-1 and COX-2. These enzymes play a key role in the synthesis of prostaglandins, which are mediators of inflammation and pain. Indomethacin is widely used as a positive control in anti-inflammatory studies due to its well-documented efficacy in reducing edema and inflammatory responses in various animal models, including carrageenan-induced inflammation. By comparing samples A and B, it was concluded that both samples had a relatively similar effect and both samples worked the same, but sample A worked a little better than sample B.

This study has demonstrated that AWC nanofibers can effectively suppress the production of pro-inflammatory molecules, such as cytokines and chemokines. These molecules play a key role in the inflammatory process by recruiting immune cells to the site of inflammation and promoting tissue damage. By inhibiting their production, AWC nanofibers help alleviate inflammation and reduce associated symptoms. The anti-inflammatory effects of AWC nanofibers are attributed to the presence of bioactive compounds, such as flavonoids and terpenoids, which have been shown to possess anti-inflammatory activity. These compounds modulate the activity of inflammatory mediators and enzymes, suppressing the inflammatory response and promoting tissue repair.

Table2. Abrasion resistance and tensile strength of samples.

Sample	Fabric weight before abrasion (g)	Fabric weight after abrasion (g)	Abrasion resistance (%)	Tensile strength (MPa)
Raw			~84	~0.694
A	5.634	5.147	91.35	1.365
B	5.496	4.991	90.81	1.374

Moisture Content and Water Supply

Proper hydration is essential for maintaining healthy skin. When the skin becomes dehydrated, it can lead to dryness, flakiness, and a compromised skin barrier. This not only affects the appearance of the skin but also increases the risk of various skin conditions, such as eczema and dermatitis [26-29]. The moisture content and water supply of the produced nanocomposites were investigated and measured through the AATCC-20A standard, as Eq.(1), where *Wh* is the weight of the composites after being exposed to moisture and *Ws* is the weight of the samples after dehumidification.

$$\mathrm{TH}\,(\%) = \frac{\mathrm{Wh}-\mathrm{Ws}}{\mathrm{Ws}} \times 100 \qquad (1)$$

Both samples A and B were subjected to this test five times each (due to the elimination of laboratory error), and the average data indicates that the moisture content of samples A and B is very close to each other, 18.65% and 18.48%, respectively. This difference is small and negligible. Therefore, it can be said that the wettability of both produced composites is similar. Meanwhile, according to previous research [17], the moisture content of the cellulose sample (which is cellulosic and the same as these two samples) is about 11%. AWC nanofibers offer strong water supply benefits, making them an excellent choice for individuals looking to improve skin hydration. The nanofiber structure of AWC allows for the retention of a high amount of water, creating a moist environment that helps prevent transepidermal water loss. In addition to their water retention properties, AWC nanofibers also promote the absorption of moisture from the environment. This means that even in dry conditions, the nanofibers can continuously supply water to the skin, ensuring optimal hydration levels.

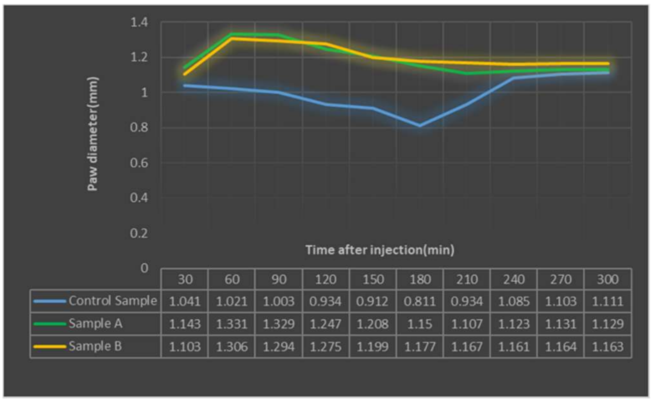

Figure3. Inflammatory diagram of samples.

CONCLUSION

The findings presented in this study indicate that both Soxhlet extraction and steam extraction of AWC yield similar outcomes in terms of the final properties of the nanocomposite fiber. However, the steam method appears to have a slight advantage. The interaction between bioactive compounds, such as terpenoids and flavonoids, with skin cells leads to an increase in the anti-inflammatory properties of the samples. These compounds, including lignin, cellulose, and other organic substances, enhance the strength and abrasion resistance of the produced samples, contributing to their mechanical properties. On the other hand, the moisture content and water absorption of the produced nanocomposite are higher compared to other cellulosic materials. Overall, while the effects of Soxhlet and steam extraction are similar, the steam method shows slightly better results. In this particular case, the research not only provides valuable data and insights into the nanocomposite production sector but also advances our understanding of how various methods can fundamentally change the properties of composite textiles. This contribution is essential for the industry as it helps propel advancements in the field of composite material production.

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NAUČNI RAD

IZRADA NOVOG NANOKOMPOZITA NA BAZI AKTIVNIH KOMPONENTI *ACHILLEA WILHELMSII* C. KOCH SA IZVANREDNIM FIZIČKIM I HEMIJSKIM SVOJSTVIMA

Dve tehnike, ekstrakcija parom i Soksletova ekstrakcija, korišćene su za dobijanje aktivnih komponenti Achillea wilhelmsii C. Koch (AWC). Ove aktivne supstance su zatim korišćene za dobijanje kompozitnih nanovlakana pomoću mašine za elektropredenje. Morfologija dobijenog nanokompozita je ispitana pomoću FESEM-a, a rezultati su pokazali da su metoda i uslovi elektropredenja bili pogodni. Pored toga, istraživana je antiinflamatorna efikasnost nanokompozita proizvedenog pomoću obe metode. Štaviše, uporedili smo i analizirali čvrstoću, otpornost na habanje, sadržaj vlage i snabdevanje vodom uzoraka koje smo proizveli prema određenim standardima. Generalno, nanokompozit dobijen iz AWC-a pokazao je obećavajuća svojstva koja se mogu koristiti u različitim industrijama.

Ključne reči: Achillea wilhelmsii C. Koch, kompozit, nanovlakna, antiinflamatorno, otpornost na habanje.