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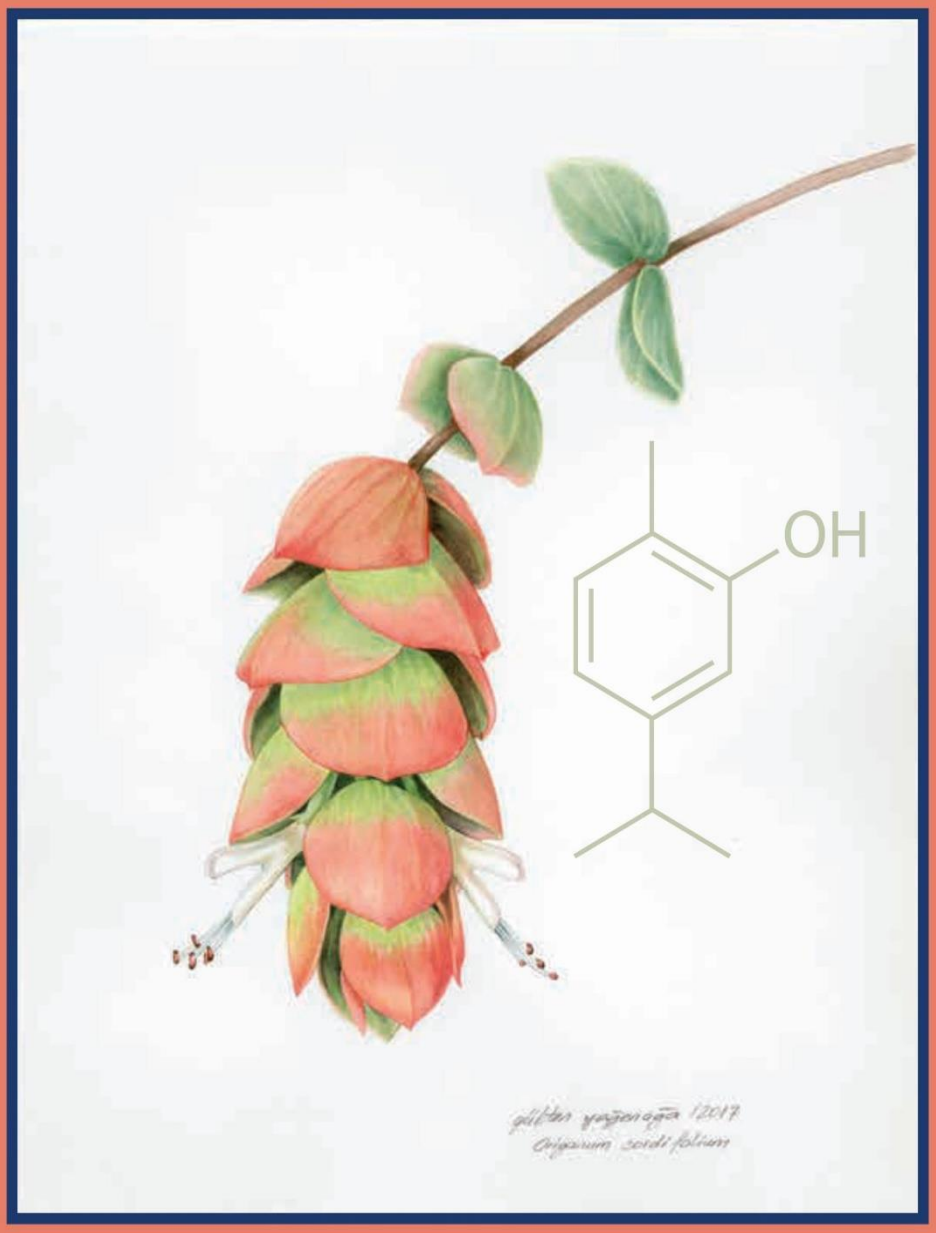


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a) The abstract appears on its own page.
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State the objectives of the work and provide a brief background of the literature related with the topic. The novelty and the aim of the study should be clearly stated.

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A combined Results and Discussion section is often appropriate. Results should be concise.

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## *Review*

Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

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# Development of Zinc-loaded Hydrogel Infused with *Aloe barbadensis* Mucilage for Wound Healing

Ibilola Mary Cardoso-daodu*, Emmanuel Chibuike Agbarakwe, Margaret Okonawan Ilomuanya, Chukwuemeka Paul Azubuike, Boladale Olanrewaju Silva

[1] University of Lagos, Faculty of Pharmacy, Department of Pharmaceutics and Pharmaceutical Technology, Lagos, Nigeria.

## Abstract

This study aims to formulate and characterize zinc-loaded hydrogel infused with *Aloe barbadensis* mucilage for wound dressing. Five formulations containing varying proportions of carbopol, zinc, Aloe and water (as vehicle) were developed via physical crosslinking using triethanolamine. All formulations had a translucent off-white colour while the control gave a transparent gel. The viscosity was the highest in the control, 30000.00 ± 2.07 PaS. The pH of the formulations was between 5.7 and 5.8. formulation 2 which was composed of 30 mg of Zinc and 1.4 mg of *Aloe barbadensis* incorporated into 1% w/v Carbopol Ultrez hydrogel polymer had the lowest swelling index of 79.2 ± 1.95% implying that it had the fastest drug release rate. The wounds treated with formulation 2 had the most rapid healing with no sign of scars in the wound area. Histomorphometric evaluation reflected a high re-epithelisation rate of 70%, a significant percentage occupied by collagen in granulation tissue of 85%. The thickness of the tissue's central region was 10 mm. The inflammatory cells /mm$^2$ tissue was 200 cells/mm$^2$ while the number of microvessels in granulation tissue was 1.0 microvessels/mm$^2$. Zinc-loaded hydrogel infused with *Aloe barbadensis* mucilage shows great potential as a modern wound dressing.

**Keywords**

*Aloe barbadensis*, wound healing, hydrogel, zinc.

**Article History**

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## INTRODUCTION

A wound is a break in the epithelial lining or mucosa in any anatomical part of the body due to trauma (Dhivya, 2015; Sarp *et al.,* 2021). Wound dressings are fabricated to aid healing by creating an optimal microenvironment by shielding the wound from bacteria and other microorganisms (Ilomuanya *et al.,* 2019). Hydrogels are Hydrogels are biocompatible, biodegradable, and water-soluble. Moreover, the structural network of hydrogels imitates that of the body's extracellular matrix making them ideal for wound dressing (Liu *et al.,* 2022). Hydrogels provide an ideal microenvironment for wound healing by allowing proper penetration of oxygen while shielding the wound from dust, particles, and bacteria. Hydrogels show moderate attachment to the wound bed allowing easy separation from the wound surface while removing the wound dressings making it less painful for the patient (Soleimanpour *et al.,* 2022). One prominent advantage of hydrogels is the fact that they can house bioactive medicaments in their polymer matrices and provide sustained release of the medicaments into the wound bed over a specific period (Avila-Salas *et al.,* 2019). well-established examples of second-generation wound dressings. They are three-dimensional polymer networks that can absorb large amounts of water into their matrices whilst retaining their structure due to chemical and physical crosslinking of polymer chains (Yiyang *et al.,* 2016; Bahram *et al.,* 2016).

Zinc is an important micronutrient that should be present in minute quantities in the human body. It plays a vital role in growth, development, bone formation, immune function and wound healing. Low zinc levels in the body have been associated with poor wound healing. Zinc influences wound healing positively through extracellular matrix regulation and antioxidant defense (Lin *et al.,* 2017). Antioxidants like zinc are important in proper wound therapy because they help to reduce the excessive presence of oxidants at the wound site and clear the accumulation of products of inflammation. Zinc participates in extracellular matrix re-modeling through the regulation of matrix metalloproteinases and interacts with the tripartite motif family of proteins to facilitate membrane repair and angiogenesis (Ilomuanya *et al.,* 2019). Zinc can be incorporated into the hydrogel matrix to allow sustained micronutrient

activity at the wound bed facilitating wound healing. It is vital that while facilitating wound healing, the wound bed is also actively protected from microorganisms. Further incorporation of a bioactive material with antibacterial properties into the polymer hydrogel matrices is essential (Arab *et al.,* 2020).

*Aloe barbadensis* is a tropical plant that is about 1-2 feet in height. It is perennial and can be described as a spiky cactus-like xerophytes clump with a thick fibrous root belonging to the *Asphodelaceae* family. Aloe gel consists of about 98.5% - 99.5% water (Adom *et al.,* 2020). Its solid component contains more than 200 different phyto-constituents with polysaccharides being the most abundant compound. Other phytochemical constituents present include chemical compounds such as soluble sugar, glycoprotein, phenolic anthraquinones, flavonoids, flavonols, enzymes, minerals, sterols, saponins, and vitamins (Zeng *et al.,* 2020). Aloe is a popular plant with well-established health benefits. It acts as an antioxidant, reduces constipation and increases insulin sensitivity thereby reducing blood sugar levels in type 2 diabetes. Aloe also possesses antibacterial properties and can be applied to wounds to facilitate healthy wound healing. Aloe possesses anthraquinones which exhibit antibacterial properties via direct interference with solute transport through the cellular membrane of a bacterium (Zeng *et al.,* 2020).

This study aims to co-formulate and characterize zinc-loaded hydrogel infused with *A. barbadensis* for wound dressing. Based on the current trend in the field of wound healing there is a need for an ideal dressing that is cost-effective (Okur *et al.,* 2020). This study bridges the gap between conventional dressing and second-generation dressing as hydrogel possesses all the functional properties of a conventional dressing. A step further would be the advancement of the hydrogel polymer by the inclusion of the bioactive materials such as zinc and *A. barbadensis* mucilage into its polymer matrices upgrading it to function as a smart wound dressing. The study particularly takes advantage of the antimicrobial and antioxidant properties of zinc and *A. barbadensis* by fabricating a potent hydrogel formulation that can serve as an excellent wound dressing for trauma wounds when impregnated on a gauze sponge (Rasli *et al.,* 2020).

## MATERIALS AND METHODS

### Materials

The materials used in this study include *A. barbadensis* mucilage (grown and harvested in Lagos South-west Nigeria), zinc (Hebei Co Ltd, China), phosphate buffer (Nanjing Biotech, China), 1% cremophor (RH 40) (Shandong Ltd, China), triethanolamine (Xingtai Dakun Technology Ltd, China), urethane (Selleck, USA), Carbopol® Ultrez (Qingdao Co Ltd China).

### Plant collection and authentication

The leaves of *A. barbadensis* was obtained from Yaba, Lagos state, Nigeria in August 2022. They were identified taxonomically and authenticated at a Herbarium in the Department of Botany, University of Lagos. The leaves were designated voucher number LUH 5977.

### Preparation of *A. barbadensis* mucilage

Mature leaves cut from the *Aloe barbadensis* were rinsed with an adequate quantity of water to remove dirty particles. The plant leaves were allowed to dry at room temperature without direct contact with sunlight, to make the gel thicker. The rind was removed gently after it was cut open transversely and the mucilage was carefully scrapped out (Saleem *et al.*, 2022).

### Preparation of zinc and *A. barbadensis*-loaded hydrogel

The hydrogel polymer was prepared at 24°C by dissolving Carbopol® Ultrez in distilled water using a mechanical stirrer at 100 rpm. Then it was left to soak for 24 hours. The required quantities of zinc (Table 1) were incorporated into the hydrogel. The stipulated quantity of *Aloe* mucilage was incorporated using the mechanical stirrer (Chang Bioscience CA, U.S) at a constant stirring rate of 100 rpm to achieve proper mixing. The pH was modified by adding the cross-linking agent triethanolamine. Distilled water was used to make up the final volume (Ilomuanya *et al.,* 2019).

Table 1: Composition of alkyl acrylate cross polymer hydrogels and control.

| Ingredient | F1 | F2 | F3 | F 4 | F5 | Control |
|---|---|---|---|---|---|---|
| **Carbopol® (g)** | 1.5 | 1 | 0.75 | 1.5 | 1 | 1 |
| **Triethanolamine (ml)** | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| **Zinc (mg)** | 40 | 30 | 25 | 10 | - | - |
| ***Aloe babadensis* mucilage (mg)** | - | 1.4 | 1.4 | 1.4 | 1.4 | - |
| **Water (ml) to** | 100 | 100 | 100 | 100 | 100 | 100 |

**Physical examination and pH assessment of the hydrogels**

Physical examination of the formulations was carried out after preparation and pH assessment was done using a pH meter (Mettler Toledo, Columbus USA). The hydrogels were optically evaluated for color, consistency and homogeneity. The pH of the formulations was determined by immersing the electrode in the formulation (Ilomuanya *et al.*, 2020).

**Rheology test**

The viscosity of the formulations was determined at 24°C at 20 rpm using Spindle 7.0 cone and plate viscometer (BYK Instruments Shanghai, China) (Elegbede *et al.*, 2020).

**Skin patch test**

This study is covered by ethical approval number CMUL/HREC/0974/19. The formulations (0.4 g) were applied to the shaved dorsal surface (1.5 $cm^2$) of male Wistar rats. The skin appearance was visually examined for redness and swelling 1h post application (Ternullo *et al.*, 2019).

**Swelling test**

The degree of water absorption by hydrogel formulations was evaluated by incubating 100 mg of hydrogel in 50 ml of phosphate buffer saline (pH 7.4) at 37 °C for 1h. Initial dry weights of the formulations were recorded as "SWa" and equilibrium swelling weight as "SWb". Measurements were carried out in triplicate (Elegbede *et al.*, 2020).

The swelling ratio was expressed as

$$\% \text{ Swelling ratio } = \frac{(\text{SWb} - \text{SWa})}{\text{SWa}} \, x \, 100$$

(Equation 1)

**Formulation stability testing**

To assess the stability of the formulations, stability testing was carried out after storage for one month at room temperature and relative humidity (24 °C and 40%, respectively). The appearance, texture properties and bio-adhesiveness of the formulations were determined before (one day after the formulation was prepared) and after storage (on days 1, 3, 7, 14 and 30) (Ilomuanya *et al.*, 2020b).

***In-vivo* wound healing studies**

Eighteen male Wistar rats, each weighing 350–400 g, were purchased at the start of the experiment from Komad Farms® Lagos, Nigeria. The rats were allowed to adapt to their new environment for one week before commencing the experiment. The required feeding, housing and diet conditions were provided. Ethical approval for the study was obtained with approval number CMUL/ACUREC/02/24/1387. Rats were divided into six sets of three rats each. All rats were anaesthetized intraperitoneally with urethane (0.03 mL/kg). The dorsal area was completely

shaved and cleaned with 70% ethanol. A 20 mm excision wound was incised on the upper back of each animal with a scalpel. The bioactive dressing containing formulations F1-F5 and control was used to dress the wounds of the rat groups. The pictures of the wound surface were taken, and wound contraction was measured on days 0, 3, 7, and 14 post-treatment. The wound dressing was changed on days 3, 7, and 14 (wound size was measured using a caliper). Data was reported as a percentage of wound contraction against time. The percentage of wound contraction was calculated using equation (2) (Ilomuanya *et al.*, 2020, a)

$$\%\ \text{wound closure} = \frac{A_0 - A_1}{A_0} \times 100$$

(Equation 2)

$A_0$ = Wound area on day zero $A_1$ = Wound area on day 3, 7, 14 and 21 after-treatment.

**Histological examination**

Fourteen days after the operation, tissues from the wound area (containing the dermis and hypodermis) of rats representing each group were sampled and fixed in 10% neutral buffered formalin. Paraffin embedding was carried out after which, 3–4 μm sections were prepared and stained with haematoxylin and eosin (H&E) and Masson's trichome. Light microscopic examination on histological profiles of individual skin was performed using a Leica Microsystems microscope (Mannheim, Germany) (Elegbede *et al.*, 2020).

**Histomorphometry**

The thicknesses of central regions of granulation tissues (in mm from the epidermis to dermis), numbers of infiltrated inflammatory cells in granulation tissues (cells/$mm^2$ of field), numbers of micro-vessels in granulation tissues (vessels/$mm^2$ of field), percentages of re-epithelization rates (%/$mm^2$ of field), and percentage collagen-occupied regions in granulation tissues, were measured on the histological Wistar rat skin samples using a digital image analyzer (Image Pro, Media Cybernetics, U.S) (Ilomuanya *et al.*, 2020 a).

**Statistical analysis**

Measurements were carried out in triplicates. The statistically significant difference was determined using a one-way ANOVA test ($p < 0.05$). Bonferroni's multiple comparisons test was carried out, when necessary (Sinjari *et al.*, 2019).

## RESULTS

The physicochemical properties of the hydrogel formulations are presented in Table 2.0. All formulations had a pH within the range of 5.6 to 5.8 which is close to the natural pH of the skin 5.5. The viscosity was the highest in the control formulation. This may be due to the absence of Aloe mucilage and zinc in the formulation. The second highest was formulation F4 which did not contain Aloe mucilage as well. The swelling index that indicates the release rates of the formulations is indirectly proportional to the release rate.

Table 2: Physicochemical properties of formulations, F1-F5 and control.

| Hydrogel Formulation | Dynamic Viscosity (PaS) (20 rpm) | pH | Swelling Index % | Skin Irritancy |
|---|---|---|---|---|
| **F1** | 21250 ± 1.9 | 5.7 ± 1.7 | 80.7 ± 1.7 | Nil |
| **F2** | 22900 ± 1.1 | 5.8 ± 1.1 | 79.2 ± 1.9 | Nil |
| **F3** | 20500 ± 2.8 | 5.6 ± 1.3 | 83.4 ± 0.8 | Nil |
| **F4** | 28860 ± 1.2 | 5.8 ± 1.7 | 82.3 ± 1.4 | Nil |
| **F5** | 22950 ± 1.0 | 5.7 ± 1.1 | 80.4 ± 1.0 | Nil |
| **CONTROL** | 30000 ± 2.1 | 5.8 ± 1.3 | 84.5 ± 1.2 | Nil |

A pictorial representation of the progress in wound healing in rats treated with the different formulations (F1-F5) and control is displayed in Figure 1. Wounds treated with formulations F2-F4 showed 100 % contraction, complete re-epithelization, and tissue remodeling by day fourteen. Healing was also without any scarification. Scars were observed on wounds treated with F1, F5 and control after wound healing on day fourteen.


Figure 1: The contraction and re-epithelialization of wounds in rat groups treated with formulations (F1- F5) and control.

The histological evaluation of the skin tissues in the wound area post-wound healing is displayed in Figure 2A. Figure 2A provides an overview of the anatomical state of the epidermis, dermis and hypodermis after angiogenesis. The black two-way arrows show the thickness of the epidermis, while the blue arrows point the mature pink granulation tissues which is evidence that the wound healing pattern is healthy. The red arrows indicate the micro-vessels in granulation tissue. The percentage of wound closure over fourteen days is displayed in Figure 2B. The graph of percentage wound contraction against time showed that healing occurred over fourteen days for most of the formulation-treated wounds. The wound contraction phase took place after the prior inflammation and proliferative phases by the end of the first week allowing the commencement of the tissue re-modeling phase of wound healing which was within the second week for the treated wounds. Figures 3A-E display the number of microvessels in granulation tissue, the thickness of the central region between the dermis and epidermis, re-epithelization percentage, the percentage occupied by collagen in granulation tissue and the number of inflammatory cells.

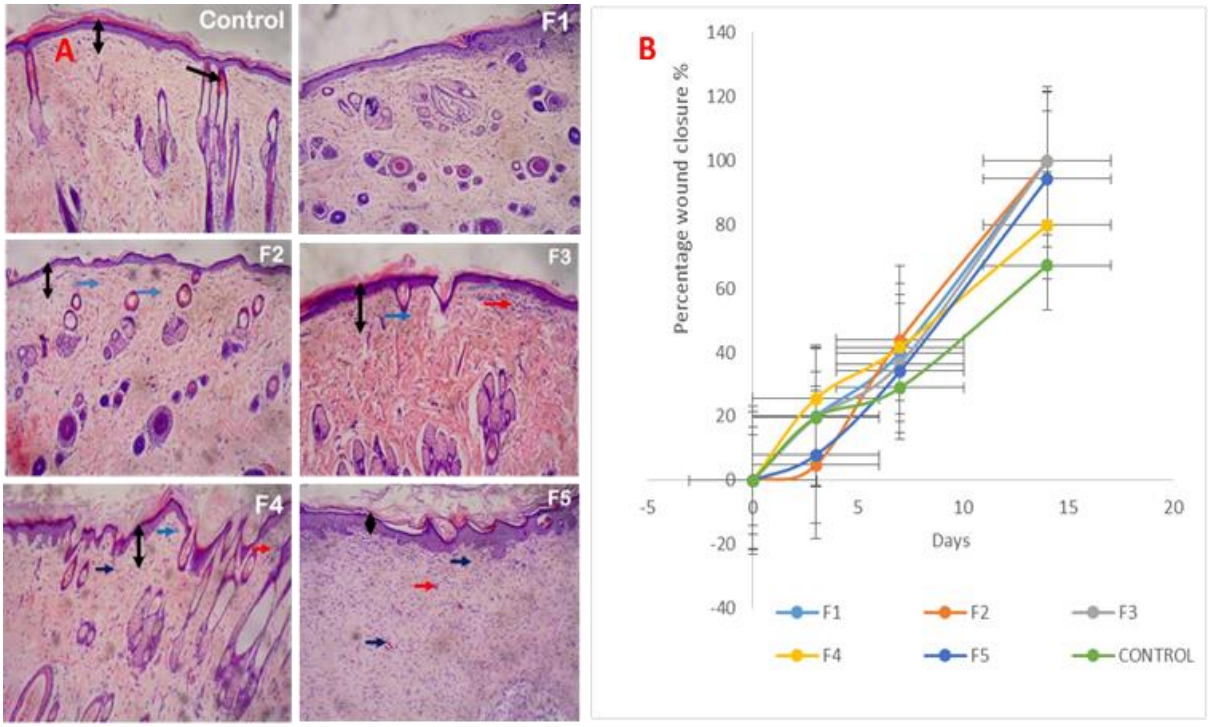


Figure 2A and B: The histological images of the tissue sections from the wound area (A). The percentage relative wound contraction from day 1 to day 14 (post-surgery) (B).

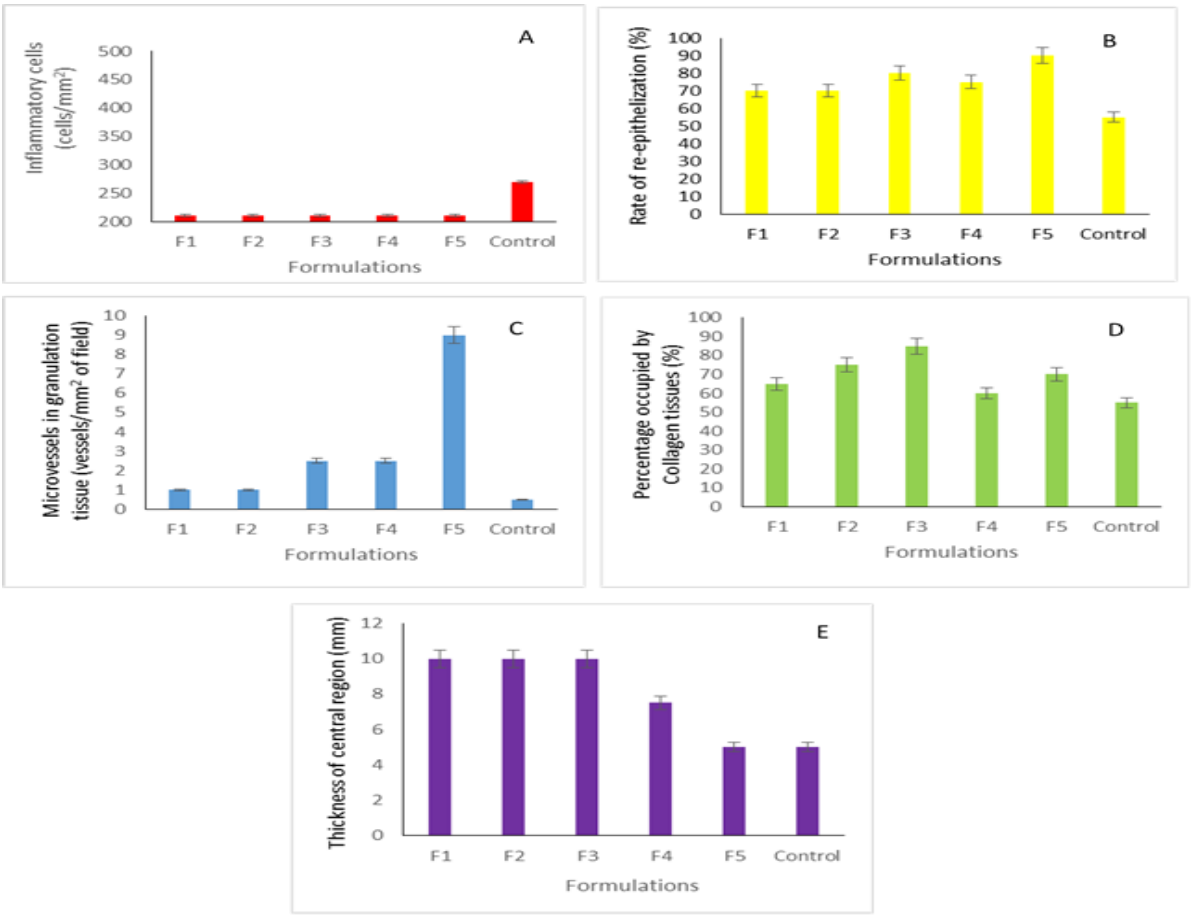


Figure 3 (A - E). Histomorphometrical values for diabetic wound tissues 14 days post wound incision. Results are expressed as mean ± S.D (n=3). For all data sets ($p<0.05$).

## DISCUSSION

Hydrogels have reflected great potential as wound dressings. They can control the moisture level in the wound environment while serving as a barrier thereby preventing exposure of the wound's microenvironment to microorganisms (Parhi *et al.,* 2017). Hydrogels have acquired some level of prominence because of their similarity to the extracellular matrix. Their ability to house bioactive compounds in their polymer matrix has placed them in an indispensable position. In this present study, hydrogel polymer was loaded with *A. barbadensis* and zinc. The wound-enhancing potential of developed and characterized *A. barbadensis* and zinc-loaded alkyl acrylate cross-polymer hydrogel was investigated (Ilomuanya *et al.*, 2020b).

Formulations F1-F5 and control were physicochemically examined. All formulations gave no characteristic odor. Formulations F1-F4 had a translucent off-white color while F5 and control gave a transparent gel. No erythema or edema was observed on the application of the formulations to the bare skin of the rat model after observation for one hour. Hence, all formulations were safe for dermal application (Niu *et al.*, 2022). The pH of the wound's microenvironment is usually basic (7.2-8.5) and a basic pH encourages the growth of microorganisms. The pH of the formulations as seen in Table 2, was between 5.7-5.8 (slightly acidic) (Rippke *et al.,* 2018). An acidic pH is necessary to cause a regression in the growth of bacteria in the wound environment to allow a healthy healing pattern and proper wound re-epithelisation and tissue remodeling (Chen *et al.,* 2019).

Hydrogels are non-Newtonian and display pseudoplastic behaviour with thixotropy reflecting the fact that the hydrogel remains thick when static but becomes thinner on the introduction of shear stress. The components of a formulation influence its rheological properties. The control had the highest dynamic viscosity of 30000.00 ± 2.07 PaS. This may be because it did not contain zinc or *A. barbadensis* in its formulation because the inclusion of these bioactive materials affects the thickness and texture of the hydrogel formulation. A good swelling property indicates that the hydrogel is an ideal medium for prompt release of the loaded medicaments. The swelling index has an inversely proportional relationship with the release rate (Gupta and Kumar, 2015). The formulation with the highest swelling index was the control, 84.5 ± 1.21 %. This

shows that the release rate will be the slowest in the control formulation. Formulation 2 had the lowest swelling index of 79.2 ± 1.95 % which means that it had the fastest release rate. All formulations were stable throughout the storage. There was no physical or chemical decomposition nor changes in the appearance or odor (Tsumura *et al.,* 2016). Angiogenesis starts with homeostasis at the point of injury and proceeds to an inflammatory phase. Subsequently, proliferation of the epithelial and extracellular matrix components occurs. Finally a scar tissue that is marked by an array of a highly organized collagen matrix is formed. Figure 1 portrays a pictorial representation of the wound healing progression in rat models. The wounds treated with formulations F2, F3, F4 had a healthy wound healing trajectory as the wound healed completely by day 14 with no sign of scars in the wound area. This may be due to the presence of both bioactive components, zinc and *A. barbaensis,* in these formulations creating a synergistic wound healing enhancing effect. F1, F5 and control showed a less healthy pattern of angiogenesis that can be due to the absence of either of the main active ingredients, zinc or *A. barbadensis* (Alves *et al.,* 2018).

Histology can be described as the evaluation of tissue structure in relation to its function through microscopic evaluation. The tissues of the healed wound site were observed microscopically 14 days post-surgical incision. The anatomy and structural integrity of the tissues are showen in Figure 2A, as an indication of the depth and quality of wound healing. As in all formulations, the presence of matured granulation tissues, an intact epidermis featuring its five main layers as well as the presence of the dermis and micro-vessels indicate that proper and healthy wound healing had taken place at the wound bed. However, the tissue architecture was less defined in the control sample compared to the wound tissues treated with formulations F1-F5. Figure 2B shows a positive trend for the wound healing progression with the control having the slowest wound contraction (Wynn and Vannella, 2016). The histomorphometric evaluation can be described as a quantitative study of the microscopic array and architecture of a tissue especially by computer-assisted analysis of images formed by a microscope (Zhong *et al.,* 2022). In tissues sampled from wound sites, histomorphometric evaluation indicated the nature of wound healing processes, cues and pathways. Macrophages are the most important

inflammatory cells during wound healing. They may function to assist cell proliferation and tissue restoration, but their primary function is to provide a host defense against foreign invasion, remove cellular debris and microorganisms (Rouselle *et al.,* 2019). A high level of inflammatory cells at the latter stages of wound healing may indicate that the wound is infected. All treated formulations showed a lower number of inflammatory cells at the first stages of wound healing compared to the control (Lin *et al.,* 2022). The high number of inflammatory cells in the control at day 14 may be because of bacterial infection (Heras *et al.,* 2022).

Re-epithelisation can be described as the layering of a wound surface by the formation of new epithelium. The molecular and cellular pathways that initiate the stimulation, maintenance and resolution of epithelisation are important for complete wound contraction and healthy wound healing. The re-epithelialisation rate was higher in wounds treated with formulations F1-F5 compared to the control. The thickness of the central region from the epidermis to the dermis that was the least in the control group and the highest in wounds treated with formulations F1, F2, and F3 indicates the level of advancement of angiogenesis (Reilly and Lozano, 2021). Granulation tissue is newly formed connective tissue and micro blood vessels on the surface of the wound bed during the wound repair. The number of micro-vessels in granulation tissue indicates the structural depth of wound healing (Sadoyu *et al.*, 2020). The treated wound had a higher number of micro-vessels when compared to the control. The percentage of collagen tissue was the highest in the wounds treated with formulation F2 and F3 but the lowest in the control (Wallace *et al.*, 2019). Collagen is a vital building block of the cutaneous layer that initiates the recruitment of fibroblast. It is also involved in the degradation of matrix metalloproteinase allowing preservation of the extracellular matrix structure that faciliates wound healing (Mei *et al.,* 2022).

It is important to note that the formulations developed in this study are to be applied on a wound surface and not on an intact skin. This means that the dermal layer which is to act as a semi-permeable membrane for absorption of the bioactive molecules is already compromised. One of the limitations of the present study is that, Franz cell may not be a perfect model as the membrane is still intact. Future studies will be conducted towards developing an ideal model for drug release on wound surfaces.

## CONCLUSION

Formulation, F2, containing both *A. barbadensis* and zinc gave the best performance regarding the enhancement of wound healing with an ideal pH, good viscosity and an ideal swelling index that brings about rapid release of bioactive agents enhnancing wound-healing. Histomorphometrical evaluation of wound dressing of F2, reflected a re-epithelisation rate of 70 %, percentage occupied by collagen in granulation tissue of 85 %, thickness of the tissue central region from the epidermis to dermis 10 mm. The inflammatory cells /mm$^2$ tissue was 200 cells/mm$^2$ while the number of microvessels in granulation tissue was 1.0 microvessel/mm$^2$. This spotlights and establishes the wound healing enhancing abilities of 'F2' zinc-loaded alkyl acrylate cross polymer hydrogel infused with *A. barbadensis* mucilage, providing a useful and affordable formulation for filling the gap of an urgent need for a novel and smart formulation for dressing wounds evidently. The findings of the present study fills an existing knowledge gap in the field of wound healing.

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## REFERENCES

Adom D, Appiah S, Mohan K (2020). The Chemical Constituents, Anti-Inflamatory, Anti-Oxidant and Ethno medicinal Properties of Aloe barbadensis: Ethnomedicinal Plant Use and Practice in Traditional Medicine. Publisher: *IGI Global. Information Resources Management Association*.

Alves A, Attik N, Bayon Y, Royet E, Wirth C (2018). Devising tissue ingrowth metrics: a contribution to the computational characterization of engineered soft tissue healing. *Biomed Mater* **13**(3): 035010.

Arab M, Jallab M, Ghaffari M, Moghbelli E, Saeb M (2021). Synthesis, rheological characterization, and antibacterial activity of polyvinyl alcohol (PVA)/ zinc oxide nanoparticles wound dressing, achieved under electron beam irradiation. *Iran Polym J* **30**(10): 1019–28.

Ávila-Salas F, Marican A, Pinochet S, Carreño G, Valdés O, *et al.* (2019). Film Dressings Based on Hydrogels: Simultaneous and Sustained-Release of Bioactive Compounds with Wound Healing Properties. *Pharmaceutics* **11**(9): 447-466.

Bahram M, Mohseni N, Moghtader M (2016). An Introduction to Hydrogels and Some Recent Applications. *IntechOpen*. London.

Cardoso-Daodu, I.M., Ilomuanya, M.O. and Azubuike, C.P (2022). Development of curcumin-loaded liposomes in lysine–collagen hydrogel for surgical wound healing. *BJBAS* **11**(1): 1-13.

Dhivya S, Padma V, Santhini E (2015) Wound dressings – a review. *BioMedicine* **28** (4): 5-15.

Gupta A, Kumar P (2015). Assessment of the histological state of the healing wound. *PAR* **1**(2): 239–42.

Heras K, Igartua M, Santos-Vizcaino E, Hernandez R (2022). Cell-based dressings: A journey through chronic wound management. *Biomater* **17**: 212738.

Ilomuanya M, Adeyinka O, Aghaizu C, Cardoso-Daodu I, Akhimien T, *et al.* (2019). Co-formulation and characterisation of gentamicin-loaded alkyl acrylate cross polymer hydrogel infused with ethanol extract of Tetracarpidium conophorum impregnated on gauze sponge for wound dressing. *WHSA* **1**(12): 22-28.

Ilomuanya M, Okafor P, Amajuoyi J, Onyejekwe J, Okubanjo O, *et al.* (2020). Polylactic acid-based electrospun fiber and hyaluronic acid-valsartan hydrogel scaffold for chronic wound healing. *BJBAS* **9**(1): 515-530.

Ilomuanya M, Adebona A, Wang W, Sowemimo A, Eziegbo C, *et al.* (2020). Development and characterization of collagen-based electrospun scaffolds containing silver sulphadiazine and Aspalathus linearis extract for potential wound healing applications. *SN Appl Sci* ***2***(5): 811-823.

Lin H, Lin H, Yin C, Mo A, Hong G (2019). Applications of Hydrogels with Special Physical Properties in Biomedicine. *Polymers* **11**(9): 1420-38.

Lin PH, Sermersheim M, Li H, Lee P, Steinberg S, *et al.* (2017). Zinc in Wound Healing Modulation. *Nutrients* **10**(1): 16-36.

Liu Y, Song S, Liu S, Zhu X, Wang P (2022). Application of Nanomaterial in Hydrogels Related to Wound Healing. *Journal of Nanomaterials* **4**: 1–11.

Mei L, Zhang D, Shao H, Hao Y, Zhang T, *et al.* (2022). Injectable and Self-Healing Probiotics-Loaded Hydrogel for Promoting Superbacteria-Infected Wound Healing. *ACS Appl Mater Interfaces* **14**(18): 20538–50.

Niu C, Wang L, Ji D, Ren M, Ke D, *et al.* (2022). Fabrication of SA/Gel/C scaffold with 3D bioprinting to generate micro-nano porosity structure for skin wound healing: a detailed animal in vivo study. *Cell Regeneration* **11**(1): 1-12.

Okur M, Karantas I, Senyigit Z, Ustundag Okur N, *et al.* (2020). Recent trends on wound management: New therapeutic choices based on polymeric carriers. *Asian J Pharm* **15**(6): 661-684.

Parhi R (2017). Cross-Linked Hydrogel for Pharmaceutical Applications: A Review. *Advanced Pharmaceutical Bulletin* **7**(4): 515–530.

Rasli NI, Basri H, Harun Z (2020). Zinc oxide from aloe vera extract: two-level factorial screening of biosynthesis parameters. *Heliyon* **6**(1): e03156.

Reilly D, Lozano J (2021). Skin collagen through the lifestages: importance for skin health and beauty. *PAR* **1**(2):2-26.

Rippke F, Berardesca E, Weber T (2018). pH and Microbial Infections. *Current Problems in Dermatology* **21**(54): 87-94.

Rousselle P, Braye F, Dayan G (2019). Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. *Adv Drug Deliv Rev* **146**: 344–365.

Sadoyu S, Rungruang C, Wattanavijitkul T, Sawangjit R, Thakkinstian A, *et al.* (2020). Aloe vera and health outcomes: An umbrella review of systematic reviews and meta-analyses. *Phytother Res* **35**(1): 555-576.

Saleem A, Naureen I, Naeem M, Murad HS, Maqsood S, *et al.* (2022). Aloe Vera Gel Effect on Skin and Pharmacological Properties. *SIJAP* **5**(1):1–8.

Sarp S, Kuzlu M, Wilson E, Cali U, Guler, O (2021). The Enlightening Role of Explainable Artificial Intelligence in Chronic Wound Classification. *Electronics* **10**(12): 1406.

Soleimanpour M, Mirhaji SS, Jafari S, Derakhshankhah H, Mamashli F, *et al*. (2022). Designing a new alginate-fibrinogen biomaterial composite hydrogel for wound healing. *Sci Rep* **12**(1): 1-17.

Ternullo S, Schulte Werning L, Holsæter A, Škalko-Basnet N (2019). Curcumin-In-Deformable Liposomes-In-Chitosan-Hydrogel as a Novel Wound Dressing. *Pharmaceutics* **12**(1): 8-22.

Tsumura R, Takishita Y, Fukushima Y, Iwata H (2016). Histological evaluation of tissue damage caused by rotational needle insertion. *Annu Int Conf IEEE Eng Med Biol Soc* **1**: 5120-5123.

Wallace H, Zito P, Basehore B (2019). Wound Healing Phases. *StatPearls Publishing*.

Wynn T, Vannella K (2016). Macrophages in Tissue Repair, Regeneration, and Fibrosis. *Immunity* **44**(3): 450–62.

Yi-Yang P, Shruti S, Ravin N (2020). Polymer Science and Nanotechnology. *Elsevier*. Canada.

Zeng W, Parus A, Barnes C, Hiro M, Robson M, *et al.* (2020). Aloe vera—Mechanisms of Action, Uses, and Potential Uses in Plastic Surgery and Wound Healing. *Surg Sci* **11**(10): 312–28.

Zhong J, Wang H, Yang K, Wang H, Duan C, *et al*. (2022). Reversibly immortalized keratinocytes (iKera) facilitate re-epithelization and skin wound healing: Potential applications in cell-based skin tissue engineering. *Bioact Mater* **9**(1): 523–40.

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# Screening Cholinesterase Inhibitory Potential of Selected Amines

Acelya Mavideniz*, Tugba Ercetin, Aybike Yektaoglu, Zahra Nobavar, Jale Yuzugulen, Emine Dilek Ozyilmaz, Hayrettin Ozan Gulcan

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10 Turkiye.

## Abstract

Cholinesterase inhibition has gained attention in the treatment of some disease states, covering cholinergic deficiency. Alzheimer's disease (AD) can be counted as the most important one among them. Indeed, the current drugs used in the treatment of AD are cholinesterase inhibitor molecules, besides memantine, and biological new drugs. Many pharmacophores have been suggested so far for cholinesterase inhibition and many of them possess a basic center with an amine function. In the present study, we have selected some simple amines and investigated their potential to inhibit acetylcholinesterase and butyrylcholinesterase enzymes. The results indicated that simple amines by themselves do not have strong potential unless they are used with other pharmacophores.

**Keywords**

Alzheimer's Disease, cholinesterases, selected amines, pharmacophore.

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***Corresponding author:** Acelya Mavideniz **email:** acelya.mavideniz@emu.edu.tr

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## INTRODUCTION

Athough Alzheimer's disease (AD) was first discovered and diagnosed over a century ago, there are still a lot of unanswered concerns, particularly in relation to its pathophysiology (Gulcan and Orhan 2020). Unfortunately, AD-related dementia, which impairs cognition, is the most prevalent kind of dementia. Given that AD is a progressive disease, symptoms of dementia deteriorate over time and can be classified as mild, moderate, or advanced (Pagani *et al.*, 2017).

Many validated and non-validated targets have been proposed so far for the therapy of AD, despite the fact that the pathophysiology of the illness is too complex to completely comprehend. However, cholinesterase inhibition still stays as the major validated system. From this perspective, cholinesterase inhibitors are still important, and they are the only drugs used in the treatment of AD, besides memantine, and the new biological drugs offered for amyloid beta clearance in the mild stage of the disease (Krall *et al.*, 1999).

Four cholinesterase inhibitors have been available on the market since the 1980s to treat dementia associated with AD (Gulcan and Orhan 2021). Tacrine was the first one, however it was withdrawn from the clinic with respect to its hepatotoxicity (Blackard *et al.*, 1998). From the perspectives of source, target, dose, pharmacokinetics, and pharmacodynamics, the remaining three (i.e., donepezil, galantamine, rivastigmine) exhibit a variety of characteristics (Figure 1). Therefore, there has been a continuous interest in the screening of diverse structures (Gao *et al.*, 2021).

There have been many scaffolds used so far with diverse heterocycles to obtain potent cholinesterase inhibitor molecules. Among the pharmacophores employed, amine portion is indispensable in the majority of them (Norouzbahari *et al.*, 2018). Within this study, we have employed ten simple amine molecules and aimed to investigate their potential to inhibit cholinesterase enzymes.

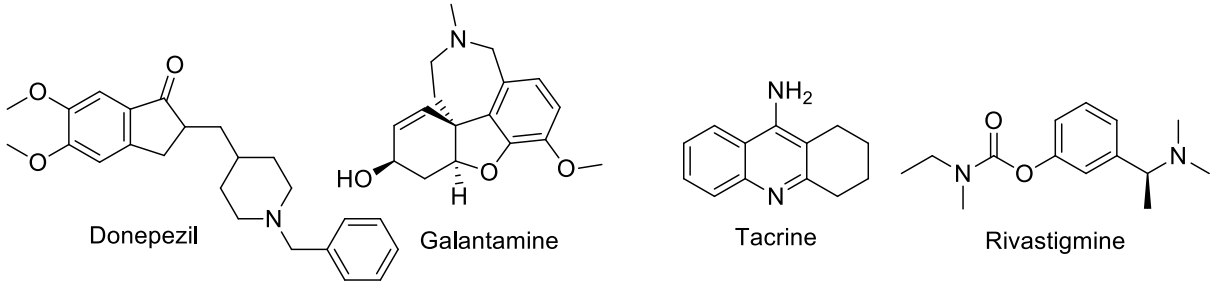


**Figure 1:** Cholinesterase inhibitor drugs.

## MATERIALS AND METHODS

All reagents and organic solvents were obtained from Sigma Aldrich through the aid of local vendors and used directly unless otherwise stated.

**Enzyme assays**

The title compounds' potential to inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes was measured employing Ellmann's method. Accordingly, each enzymatic reaction was prepared with a 200 µL total volume. 168 µL of 50 mM Tris HCl buffer (pH 8.0), 10 µL of 6.8 mM DTNB solution (0.34 mM final), 20 mM $MgCl_2$, and 100 mM NaCl, and 10 µL of AChE or BuChE solution were mixed. The reactions were initiated by the addition of 10 µL of either 10 mM acetylthiocholine iodide or 10 µL of 1.5 mM butyrylthiocholine iodide. Measurements were achived using UV absorptions at 412 nm following incubation for 15 minutes at 27°C (Varioskan Flash, Thermo Scientific, USA). By comparing the rates of reaction of samples relative to blank samples (DMSO and methanol), the percentage of inhibition of AChE and BuChE was calculated. The compounds were tested at 40 µM level. Each concentration was evaluated in triplicate using each measurement. The mean ± standard deviation was calculated.

## RESULTS AND DISCUSSION

The title compounds employed and the percent inhibition results obtained are summarized in Table 1.

**Table 1:** The compounds and inhibitions of AChE and BuChE (%).

| Compound | AChE | BuChE |
|---|---|---|
| 1-Naphtylamine HCl | 10.63 ± 0.011 | 34.84 ± 0.038 |
| Dimethylamine HCl | 18.33 ± 0.035 | 26.04 ± 0.021 |
| N-benzylpiperazine 2HCl | 25.12 ± 0.024 | 21.86 ± 0.012 |
| 6,7-Dimethoxy-1,2,3,4-tetrahydoisoquinoline | 41.74 ± 0.019 | 41.85 ± 0.014 |
| Hydroxylamine HCl | 32.83 ± 0.026 | 11.40 ± 0.024 |
| N benzyl methyl amine | 39.38 ± 0.032 | 13.92 ± 0.027 |
| Aniline | 21.51 ± 0.048 | 6.06 ± 0.011 |
| Morpholine | 31.61 ± 0.036 | 11.72 ± 0.025 |
| Triethylamine | 40.69 ± 0.002 | 34.59 ± 0.027 |
| 1,2,3,4-Tetrahidraisoquinoline | 44.69 ± 0.051 | 35.22 ± 0.028 |
| **Donepezil 10 µM** | 100 ± 0.007 | 83.10 ± 0.002 |
| **Galantamine 10 µM** | 96.11 ± 0.006 | 54.59 ± 0.002 |

Accordingly, none of the simple amines displayed superior activity over the standards (donepezil and galantamine) employed. The most active amines were

found to be the ones possessing benzylamine moiety. As seen in 1-naphtylamine and aniline examples, direct amine attached substituents generated lower potential. The most active compound was found to be an isoquinoline derivative.

Overall, the results definitely displayed that those simple amines alone are not potential inhibitors of cholinesterases. However, they are important components of pharmacophores in drug design to inhibit cholinesterase enzymes.

## CONCLUSION

Within this limited research work, 10 amines have been selected and screened for their potential to inhibit acetylcholinesterase and butyrylcholine-esterase enzymes. With respect to the standards employed, low potential inhibitions were measured. The results clearly stated their function as a pharmacophore, since they are employed as an important scaffold in cholinesterase inhibitors. However, they have limited potential by themselves to inhibit cholinesterase enzymes.

## REFERENCES

Blackard Jr, WG, Sood GK, Crowe DR, Fallon MB (1998). Tacrine: a cause of fatal hepatotoxicity?. *J Clin Gastroenterol* **26**(1): 57-59.

Gao H, Jiang Y, Zhan J, Sun Y (2021). Pharmacophore-based drug design of AChE and BChE dual inhibitors as potential anti-Alzheimer's disease agents. *Bioorg Chem* **114**: 105149.

Gulcan HO, Orhan IE (2020). The main targets involved in neuroprotection for the treatment of Alzheimer's disease and Parkinson disease. *Curr Pharm Des* **26**(4): 509-516.

Gulcan HO, Orhan IE (2021). Dual Monoamine Oxidase and Cholinesterase Inhibitors with Different Heterocyclic Scaffolds. *Curr Med Chem* **21**(30): 2752-2765.

Krall WJ, Sramek JJ, Cutler NR (1999). Cholinesterase inhibitors: a therapeutic strategy for Alzheimer disease. *Ann Pharmacother* **33**(4): 441-450.

Norouzbahari M, Burgaz EV, Ercetin T, Fallah A, Foroumadi A, *et al.* (2018). Design, synthesis and characterization of novel urolithin derivatives as cholinesterase inhibitor agents. *Lett Drug Des Discov*, **15**(11): 1131-1140.

Pagani M, Giuliani A, Oberg J, De Carli F, Morbelli S, *et al.* (2017). Progressive disintegration of brain networking from normal aging to Alzheimer disease: analysis of independent components of 18F-FDG PET data. *J Nucl Med* **58**(7): 1132-1139.

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# Antibacterial Potency of Ibuprofen and Its Interaction with Ciprofloxacin Against Gram Positive and Gram Negative Bacteria

Ertugrul Ozbil, Sultan Ogmen, Acelya Mavideniz, Mehmet Ilktac*

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10 Turkiye.

## Abstract

Ibuprofen, a nonsteroidal anti-inflammatory drug (NSAID), acts by reducing hormones for the treatment of fever, inflammation, and pain. Previously, it was only shown that the ibuprofen inhibits the effect of various bacteria that are stimulated by bacterial infections and not directly on bacterial cells. In this study, we aimed to investigate antibacterial and synergistic activities of ibuprofen against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603. The results revealed promising antibacterial activity against tested Gram-positive bacteria, but there was no effect on Gram-negative bacteria. Furthermore, checkerboard assay did not reveal any additive or synergistic activity when ibuprofen was combined with ciprofloxacin against tested Gram-positive bacteria. Collectively, our data reveal the selective antibacterial activity of ibuprofen against Gram-positive bacteria which suggest that ibuprofen can further be investigated as a potential source for new therapeutic options.

**Keywords**

Antibacterial, **c**heckerboard, ciprofloxacin, *Enterecoccus faecalis*, ibuprofen, *Staphylococcus aureus*.

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## INTRODUCTION

Ibuprofen (Figure 1) is (2RS)-1[4-(2-methyl propyl) phenyl] propionic acid. Ibuprofen was the first member of propionic acids that is a nonsteroidal anti-inflammatory drug (NSAID) of the 2-aryl propionic acid family. It is the most commonly used and prescribed NSAID. Ibuprofen is a non-selective inhibitor of cyclooxygenase-1 and cyclooxygenase-2 that functions by reducing hormones for the treatment of inflammation, fever, and pain (Bushra and Aslam, 2010).

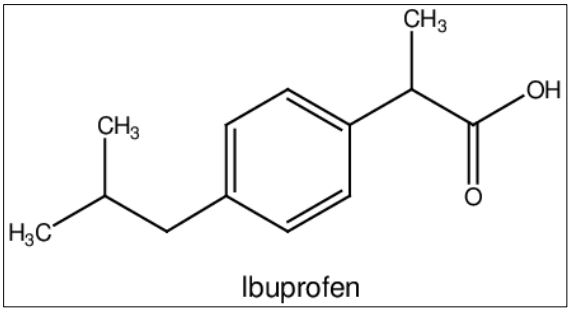


**Figure 1**: Structural formula of ibuprofen.

Previously, the studies that analyzed the relationship between bacteria and ibuprofen did not reveal any direct antibacterial activity but reported the anti-inflammatory action of the drug reducing the inflammation stimulated during various bacterial infections (Al-Janabi, 2010). Ibuprofen was shown to reduce the inflammation in mouse lung resulting from *Pseudomonas aeruginosa* infection but with no direct effect on the bacterium itself (Sordelli *et al.,* 1985). In this study, we investigated the antibacterial activity of ibuprofen against various pathogenic bacteria, while also demonstrating its possible additive or synergistic activity in combination with one of the most frequently prescribed antibiotics, ciprofloxacin.

## MATERIALS AND METHODS

### Extraction of ibuprofen from the commercial pills

The coating of the pills (Brufen, Abbott Laboratuvarlari, Turkiye) was removed by treating them with water. Then, the pills were ground into powder. Acetone was added to the powder and filtered. Acetone was removed from the filtrate by using a rotary evaporator. The residue was scratched and pure ibuprofen was obtained.

### Inoculum and sample preparation

The antibacterial activity of ibuprofen was investigated against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC

700603. The bacteria were subcultured on Mueller-Hinton Agar (MHA). The media were incubated at 37 $^{o}$C under an aerobic atmosphere for 24 hours. After 24 hours, pure culture of the bacteria was obtained on MHA. 0.5 Mc Farland ($1x10^{8}$ cfu/ml) standard solutions of bacteria were prepared in Mueller-Hinton broth (MHB).

The stock solution of the sample was prepared using pure dimethyl sulfoxide (DMSO). The final concentration of DMSO was 3% for antibacterial activity tests and checkerboard assays.

**Minimum inhibitory concentration (MIC) determination**

Antibacterial activity of ibuprofen was investigated by the broth microdilution method (Wikler, 2006). Final inocula of the bacteria in the U-bottomed 96 well plates were 1 x $10^{6}$ cfu/mL and the final concentrations of the sample ranged from 0.125 to 4 mg/mL. Ciprofloxacin was utilized as the positive control and the highest concentration of the sample in MHB was negative control for all replicates. Incubation of the microplates was carried out at 37 $^{o}$C for 18h. The MIC was regarded as the minimum concentration of the sample that the growth of bacteria was inhibited. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide test (MTT) was used for the confirmation of the MIC.

**Minimum bactericidal concentration (MBC) determination**

MBC was determined as the lowest concentration of ibuprofen that killed bacteria. For this aim, 10 µL of the sample from the wells that have the concentration equal to MIC and higher was inoculated onto MHA. The media were incubated under an aerobic atmosphere at 37 $^{o}$C for 18 hours.

**Interaction of ibuprofen with ciprofloxacin - Checkerboard assay**

Checkerboard assay was used for the determination of the interaction of ibuprofen with ciprofloxacin as previously described (Bellio *et al.*, 2021). The final concentration of the sample ranged from 4 to 0.06 mg/mL, whereas ciprofloxacin ranged from 0.001 to 1 mg/L. Ciprofloxacin and the sample each alone in MHB were used as controls. Incubation was carried out under aerobic atmosphere at 37 $^{o}$C for 18h. Fractional inhibitory concentration index (FICI) was calculated using the formula:

$$FICI = A / MICA + B / MICB$$

where ‘A’ and ‘B’ are the MIC of each antimicrobial agent in combination within a single well plate; and MICA and MICB are the MIC of each drug individually. The interaction was interpreted as follows;

Synergy when FICI was $< 0.5$,

Additive when $0.5 \leq FICI \leq 0.9$,

Indifference when $1 \leq FICI \leq 4$

Antagonistic when $FICI > 4$.

### Statistical analyses

All of the experiments were performed in triplicates. Statistical analyses were performed by Students t-test.

## RESULTS AND DISCUSSION

### Antibacterial and checkerboard assays

To assess the antimicrobial activities associated with ibuprofen, the microdilution method was used to measure MICs against *E. faecalis*, *S. aureus*, *E. coli*, and *K. pneumoniae*. MIC of ibuprofen was detected as 1 and 2 mg/ml against *S. aureus* and *E. faecalis*, respectively (Table 1).

**Table 1:** MICs of ibuprofen against Gram-positive and Gram-negative bacteria.

| Agents | | Gram- positive bacteria | | Gram-negative bacteria | |
|---|---|---|---|---|---|
| | | ***S. aureus* ATCC 25923** | ***E. faecalis* ATCC 29212** | ***E. coli* ATCC 25922** | ***K. pneumoniae* ATCC 700603** |
| **Sample (mg/mL)** | Ibuprofen | 1 ± 0 | 2 ± 0 | - | - |
| **Control (mg/L)** | Ciprofloxacin | 0.25 ± 0 | 0.5 ± 0.083 | 0.008 ± 0 | 0.25 ± 0.021 |

Data represented as the standard error of mean (±S.E.M). ; -: Not inhibited.

On the other hand, no antimicrobial activity was detected against the tested Gram-negative bacteria (Table 1). This result may be due to the presence of an outer membrane in Gram-negative bacteria that can limit the entry of large molecules (Zgurskaya and Rybenkov, 2019). MBC of ibuprofen against *S. aureus* and *E. faecalis* was 1 and 2 mg/mL, respectively. In another study carried out, ibuprofen was found to limit the physiological activities of *E. coli* endotoxin on rabbits (Celik *et al.*, 2002) and humans (Bernard *et al*., 1997). In another study, ibuprofen showed no antibacterial effect on *Helicobacter pylori* in human body (Graham *et al.*, 1989) and on *Mycobacterium tuberculosis* in mice (Byrne *et al*., 2006). However, direct action of ibuprofen on bacterial cells has not been clearly illustrated until now.

Checkerboard assay was conducted only against *E. faecalis* and *S. aureus* because antibacterial activity was only detected against the two bacteria. The interaction was indifference for all the combinations.

**Table 2:** FICI of ibuprofen and ciprofloxacin combinations against *S. aureus* and *E. faecalis*.

| Samples | Optimal Combination | | FICI | |
|---|---|---|---|---|
| | **Ciprofloxacin (mg/L)** | **Ibuprofen (mg/mL)** | **< 0.5** | **> 0.5** |
| ***S. aureus*** | 0.001 | 1 | | 1.0 (I) |
| ***E. faecalis*** | 0.001 | 2 | | 1.0 (I) |

A: Additive. I: Indifference. S: Synergy.

When different concentrations of ciprofloxacin and ibuprofen were used against *S. aureus* and *E. faecalis*, no additive or synergistic effect was observed. None of the combinations showed any antagonistic activity against any of the tested bacteria (Table 2).

## CONCLUSION

Ibuprofen, an NSAID, functions by reducing hormones for the treatment of fever, inflammation, and pain. Previously, it was shown that ibuprofen has activity against bacterial infections due to its anti-inflammatory action. Although, no additive or synergistic activity was observed with ciprofloxacin, our results demonstrate promising selective antibacterial activity of ibuprofen against Gram-positive bacteria.

## REFERENCES

Bellio P, Fagnani L, Nazzicone L, Celenza G (2021). New and simplified method for drug combination studies by checkerboard assay. *MethodsX* **8**: 101543.

Bernard GR, Wheeler AP, Russell JA, Schein R, Summer WR, *et al.* (1997). The effects of ibuprofen on the physiology and survival of patients with sepsis. *New Engl J Med* **336**: 912-918.

Byrne ST, Denkin SM, Zhang Y (2006). Aspirin and ibuprofen enhance pyrazinamide treatment of murine tuberculosis. *J Antimicrob Chemother* **59**: 313-316.

Celik I, Akbulut A, Kilic SS, Rahman A, Vural P, *et al.* (2002). Effects of Ibuprofen on the physiology and outcome of rabbit endotoxic shock. *BMC Infect Dis* **2**: 26-38.

Graham DY, Klein PD, Opekun AR, Smith KE, Polasani RR, *et al.* (1989). In vivo susceptibility of *Campylobacter pylori*. *Am J Gastroenterol* **84**: 233-238.

Sordelli DO, Cerquetti MC, El-Tawil G, Ramwell PW, Hooke AM, *et al.* (1985). Ibuprofen modifies the inflammatory response of the murine lung to *Pseudomonas aeruginosa*. *Eur J Respire Dis* **67**: 118-127.

Wikler MA (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, vol. 26, p M7–A7. CLSI, Wayne, Pennsylvania.

Zgurskaya HI, Rybenkov V (2019). Permeability barriers of Gram-negative pathogens. *Ann N Y Acad Sci* **1459**: 5-18.

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# In vitro Antibacterial Activity of Naproxen and its Combination with Ciprofloxacin

Sultan Ogmen, Ertugrul Ozbil, Acelya Mavideniz, Mehmet Ilktac*

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10 Turkiye.

## Abstract

Naproxen, a nonsteroidal anti-inflammatory drug (NSAID), is commonly used to reduce fever, and to treat pain and inflammation caused by several conditions. Previously, naproxen was evaluated for its antimicrobial potency in various studies. In our study, we aimed to demonstrate the antibacterial and synergistic activities of naproxen and ciprofloxacin against various Gram-positive and Gram-negative bacteria including, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603. The results showed promising antibacterial activity against the tested Gram-positive bacteria. However, there was no effect on Gram-negative bacteria. Additionally, checkerboard assay did not reveal any additive or synergistic activity when combined with ciprofloxacin. Collectively, our study's data show naproxen's selectivity against Gram-positive bacteria. This result suggests that naproxen can further be used as a potential source of antibiotics against Gram-positive bacteria.

**Keywords**

Antibacterial, **c**heckerboard, ciprofloxacin, *Enterococcus faecalis*, naproxen, *Staphylococcus aureus*.

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***Corresponding author:** Mehmet Ilktac **email:** mehmet.ilktac@emu.edu.tr

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## INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used mainly in the treatment of pain and inflammation. Naproxen (Figure 1), 2-(6-methoxynaphthalen-2-yl) propionic acid, which is an NSAID and one of the most utilized propionic acid derivatives is used as the first-line treatment of musculoskeletal pain, acute gout, and ankylosing spondylitis. Additionally, it is commonly used to reduce fever, treat headache, muscle and tooth pains, and inflammation caused by several conditions (Stoev et al., 2021).

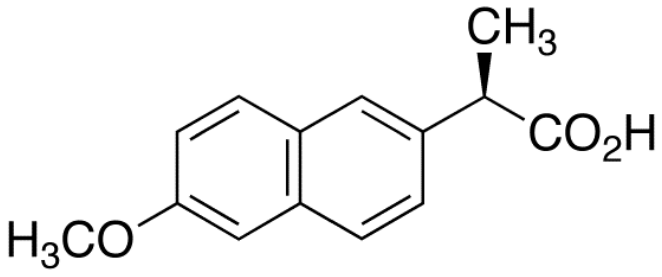


**Figure 1:** Structural formula of naproxen.

NSAIDs are the widest pharmacological group that has been researched for anticancer and antimicrobial activities (Hasan and Das, 2019). Especially, antibacterial activity has been of importance due to increased resistance to currently available antibiotics and a decrease in the rate of novel antibiotic discovery. Hussein and Al-Janabi (2011) demonstrated a promising antimicrobial activity of naproxen against several microorganisms.

In our study, we aim to reveal the potential antibacterial activity of naproxen, while also demonstrating its possible additive or synergistic activity with ciprofloxacin, one of the most frequently prescribed commercial antibiotics against which the resistance has been increasing worldwide.

## MATERIALS AND METHODS

### Extraction of naproxen sodium and its conversion to naproxen

The coating of the pills (Apranax Ford, Abdibrahim, Turkiye) was removed using methanol. The pills were then crushed into powder. Following the addition of 15 mL of water to the powder, a filtration process was carried out. At this stage, the filtrate was acidified with HCl. After filtering the precipitate by vacuum filtration, pure naproxen was obtained (Figure 2).

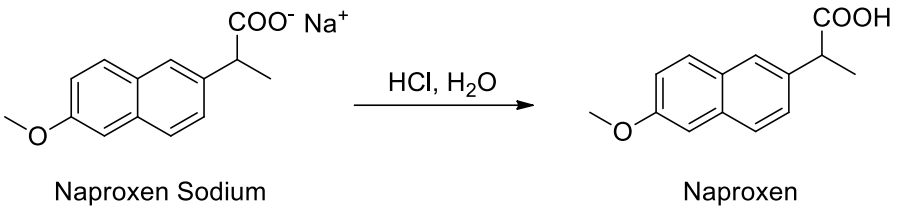


**Figure 2:** Synthesis scheme of naproxen.

**Bacterial inoculum and the sample preparation**

*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Klebsiella pneumoniae* ATCC 700603 were sub-cultured on Mueller-Hinton agar (MHA). The media were incubated at 37 $^{o}$C overnight. Upon incubation, strains from individual colonies were inoculated into Mueller-Hinton broth (MHB), and the turbidity was adjusted to 0.5 McFarland standard for each bacterial strain.

The stock solution of the sample was prepared using pure dimethyl sulfoxide (DMSO). For detecting minimum inhibitory concentration (MIC) of naproxen by microdilution test and investigating its interaction with ciprofloxacin by checkerboard assay, the concentration of DMSO of the sample was adjusted to 3% using sterile distilled water.

**Microdilution test**

**MIC determination**

MIC was investigated by broth microdilution method (Wikler, 2006). The inocula of four quality control strains were adjusted to 1 x $10^6$ cfu/mL using MHB. The final concentration of the sample ranged from 0.125 to 4 mg/mL. Ciprofloxacin was used as the positive control and the highest concentration of sample in MHB was used as the negative control for all tests.

The microplates were incubated at 37 $^{o}$C for 18 hours. The MIC was regarded as the minimum concentration of the sample that inhibited the growth of bacteria. MIC was confirmed by addition of 10 µl of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Formation of blue color after 2 hour incubation at 37 $^{o}$C was regarded as bacterial growth.

**Minimum bactericidal concentration (MBC) determination**

To determine the MBCs of the sample against the tested bacteria, 10 µL of the sample taken from the wells of each of the concentrations that are equal to and greater than MIC were inoculated onto MHA. The media were incubated at 37 $^{o}$C for 18 hours. The MBC was regarded as the minimum concentration of the sample that prevented bacterial growth on MHA.

**Checkerboard assay**

The interaction between naproxen and ciprofloxacin was investigated by checkerboard assay as previously proposed (Bellio *et al*, 2021). The final concentration of the sample ranged from 4 to 0.06 mg/mL, whereas that of ciprofloxacin ranged from 0.001 to 1 mg/L. Ciprofloxacin and the sample in MHB were used as controls. The microplates were incubated at 37 $^{o}$C for 18h.

To determine the interaction of the naproxen and ciprofloxacin tested in combination, fractional inhibitory concentration index (FICI) calculation (FICI = A / MICA + B / MICB) was used where 'A' and 'B' are respectively the MIC of ciprofloxacin and naproxen in combination within a single well plate, and MICA and MICB are the MIC of ciprofloxacin and naproxen individually, respectively. The interaction is synergistic at < 0.5, additive between 0.5-0.9, indifference between 1-4, and antagonistic at >4.

**Statistical analyses**

All of the experiments were performed in triplicates and the data were examined as means ± standard error of mean (SEM). Students t-test was carried out to determine the statistical significance ($p \geq 0.05$).

## RESULTS AND DISCUSSION

**MIC and MBC determinations**

The microdilution method was used to assess MIC of naproxen against *E. faecalis* ATCC 29212, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603. Naproxen showed promising antibacterial activity against the Gram-positive bacteria*: S. aureus* and *E. faecalis* with 2 mg/mL and 4 mg/mL, respectively (Table 1).

**Table 1:** MIC values of naproxen and ciprofloxacin against the tested bacteria.

| Agents | | Gram- positive bacteria | | Gram-negative bacteria | |
|---|---|---|---|---|---|
| | | ***S. aureus*** **ATCC 25923** | ***E. faecalis*** **ATCC 29212** | ***E. coli*** **ATCC 25922** | ***K. pneumoniae*** **ATCC 700603** |
| **Sample (mg/mL)** | Naproxen | 2 ± 0.33 | 4 ± 0.67 | NC | NC |
| **Control (mg/L)** | Ciprofloxacin | 0. 25 ± 0 | 0.5 ± 0.083 | 0.008 ± 0 | 0.25 ± 0.021 |

Data represented as the standard error of mean (±S.E.M). NC: No change.

However, naproxen did not have any antibacterial activity against the tested Gram-negative bacteria (Table 1). Additionally, no bactericidal effect was observed with naproxen against tested Gram-positive bacteria at the MIC concentrations and above. In line with our results, naproxen showed considerable antibacterial activity against various Gram-positive bacteria (Hasan and Das, 2019).

Furthermore, Mamatha *et al*. (2011) synthesized a title compound, by the reaction of naproxen and 4-methylpentan-2-one to evaluate the potential in vitro antibacterial activity against various Gram-positive and Gram-negative bacteria. The results of the title compounds revealed promising antibacterial activity towards various strains of bacteria including *S. aureus*.

**Checkerboard assays**

Checkerboard assay was not conducted for Gram negative bacteria because no antibacterial activity was detected. FICI was used to assess the interaction of naproxen and ciprofloxacin against the two Gram-positive bacteria: *E. faecalis* and *S. aureus*.

**Table 2:** FIC of naproxen in combination with ciprofloxacin against *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212.

| Samples | Optimal Combination | | FIC Index | |
|---|---|---|---|---|
| | Ciprofloxacin (mg/L) | Naproxen (mg/mL) | < 0.5 | > 0.5 |
| ***S. aureus*** | 0.001 | 1 | | 1.0 (I) |
| ***E. faecalis*** | 0.001 | 2 | | 1.0 (I) |

A: Additive. I: Indifference. S: Synergy.

When different concentrations of ciprofloxacin and naproxen were used against *S. aureus* and *E. faecalis*, the results revealed an indifference effect. None of the combinations showed any antagonistic activity against any of the tested bacteria as shown in Table 2.

## CONCLUSION

Naproxen, an NSAID, is commonly used to reduce fever, and to treat pain and inflammation caused by several conditions. Previously, naproxen was evaluated for its antimicrobial potency in various studies (Hasan and Das, 2019; Han and Kucukguzel, 2020). In parallel to the previous studies, our results reveal promising antibacterial activity of naproxen against Gram-positive bacteria. However, no additive or synergistic activity was observed when combined with ciprofloxacin. Collectively, the data from our study show the selectivity of naproxen against Gram-positive bacteria in terms of antibacterial activity. This result suggests that naproxen can further be used as a potential source for novel antibiotics against Gram-positive bacteria by various modifications and/or combinations.

## REFERENCES

Bellio P, Fagnani L, Nazzicone L, Celenza G (2021). New and simplified method for drug combination studies by checkerboard assay. *MethodsX* **8**: 101543.

Han IM, Kucukguzel G (2020). Anticancer and antimicrobial activities of Naproxen and Naproxen derivatives. *Mini-Rev Med Chem* **20**: 1300-1310.

Hasan MS, Das N (2019). A detailed in vitro study of naproxen metal complexes in quest of new therapeutic possibilities. *Alexandria Med J* **53**: 157-165.

Hussein AA, Al-Janabi S (2011). Investigation of antidermatophytic effects of non-steroidal anti-inflammatory drugs on trichophyton mentagrophytes and epidermophyton floccosum. *Iran J Pharm Res* **10**: 547-552.

Mamatha N, Babu NS, Mukkanti K, Pal S (2011). 2-(6-methoxynaphthalen-2-yl)propionic acid (1,3-dimethyl-butylidene)-hydrazide. *Molbank* **4**: 1-4.

Stoev SS, Gueorguiev SS, Madzharov VG and Lebanova HV (2021). Naproxen in pain and inflammation – a review. *Int J Pharm Clin* **11**: 142-148.

Wikler MA (2006). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard, vol. 26, p M7–A7. CLSI, Wayne, Pennsylvania.

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# Antimicrobial Activity of *Pimpinella* – an Overview

Mehmet Ilktac[1], Zeynep Pelin Kutlu[2], Yanki Celebi[2], Gulden Celik[2]

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus, Mersin 10 Turkey.

[2] Bahcesehir University, Faculty of Medicine, Istanbul, Turkey.

## Abstract

*Pimpinella* species that are classified under *Apiaceae* (*Lindl*.) family are utilized in many fields of industry as spice, fruit, vegetable, and beverage and have especially been used in traditional medicine as a remedy in various countries. Essential oils of various species including *Pimpinella cypria, Pimpinella kotshchyana, Pimpinella saxifraga, and Pimpinella anisum* were reported to have antibacterial, antiviral, and antifungal activities. Along with its antimicrobial activity, *Pimpinella* species were found to have antioxidant, anti-inflammatory, anticonvulsant, antispasmodic, estrogenic, cytotoxic, insecticidal, and repellent properties. This review aims to provide an enhanced understanding on the morphology, chemical constituent, industrial and medicinal use, and antimicrobial activities of some important *Pimpinella* species against bacteria, fungi, and viruses.

**Keywords**

Antibacterial, antiviral, antifungal, essential oil, *Pimpinella*.

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***Corresponding author:** Mehmet Ilktac **email:** mehmet.ilktac@emu.edu.tr

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## INTRODUCTION

Numerous health problems that were experienced throughout the hiory were solved out by humankind using natural products. As human-being has steadily been in touch with the nature during their daily lives, medicinal benefits of various plants have been experienced and passed from one generation to another. The increase in the use of natural products as complementary and alternative medicine for a variety of health problems has attracted the attention of scientists to investigate the beneficial effects of medicinal plants. *Pimpinella* that belongs to *Apiaceae* (*Lindl*.) family was used as a herbal remedy for treating various disorders in several countries including Turkey (Tepe Sihoglu and Tepe, 2015; Tas *et al.,* 2006; Kuruuzum-Uz *et al.,* 2010; Demirezer *et al.,* 2012; Tetik *et al.,* 2013), China (Kang *et al.,* 2012; Kang *et al.,* 2013), Iran (Fard and Shojaii, 2013), Korea (Lee *et al.,* 2012), Palestine (Sawalha *et al.,* 2008), Lebanon (Kreydiyyeh *et al.,* 2003) and the United Kingdom (Yoney *et al.,* 2010). In addition to their conventional medicinal properties, *Pimpinella* species have economically of great importance because they are used as a spice, fruit and vegetable especially in the Mediterranean countries. *Pimpinella anisum* is frequently encountered in the beverage industry as “raki” in Turkey, “pastis” in France, “anesone” in Spain and “arak” in Syria (Anli and Bayram, 2010; Jurado *et al.,* 2007).

The genus *Pimpinella* includes around 150 species, the most perennial and permanent of which are full and needle-like, with graceful, star-shaped flowers carrying umbrella-like heads. They have small, oval-shaped fruits (Tepe *et al.,* 2005).

*Pimpinella* species contain monoterpene and sesquiterpene hydrocarbons, trinorsquiterpenes, phenylpropanoids, and 2-hydroxy-5-methoxy-1- (E) - propenylbenzene skeleton (pseudoisougenol) as the main essential oils (Tabanca *et al.,* 2003; Tabanca *et al.,* 2005; Tabanca *et al.,* 2006; Joshi, 2013; Tabanca *et al.,* 2016). Pharmacological and biological studies have shown that various *Pimpinella* species have antimicrobial, antifungal, antioxidant, anti-inflammatory, anticonvulsant, antispasmodic, estrogenic, cytotoxic, insecticidal, and repellent properties (Tepe Sihoglu A and Tepe B, 2015). Especially, *P. anisum* has been used as a remedy for asthma, bronchitis, cancer, diarrhea, cough, and nausea (Tabanca *et al.,* 2004; Baser *et al.,* 2007).

In this review, it was aimed to revise the plant morphology, chemical constituents, traditional and industrial uses, and antibacterial/antiviral activities of *Pimpinella* species.

**Morphology**

*Pimpinella* is one of the largest genus (170–180 species) classified under the family of *Umbelliferae/Apiaceae*. *Pimpinella* species that are found primarily in the subtropical/temperate locations of northern hemisphere and the Mediterranean region are biennial, permanent and grow yearly in the rocky cracks, fields, wilderness, hill pastures and meadows on dry rocky areas. They comprise mostly long-term herbs with slight and laterally compressed fruits that are cordate or oblong-ovoid, each with five filiform ribs constricted at its commissures. On the commissural side, the fruit generally has two vittae. The fruit is recognized on the commissural side with two major vittae. In the transverse section, mericarps are two and have elliptical, semi-round, or pentagonal shapes. Epidermal surface is pubescent, papillate or tubercuate. The seeds contain a thick-walled testa and an endosperm that includes a plenty of oils and proteins (Akalin *et al.,* 2016). The aerial and fluorescent parts of *Pimpinella cypria* are shown in Figure 1.

**Figure 1:** *P. cypria* and its florescence in the natural habitat (Muti and Ozhatay, 2020).

**Chemical constituents**

Biological activities of *Pimpinella* species are primarily due to the phenylpropanoid derivatives. The structural moiety of these compounds, 2-hydroxy-5-methoxy-1-(E)-propenylbenzene, that is known as pseudoisoeugenol has only been discovered in the *Pimpinella* genus. Previous studies on the *Pimpinella* identified antigermination, insecticidal, acaricidal, and poor antitumor activities of phenylpropanoids (Tabanca *et al.,* 2003). Monoterpenes, sesquiterpenes, trinorsesquiterpenes and phenylpropanoids (propenylphenols, pseudoisoeugenols) were reported to be the main volatile compounds (Tabanca *et al.*, 2006). Table 1 shows the chemical constituents and their biological effects. Some important essential oil components and their biological activities are given Table 2.

**Table 1:** Chemical constiutents of *Pimpinella* species and their effects (Muti S and Ozhatay FN, 2020).

| Constituents | Effect |
|---|---|
| **Flavonoids (e.g. falcarinol)** | Cytotoxic against acute lymphoblastic leukemia cells<br>Antioxidant<br>Antispasmodic<br>Anti-inflammatory |
| **Caumarin** | Vascular effect<br>Hepatoprotective<br>Anticancer<br>Antispasmodic<br>Hormonal<br>Immune enhancement |
| **Umbelliferone** | Antiproliferative effect on vascular smooth muscles<br>Anti-hyperlipidaemic<br>Anticancer<br>Antiviral |

**Table 2:** Essential oil components of *Pimpinella* and their biological effects (Tabanca *et al.,* 2005).

| Essential Oil | Antimicrobial Effect |
|---|---|
| **Pseudoisoeugenol 2-methylbutanoate** | Potent activity against fungi species<br>Potent activity against *Mycobacterium intracellulare* |
| **Traginone** | Negligible antifungal activity |
| **Dictamnol** | Negligible antifungal activity |
| **2- methoxy-4-(1-propenyl) phenyl tiglate** | Potent activity against *M. intracellulare* |
| **Epoxyisoeugenol** | Antimycobacterial activity |

## Traditional and industrial use of *Pimpinella* species

Various *Pimpinella* species have been used in both traditional medicine and industry. Anise seeds have been utilized in traditional Iranian medicine to treat epilepsy. Seeds of *P. anisum* are also used as a diuretic, carminative, fragrant, antiseptic, and analgesic for migraines in traditional medicine (Sun *et al.,* 2019). To increase milk secretion, essential oil of anise seed is used in lactating women whereas that of *Pimpinella isaurica*, *Pimpinella aurea*, and *Pimpinella corymbosa* are used as animal feed (Mahboubi, 2021; Tabanca *et al.*, 2003, Baser *et al.*, 2007). The roots of *Pimpinella saxifraga* are used as a demulcent, stomachic, expectorant, and tonic (Baser *et al.*, 2007). Fruits and seeds of anise are used in the food industry such as making bread, cookies and sugar, as well as in the production of alcoholic beverages such as raki, ouzo, pastis, pernod, anisette, ricard, and granier (Boztas and Bayram, 2020; Baser *et al.*, 2007). Additionally, anise is used in the cosmetic industry in the production of toothpastes, soaps, lotions, and dermal creams (Boztas and Bayram, 2020).

## Antimicrobial Activity of the Essential Oils of *Pimpinella*: Action of Mechanism

The chemical composition and functional attachments that target various bacterial pathways play a major role in the

antibacterial efficacy and mode of action of crude or individual essential oils. The essential oils of *Pimpinella* results in the loss of vital processes such ion homeostasis and the electron transport chain by disrupting the permeability of the outer membrane of Gram negative bacteria and damaging bacterial cell membrane of both Gram positive and Gram negative bacteria. The hydrophobic nature of the essential oils is the key chemical characteristic for cell membrane disruption (Tang *et al.,* 2020). Additionally, the essential oils may stop bacterial cell division and inhibit bacterial protein synthesis. The deficiency in ATP synthesis is among other fundamental mechanisms of antimicrobial and antifungal activities (Turgis *et al.,* 2009). In general, it is difficult to attract an antibacterial activity to one specific mechanism due to the numerous essential oil components and two or more mechanisms are thought to be responsible for antimicrobial activity.

**A review of Literature: Antimicrobial Activity**

In the study conducted by Tabanca *et al.* (2016), aerial parts of *P. cypria*, which is an endemic plant specific to Cyprus, were subjected to hydrodistillation for 3 hours after which yellowish essential oil was obtained. The essential oil was subjected to gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionisation detector (GC-FID). As a result, oxygenated sesquiterpenes, sesquiterpenes and monoterpens were found to be the most prevalent compounds (33.9%, 22% and 11.4%, respectively). The main components were (Z)-β-farnesene (6.0%), spathulenol (5.9%), ar-curcumene (4.3%), and 1,5-epoxy-salvial(4)14-ene (3.8%). Although the essential oil was found to be less effective than l N,N-diethyl-3-methylbenzamide (DEET), a commonly used insect repellent, it was reported to act as a repellent against *Aedes aegypti* (a vector for yellow fever) and star tick, *Amblyomma americanum*. Moreover, moderate antibacterial activity was detected against various Gram positive and Gram negative bacteria including *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterococcus faecium*, and Salmonella *Typhimurium.* On the other hand, the essential oil was found to have similar antifungal activity against *Candida albicans* compared to flucytosine.

In another research conducted by Askari *et al.* (2010), essental oils were isolated from different parts (including aerial parts, stem/leaf, flowers, unripe and ripe seeds) of *Pimpinella barbata* collected from the south of Iran. The highest yield of oil was extracted from unripe and ripe seeds followed by inflorescence, aerial parts and stem/leaf, in order. Sixteen, 22, 28, 22 and 12 components were detected in the aerial

parts, stem/leaf, inflorescence, unripe seed and ripe seed essential oils, respectively. The major components were pregeijerene that comprised 32.7% of the essential oil extracted from aerial parts, g-muurolene that made up 28.2% of stem/leaf oil and methyl eugenol (18.7% in unripe seed oil). Although the antibacterial activities of the essential oils detected by disk diffusion method were much weaker than tetracycline and gentamycin, the oil showed higher activity against Gram positive bacteria (*Bacillus subtilis, Bacillus cereus, Micrococcus luteus and S. aureus*) than Gram negative bacteria (*Yersinia enterocolitica, Klebsiella oxytoca, Klebsiella pneumoniae, Serratia marcescens, E. coli* and *Pseudomonas aeruginosa*). On the other hand, unriped and ripe seeds revealed high antifungal activity against *C. albicans*. The essential oils extracted from the inflorescence and seeds of *P. kotschyana*, a species that is spread in Iran and north of Iraq, were reported to be rich in β-caryophyllene, germacrene D and langipinanol. Essential oils extracted from seeds that were collected after their colors changed to brown were effective against *B. subtilis, P. aeruginosa*, and *E. coli* and that extracted from inflorescence against *E. coli* (Asgari *et al*., 2011).

In a study carried out by Ksouda *et al.* (2019), the main components of the essential oil of *P. saxifraga* were found to be anethole, pseudoisoeugenol and p-anisaldehyde by gas chromatography-high resolution mass spectrometry (GC–HRMS) analysis. Along with strong total antioxidant and radical scavenging activity, β-carotene bleaching inhibition, ferric reducing power and potential DNA protection, the essential oil was shown to have potent antibacterial activity against Gram negative bacteria such as *E. coli, P. aeruginosa*, S. *Typhimurium* and Gram positive bacteria including *Listeria monocytogenes, M. luteus* and *B. cereus*. MICs and MBCs ranged from 0.78 to 3.125 mg/ml and 3.125 to 6.25 mg/ml against Gram negative bacteria whereas they were 1.56-3.125 mg/ml and 3.125-12.5 mg/ml against Gram positive bacteria, respectively. Considering MBC/MIC ratio (eg., MBC/MIC <4 bactericidal; MBC/MIC >4 bacteriostatic effect), the essential oil was reported to exert bactericidal activity against S. *Typhimurium, B. cereus* and *M. luteus* whereas it had bacteriostatic effect against *E. coli, P. aeruginosa* and *L. monocytogenes.* Antibacterial activity was attributed to anethole. Similar to the findings of Ksauda *et al.* (2019), Tepe *et al.* (2006) reported that the main components of the essential oil of *P. anisetum* were (E)-anethole (82.8%) and methyl chavicol and that of *P. flabellifoli* were limonene (47.0%), (E)-anethole (37.9%) and α-pinene (6.0%). The essential oils of both species had moderate antibacterial and antifungal activities.

*Pimpinella anisetum* essential oils had stronger antimicrobial activity than *Pimpinella flabellifolia* essential oil. *Clostridium perfringens* was the most sensitive bacterium to both of the essential oils followed by *Streptococcus pneumoniae* by microdilution method. *Anethole, as the highest* component of the essential oils, was thought to play a fundemental role in the antimicrobial activity.

The composition and antimicrobial activity of essential oils of air-dried and grained *P. anisum* ripe seeds were investigated by Abdel-Reheem and Oraby (2015). Propanoids and monoterpens were the major consituents of the essential oil. The major compounds were determined by ultra performance liquid chromatography (UPLC) coupled with tandem mass spectrometry (MS/MS) as trans-anethole (82.1%) followed by cis-anethole (5.8%), estragole (2.5%), linalool (2.3%), aterpineol (1.5%), and methyl eugenol (1.3%). A strong antibacterial acitivity was observed against Salmonella *Typhi* (zone of inhibition: 17 mm) and *E. faecalis* zone of inhibition: 16 mm) by disk diffusion method. Zones of inhibition of the essential oil were greater than corn oil against *E. coli* and *M. luteus*. MIC of the essential oil was found to be 2 µg/ml against S. Typhi, *E. faecalis* and *M. luteus.* Similar to the findings by disk diffusion method, the essential oil revealed stronger effect with MICs of the essential oil smaller than that of corn oil against *E. coli* and *M. luteus*.

In contrast to antibacterial and antifungal activities, studies investigating the antiviral activities of antimicrobial activities of various *Pimpinella* species are limited. Lee *et al.* (2011), isolated three antiviral and immuno-stimulating complexes identified as lignin-carbohydrate-protein complexes (LC1, LC2 and LC3) from the hot water extracts of *P. anisum* seeds using combination of anion-exchange, gel filtration and hydrophobic interaction column chromatography. The lignin-carbohydrate complexes included neutral sugars and uronic acids. All three LCs exerted antiviral activities against Herpes simplex type 1 and 2, cytomegalovirus and measles viruses. Additionally, respective selective toxicity was found for LC1 and LC3 against human coronaviruses and Coxsackie viruses. The antiviral activity was most probably due to interference with the adhesion of the virus to host cell and decrease in the infectivity of viruses which are two early phases of virus replication. The immunomodulatory effect of LCs were attributed to enhanced nitric oxide production, IL-1β and IL-10 production.

In a recent in silico study carried out in 2021, isovitexine, a flavone, of *P. anisum* was reported as the most potent ligands against severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) spike proteins

and main proteases which are potential target for treatment or vaccine discovery because they have indispensable role during SARS-CoV-2 replication (Kumar *et al.*, 2021).

## REFERENCES


Abdel-Reheem MAT, Oraby MM (2015). Anti-microbial, cytotoxicity, and necrotic ripostes of *Pimpinella anisum* essential oil. *Ann Agric Sci* ***60***(2): 335-340.

Akalin E, Yesil Y, Akpulat A (2016). Fruit anatomy of the Turkish *Pimpinella* species. *Flora* ***223***: 62-73.

Anli R, Bayram M (2010). Traditional aniseed-flavored spirit drinks. *Food Rev Int* **26**(3): 246-269.

Askari F, Sefidkon F, Teimouri M (2010). Essential oil composition of different parts of *Pimpinella barbata* (DC.) Bois. in Iran. *J Essen Oil Res* **22**.

Askari F, Teimouri M, Sefidkon F (2011). Chemical composition and antimicrobial activity of *Pimpinella kotschyana* Boiss. oil in Iran. *J Essent Oil-Bear Plants* ***14***(1): 124-130.

Baser KHC, Tabanca N, Kirimer N, Bedir E, Khan IA, *et al*. (2007). Recent advances in the chemistry and biological activities of the *Pimpinella* species of Turkey. *Pure and Applied Chemistry* ***79***(4): 539–556.

Boztas G, Bayram E (2020). Foreign trade and production of anise (*Pimpinella anisum* L.) in Turkey. *Ziraat Fakultesi Dergisi, Ozel Sayisi*: 103–107.

Demirezer L, Kuruuzum-Uz A, Guvenalp Z, Simon A, Patócs T (2012). Further secondary metabolites from *Pimpinella kotschyana*. *Planta Med*, **78**: PI406.

Fard MA, Shojaii A (2013). Efficacy of Iranian traditional medicine in the treatment of epilepsy. *BioMed Res Inter* 2013: 692751.

Joshi RK (2013). Chemical composition of the essential oil of the flowering aerial parts of *Pimpinella monoica*. Nat Prod Commun **8**(11):1643-1644.

Jurado J, Ballesteros O, Alcazar A, Pablos F, Martin M, *et al.* (2007). Characterization of aniseed-flavoured spirit drinks by headspace solid-phase microextraction gas chromatography–mass spectrometry and chemometrics. *Talanta* **72**(2): 506-511.

Kang Y, Łuczaj Ł, Kang J, Zhang S (2013). Wild food plants and wild edible fungi in two valleys of the Qinling Mountains (Shaanxi, central China). *J Ethnobiol Ethnomed* **9**: 26.

Kang Y, Łuczaj Ł, Ye S, Zhang , Kang J (2012). Wild food plants and wild edible fungi of Heihe valley (Qinling Mountains, Shaanxi, central China): Herbophilia and indifference to fruits and mushrooms. *Acta Soc Bot Pol* **81**(4): 405-413.

Kreydiyyeh S, Usta J, Knio K, Markossian S, Dagher S (2003). Aniseed oil increases glucose absorption and reduces urine output in the rat. *Life Sci* **74**(5): 663-673.

Ksouda G, Sellimi S, Merlier F, Falcimaigne-Cordin A, Thomasset B, *et al.* (2019). Composition, antibacterial and antioxidant activities of *Pimpinella saxifraga* essential oil and application to cheese preservation as coating additive. *Food Chem* ***288***: 47-56.

Kumar B, Zaidi S, Haque S, Dasgupta N, Hussain A, *et al.* (2021). In silico studies reveal antiviral effects of traditional Indian spices on COVID-19. *Curr Pharm Des* ***27***(32): 3462–3475.

Kuruuzum-Uz A, Guvenalp Z, Yuzbasioglu M, Ozbek H, Kazaz C, *et al.* (2010). Flavonoids from *Pimpinella kotschyana*. *Planta Med*, **76**: p274.

Lee JB, Yamagashi C, Hayashi K, Hayashi (2011). Antiviral and immunostimulating effects of lignin-carbohydrate-protein complexes from *Pimpinella anisum*. *Biosci Biotechnol Biochem* ***75***(3): 459–465.

Lee S, Park J, Moon E, Kim S, Lee K (2012). Quinic acid derivatives from Pimpinella brachycarpa. *Planta Med*, **78**: PJ127.

Mahboubi M, Mahboubi M (2021). *Pimpinella anisum* and female disorders: A review. *Phytomedicine Plus*, ***1***(3): 100063.

Muti S, Ozhatay FN (2020). Morphological and leaf anatomical structure of *Pimpinella cypria* Boiss. *EMU J Pharm Sci* **3**(3):169-181.

Sawalha A, Sweileh W, Zyoud S, Jabi S (2008). Self-therapy practices among university students in Palestine: Focus on herbal remedies. *Complement Ther Med* **16**(6): 343-349.

Sun W, Shahrajabian MH, Cheng Q (2019). Anise (*Pimpinella anisum* L.), a dominant spice and traditional medicinal herb for both food and medicinal purposes. *Cogent Biology* ***5***: 1673688 .

Tabanca N, Bedir E, Ferreira D, Slade D, Wedge DE, *et al.* (2005). Bioactive constituents from Turkish *Pimpinella* species. *Chemistry Biodivers* **2**(2): 221–232.

Tabanca N, Bedir E, Kirimer N, Baser K, Khan S, *et al.* (2003). Antimicrobial compounds from *Pimpinella* species growing in Turkey. *Planta Med* ***69***(10): 933-938.

Tabanca N, Demirci B, Ozek T, Kirimer N, Baser K, *et al.* (2006). Gas chromatographic–mass spectrometric analysis of essential oils from *Pimpinella* species gathered from Central and Northern Turkey. *J Chromatogr A* ***1117***(2): 194-205.

Tabanca N, Khan S, Bedir E, Annavarapu S, Willett K, *et al.* (2004). Estrogenic activity of isolated compounds and essential oils of *Pimpinella* species from Turkey, evaluated using a recombinant yeast screen. *Planta Med* ***70***(8): 728-735.

Tabanca N, Nalbantsoy A, Bernier U, Agramonte N, Ali A, *et al.* (2016). Essential oil composition of *Pimpinella cypria* and its insecticidal, cytotoxic, and antimicrobial activity. *Nat Prod Commun* **11**(10): 1531-1534.

Tang C, Chen J, Zhang L, Zhang R, Zhang S, *et al.* (2020). Exploring the antibacterial mechanism of essential oils by membrane permeability, apoptosis and biofilm formation combination with proteomics analysis against methicillin-resistant *Staphylococcus aureus*. *Int J Med Microbiol* ***310***(5): 151435.

Tas A, Ozbek H, Atasoy N, Altug ME, Ceylan E (2006). Evaluation of analgesic and anti inflammatory activity of *Pimpinella anisum* fixed oil extract. *Indian Veterinary Journal* **83**(8): 840-843.

Tepe B, Akpulat H, Sokmen M, Daferera D, Yumrutas O, *et al.* (2006). Screening of the antioxidative and antimicrobial properties of the essential oils of *Pimpinella anisetum* and *Pimpinella flabellifolia* from Turkey. *Food Chemistry* **97**(4): 719-724.

Tepe Sihoglu A, Tepe B (2015). Traditional use, biological activity potential and toxicity of *Pimpinella* species. *Industrial Crops and Products* ***69***: 153–166.

Tetik F, Civelek S, Cakilcioglu U (2013). Traditional uses of some medicinal plants in Malatya (Turkey). *J Ethnopharmacol* **146**(1): 331-346.

Turgis M, Han J, Caillet S, Lacroix M (2009). Antimicrobial activity of mustard essential oil against *Escherichia coli* O157:H7 and *Salmonella* Typhi. *Food Control* ***20***(12): 1073–1079.

Yoney A, Prieto J, Lardos A, Heinrich M (2009). Ethnopharmacy of turkish-speaking cypriots in greater London. *Phytother Res* **24**(5): 731-740.

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