

Antioxidant, anti-tyrosinase, antidiabetic, wound healing, and cytoprotective effects of the aqueous extract of *Helichrysum odoratissimum*: *in vitro*, *ex vivo*, and *in silico* studies.

Patrick Brice Defo Deeh

Kangwon National University

Kiseok Han

Kangwon National University

Myeong-Hyeon Wang

mhwang@kangwon.ac.kr

Kangwon National University

Research Article

Keywords: Helichrysum odoratissimum, antioxidant, anti-tyrosinase, antidiabetic, wound healing, HaCat cells

Posted Date: May 7th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-6598546/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: The authors declare no competing interests.

Abstract

Helichrysum odoratissimum is commonly used in the treatment of diabetes, skin wounds, and eczema. We determined its phytochemical composition and evaluated its antioxidant, anti-tyrosinase, antidiabetic, wound healing, and cytoprotective activities. The antioxidant effect was tested by DPPH, ABTS+, cupric, and ferric methods. The anti-tyrosinase action was determined using tyrosine and L-DOPA as substrates, while the antidiabetic effect was studied by α -amylase and α -glucosidase assays. The cytoprotective activity was studied via cell viability in HaCaT cells, hemolysis using erythrocytes, and HET-CAM assay. Furthermore, drug-likeness, toxicity prediction, and molecular docking of selected phyto-compounds docked against tyrosinase, L-DOPA, α -amylase, and α -glucosidase were studied. *H. odoratissimum* contained various components of known pharmacological importance such as p-menthone, eucalyptol, palmitic acid, pulegone, and (E)-caryophyllene, and exhibited a strong antioxidant activity. It also inhibited tyrosinase, L-DOPA auto-oxidation, α -amylase and α -glucosidase in a concentration-dependent manner. *H. odoratissimum* enhanced the migration of keratinocytes and significantly ($p < 0.05$ - 0.01) increased the percentage of wound closure after 12, 24 and 48 hours of treatment. On the other hand, *H. odoratissimum* did not cause any cytotoxicity in erythrocytes and hen's egg-chorioallantoic membrane after treatment. All phyto-components from *H. odoratissimum* met the acceptable standards for drug-likeness and toxicity prediction, according to Lipinski's rule. The strong binding affinity of phyto-components with the active site of proteins recorded *in silico* supported the *in vitro* anti-tyrosinase and antidiabetic activities of *H. odoratissimum*. This plant can be exploited in the development of plant-based anti-tyrosinase, antidiabetic, and wound healing agents. These findings could justify its ethnomedicinal uses.

1. Introduction

In developed and developing countries, physical appearance is progressively emerging as one of the most important determinants of male and female attractiveness within societies undergoing rapid urbanization (Swami et al., 2008). This conquest of physical beauty pushes people to go to extremes to achieve their goal (Samson et al., 2010). In some countries, skin color is one of the main criteria that determines physical beauty. Hence, some individuals undergo voluntary skin pigmentation or depigmentation procedures (de Souza et al., 2008). Indeed, pigmentation disorders are caused by various factors, such as hormonal changes due to pregnancy, sun exposure, skin diseases, and long-term use of lighting creams or lotions, among others (Thawabteh et al., 2023; Hernández et al., 2024). On average, around 40% of Asian women use lighting agents, while in certain African countries, the usage ranges from 25–80% (Antoine et al., 2005). In some countries, the use of soft lighting agents like kojic acid and alpha hydroxy acid is allowed in cosmetics, but their action is limited. Other lighting compounds with rapid effects like hydroquinone and mercury are not allowed, due to their harmful effects on health (Couteau et al., 2016; Zheng et al., 2023). In general, skin bleaching agents induce their lightning effects by reducing melanin pigment through inhibition of the action of tyrosinase and L-DOPA, by scavenging the existing melamine, or by destroying melanocytes (Ebanks et al., 2009; Gillbro and Olsson, 2011). The action can be irreversible when melanocytes are completely destroyed (Chen et al., 2020).

In addition to skin disorders, hydroquinone and other depigmenting agents like deoxyarbutin, kojic acid, and aloesin may also cause inflammation (Zheng et al., 2023) and increase the risk of developing hypertension (Bwomda et al., 2005), renal diseases (Okoye, 2019), and diabetes mellitus (Phan and Smith, 2021). For instance, hydroquinone can directly increase the capillary glycemia (Choukem et al., 2018), while cortisol can suppress the hypothalamic-pituitary-adrenal system and inhibit the production of Antidiuretic Hormone (ADH) (Papanek and Raff, 1994), and alter the synthesis or action of insulin (by stimulating lipolysis for instance) (Anagnostis et al., 2009), leading to diabetes mellitus (Sun and Wang, 2023). Considering the adverse effects of lightening cosmetics on health, skin bleaching is a public health problem that may require special attention. The development of effective plant-based products with no side effects is of great importance.

Helichrysum odoratissimum, commonly called “Mbantchuet” in Cameroon or “Impepho” in South Africa, is an aromatic plant from the family *Asteraceae*. It is a highly fragrant and extensively branched perennial herb characterized by its petite silvery leaves and small yellow flowerheads clustered at the branch tips (Asekun et al., 2007). It can be woody at the lower part, growing upright or spreading, reaching heights of up to 50 cm. This plant is also known for its strong smell. Experimental studies revealed the antioxidant (Legoalea et al., 2013), pro-sexual (Watcho et al., 2019), antifungal (Zantanta et al., 2022), anticancer (Twilley et al., 2017), and anti-inflammatory and antidiabetic (Ngagi et al., 2015) activities of *H. odoratissimu*. Additionally, another experimental study stated the efficacy of *H. odoratissimu* against acne through its ability to prevent bacterial growth associated with acne progression (De Canha et al., 2020). In traditional medicine, this aromatic plant is used to treat diabetes, burns, wounds, and eczema (Hutchings et al., 1996; Akaberi et al., 2019). It is also used as a skin softener. However, there is no experimental study on the effect of this plant on tyrosinase and L-DOPA activities, despite its folkloric use against skin disorders. In the present study, after evaluating the phytochemical profile and antioxidant power of *H. odoratissimu*, its action on melanogenesis-related markers (tyrosinase and L-DOPA) and diabetes-related enzymes (α -amylase and α -glucosidase) was determined *in vitro* and *in silico*. Moreover, the cytoprotective effects of this plant were tested through cell viability in human keratinocyte cells (HaCat cells), hemolysis in red blood cells, and *ex vivo* HET-CAM irritation assay.

2. Materials and methods

2.1. Plant collection, preparation of aqueous extract, and GC-MS analysis

The whole plant of *H. odoratissimum* was harvested in March 2023 in Dschang (a city in the West Region of Cameroon, GPS coordinates: 5° 26' 38.29" N, 10° 03' 11.95" E), and identified at the Cameroon National Herbarium (specimen N°: HNC 1640). After collection, the plant underwent a three-week period of shade drying, followed by grinding. The aqueous extract was prepared by macerating 100 g of powder in distilled water (2L) for 48 hours. The mixture was filtered using filter paper (Whatman N°1) and the

filtrate was evaporated at 35°C. 18.45 g of extract was obtained (extraction yield: 18.45). The GC (Agilent 789A, Agilent, Santa Clara, CA, USA)/MS (Agilent 5975C; GC-MSD) system was utilized to identify the volatile compounds in *H. odoratissimum*, following the previously outlined procedure (Saravanakumar et al., 2021; Deeh et al., 2024). Briefly, we injected 1 mg/mL of extract dissolved in methanol into the HP-5 capillary column at 250°C, with helium gas (99.99%) serving as the carrier at a flow rate of 1 mL/min. The sample was run for 73 minutes. The phytochemicals were identified by comparing the GC-MS data of the samples with the electronic library of W8N05ST.L.

2.2. Determination of total phenolics (TP) and total flavonoids (TF)

The measurement of TP in *H. odoratissimum* was done using the Folin Ciocalteu method as described earlier (Mehrabani et al., 2023). Briefly, 100 µL of sample, at a concentration of 1 mg/mL, was gently mixed with 200 µL of Folin-Ciocalteu's phenol reagent and 500 µL of Na₂CO₃. Then, the mixture was incubated for 50 min at 25°C, and the absorbance was measured at 760 nm. Gallic acid was used as standard, and TP content was presented as milligram gallic acid equivalents per 100 g of dry weight (GAE/g DW).

The TF in the extract was quantified using the AlCl₃ method. Indeed, after mixing the sample (100 µL) with 250 µL of distilled water and 150 µL of 95% ethanol, we added 25 µL of AlCl₃ (10%) and 25 µL of CH₃COOK (1M). The mixture was then incubated for 30 minutes at 25°C, the optical density was measured at 415 nm, and the TF level was estimated as milligram quercetin equivalent/gram of dry weight (mg QE/g DW) (Naveen et al., 2023).

2.3. Antioxidant study and measurement of lipid peroxidation inhibition

The estimation of DPPH (Naveen et al., 2023) and ABTS⁺ (Blois, 1958) free radical scavenging activity, as well as CUPRAC (Apak et al., 2008) and FRAP (Gao et al., 2020; Sathiyaseelan et al., 2021) reducing power, and the level of lipid peroxidation inhibition (Roberto and Baratta 2000) were determined as described in the supplementary materials (S1).

2.4. Anti-tyrosinase assay

The ability of *H. odoratissimum* to inhibit tyrosinase was determined in a 96-well plate as described earlier (Fawol et al., 2012). In brief, 70 µL of various concentrations (7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000, 2000 µg/mL) of plant extract or ascorbic acid diluted in DMSO was mixed with 30 µL mushroom tyrosinase (333 units/mL), and the mixture was incubated for 5 min. Then, 110 µL of L-DOPA (12 mM) or tyrosine (2 mM) used as substrate was added, and the absorbance of the mixture was recorded at 490 nm. The mixture was incubated for 30 min at room temperature and the absorbance was measured again at 492 nm, and the percentage of tyrosinase inhibition was estimated as previously reported

(Klomsakul et al., 2022). Tyrosinase, L-DOPA and tyrosine were prepared in sodium phosphate buffer (0.05 mM, pH 6.5), and all samples were prepared in triplicate.

2.5. Anti-L-DOPA auto-oxidation assay

The inhibition of L-DOPA auto-oxidation was measured as described by Klomsakul et al. (2022). Briefly, 500 μ L of serial concentrations (7.8–2000 μ g/ml) of *H. odoratissimum* or ascorbic acid were mixed with 500 μ L of L-DOPA (12 mM). The mixture was incubated for 48 hours at 30°C and the optical density of the samples was recorded at 475 nm. Then, the percentage of L-DOPA inhibition was estimated (Klomsakul et al., 2022).

2.6. *In vitro* anti-diabetic assay

2.6.1. α -Amylase activity

50 μ L of *H. odoratissimum* or Acarbose (positive control) in serially diluted concentrations (7.8–1000 μ g/mL) was separately added to 50 μ L of α -amylase (2 Units) and further mixed with 100 μ L of phosphate buffer (1 mM, 6.9 pH). The mixture was incubated for 15 min at 37°C and 50 μ L of starch solution (0.5%) was added. It was further incubated for 15 min at the same temperature. At the end of the incubation period, 500 μ L of DNS reagent (0.1 M) was added to the reaction mix and incubated in a water bath for 5 min at 95°C. Then, the mixture was diluted 5 folds, the absorbance was recorded at 540 nm, and the percentage of α -amylase inhibition was estimated (Alshehri et al., 2022).

2.6.2. α -Glucosidase activity

50 μ L of serial concentrations (7.8–1000 μ g/mL) of *H. odoratissimum* was separately added to 25 μ L of α -glucosidase (1 Unit), and further mixed with 100 μ L of phosphate buffer (1 mM, 6.9 pH). Acarbose (7.8–1000 μ g/mL) was used as experimental positive control. The mixture was incubated for 20 min at 37°C, added with 50 μ L of PNPG solution (5 mM), and further incubated for 20 min in the dark at 37°C. Following incubation, the reaction was stopped by adding 100 μ L of Na₂CO₃ (0.1 M), and the optical density was recorded at 405 nm. PBS (6.9 pH) was used as experimental control to calculate the percentage of α -glucosidase inhibition (Naveen et al., 2023).

2.7. Cell culture and scratch wound healing assay in HaCaT cells

HaCaT cells were provided from the Korean Cell Line Bank (KCLB, Seoul, ROK), and cultured according to the suppliers' guidelines. At about 80–90% growth confluence, the cells were trypsinized and transferred to a 12-well plate (2×10^5 cells per well) containing DMEM with 10% FBS medium. After 24 hours of incubation, the media was removed and the adherent cell layer was scratched with a sterile 100 μ L pipette tip. The wells were rinsed with phosphate-buffered saline (PBS) in order to eliminate the cellular debris. The cells were treated with various concentrations of *H. odoratissimum*, and the scratch area images were taken at 0, 12, 24 and 48 hours. The results were analyzed with Image J version 1.52v

software. The group without treatment (only media) was used as a positive control (Saravanakumar et al., 2024; Li et al., 2024).

2.8. Cytotoxicity assay

2.8.1. Cell viability and evaluation of the protective effect of *H. odoratissimum* against H₂O₂ toxicity

The cell viability was determined by using 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described earlier (Deeh et al., 2019; 2022). In brief, the HaCaT cells were exposed to various concentrations (7.8–2000 µg/mL) of *H. odoratissimum* for 24 hours. At the end of the treatment period, 10 µL of MTT working solution at a concentration of 5 mg/mL was added to each well. The plate was then incubated for 3 hours in the dark. After removing the media, 100 µL of DMSO was added to each well, and absorbance was recorded at 570 nm. Then, the cell viability (%) was estimated (Deeh et al., 2022). The protective effect of *H. odoratissimum* against H₂O₂ cytotoxicity was determined by using MTT assay as described earlier (Naveen et al., 2023). Briefly, the cells were pretreated with H₂O₂ (1.6 mM) for 30 min and later incubated for 12 hours with various concentrations (7.8–2000 µg/mL) of *H. odoratissimum* or ascorbic acid. Then, the cell viability was measured (Naveen et al., 2023). Moreover, the morphology of the HaCaT cells was visualized by an inverted microscope after 24 hours of treatment.

2.8.2. Hemolysis

The effects of *H. odoratissimum* on hemolysis were tested on red blood cells (RBCs). In brief, the mixture of 1 mL of defibrinated blood and 10 mL of PBS was centrifuged (2000 rpm/10 min) to isolate the RBCs. Subsequently, different concentrations (7.8–2000 µg/mL) of *H. odoratissimum*, PBS (used as negative control) and 1% Triton X-100 (used as positive control) were mixed with 200 µL of RBCs suspended in PBS, and the mixture was incubated at 37°C for one hour. Then, the samples were centrifuged (2000 rpm/10 min), and the absorbance of the supernatant was measured. The hemolytic percentage was calculated as described earlier (Li et al., 2024).

2.8.3. HET-CAM irritation *ex vivo* toxicity assay

This experiment was conducted following the previously described methodology (Jin et al., 2023). Briefly, prior to the experiment, the eggs were examined by candling to ensure the presence of live embryos. Fifteen eggs (3/group) were divided as follows: (1) negative control group treated with 0.9% NaCl; (2) positive control group administered with NaOH (0.1M); and (3–5) plant-treated groups exposed to *H. odoratissimum* at concentrations of 500, 1000, 2000 µg/mL, respectively. The shells of the eggs were carefully removed, and the outer membrane was taken off using 30 cm blunt tweezers to expose the chorioallantoic membrane. 200 µL of each sample was applied to the membrane and left for 20 seconds before being wiped off with tissue paper. Toxicity-related parameters such as hemorrhage, vascular damage, and coagulation were observed at 0 s (before treatment), 30 s, 2 min, and 5 min.

2.9. *In silico* studies

2.9.1. Drug likeness and toxicity prediction

The drug-likeness and toxicity of all the phytocompounds identified in *H. odoratissimum* were assessed using Lipinski's rule through SwissADME, as previously described (Benet et al., 2016). In brief, the phyto-components were converted into SMILES format, and the SwissADME package was utilized to calculate various properties, including molecular weight, number of hydrogen bonds (donor and acceptor), gastrointestinal (GI) absorption, lipophilicity, molar refractivity, and Lipinski violations. Compounds satisfying all Lipinski's rules (5/5) were selected for molecular docking against tyrosinase, L-DOPA, α -amylase and α -Glucosidase.

2.9.2. Molecular docking

At the end of the drug likeness and toxicity prediction essay, 5 phytocompounds were selected for molecular docking against tyrosinase (PDB ID: 2Y9X), L-DOPA (PDB ID: 6ON3), α -amylase (PDB ID: 1OSE), and α -glucosidase (PDB ID: 5NN8), to determine their binding energy and predict their affinity. The proteins were downloaded from the protein data bank (PDB; www.rcsb.org) and prepared for the study. Water molecules and ligands were removed, and polar hydrogen atoms were added. Their energy was minimized, and their active sites were built. They were saved as protein data bank (PDB) files. The ligands were created using UCSF Chimera software (version 1.16, San Francisco, CA, USA), and stored as Mol2 files. Subsequently, selected ligands were subjected to docking with the active site of proteins using ArgusLab (version 4.0.1), and the results were analyzed to understand the binding interaction by using the BIOVIA Discovery Studio Visualizer 2021 client.

2.10. Statistical analysis

All tests were repeated three times. The results are presented as the mean plus or minus the standard error of the mean (SEM). Differences among the means were analyzed using ANOVA one-way, followed by the Tukey HSD post hoc test using STATISTICA software (version 8.0, StatSoft, Inc., Tulsa, USA). The significance threshold was established at 0.05 or lower.

3. Results and discussion

3.1. Phytochemical screening and total phenolic and total flavonoid contents in *H. odoratissimum*

A previous report documented the identification of non-volatile compounds in this plant (Serabele et al., 2021). The volatile compounds identified in *H. odoratissimum* are presented in Table S2 and Fig. 1. Indeed, p-Menthone, Eucalyptol, n,n-Dimethylacetamide, Octane, Nonane, Palmitic acid, 5-Methyl-2(5H)-furanone, 1-Pentadecanamine, 2-Cyanoacetamide, Propane-2-13c, Oxirane, Pulegone and (E)-Caryophyllene were detected. These results are consistent with the study of Asekun et al. (2007) who

reported the presence of α -Pinene, (E)-Caryophyllene, p-Menthone, and other volatile compounds in *H. odoratissimum* harvested in July, 2005 in the Cap Region of South Africa. Total phenolic and total flavonoid contents in *H. odoratissimum* were 126.787 ± 10.72 mg of GAE/g DW and 4.56 ± 0.71 mg of QE/g DW, respectively (Table S3). Matrose et al. (2022) obtained a total phenolic content of 193 ± 7.6 mg of GAE/g DW and 145 ± 4.5 mg of GAE/g DW in the acetone and ethanolic extracts of *H. odoratissimum*, respectively. The slight variation could be attributed to the extraction procedure and period and place of plant collection, as reported earlier (Yao et al., 2005; Matrose et al., 2021). In the present work, selected phytocompounds found in *H. odoratissimum* were used to predict the protein ligand interaction using the molecular docking method.

3.2. Antioxidant activity of *H. odoratissimum*

The DPPH and ABTS⁺ free radical scavenging activity, as well as CUPRAC and FRAP of *H. odoratissimum* were increased in a concentration-dependent manner. Indeed, the DPPH and ABTS⁺ values at doses 125–2000 $\mu\text{g/mL}$ were significantly ($p < 0.001$) increased, compared to the lowest dose (7.8 $\mu\text{g/mL}$). At doses 500–2000 $\mu\text{g/mL}$, we also noticed a significant ($p < 0.001$) improvement in CUPRAC of the plant extract, compared with dose 7.8 $\mu\text{g/mL}$. However, ascorbic acid used as a positive control showed the highest antioxidant activity. The FRAP (at doses 31.3–2000 $\mu\text{g/mL}$) and CUPRAC (at doses 500 and 1000 $\mu\text{g/mL}$) of ascorbic acid were significantly ($p < 0.05 - 0.001$) elevated, compared with that of *H. odoratissimum* (Fig. 2A-D). Moreover, the ability of *H. odoratissimum* to inhibit lipid peroxidation by using egg yolk homogenate as a lipid-rich media was tested. The lipid peroxidation inhibitory activity of *H. odoratissimum* and ascorbic acid was dose-dependent, which correlated with DPPH, ABTS⁺, CUPRAC, and FRAP values. There was no significant ($p > 0.001$) change in the percentage of lipid peroxidation inhibition between *H. odoratissimum* and ascorbic groups (Fig. 2E). In parallel, an early study by Matrose et al. (2022) reported a strong DPPH free radical scavenging activity of *H. odoratissimum*, with the highest activity observed with acetone extract. The potent antioxidant activity of *H. odoratissimum* could be due to its phytocomponents. For instance, dimethylacetamide has been reported as a good antioxidant agent to prevent oxidative stress during sperm cryopreservation (Mehaisen et al., 2022), while Eucalyptol exhibited a potent antioxidant action in hyperglycemic rats by enhancing the production of endogenous antioxidant enzymes (Peng et al., 2021). In addition, several studies indicate a positive correlation between antioxidant power and high TP and TF contents (Muflihah et al., 2021; Wairata et al., 2022).

3.3. Effect of *H. odoratissimum* on tyrosinase and L-DOPA auto-oxidation

During the process of melanogenesis, the enzyme tyrosinase first catalyzes the crucial step of converting L-tyrosine to L-DOPA, followed by the oxidation of L-DOPA to DOPA-quinone and other substances, leading to the synthesis of melanin (Ohbayashi et al., 2020). Skin lightening products primary target tyrosinase and L-DOPA metabolism, as hyperpigmentation is associated with high tyrosinase activity. Many lotions and creams used as skin lightening agents are usually associated with

various side effects such as itching and acne (Couteau and Coiffard, 2016). Moreover, several toxic agents like hydroquinone and mercury present in some lightning cosmetics can affect the reproductive, immune, digestive, and nervous systems, leading to serious illnesses (Zheng et al., 2023). The development of effective plant-based products with no side effects is attractive. *H. odoratissimum* is commonly used to soften the skin, but there is no scientific proof. Thus, in this study, we evaluated the action of this plant on tyrosinase inhibition (using L-DOPA and L-tyrosine as substrates), and L-DOPA auto-oxidation. From the results obtained, we found that *H. odoratissimum* inhibited tyrosinase and L-DOPA auto-oxidation in a dose-dependent manner. Indeed, the tyrosinase inhibition and L-DOPA auto-oxidation values of *H. odoratissimum* (dose 62.5–2000 µg/mL) were higher than those of ascorbic acid (used as positive control) (Figs. 3A-C). We also noticed that the percentage of tyrosinase inhibition was higher when L-DOPA was used as substrate, compared to the values obtained when tyrosine was used as substrate (Figs. 3A, B). *H. odoratissimum* mainly acts as a tyrosinase cofactor, as L-tyrosine and L-DOPA are known as substrate and cofactor for tyrosinase, respectively. Tyrosinase uses distinct locations for its tyrosine hydroxylase and DOPA oxidase functions, along with a separate third binding site for DOPA (Hearing and Jimenez, 1987). Flavonoids are known as potent tyrosinase inhibitors (El-Nashar et al., 2021). The anti-tyrosinase action of *H. odoratissimum* could be linked to its high TP and TF contents as well as its potent antioxidant power, as previously reported (Park et al., 2015; Zuo et al., 2018).

3.4. *In vitro* anti-diabetic and wound healing effects of *H. odoratissimum*

Skin bleaching has been reported to increase the risk of developing diabetes mellitus, due to the detrimental effects of toxic compounds like hydroquinone and mercury, which can directly increase the capillary glycemia or inhibit Antidiuretic Hormone production at the hypothalamic-pituitary-adrenal system, resulting in the suppression of the synthesis or action of insulin (Papanek and Raff, 1994; Sun and Wang, 2023). Thus, the *in vitro* anti-diabetic potential of *H. odoratissimum* was studied by estimating the α -amylase and α -glucosidase activities. It is well known that the inhibition of these enzymes decreases glucose absorption at the level of intestine (Benrahou et al., 2022). No previous studies on the *in vitro* antidiabetic activity of *H. odoratissimum* have been found in the literature, despite its use in traditional medicine in the treatment of diabetes. In the present study, we found that *H. odoratissimum* exhibited a potent antidiabetic activity *in vitro* by inhibiting the activity of α -amylase and α -glucosidase in a dose-dependent manner. However, acarbose, used as a reference drug showed the highest activity (Fig. 3D, E). This is the first time to report the *in vitro* antidiabetic activity of *H. odoratissimum*, which could support its use as an antidiabetic herb in phytomedicine. Moreover, Ngagi et al. (2015) conducted an *in vivo* study on the effect of this plant on alloxan-induced diabetic mice and found a strong antidiabetic activity at doses 50–150 mg/kg.

In this work, we found that *H. odoratissimum* inhibited the activity of tyrosinase and L-DOPA auto-oxidation, which may justify its use as a skin softener. Skin bleaching is harmful to skin health because it can cause skin burns and/or affect the strength and elasticity of the skin, which may lead to chronic skin wounds (Jeong et al., 2010). Therefore, it was of great interest to evaluate the action of *H.*

odoratissimum on wound healing. HaCat cells are widely used as an experimental model to test the effect of drugs on wound healing (Karatay et al., 2023; Payuhakrit et al., 2024). In all groups, the percentage of wound closure was time-dependent. Compared to the control group, we noticed that *H. odoratissimum* significantly ($p < 0.05 - 0.01$) enhanced the migration of keratinocytes after 12, 24, and 48 hours of treatment. For instance, the wound closure ratio was 29%, 83%, and 100% after 12, 24 and 48 hours of treatment with *H. odoratissimum* (at a dose of 125 $\mu\text{g/mL}$), respectively. At the end of the incubation period (48 hours), *H. odoratissimum* at dose 125 $\mu\text{g/mL}$ was the most active, since 100% of wound closure was recorded. In the control group, 19.45% of the wound area remained open after 48 hours of incubation (Fig. 4A, B). These findings corroborate the cell viability results. Various phytochemicals found in *H. odoratissimum* could be responsible for the wound healing effect of this plant. For instance, it has been reported that *N,N*-dimethylacetamide prevents inflammation through NF- κB pathway (Pekson et al., 2016), which may have a positive impact on skin repair. In addition to its anti-tyrosinase and antidiabetic activity, our finding revealed that *H. odoratissimum* exhibited a potent wound healing activity in HaCat cells. These results support the use of *Helichrysum* species as wound healing enhancer in traditional medicine documented previously (Grčić et al., 2017).

3.5. *In vitro* and *ex vivo* cytotoxicity study of *H. odoratissimum*

In the present work, the cytotoxicity study of *H. odoratissimum* was carried out through cell viability, hemolysis, and HET-CAM irritation assay. After exposing the HaCat cells (normal human keratinocytes) to various concentrations of *H. odoratissimum* for 24 hours, we found that low or moderate concentrations (7.8–250 $\mu\text{g/mL}$) of plant extract increased the cell viability after treatment (Fig. 5A). Several studies have reported the cytotoxicity of this plant on cancer cells. The ethanolic extract of *H. odoratissimum* exhibits hepatoprotective activity and low cytotoxicity in HepG2 cells, but high cytotoxicity in HEK-293, A431, HeLa and A375 cells (Twilley et al., 2017). The protective effect of *H. odoratissimum* against H_2O_2 -induced cytotoxicity in HaCat cells was also tested in this study. *H. odoratissimum* (at all doses) significantly ($p < 0.001$) increased the viability of HaCaT cells, compared to H_2O_2 group (Fig. 5B). This may be attributed to its strong antioxidant potential. We also found that the untreated cells (control) were well organized and had a unique cell shape and size, as reported previously (Rezk et al., 2015; Campos et al., 2022). H_2O_2 negatively affected the morphology of HaCat cells after treatment. Indeed, H_2O_2 -treated cells were shrunk, had irregular contours, and an elongated shape compared to the control. Many apoptotic bodies were also visible. Interestingly, these abnormal features were improved by *H. odoratissimum* (Fig. 5C). This finding may support the folkloric use of this plant for skin repair after skin bleaching. Hemolysis assay using RBCs revealed that *H. odoratissimum* was non-hemolytic at doses 7.8 to 500 $\mu\text{g/mL}$ and hemolytic at doses 1000 to 2000 $\mu\text{g/mL}$, according to ASTM F756-00 rules (Elahi et al., 2014) (Fig. 5D). However, the hemolytic activity of *H. odoratissimum* was significantly ($p < 0.01$) low, compared to the positive control (Triton X-100). Thus, low or moderate doses of *H. odoratissimum* did not affect the RBC membrane stability *in vitro*. The HET-CAM method is a reliable method for assessing the safety of skin products (Pardeike and Müller, 2009). As shown in

Fig. 5E and Table S4, NaOH (0.1M) displayed a strong irritation (presence of vascular damage, hemorrhage, and coagulation), while no signs of toxicity were observed after application of 0.9% NaCl or *H. odoratissimum*.

3.6. Drug likeness and toxicity prediction, and molecular docking

The results of the drug likeness and toxicity prediction of phytochemicals detected in *H. odoratissimum* are presented in Table S5. Compounds 3, 4, 5, 10, 11 and 13 showed low GI absorption, which can be correlated with the absence or low hydrogen bond donor and low permeation potential (Lipinski et al., 2001), while compounds 1, 2, 6, 7, 8, 9 and 12 possessed high GI absorption. However, all compounds met the acceptable ADME standards, according to Lipinski's rules (Lipinski et al., 2001). Log P values of all compounds were ≤ 5 . Compounds with a Log P value of 5 or less exhibit favorable solubility and absorption characteristics, enabling them to readily enter cells and trigger physiological reactions. Compounds with high GI absorption and satisfying all Lipinski's rules (5/5) were selected for molecular docking against tyrosinase (PDB ID: 2Y9X), L-DOPA (PDB ID: 6ON3), α -amylase (PDB ID: 1OSE), and α -glucosidase (PDB: 5NN8) (Fig. 6).

3.7 In silico anti-tyrosinase studies: molecular docking between selected compounds from *H. odoratissimum* against tyrosinase and L-DOPA

Molecular docking is an effective method in computational research to determine the interactions and binding energies between a drug and its target. Tyrosinase and L-DOPA are key compounds involved in the production of melanin by melanocytes and therefore, are the key targets of lightning cosmetics (Ebanks et al., 2009; Gillbro and Olsson, 2011). Hence, in the present study, molecular docking of selected phyto-constituents identified in *H. odoratissimum* against tyrosinase (PDB ID: 2Y9X) and L-DOPA (PDB ID: 6ON3) was investigated to analyse the ligand-protein interaction. As shown in Fig. 7, the binding pattern between mushroom tyrosinase or L-DOPA and each phytochemical varies depending on the ligand structure. In general, all ligands exhibited a good binding energy with the active pocket of the enzymes (Table S6; Fig. 7). For instance, p-Menthone exhibited a strong binding affinity with tyrosinase (-8.76 kcal/mol) and interacted with the active site of protein through 4 Alkyl/Pi-Alkyl bonds and several van der Waals interactions (Fig. 7A). However, Eucalyptol was more stable, due to the presence of 2 conventional hydrogen bonds and several van der Waals and Alkyl/Pi-Alkyl interactions (Fig. 7B). 2-Cyanoacetamide was attached to the binding site of mushroom tyrosinase via 2 conventional hydrogen bonds, while 2(5h)-furanone, 5-methyl- was linked by one conventional hydrogen bond (Fig. 7C-D). Pulegone interacted with tyrosinase via one conventional hydrogen bond with Asn-2029 residue and strong van der Waals and Alkyl/Pi-Alkyl interactions, which can justify its high affinity (-8.39 kcal/mol) (Fig. 7E). As shown in Fig. 7F, p-Menthone interacted with the active pocket of L-DOPA via 2 conventional hydrogen bonds with Arg-640 and Thr-637 residues, with a binding energy of -10.07 kcal/mol. On the other hand, 2(5h)-furanone, 5-methyl and 2-Cyanoacetamide interacted with L-DOPA via one conventional hydrogen bond with the binding affinity of -6.43 kcal/mol and - 5.69 kcal/mol, respectively

(Fig. 7H, I). There were no conventional hydrogen bonds between Eucalyptol, Pulegone and L-DOPA, but these ligands exhibited strong Alkyl/Pi-Alkyl and Van-der Waals interactions with the enzyme (Fig. 7G and J). Overall, among all selected phytochemicals, we found that p-Menthone showed the strongest binding affinity with Tyrosinase (-8.76 kcal/mol) and L-DOPA (-10.07 kcal/mol). This result corroborated the *in silico* anti-tyrosinase activity of p-Menthone described earlier (Bellou et al., 2023). These findings clearly showed that all selected bioactive compounds from *H. odoratissimum* are able to inhibit tyrosinase and L-DOPA activities, which could justify the folkloric use of this plant as a skin smoother.

3.8 *In silico* antidiabetic studies: molecular docking between selected compounds from *H. odoratissimum* against α -amylase and α -glucosidase

The molecular interaction of identified compounds from *H. odoratissimum* against α -amylase is presented in Fig. 8A-F. His-305 residue of α -amylase interacted with p-Menthone or Pulegone through one conventional hydrogen bond (Fig. 8A, E). No conventional hydrogen bonds between Eucalyptol or 2(5h)-furanone, 5-methyl and the active site of α -amylase were detected, but they displayed strong hydrophobic π -alkyl and Van-der Waals interactions, respectively (Fig. 8BC). On the other hand, 2-Cyanoacetamide interacted with Arg-124, Asp-138 and Asn-137 of the enzyme via 3 conventional hydrogen bonds, confirming its best stability (Fig. 8D). The binding affinity of p-Menthone (-8.49 kcal/mol), Eucalyptol (-7.90 kcal/mol) or Pulegone (-8.59 kcal/mol) was higher than that of Acarbose (-7.54 kcal/mol) used as a reference inhibitor, but Acarbose exhibited the highest stability with α -amylase, due to the presence of 4 conventional hydrogen bonds with Thr-376, Asp-375, Asp-456, and Arg-392 residues (Figure A-F; Table S7). Acarbose is widely used as a reference drug for *in vivo*, *in vitro*, and *in silico* antidiabetic assays. The binding strategy of the selected phytochemicals against the binding pocket of α -glucosidase was similar to that of α -amylase (Table S7, Fig. 8). For instance, the binding affinity of p-Menthone (-9.26 kcal/mol), Eucalyptol (-8.61 kcal/mol) and Pulegone (-9.72 kcal/mol) with α -glucosidase was higher than that of Acarbose (-8.54 kcal/mol) (Table S7). Eucalyptol and p-Menthone interacted with the binding site of α -glucosidase via one and two conventional hydrogen bonds, respectively, while 2-Cyanoacetamide showed 3 conventional hydrogen bonds with the enzyme (Fig. 8A-D). During docking analysis, higher negative values indicate strong binding affinity between ligand and protein, suggesting greater effectiveness of the phytochemicals. Thus, Pulegone had the best affinity (-9.72kcal/mol) with α -glucosidase. Pharmacologically, the strong binding interactions between phyto-components and enzymes indicate their ability to inhibit α -amylase and α -glucosidase activities, which corroborates the antidiabetic potential of this plant reported previously (Ngagi et al., 2015). These findings are consistent with previous studies which reported the *in silico* inhibitory action of Eucalyptol (Dar et al., 2024), p-Menthone (Złotek et al., 2020) and Pulegone (El-Omari et al., 2024) against α -amylase and α -glucosidase. Moreover, the inhibitory effect of non-volatile compounds like helichrysetin isolated from *Helichrysum* species has been revealed (Jadalla et al., 2022). These *in silico* results, along with *in vitro* findings offer a solid foundation for testing this plant in experimental animals.

The possible mechanism of action of *H. odoratissimum* is illustrated in Fig. 9. Based on the results obtained, the antioxidant effect of *H. odoratissimum* is linked to its ability to prevent lipid peroxidation

(i). This action could improve mitochondrial fatty acid oxidation (ii) and prevent ROS overproduction (iii), which may finally prevent oxidative stress. *H. odoratissimum* could also act by directly enhancing the antioxidant markers (iv). It is well known that L-DOPA overproduction induces oxidative stress (Stansley and Yamamoto, 2013; Nikolova et al., 2019). Under normal conditions, tyrosinase initiates melanogenesis by stimulating the conversion of L-tyrosine to L-DOPA, which is later transformed into DOPA quinone, leading to the production of melanin through several polymerized reactions (Videira et al., 2013). In the current study, *H. odoratissimum* strongly inhibited tyrosinase and L-DOPA activities (v), which are precursors of melanogenesis, and therefore suggesting the anti-melanogenic action of this plant. However, further *in vivo* and *in vitro* studies are required to quantify melanin synthesis after treatment with this plant. *H. odoratissimum* may also exhibit its antioxidant activity by inhibiting L-DOPA activity, which may enhance the production of antioxidant enzymes and prevent oxidative stress (vi). On the other hand, we found that *H. odoratissimum* exhibited a powerful antidiabetic action through its inhibitory activity on α -amylase and α -glucosidase. Indeed, salivary and pancreatic α -amylase initiate the chemical digestion of carbohydrates. At the level of the small intestine, α -glucosidase breaks polysaccharides into simple absorbable sugars (Bischoff, 1994). *H. odoratissimum* inhibited α -amylase activity, which may reduce the availability of oligosaccharides at the level of intestine (vii). In addition, the inhibition of α -glucosidase activity by this extract may decrease or suppress the amount of simple absorbable sugars, leading to the reduction in blood glucose level (viii). Since α -glucosidase inhibitors used in the treatment of diabetes are known to stimulate insulin secretion at the level of the pancreas (Bischoff, 1995; Kalra 2014), *H. odoratissimum* may also display its antidiabetic action through this pathway (ix). However, more *in vivo* studies are required to clarify this mechanism.

4. Conclusion

H. odoratissimum exhibited a potent antioxidant activity. *In vitro* studies revealed the anti-tyrosinase, anti- α -amylase, anti- α -glucosidase and wound healing activities of *H. odoratissimum*, which were also supported by the *in silico* findings. *H. odoratissimum* improved the viability of HaCat cells and protected the cells against H_2O_2 cytotoxicity. Moreover, *H. odoratissimum* did not cause any cytotoxicity in red blood cells (at doses 7.8 to 500 μ g/mL) and hen's egg-chorioallantoic membrane (at all doses) after treatment. The phyto-components found in *H. odoratissimum* met the acceptable standards for drug-likeness and toxicity prediction. *H. odoratissimum* can be exploited in the development of plant-based anti-tyrosinase, antidiabetic and wound healing agents. This could justify the ethnomedicinal uses of *H. odoratissimum*.

Abbreviations

ABAP: 2,2'-azo-bis(2-amidino propane dihydrochloride, ABTS: (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), ADH: Antidiuretic Hormone, $AlCl_3$: aluminum chloride, CUPRAC: cupric reducing antioxidant capacity, CH_3COOK : potassium acetate, DNS: 3,5-Dinitrosalicylic acid, FBS: fetal bovine serum, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: Ferric-reducing antioxidant power, $FeSO_4$: ferrous

sulfate, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl sulfoxide, GST: glutathione S-transferase, HET-CAM: Hen's Egg Test-Chorioallantoic Membrane, FeCl₃: ferric chloride, NF-κB: Nuclear Factor Kappa B, RBCs: red blood cells, PBS: Phosphate-buffered saline, NH₄CH₃COO: ammonium acetate, ROS: Reactive Oxygen Species, GC-MS: Gas chromatography-mass spectrometry, H₂O₂: hydrogen peroxide, K₂S₂O₃: potassium persulphate, TBARS: thiobarbituric acid reactive substances, SDS: Sodium dodecyl sulfate, TBA: thiobarbituric acid, L-DOPA: 3,4-dihydroxyphenylalanine, TCA: trichloroacetic acid, Na₂CO₃: sodium carbonate, PDB: protein data bank, PNPg: 4-Nitrophenyl-β-D- glucopyranoside, PS: penicillin–streptomycin, TF: total flavonoid, TP: total phenolic.

Declarations

CRedit authorship contribution statement

PBDD, Validation, Visualization, Data curation, Methodology, Investigation, Formal analysis, Software, Writing – original draft. **KH**, Methodology, Investigation, Data curation, Formal analysis, Manuscript writing. **MHW**, Supervision, Data curation, Methodology, Project administration, Validation. Writing – final draft, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Availability of data and material

All data and materials are available upon request.

Acknowledgements

This work was supported by the National Research Foundation of Korea (2022R1A2C2091029; RS-2023-00221241).

References

1. Akaberi M, Sahebkar A, Azizi N, Emami SA (2019) Everlasting flowers: phytochemistry and pharmacology of the genus *Helichrysum*. *Ind Crops Prod* 138:1–21.
<http://dx.doi.org/10.1016/j.indcrop.2019.111471>
2. Alshehri OM, Mahnashi MH, Sadiq A, Zafar R, Jan MS, Ullah F, Alshehri MA, Alshamrani S, Hassan EE (2022) Succinimide derivatives as antioxidant anticholinesterases, anti- α-amylase, and anti- α-glucosidase: *In vitro* and *in silico* approaches. *Evid Based Complement Alternat Med* 2022, 6726438.
<https://doi.org/10.1155/2022/6726438>

3. Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP (2009) Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab* 94(8):2692–2701. <https://doi.org/10.1210/jc.2009-0370>
4. Antoine M, Fatimata L, Jean-Luc P (2005) Systemic complications of the cosmetic use of skin-bleaching products. *Int J Dermatol* 44:37–38. <https://doi.org/10.1111%2Fj.1365-4632.2005.02810.x>
5. Apak R, Güçlü K, Ozyüre M, Oğlu BB, Bener M (2008) Cupric ion reducing antioxidant capacity assay for food antioxidants: vitamins, polyphenolics, and flavonoids in food extracts. *Methods Mol Biol* 477, 163–93. https://doi.org/10.1007/978-1-60327-517-0_14
6. Asekun OT, Grierson DS, Afolayan AJ (2007) Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. *J Appl Sci* 7(7):1005–1008. <https://doi.org/10.3923/jas.2007.1005.1008>
7. Bellou MG, Patila M, Fotiadou R, Spyrou K, Yan F, Rudolf P, Gournis DP, Stamatis H (2023) Tyrosinase magnetic cross-linked enzyme aggregates: Biocatalytic study in deep eutectic solvent aqueous solutions. *Biomolecules* 13(4):643. <https://doi.org/10.3390/biom13040643>
8. Benet LZ, Hosey CM, Ursu O, Oprea TI (2016) BDDCS, the Rule of 5 and drug ability. *Adv Drug Deliv Rev* 101:89–98. <https://doi.org/10.1016/j.addr.2016.05.007>
9. Benrahou K, Mrabti HN, Bouyahya A, Daoudi NE, Bnouham M, Mezzour H, Mahmud S, Alshahrani MM, Obaidullah AJ, Cherrah Y, Faouzi MEA (2022) Inhibition of α -amylase, α -glucosidase, and lipase, intestinal glucose absorption, and antidiabetic properties by extracts of *Erodium guttatum*. *Evid Based Complement Alternat Med* 2022, 5868682. <https://doi.org/10.1155/2022/5868682>
10. Bischoff H (1994) Pharmacology of alpha-glucosidase inhibition. *Eur J Clin Invest* 24(3):3–10
11. Bischoff H (1995) The mechanism of alpha-glucosidase inhibition in the management of diabetes. *Clin Invest Med* 18(4):303–311
12. Blois MS (1958) Antioxidant determinations by the use of a stable free radical. *Nature* 181:1199–1200. <https://www.nature.com/articles/1811199a0>
13. Bwomda P, Sermijn E, Lacor P, Velkeniers B (2005) Glucocorticoid hypertension due to the use of bleaching skin cream, a case report. *Acta Clin Belg* 60(3):146–149. <https://doi.org/10.1179/acb.2005.027>
14. Campos ADS, Silva ACG, Braga PAC, Reyes FGR, Fernandes PM, Botelho PB, Valadares MC, Horst MA (2022) Organic *Curcuma caesia* Roxb. extract induces p21 expression and G0/G1 cell cycle arrest in FaDu oropharyngeal cancer cells. *Nov Tech Nutri Food Sci* 6(4):000645. <http://dx.doi.org/10.31031/NTNF.2022.06.000645>
15. Chen J, Ye Y, Ran M, Li Q, Ruan Z, Jin N (2020) Inhibition of tyrosinase by mercury chloride: spectroscopic and docking studies. *Front Pharmacol* 11:81. <https://doi.org/10.3389%2Ffphar.2020.00081>
16. Choukem SP, Efié DT, Djiogue S, Kaze FF, Mboue-Djieka Y, Boudjeko T, Dongo E, Gautier JF, Kengne AP (2018) Effects of hydroquinone-containing creams on capillary glycemia before and after serial

- hand washings in Africans. PLoS ONE 13(8):e0202271.
<https://doi.org/10.1371/journal.pone.0202271>
17. Couteau C, Coiffard L (2016) Overview of skin whitening agents: Drugs and cosmetic products. *Cosmetics* 3(3):27. <https://doi.org/10.3390/cosmetics3030027>
 18. Dar MA, Siddiqui NA, Mir SR, Akbar S, Mothana RA, Masoodi MH (2024) Anti-diabetic activity-guided isolation of α -amylase and α -glucosidase inhibitory terpenes from *Capsella bursa-pastoris* Linn. *Open Chemistry*, 22, (1), 2024-0025. <https://doi.org/10.1515/chem-2024-0025>
 19. De Canha MN, Komarnytsky S, Langhansova L, Lall N (2020) Exploring the anti-acne potential of impepho [*Helichrysum odoratissimum* (L.) Sweet] to Combat *Cutibacterium acnes* Virulence. *Front Pharmacol* 10:1559. <https://doi.org/10.3389/fphar.2019.01559>
 20. de Souza MM (2008) The concept of skin bleaching in Africa and its devastating health implications. *Clin Dermatol* 26(1):27–29. <https://doi.org/10.1016/j.clindermatol.2007.10.005>
 21. Deeh PBD, Arumugam M, Alagarsamy K, Karanam G, Natesh NS, Watcho P, Vishwakarma V (2022) *Phyllanthus muellerianus* and *Ficus exasperata* exhibit anti-proliferative and pro-apoptotic activities in human prostate cancer PC-3 cells by modulating calcium influx and activating caspases. *Biologia* 77, 1981–1994. <https://doi.org/10.1007/s11756-022-01065-z>
 22. Deeh PBD, Sathiyaseelan A, Naveen KV, Wang MH (2024) Phytochemical analysis and antioxidant potential of *Mondia whitei* and *Guibourtia tessmannii* against H. cells *J Biomol Struct Dyn* 1–15. <https://doi.org/10.1080/07391102.2024.2335299>
 23. Deeh PBD, Watcho P, Wankeu-Nya M, Ngadjui E, Usman UZ (2019) The methanolic extract of *Guibourtia tessmannii* (caesalpiniaceae) and selenium modulate cytosolic calcium accumulation, apoptosis and oxidative stress in R2C tumour Leydig cells: Involvement of TRPV1 channels. *Andrologia* 51(3):e13216. <https://doi.org/10.1111/and.13216>
 24. Ebanks JP, Wickett RR, Boissy RE (2009) Mechanisms regulating skin pigmentation: The rise and fall of complexion coloration. *Int J Mol Sci* 10(9):4066–4087
 25. Elahi MF, Guan G, Wang L (2014) Hemocompatibility of surface modified silk fibroin materials: a review. *Rev Adv Mater Sci* 38:148–159
 26. El-Nashar HAS, El-Din MIG, Hritcu L, Eldahshan OA (2021) Insights on the inhibitory power of flavonoids on tyrosinase activity: a survey from 2016 to 2021. *Molecules* 26(24):7546. <https://doi.org/10.3390/molecules26247546>
 27. El-Omari N, Chamkhi I, Bakrim S, Aanniz T, Benali T, Akhazzane M, Ullah R, Alotaibi A, Bari A, Elhrech H, Zengin G, Bouyahya A (2024) Biological properties of *Mentha viridis* L. essential oil and its main monoterpene constituents. *Chem Biodivers* 21(9):e202401209. <https://doi.org/10.1002/cbdv.202401209>
 28. Fawole OA, Makunga NP, Opara UL (2012) Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complement Altern Med* 12:200. <https://doi.org/10.1186/1472-6882-12-200>

29. Gao X, Xue Z, Ma Q, Guo Q, Xing L, Santhanam RK, Zhang M, Chen H (2020) Antioxidant and antihypertensive effects of garlic protein and its hydrolysates and the related mechanism. J Food Biochem 44:e13126. <https://doi.org/10.1111/jfbc.13126>
30. Gillbro JM, Olsson MJ (2011) The melanogenesis and mechanisms of skin-lightening agents-existing and new approaches. Int J Cosmet Sci 33(3):210–221. <https://doi.org/10.1111/j.1468-2494.2010.00616.x>
31. Grčić N, Esch S, Hensel A, Dias ACP (2017) Assessment of *Helichrysum sp.* extracts on *in vitro* keratinocyte proliferation and differentiation: potential use of plants for improved wound healing. Zeitschrift für Phytotherapie 38(S01), S1-S44. 10.1055/s-0037-1607159
32. Hearing VJ, Jimenez M (1987) Mammalian tyrosinase—The critical regulatory control point in melanocyte pigmentation. Int J Biochem 19:1141–1147. [https://doi.org/10.1016/0020-711x\(87\)90095-4](https://doi.org/10.1016/0020-711x(87)90095-4)
33. Hernández BC, Rives IM, Ruiz AB, Cazaña TG (2024) Pigmentation disorders as a guide sign of autoimmune diseases. Reumatol Clin (Engl Ed) 20(1):45–46. <https://doi.org/10.1016/j.reumae.2023.12.003>
34. Hutchings A, Scott AH, Lewis G, Cunningham AB (1996) Zulu medicinal plants: An inventory. University of Natal, Pietermaritzburg, South Africa
35. Hwang JH, Lee BM (2007) Inhibitory effects of plant extracts on tyrosinase, L-DOPA oxidation, and melanin synthesis. J Toxicol Environ Health A 70(5):393–407. <https://doi.org/10.1080/10937400600882871>
36. Jadalla BMIS, Moser JJ, Sharma R, Etsassala NGER, Egieyeh SA, Badmus JA, Marnewick JL, Beukes D, Cupido CN, Hussein AA (2022) *In Vitro* alpha-glucosidase and alpha-amylase inhibitory activities and antioxidant capacity of *Helichrysum cymosum* and *Helichrysum pandurifolium* Schrank Constituents. Separations 9(8), 190; <https://doi.org/10.3390/separations9080190>
37. Jeong MS, Lee CM, Jeong WJ, Kim SJ, Lee KY (2010) Significant damage of the skin and hair following hair bleaching. J Dermatol 37(10):882–887. <https://doi.org/10.1111/j.1346-8138.2010.00916.x>
38. Jin Y, Lu Y, Sathiyaseelan A, Zhang X, Naveen KV, Wang MH (2023) A facile green synthesis of multifunctional silver nanoparticles using *Paeonia suffruticosa* seed extracts. Mater Lett 348:134653. <https://doi.org/10.1016/j.matlet.2023.134653>
39. Kalra S (2014) Alpha glucosidase inhibitors. J Pak Med Assoc 64(4):474–476
40. Karatay KB, Muftuler FZB, Law B, Aras O (2023) Methanolic extract of *Kigelia africana* and wound healing: an *in vitro* study. J Wound Care 32(6):392–398. <https://doi.org/10.12968/jowc.2023.32.6.392>
41. Klomsakul P, Aiumsubtub A, Chalopagorn P (2022) Evaluation of antioxidant activities and tyrosinase inhibitory effects of *Ginkgo biloba* tea extract. Sci. World J 2022, 4806889. <https://doi.org/10.1155/2022/4806889>

42. Legoalea PB, Mashimbyeb MJ, van Rec T (2013) Antiinflammatory and antioxidant flavonoids from *Helichrysum kraussii* and *H. odoratissimum* flowers. *Nat Prod Commun* 8(10):1403–1404
43. Li Z, Saravanakumar K, Yao L, Kim Y, Choi SY, Yoo G, Keon K, Lee CM, Youn B, Lee D, Cho N (2024) *Acer tegmentosum* extract-mediated silver nanoparticles loaded chitosan/alginate acid scaffolds enhance healing of *E. coli*-infected wounds. *Int J Biol Macromol* 267(Pt 2):131389. <https://doi.org/10.1016/j.ijbiomac.2024.131389>
44. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 46(1–3):3–26. [https://doi.org/10.1016/s0169-409x\(00\)00129-0](https://doi.org/10.1016/s0169-409x(00)00129-0)
45. Matrose NA, Belay ZA, Obikeze K, Mokwena L, Caleb OJ (2022) Bioprospecting of *Helichrysum* Species: Chemical profile, phytochemical properties, and antifungal efficacy against *Botrytis cinerea*. *Plants (Basel)* 12(1):58. <https://doi.org/10.3390/plants12010058>
46. Matrose NA, Obikeze K, Belay ZA, Caleb OJ (2021) Impact of spatial variation and extraction solvents on bioactive compounds, secondary metabolites and antifungal efficacy of South African *Impepho* [*Helichrysum odoratissimum* (L.) Sweet]. *Food Biosci* 42:101139. <https://doi.org/10.1016/j.fbio.2021.101139>
47. Mehaisen GMK, Elomda AM, Hamad SK, Ghaly MM, Sun Y, Li Y, Zong Y, Chen J, Partyka A, Nazmi A, Abbas AO, Stino FKR (2022) Effect of dimethylacetamide concentration on motility, quality, antioxidant biomarkers, anti-freeze gene expression, and fertilizing ability of frozen/thawed rooster sperm. *Anim (Basel)* 12(20):2739. <https://doi.org/10.3390/ani12202739>
48. Mehrabani M, Lotfian SM, Amirkhosravi A, Farhadi S, Vasei S, Raeiszadeh M, Mehrabani M (2023) The influence of harvest time on total phenolic and flavonoid contents, antioxidant, antibacterial and cytotoxicity of *Rheum khorasanicum* root extract. *Ann Pharm Fr* 81(3):475–483. <https://doi.org/10.1016/j.pharma.2022.11.010>
49. Muflihah YM, Gollavelli G, Ling YC (2021) Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 Indonesian indigenous herbs. *Antioxid (Basel)* 10(10):1530. <https://doi.org/10.3390/antiox10101530>
50. Naveen KV, Saravanakumar K, Sathiyaseelan A, Wang MH (2023) Comparative analysis of the antioxidant, antidiabetic, antibacterial, cytoprotective potential and metabolite profile of two endophytic *Penicillium* spp. *Antioxid (Basel)* 12:248. <https://doi.org/10.3390/antiox12020248>
51. Ngagi JM, Ngugi MP, Kibiti CM, Ngeranwa J, Njue W, Gathumbi P, Njagi E (2015) Hypoglycemic effect of *Helichrysum odoratissimum* in alloxan induced diabetic mice. *J Pharmacol* 4:30–33
52. Nikolova G, Karamalakova Y, Gadjeva V (2019) Reducing oxidative toxicity of L-dopa in combination with two different antioxidants: an essential oil isolated from *Rosa Damascena* Mill., and vitamin C. *Toxicol Rep* 6:267–271. <https://doi.org/10.1016/j.toxrep.2019.03.006>
53. Ohbayashi N, Fukuda M (2020) Recent advances in understanding the molecular basis of melanogenesis in melanocytes. *F1000Res* 9, F1000. <https://doi.org/10.12688/f1000research.24625.1>. Faculty Rev-608

54. Okoye O (2019) Skin lightening and chronic kidney disease: A Tale or A Fact? *Trop J Nephro* 14(1):15–23
55. Papanek PE, Raff H (1994) Chronic physiological increases in cortisol inhibit the vasopressin response to hypertonicity in conscious dogs. *Am J Physiol* 267(5 Pt 2):R1342–R1349. <https://doi.org/10.1152/ajpregu.1994.267.5.r1342>
56. Pardeike J, Müller RH (2009) Dermal and ocular safety of the new phospholipase A2 inhibitors PX-18 and PX-13 formulated as drug nanosuspension. *J Biomed Nanotechnol* 5(4):437–444. <https://doi.org/10.1166/jbn.2009.1054>
57. Park KM, Kwon KM, Lee SH (2015) Evaluation of the antioxidant activities and tyrosinase inhibitory property from mycelium culture extracts. *Evid Based Complement Alternat Med* 2015, 616298. <https://doi.org/10.1155%2F2015%2F616298>
58. Payuhakrit W, Panpinyaporn P, Khumsri W, Yusakul G, Praphasawat R, Nuengchamnong N et al (2024) Enhancing chronic wound healing with Thai indigenous rice variety, Kaab Dum: Exploring ER stress and senescence inhibition in HaCaT keratinocyte cell line. *PLoS ONE* 19(5):e0302662. <https://doi.org/10.1371/journal.pone.0302662>
59. Pekson R, Poltoratsky V, Gorasiya S, Sundaram S, Ashby CR, Vancurova I et al (2016) N,N-dimethylacetamide significantly attenuates LPS- and TNF α -induced proinflammatory responses via inhibition of the nuclear factor kappa B pathway. *Mol Med* 22:747–758. <https://doi.org/10.2119/molmed.2016.00017>
60. Peng J, Jiang Z, Wu G, Cai Z, Du Q, Tao L, Zhang Y, Chen Y, Shen X (2021) Improving protection effects of eucalyptol via carboxymethyl chitosan-coated lipid nanoparticles on hyperglycaemia-induced vascular endothelial injury in rats. *J Drug Target* 29(5):520–530. <https://doi.org/10.1080/1061186x.2020.1859514>
61. Phan K, Smith SD (2021) Topical corticosteroids and risk of diabetes mellitus: systematic review and meta-analysis. *J Dermatolog Treat* 32(3):345–349. <https://doi.org/10.1080/09546634.2019.1657224>
62. Rezk A, Al-Hashimi A, John W, Schepker H, Ullrich MS, Brix K (2015) Assessment of cytotoxicity exerted by leaf extracts from plants of the genus *Rhododendron* towards epidermal keratinocytes and intestine epithelial cells. *BMC Complement Altern Med* 15:364. <https://doi.org/10.1186/s12906-015-0860-8>
63. Roberto G, Baratta MT (2000) Antioxidant activity of selected essential oil components in two lipid model system. *Food Chem* 69:167–174. [https://doi.org/10.1016/S0308-8146\(99\)00247-2](https://doi.org/10.1016/S0308-8146(99)00247-2)
64. Samson N, Fink B, Matts PJ (2010) Visible skin condition and perception of human facial appearance. *Int J Cosmet Sci* 32(3):167–184. <https://doi.org/10.1111/j.1468-2494.2009.00535.x>
65. Saravanakumar K, Li Z, Kim Y, Park S, Keon K, Lee CM, Ahn G, Cho N (2024) Fucoïdan-coated cotton dressing functionalized with biomolecules capped silver nanoparticles (LB-Ag NPs-FN-OCG) for rapid healing therapy of infected wounds. *Environ Res* 246:118004. <https://doi.org/10.1016/j.envres.2023.118004>

66. Saravanakumar K, Park S, Sathiyaseelan A, Kim KN, Cho SH, Mariadoss AVA, Wang MH (2021) Metabolite profiling of methanolic extract of *Gardenia jaminoides* by LC-MS/MS and GC-MS and its anti-diabetic, and anti-oxidant activities. *Pharmaceuticals (Basel)* 14:102. <https://doi.org/10.3390/ph14020102>
67. Sathiyaseelan A, Saravanakumar K, Mariadoss AVA, Kim KM, Wang MH (2021) Antibacterial activity of ethyl acetate extract of endophytic fungus (*Paraconiothyrium brasiliense*) through targeting dihydropteroate synthase (DHPS). *Process Biochem* 111:27–35. <https://doi.org/10.1016/j.procbio.2021.10.010>
68. Serabele K, Chen W, Tankeu S, Combrinck S, Veale CGL, van Vuuren S, Chaudhary SK, Viljoen A (2021) Comparative chemical profiling and antimicrobial activity of two interchangeably used 'Imphepho' species (*Helichrysum odoratissimum* and *Helichrysum petiolare*). *S Afr J Bot* 137:117–132. <https://doi.org/10.1016/j.sajb.2020.09.023>
69. Stansley BJ, Yamamoto BK (2013) L-dopa-induced dopamine synthesis and oxidative stress in serotonergic cells. *Neuropharmacology* 67:243–251. <https://doi.org/10.1016/j.neuropharm.2012.11.010>
70. Sun S, Wang Y (2023) Relationship between cortisol and diabetic microvascular complications: a retrospective study. *Eur J Med Res* 28(1):391. <https://doi.org/10.1186/s40001-023-01325-x>
71. Swami V, Furnham A, Joshi K (2008) The influence of skin tone, hair length, and hair colour on ratings of women's physical attractiveness, health and fertility. *Scand J Psychol* 49(5):429–437. <https://doi.org/10.1111/j.1467-9450.2008.00651.x>
72. Thawabteh AM, Jibreen A, Karaman D, Thawabteh A, Karaman R (2023) Skin pigmentation types, causes and treatment-a review. *Molecules* 28(12):4839. <https://doi.org/10.3390/molecules28124839>
73. Twilley D, Kishore N, Meyer D, Moodley I, Kumar V, Lall N (2017) The effect of *Helichrysum odoratissimum* (L.) sweet on cancer cell proliferation and cytokine production. *Int J Pharmacogn Phytochem Res* 9(5):621–631. <http://hdl.handle.net/2263/66287>
74. Videira IFDS, Moura DFL, Magina S (2013) Mechanisms regulating melanogenesis. *Bras Dermatol* 88(1):76–83. <https://doi.org/10.1590/s0365-05962013000100009>
75. Wairata J, Fadlan A, Purnomo AS, Taher M (2022) Total phenolic and flavonoid contents, antioxidant, antidiabetic and antiplasmodial activities of *Garcinia forbesii* King: A correlation study. *Arab J Chem* 15(2):103541. <https://doi.org/10.1016/j.arabjc.2021.103541>
76. Watcho P, Mpeck IR, Defo PBD, Wankeu-Nya M, Ngadjui E, Fozin GRB, Kamtchouing P, Kamanyi A (2019) Cyclophosphamide-induced reproductive toxicity: Beneficial effects of *Helichrysum odoratissimum* (Asteraceae) in male Wistar rats. *J Integr Med* 17(5):366–373. <https://doi.org/10.1016/j.joim.2019.07.002>
77. Yao L, Caffin N, D'Arcy B, Jiang Y, Shi J, Singanusong R et al (2005) Seasonal variations of phenolic compounds in Australia-grown tea (*Camellia sinensis*). *J Agric Food Chem* 53:6477–6483. <https://doi.org/10.1021/jf050382y>

78. Zantanta N, Kambizi L, Etsassala NGER, Nchu F (2022) Comparing crop yield, secondary metabolite contents, and antifungal activity of extracts of *Helichrysum odoratissimum* cultivated in aquaponic, hydroponic, and field systems. *Plants (Basel)* 11(20):2696. <https://doi.org/10.3390/plants11202696>
79. Zheng Y, Du X, Zhang L, Jia T, Zhang H, Peng B, Hao Y, Tong Z, Che D, Geng S (2023) Hydroquinone-induced skin irritant reaction could be achieved by activating mast cells via mas-related G protein-coupled receptor X2. *Exp Dermatol* 32(4):436–446. <https://doi.org/10.1111/exd.14723>
80. Złotek U, Rybczyńska-Tkaczyk K, Michalak-Majewska M, Sikora M, Jakubczyk A (2020) Potential acetylcholinesterase, lipase, α -glucosidase, and α -amylase inhibitory activity, as well as antimicrobial activities, of essential oil from Lettuce leaf basil (*Ocimum basilicum* L.) elicited with jasmonic acid. *Appl Sci* 10(12):4315. <https://doi.org/10.3390/app10124315>
81. Zuo AR, Dong HH, Yu YY, Shu QL, Zheng LX, Yu XY, Cao SW (2018) The antityrosinase and antioxidant activities of flavonoids dominated by the number and location of phenolic hydroxyl groups. *Chin Med* 13:51. <https://doi.org/10.1186/s13020-018-0206-9>

Figures

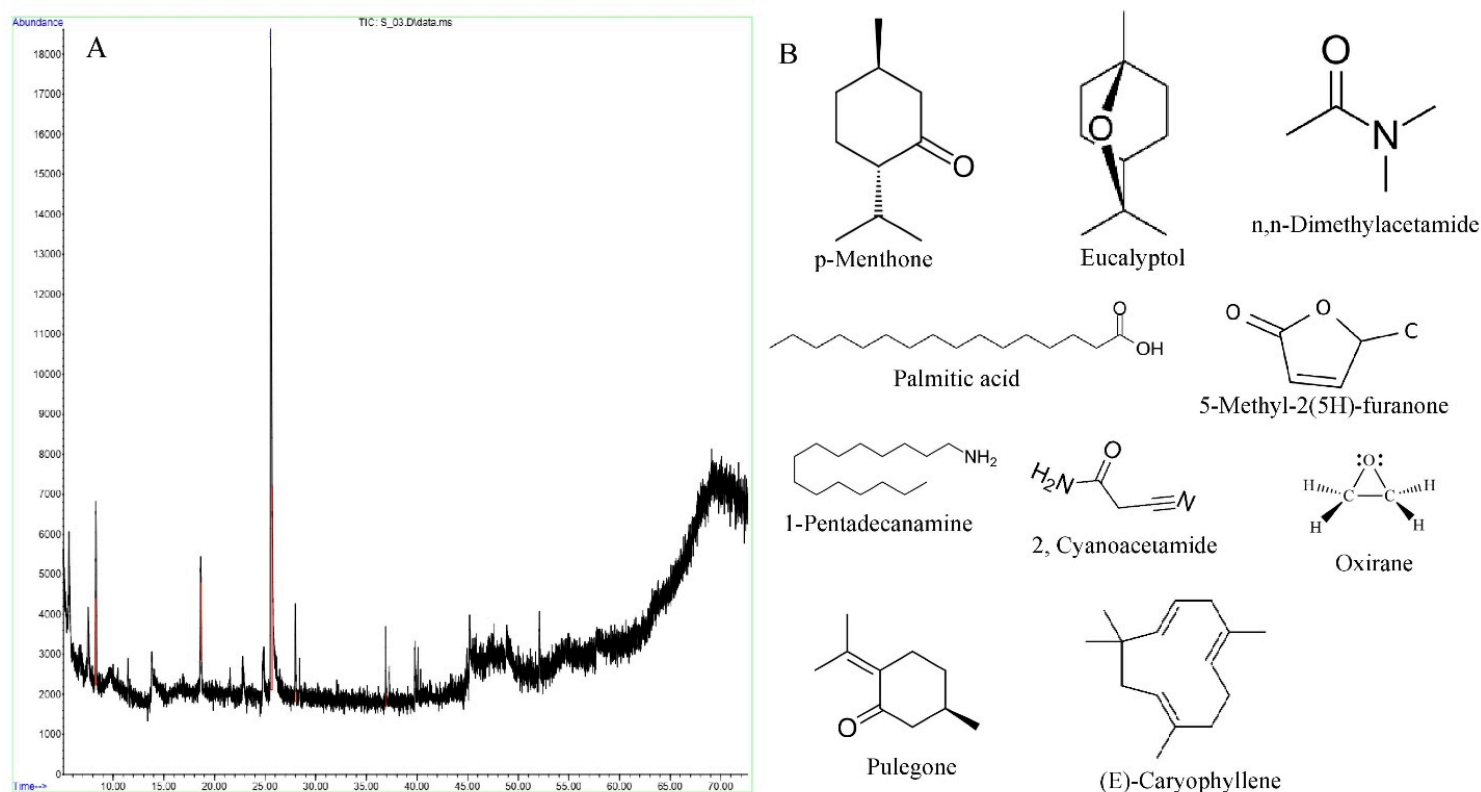


Figure 1

GC-MS analysis of *H. odoratissimum*. GC–MS chromatogram graph **(A)** and structure of some selected compounds detected in *H. odoratissimum* **(B)**.

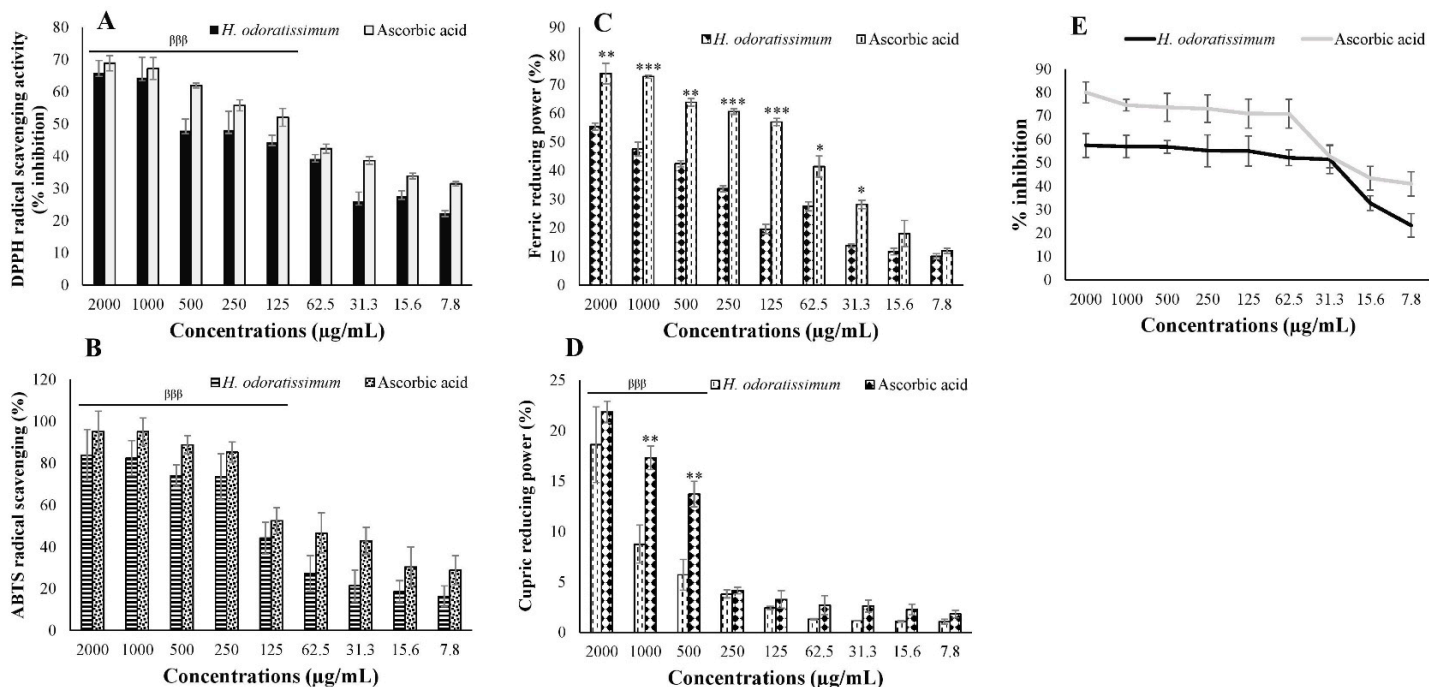


Figure 2

Antioxidant activity of *H. odoratissimum*. (A) DPPH and (B) ABTS free radical scavenging activity; (C) Cupric and (D) Ferric reducing power; (E) inhibition of lipid peroxidation. The DPPH and ABTS⁺ free radical scavenging activity, as well as CUPRAC and FRAP of *H. odoratissimum* were increased in a concentration-dependent manner. Indeed, the DPPH and ABTS⁺ values at doses 125-2000 µg/mL were significantly ($p<0.001$) increased, compared to the lowest dose (7.8 µg/mL). At doses 500-2000 µg/mL, we also noticed a significant ($p<0.001$) improvement in CUPRAC of the plant extract, compared with dose 7.8 µg/mL. The FRAP (at doses 31.3-2000 µg/mL) and CUPRAC (at doses 500 and 1000 µg/mL) of ascorbic acid were significantly ($p<0.05-0.001$) elevated, compared with that of *H. odoratissimum*. In the lipid peroxidation analysis, egg yolk homogenate was used as lipid-rich media and the ability of plant extracts to inhibit lipid peroxidation was evaluated using TBA reactive substances (TBARS) assay. *H. odoratissimum* and ascorbic acid inhibited lipid peroxidation in a dose-dependent manner. Experiments were performed in triplicate. Each bar represents the mean $\pm$ SEM. Data represent mean $\pm$ standard error of the mean (SEM). * $p<0.05$; ** $p<0.01$; *** $p<0.001$: compared to AA. $\beta\beta$ $p<0.01$; $\beta\beta\beta$ $p<0.001$: compared to dose 7.8 µg/mL.

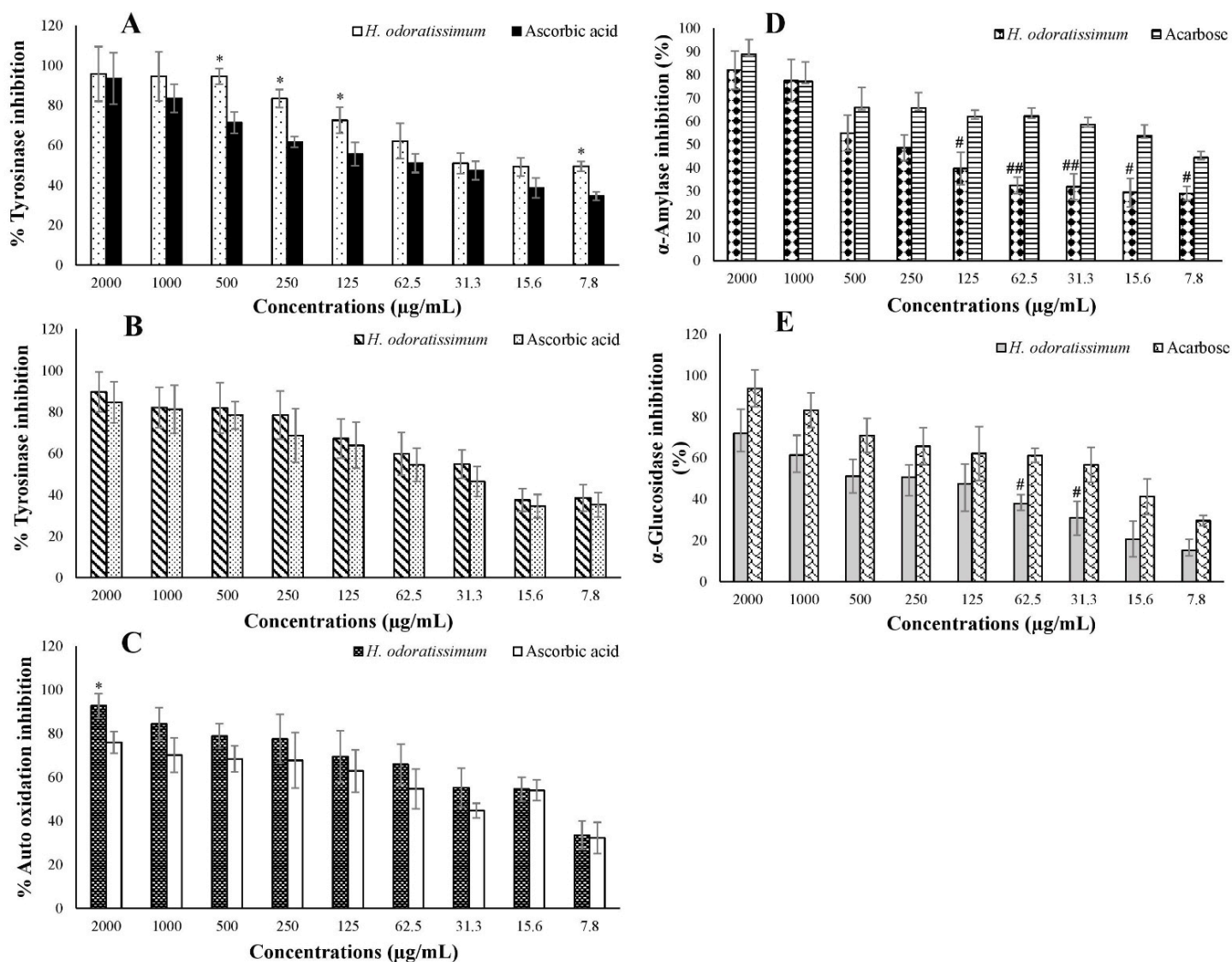


Figure 3

Anti-tyrosinase and antidiabetic effects of *H. odoratissimum*. **A** and **B** represent the tyrosinase inhibitory effect using L-DOPA or tyrosine as substrate, respectively. Various concentrations (7.8-2000 µg/mL) of *H. odoratissimum* and ascorbic acid were mixed with 30 µL of mushroom tyrosinase (333 units/mL). Then, 110 µL of L-DOPA (12 mM) or tyrosine (2 mM) was added, and the absorbance of the mixture was recorded at 490 nm. **C** represents the anti-L-DOPA auto-oxidation activity. Various concentrations (7.8-2000 µg/mL) of *H. odoratissimum* and ascorbic acid were mixed 500 µL of L-DOPA (12 mM) and the absorbance (at 475 nm.) was measured after 48 hours of incubation at 30°C. *H. odoratissimum* exhibited a potent anti-tyrosinase activity. **C** and **D** represent the effects of *H. odoratissimum* on α-amylase, and anti-α-glucosidase inhibitions, respectively. A concentration-dependent effect was recorded. Experiments were performed in triplicate. Each bar represents the mean ± SEM. *p<0.05: compared to AA. #p<0.05; ##p<0.01: compared to acarbose.

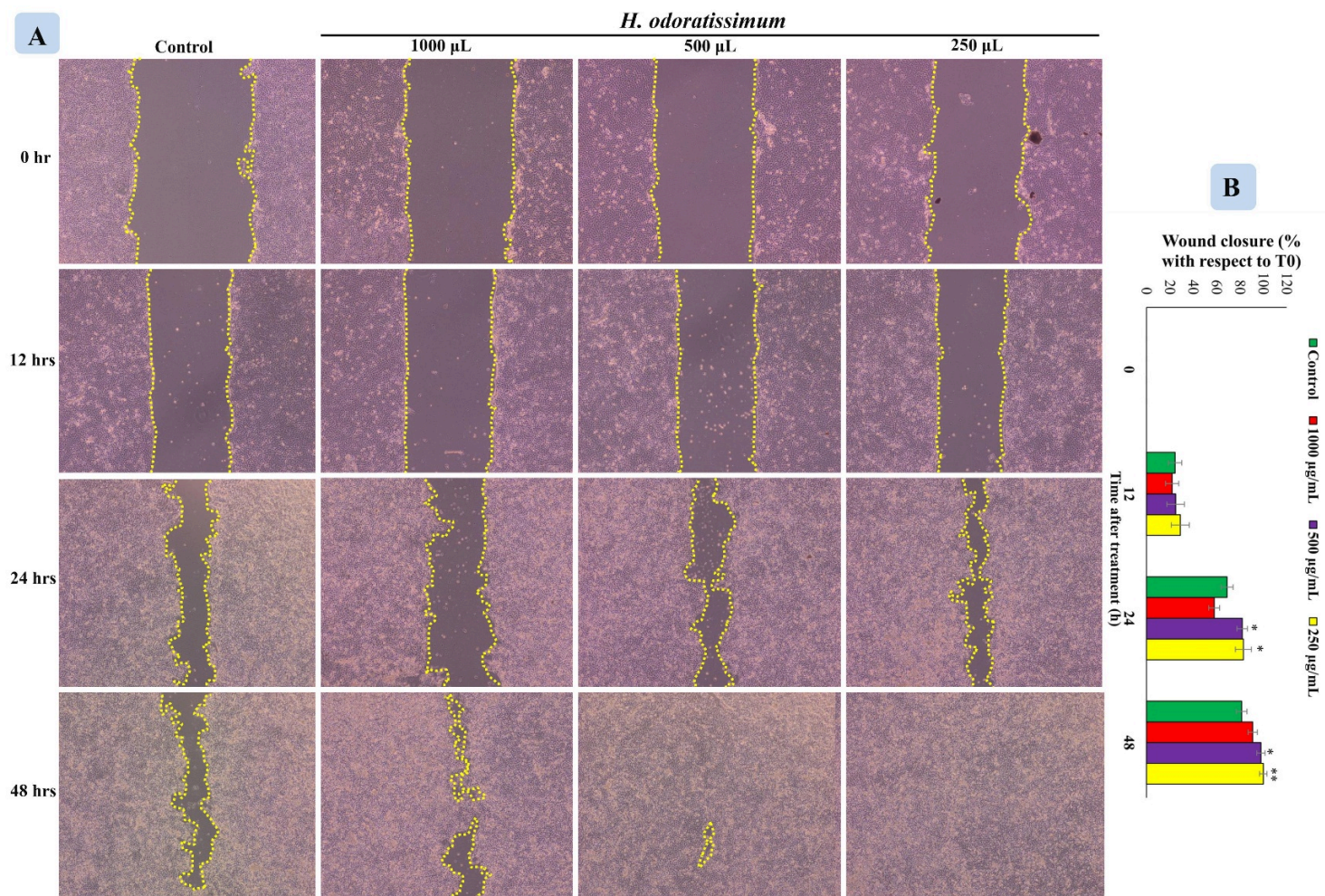


Figure 4

Wound healing effect of *H. odoratissimum*. (A): HaCaT images were captured at 0, 12, and 24 hours. (B): The percentage of the scratch area in HaCaT cells control and treated with *H. odoratissimum* at times 0, 12, 24, and 48 h. The HaCaT cells were incubated in the absence or presence of various concentrations of plant extract, and images were captured at 0, 12, and 24 hours. In all groups, the percentage of wound closure was time-dependent. Compared to the control group, we noticed that *H. odoratissimum* enhanced the migration of keratinocytes after 12, 24, and 48 hours of treatment. At the end of the incubation period (48 hours), *H. odoratissimum* at dose 250 μ g/mL was the most active, since 100% of wound closure was recorded. * $p < 0.05$; ** $p < 0.01$: compared with control.

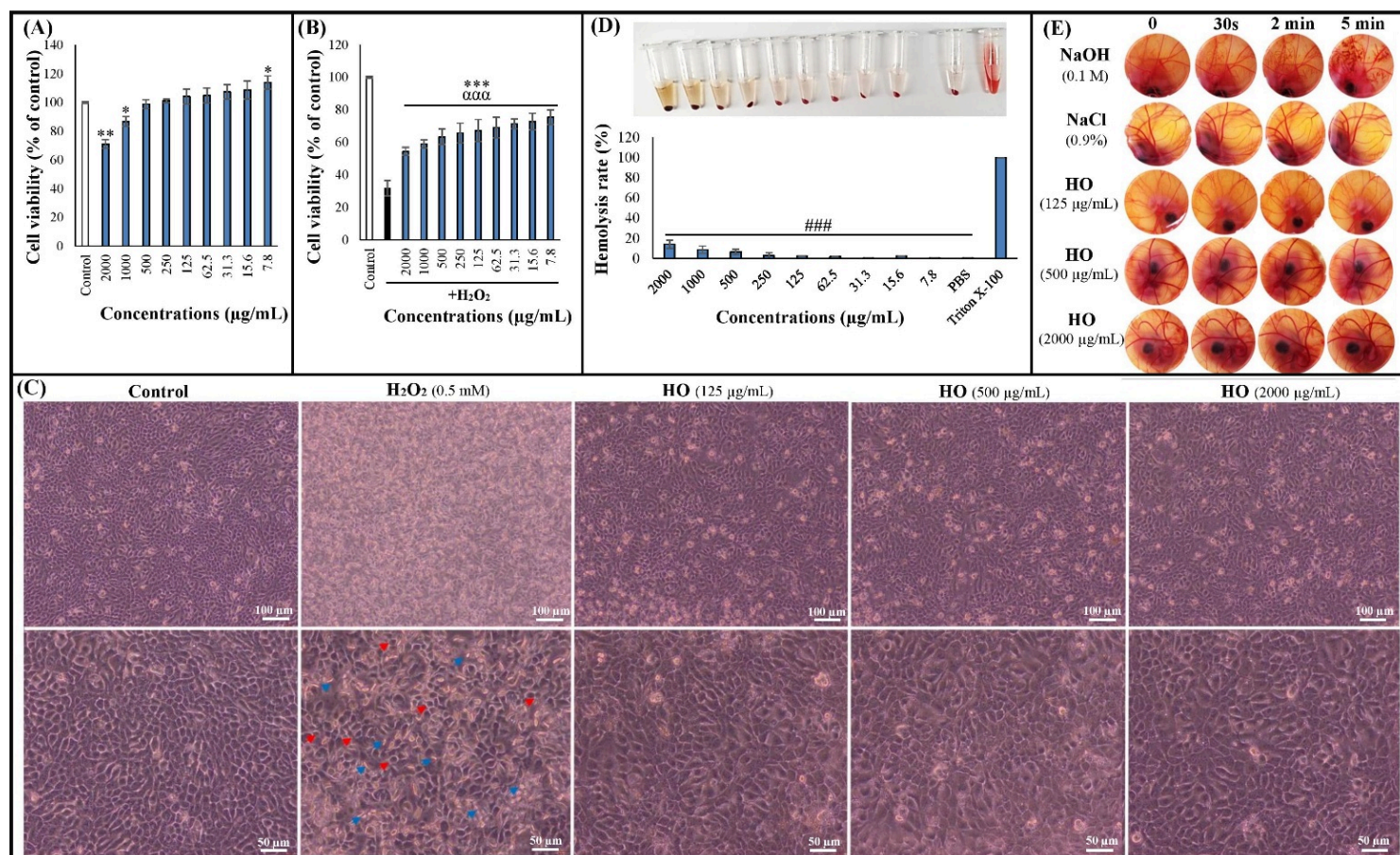


Figure 5

Cytotoxicity of *H. odoratissimum*. Cells viability (A, B), cell morphology (C), hemolytic activity (D, E) and HET-CAM irritation test (F) of various concentrations of aqueous extract of *H. odoratissimum*. In the cell viability experiment, HaCaT cells were exposed to serial concentrations (7.8-2000 µg/mL) of *H. odoratissimum*, and cell viability was estimated by MTT after 24 hours. In the absence of H₂O₂, low or moderate concentrations (7.8-250 µg/mL) of plant extract increase the cell viability after treatment. Moreover, *H. odoratissimum* (at all doses) significantly increased ($p < 0.001$) the viability of HaCaT cells, compared to H₂O₂ group. In the cell morphology essay, the control cells were well organized and had a unique cell shape and size. H₂O₂-treated cells were shrunk, had irregular contours, and an elongated shape compared to the control. Many apoptotic bodies were also visible. Interestingly, these abnormal features were improved by *H. odoratissimum*. Red and blue arrows indicate shrunk cells and apoptotic bodies, respectively. Hemolysis assay was carried out by treating RBCs with different concentrations of plant extract, PBS (negative control) and triton X-100 (positive control), and the hemolytic activity was measured. The photographs and percentage of hemolysis are presented in figure D. *H. odoratissimum* was non hemolytic at doses (7.8-500 µg/mL) and hemolytic at doses (1000 and 2000 µg/mL). The HET-CAM irritation test was conducted using fertilized eggs, as described in the materials and methods section. 200 µL of each concentration of *H. odoratissimum*, 0.9% NaCl (negative control), or 0.1M NaOH (positive control) was applied to the membrane, and toxicity-related parameters such as hemorrhage, vascular damage, and coagulation were observed at 0s (before treatment), 30 s, 2 min and 5 min. NaOH

(0.1M) displayed a strong irritation, while no sign of toxicity was observed after 0.9% NaCl or *H. odoratissimum* application. Experiments were performed in triplicate. Each bar represents the mean $\pm$ SEM. H₂O₂: Hydrogen peroxide; HO: *H. odoratissimum*. ###p<0.001: compared to TritonX-100. *p<0.05; ***p<0.01; ***p<0.001: compared to control. ^{aaa}p<0.001: compared to H₂O₂.

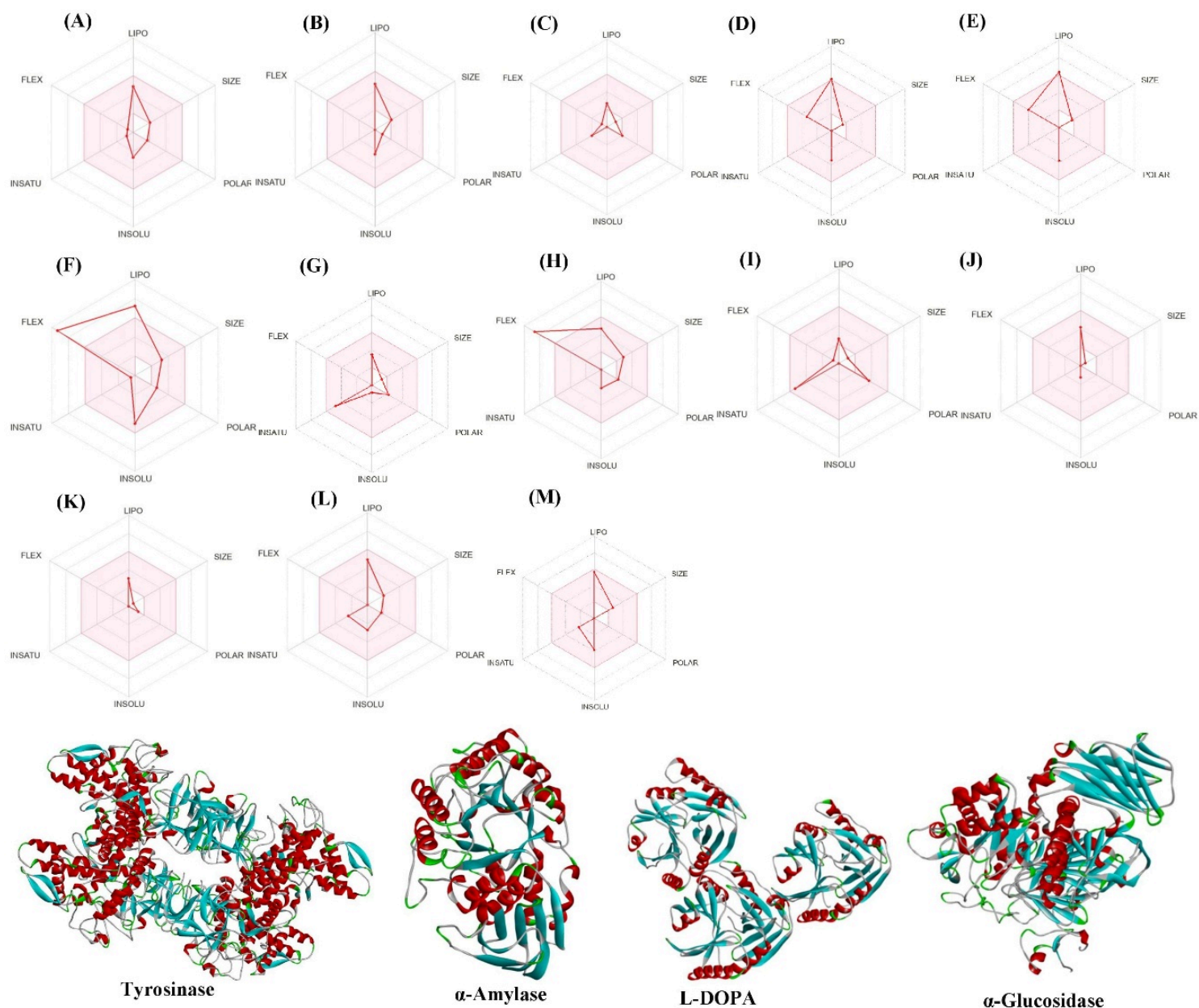


Figure 6

Bioavailability radar of selected compounds from *odoratissimum* and 3D structure of the target proteins.

(A): p-Menthone; (B): Eucalyptol; (C): n,n-Dimethylacetamide; (D): Octane; (E): Nonane; (F): Palmitic acid; (G): 2(5h)-furanone, 5-methyl-; (H): 1-Pentadecanamine; (I): 2-Cyanoacetamide; (J): Propane-2-13c; (K): Oxirane; (L): Pulegone; (M): (E)-Caryophyllene. Drug likeness and toxicity prediction of all phytocompounds identified in the aqueous extract of *H. odoratissimum* were performed. Log P values of all compounds were consistent with Lipinski's norms. Compounds with high GI absorption and satisfying

all Lipinski's rules (5/5) rules were selected for molecular docking against diabetes-related enzymes (α -amylase, PDB ID: 10SE and α -Glucosidase, PDB ID: 5NN4) and melanin-related markers (Tyrosinase, PDB ID: 2Y9X and L-DOPA, PDB ID: 6ON3).

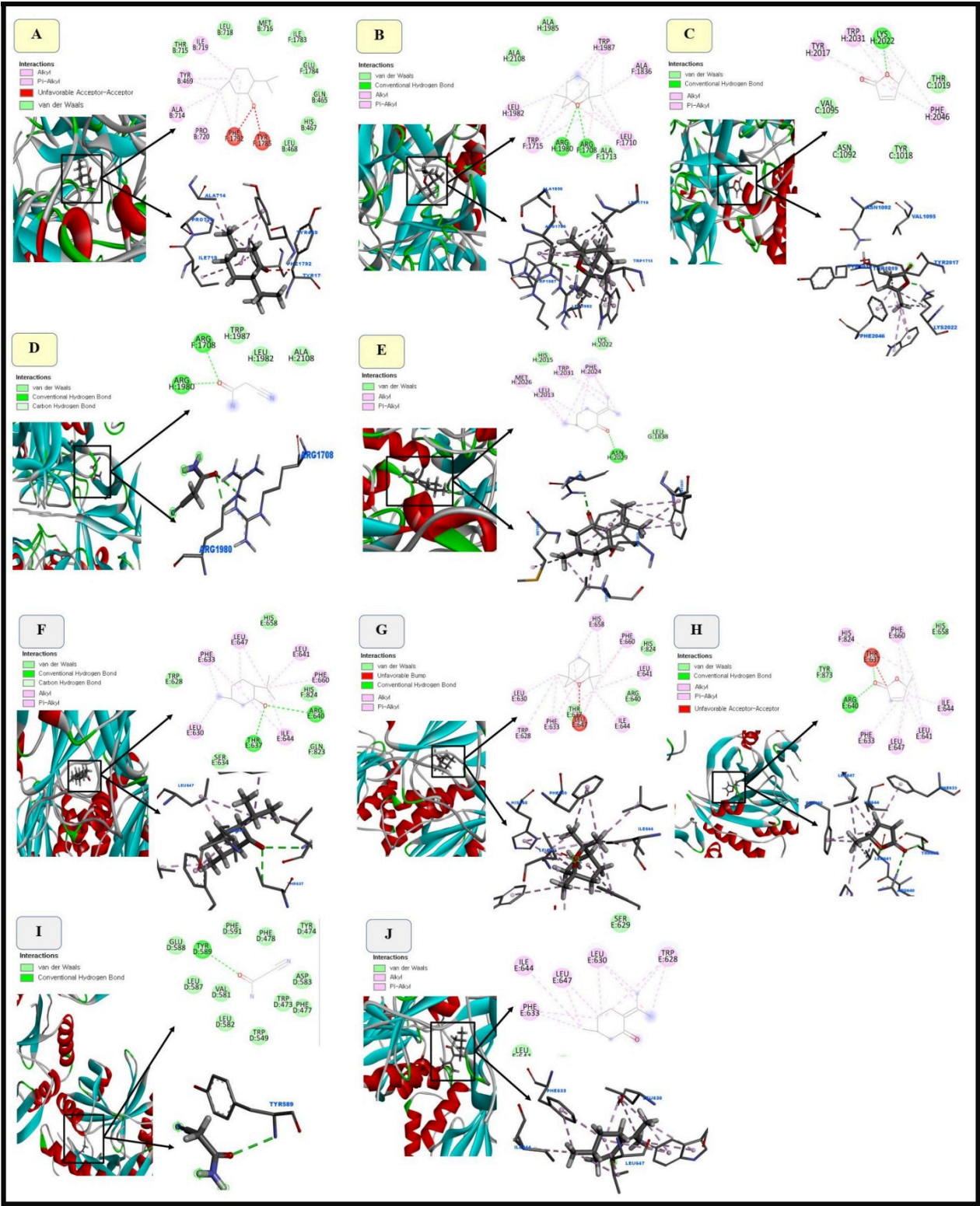


Figure 7
3D and 2D diagrams of molecular docking between selected compounds from *H. odoratissimum* and tyrosinase (A-E) or L-DOPA (F-J). A: p-Menthone interacting with the active site of tyrosinase through 4

Alkyl/Pi-Alkyl bonds and several van der Waals interactions; **B**: Eucalyptol interacting with tyrosinase through 2 conventional hydrogen bonds and several van der Waals and Alkyl/Pi-Alkyl interactions; **C**: Docking results of 2(5h)-furanone, 5-methyl interacting with tyrosinase via one conventional hydrogen bond and several van der Waals and Alkyl/Pi-Alkyl interactions; **D**: 2-Cyanoacetamide interacting with tyrosinase via 2 conventional hydrogen bonds; **E**: Docking results of Pulegone interacting with tyrosinase via one conventional hydrogen bond with Asn-2029 residue; **F**: Binding poses of p-Menthone interacting with L-DOPA showing two conventional hydrogen bonds; **G**: Docking results of Eucalyptol interacting with L-DOPA showing a strong Alkyl/Pi-Alkyl interaction; **H**: Docking results of 2(5h)-furanone, 5-methyl interacting with L-DOPA showing 2 conventional hydrogen bonds with Arg-640 and Thr-637 residues; **I**: Binding poses of 2-Cyanoacetamide interacting with L-DOPA via one conventional hydrogen bond and a strong van der Waals interaction; **J**: Molecular interaction between Pulegone and L-DOPA showing various van der Waals and Alkyl/Pi-Alkyl interactions.

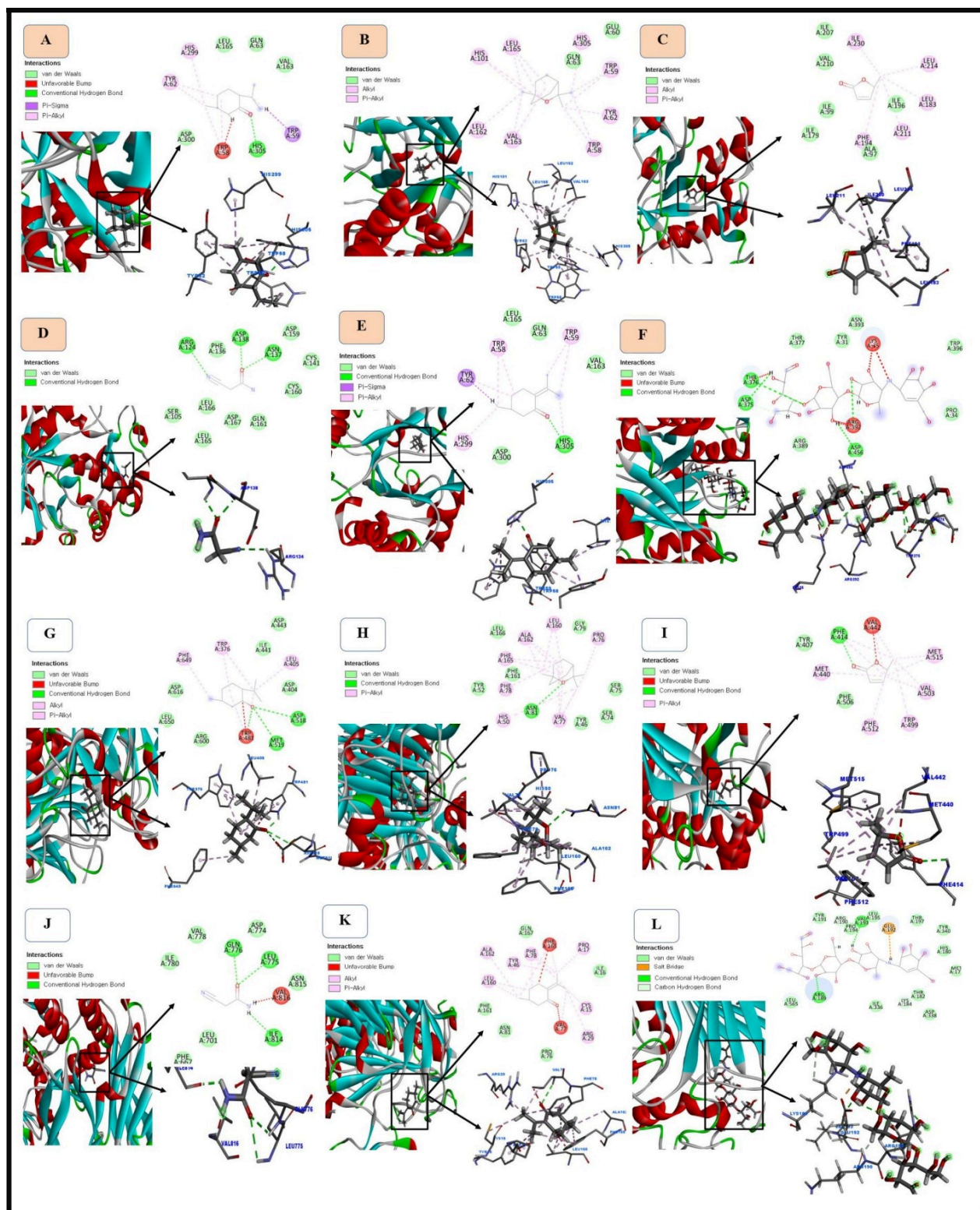


Figure 8

3D and 2D diagrams of molecular docking between selected compounds from *H. odoratissimum* and α -Amylase (A-F) and α -Glucosidase (G-L). **A:** Docking results illustrating the binding pattern of p-Menthone and α -Amylase through 1 conventional hydrogen bond; **B:** Docking results of Eucalyptol interacting with α -Amylase via several van der Waals interactions; **C:** Binding poses showing the molecular interaction between 2(5h)-furanone, 5-methyl interacting and α -Amylase; **D:** Docking results showing the strong

interaction between 2-Cyanoacetamide and α -Amylase via 3 conventional hydrogen bonds; **E**: Molecular interaction between Pulegone and α -Amylase revealing one conventional hydrogen bond; **F**: Binding poses of Acarbose interacting with α -Amylase through 4 conventional hydrogen bonds, and various Van-der Waals interactions; **G**: Docking interactions of p-Menthone and α -Glucosidase showing 2 conventional hydrogen bonds; **H**: Docking results of Eucalyptol interacting with α -Glucosidase via 1 carbon hydrogen bond and several van der Waals and Alkyl/Pi-Alkyl interactions; **I**: Docking results of 2(5h)-furanone, 5-methyl interacting with α -Glucosidase. **J**: Binding poses of 2-Cyanoacetamide interacting with α -Glucosidase via 3 conventional hydrogen bonds and various van der Waals interactions; **K**: Molecular interaction between Pulegone and α -Glucosidase; **L**: Molecular interaction between Acarbose (used as a reference inhibitor) and α -Glucosidase showing a strong interaction via 2 conventional hydrogen bonds with Arg-189 and Val-193, and various Van-der Waals interactions.

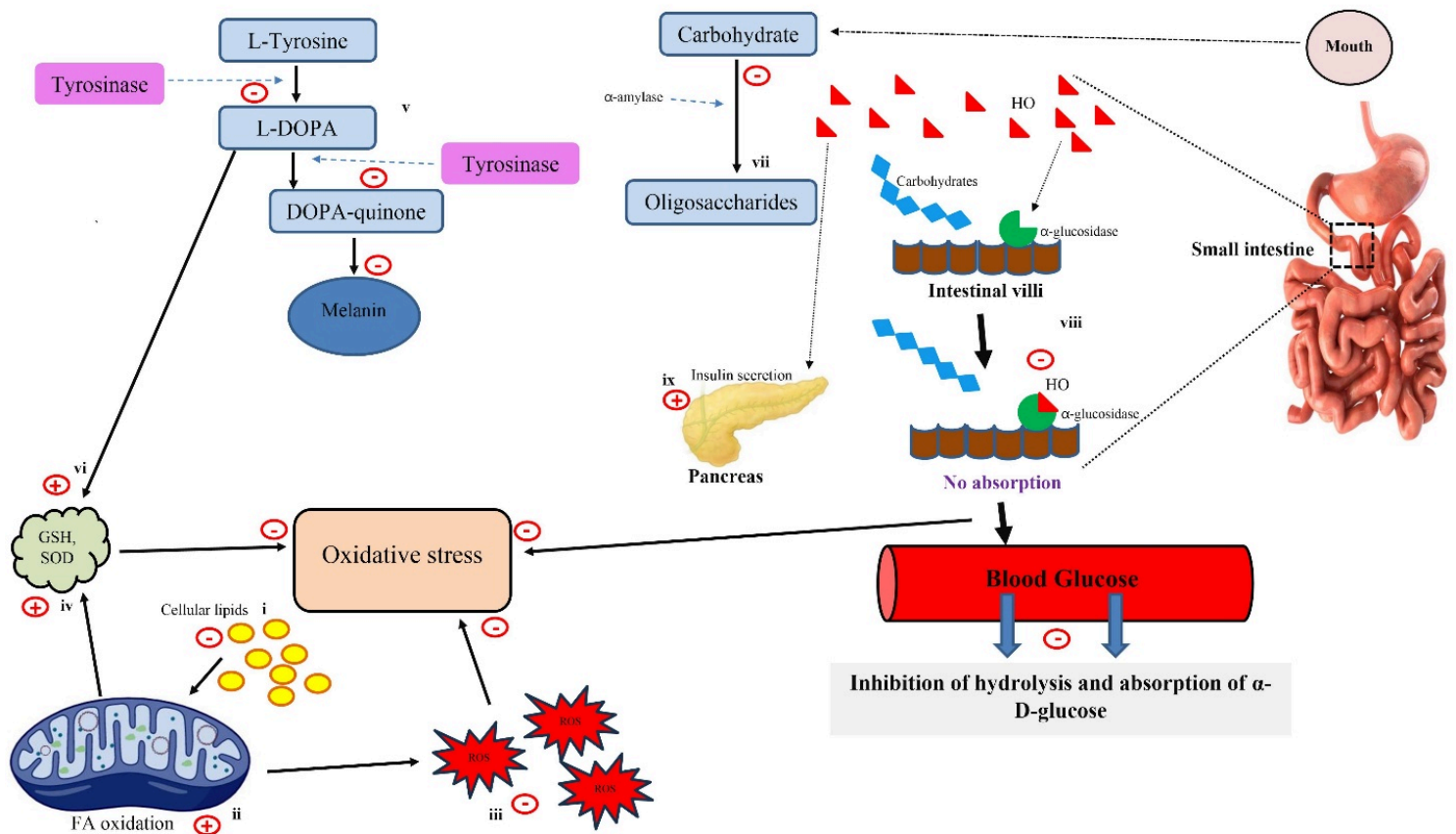


Figure 9

Possible mechanism of action of *H. odoratissimum*. The antioxidant effect of *H. odoratissimum* is linked to its ability to prevent lipid peroxidation (i). This action could improve the oxidation of mitochondrial fatty acid (ii) and prevent ROS overproduction (iii), which could finally prevent oxidative stress. *H. odoratissimum* could also act by directly enhancing antioxidant markers (iv). Under normal conditions, tyrosinase initiates melanogenesis by stimulating the conversion of L-tyrosine to L-DOPA, which is then transformed into DOPA quinone, leading to the production of melanin through several polymerized reactions. In the current study, *H. odoratissimum* strongly inhibited tyrosinase and L-DOPA activities (v), which are precursors of melanogenesis and therefore suggesting the anti-melanogenic action of this

plant. *H. odoratissimum* may also exhibit its antioxidant activity by inhibiting L-DOPA activity, which may improve the production of antioxidant enzymes and prevent oxidative stress **(vi)**. On the other hand, we found that *H. odoratissimum* exhibited a potent antidiabetic action through its inhibitory activity on α -amylase and α -glucosidase. Indeed, salivary and pancreatic α -amylase initiate the chemical digestion of carbohydrates. At the level of small intestine, α -glucosidase breaks polysaccharides into simple absorbable sugars. *H. odoratissimum* inhibited α -amylase activity, which may reduce the availability of oligosaccharides at the level of intestine **(vii)**. In addition, the inhibition of α -glucosidase activity by this extract may decrease the amount of simple absorbable sugars, leading to a reduction in blood glucose level **(viii)**. Because α -glucosidase inhibitors used in the treatment of diabetes are known to stimulate insulin secretion at the level of the pancreas, *H. odoratissimum* may also exert its antidiabetic action through this way **(ix)**.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)
- [image1.jpeg](#)