








Phytochemical Analysis of Endemic *Klasea bornmuelleri* (Azn.) Greuter & Wagenitz Extracts and Evaluation of Insecticidal and Enzyme Inhibitory Activities

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Abstract

This study investigates the phytochemical constituents of *Klasea bornmuelleri* extracts and evaluates their insecticidal and enzyme inhibitory activities. The chemical composition of flower and stem-leaf extracts was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), revealing significant amounts of fatty acid esters, hydrocarbons, and other secondary metabolites in both extracts. Liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) analysis identified phenolic acids, particularly quinic acid and chlorogenic acid, as the dominant components. The concentrations of these compounds were 3786.19 $\mu\text{g g}^{-1}$ and 3815.43 $\mu\text{g g}^{-1}$ for stem-leaf (KBSL) extracts and 2081.38 $\mu\text{g g}^{-1}$ and 4536.21 $\mu\text{g g}^{-1}$ for flower (KBF) extracts. The methanol:chloroform (1:1) (KBF-MC and KBSL-MC) extracts demonstrated significant inhibitory activity against tyrosinase, xanthine oxidase, and acetylcholinesterase (AChE) in enzyme inhibition assays. The insecticidal potential of *Klasea bornmuelleri* was evaluated on *Sitophilus granarius* for periods of 24, 48, and 72 hours, with no insecticidal activity observed at any time. In trials with *Tribolium castaneum*, hexane extracts from both flower and stem-leaf parts (KBF-H and KBSL-H) showed no significant insecticidal activity. However, KBSL-MC extracts exhibited a consistent mortality rate of $21.15 \pm 4.69\%$ across all time points. The KBF-MC extracts showed slightly higher efficacy with mortality rates of $26.07 \pm 7.40\%$ at 24 hours, $28.29 \pm 8.53\%$ at 48 hours, and $28.29 \pm 8.53\%$ at 72 hours. These findings indicate that the KBF-MC and KBSL-MC extracts derived from the *Klasea bornmuelleri* plant exhibit significant insecticidal and enzyme inhibitory properties, highlighting their potential as promising agents for the natural product-based control of storage pests and warranting further investigation.

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1. Introduction

Plants have been used by humans for food, housing, warmth, wound care, and illness treatment throughout history. Studies indicate that around 250 plant species were used for medicinal purposes as early as 5000 BCE. Ancient civilizations such as the Hittites, Egyptians, Sumerians, Assyrians, and Mesopotamians practiced herbal medicine extensively. However, with the advent of pharmaceutical drug production, the use of medicinal and aromatic plants declined. In the early 20th century, growing awareness of the side effects of synthetic drugs and the harmful impacts of synthetic additives in food and beverages led to a resurgence in the demand for natural products (Fernando, 2012).

Medicinal and aromatic plants are now widely used in various industries, including pharmaceuticals, food, cosmetics, textiles, and agriculture. Their medicinal use varies by region, with 80% of the population in developing countries relying on herbal medicine, a figure that reaches 95% in parts of the Middle East, Asia, and Africa. In contrast, developed countries report lower usage rates, such as 40–50% in Germany, 42% in the USA, and 49% in France (Akalin et al., 2020).

Türkiye, with its rich flora comprising approximately 11,000 plant taxa, is a significant source of medicinal and aromatic plants, about 500 of which have potential applications in alternative medicine. These plants are used fresh or dried, with all parts, including stems, leaves, flowers, seeds, and roots, being processed for various purposes. Turkey's unique geographic location, plant diversity, and favorable climate make it one of the leading countries in the trade of medicinal and aromatic plants (Bozyel et al., 2019).

The Asteraceae family, commonly known as Compositae, is one of the largest plant families globally, comprising

approximately 1,600 genera and over 23,600 species. In Turkey, the family is represented by about 1,350 species, with 38% endemism (Davis, 1967; Özhatay and Kültür, 2006). Among its genera, *Klasea Cass.* is notable for its 50–70 species, primarily distributed in Central Asia, Iran, Türkiye, and the Mediterranean region. In Turkey, *Klasea bornmuelleri* is an endemic species found in Central Anatolia, thriving in steppe ecosystems and arid areas at elevations between 1,000 and 1,500 meters (Davis and Kupicha, 1975).

Given the growing interest in plant-based pest control and enzyme inhibitors, this study focuses on the phytochemical analysis and bioactivities of *K. bornmuelleri*. KBSL-H and KBF-H extracts were analyzed using GC-MS, while KBSL-MC and KBF-MC extracts were evaluated quantitatively via LC-MS/MS. The enzyme inhibitory effects on α -amylase, tyrosinase, xanthine oxidase, acetylcholinesterase, and α -glucosidase were assessed, alongside the insecticidal potential against *S. granarius* and *T. confusum*, a primary pest of stored products. To the best of our knowledge, this study presents the first comprehensive analysis of *K. bornmuelleri*'s bioactive properties. These findings indicate that the KBF-MC and KBSL-MC extracts derived from the *K. bornmuelleri* plant exhibit significant insecticidal and enzyme inhibitory properties, highlighting their potential as promising agents for the natural product-based control of storage pests and warranting further investigation.

2. Material and Methods

2.1 Plant material

The plant material used in this study, *K. bornmuelleri* (Azn.) Greuter & Wagenitz, was collected by Dr. Bilal ŞAHİN from the Akçatoprak village, Malatya-Darende region (1100 m, steppe habitat, BS8155, 29 May 2023). To ensure accurate species

identification, specimens containing all plant parts were pressed in clean drying paper and prepared for herbarium storage. The plant material was separated into different parts, KBF and KBSL dried in cool, moisture-free conditions, away from direct sunlight. The dried plant parts were then ground into powder using a laboratory grinder and stored in glass containers until use.

2.2 Preparation of plant extracts

The extracts were prepared using a solid-liquid extraction method. Powdered KBSL (882 g) and KBF (188 g) parts were weighed separately and placed into pre-weighed glass containers. Initially, the plant materials were extracted with hexane (H), followed by a MeOH:CHCl₃ (MC) using maceration. Each extraction process was repeated three times independently. The resulting extracts were combined, filtered through Whatman No. 1 filter paper, and the solvents were evaporated using a rotary evaporator, ensuring the temperature did not exceed 25–30 °C (Demirtas et al., 2007; Tüfekçi et al., 2024).

2.3 Phytochemical analysis of plant extracts

2.3.1 GC-MS analysis

GC-MS analysis of the KBF-H and KBSL-H extracts was conducted at the Çankırı Karatekin University Research Laboratory. To identify the chemical composition, esterification was applied to the H extracts. For this process, 50 mg of extract was dissolved in 5 ml hexane, followed by the addition of 5 ml 1M KOH (dissolved in methanol), and the mixture was vortexed for 30 seconds. The upper hexane phase containing fatty acid methyl esters was filtered through a 0.45 µm syringe filter and analyzed using an Agilent Technologies 7890A GC system with a 5975 Triple Axis Detector mass spectroscopy. The GC-MS settings included an HP-5MS column (60 m × 320

m × 0.25 m) electron ionization energy and 70 eV energy with the as the carrier gas at a flow rate of 1 ml min⁻¹. The injection volume was set to 1 µl in splitless mode. Compounds were identified by comparing retention times and fragmentation patterns with databases (Famedb23, Famdbwax, WILLEY, and NIST05).

2.3.2 LC-MS/MS analysis

LC-MS/MS analysis of plant extracts was performed at Bingöl University Central Laboratory Application and Research Center. In order to determine the phenolic content of *Klasea bornmuelleri* plant, 10 mg of the plant extracts prepared with MC (1:1) solvent system were weighed and dissolved by adding 5 ml of methanol and 5 ml of pure water, and 10 ml was filtered through a 25 mm diameter PTFE syringe filter with a pore size of 0.22 micrometer with the help of a syringe and taken into a 15 ml falkon tube. Analyses were performed using an LC system with DIONEX UltiMate 3000 RS pump, DIONEX UltiMate 3000 RS autosampler, DIONEX UltiMate 3000 RS column oven and Exactive Plus Orbitrap (ThermoFisherScientific) high-resolution MS combination with heated electrospray ionization interface. The Orbitrap-MS instrument was calibrated with positive (Pierce™ LTQ Velos ESI PositiveIonCalibration Solution) and negative calibration solutions (Pierce™ NegativeIonCalibration Solution) using an automatic syringe injector (ThermoFisherScientific, USA). In the LC-HRMS analyses, the LC and MS sections were run simultaneously with the TraceFinder 3.2 (ThermoScientific) program installed on the system computer, and the data were collected and recorded with the Xcalibur software version 2.1.0.1140 (ThermoFisherScientific) (Dursun et al., 2025).

2.4 Enzyme activity assays

The inhibitory effects of the extracts on α -amylase, α -glucosidase, xanthine oxidase, tyrosinase, and acetylcholinesterase (AChE) enzymes were evaluated. Enzyme activities were tested following standard protocols with slight modifications (Sarikurkcu et al., 2018). All analysis was done at least three times with no significant difference.

2.4.1 α -amylase enzyme activity

Mixtures containing a total of 200 μ l of extract solution (0-50 mg ml⁻¹) and 200 μ L of α -amylase solution (porcine pancreatic α -amylase (EC 3.2.1.1), 10U ml⁻¹) prepared in 0.02 M sodium phosphate buffer (pH 6.9 containing 0.006 M NaCl) were incubated for 45 min at 37 °C. After pre-incubation, 400 ml of a 0.5% starch solution was added to each tube and incubated in a shaking bath (37 °C) for 10 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid color reagent. The test tubes were incubated in a boiling water bath for 10 min and then cooled to room temperature. The reaction mixture was then diluted by adding 3 ml of distilled water and the absorbance of the mixture was measured at 540 nm. Readings were compared to controls containing buffer instead of sample extract. Results are expressed as percent α -amylase inhibition. IC₅₀ value (mg ml⁻¹) is expressed as the concentration at which the enzyme activity was inhibited by 50%. Acarbose was used as a positive control. The percentage inhibition of the enzyme will be calculated and the IC₅₀ value was determined.

2.4.2 α -glucosidase enzyme activity

10 mM pNPG (4-Nitrophenyl α -D-glucopyranoside) solution (400 L), 400 L 1 PBS buffer and 100 L (0-50 mg ml⁻¹) were pre-incubated at 37 °C for 3 min. Then, 100 L glucosidase enzyme (EC 3.2.1.20, Type I) 0.15 U ml⁻¹) was added to each mixture and incubated for 15 min. The reaction was terminated by the addition of 2000 L

Na₂CO₃ (200 mM). The absorbance values of the mixture were measured at 405 nm. Acarbose was used as a positive control. Percent inhibition of the enzyme was calculated and IC₅₀ value was determined.

2.4.3 Xanthinoxidase enzyme activity

Enzyme and substrate concentrations were adjusted with potassium phosphate buffer (pH 7.5, 50 mM) and the tested extracts were dissolved with DMSO to 1 mg/ml. The minimum inhibition value was then determined by 10-fold dilution in case of activity with distilled water. Enzyme solution (10 μ L), 500 mM potassium phosphate buffer (50 μ L) and extracts were added to a 96-well plate and incubated at 37 °C for 15 min. Then, hypoxanthine (60 μ L, 3 mM) as substrate was added to each well and incubated at 37 °C for 20 min. Finally, the change in absorbance over 7 min was measured at 295 nm using a multi-reader spectrophotometer. Allopurinol was used as a positive control. Percent inhibition of xanthine oxidase activity was calculated by comparing the inhibitor absorbance values with the non-inhibitor control value. Percent inhibition of the enzyme was calculated and IC₅₀ value was determined.

2.4.4 Tyrosinase enzyme activity

Kojic was used as a standard tyrosinase inhibitor. Since the mode of inhibition depends on the nature of both substrate and inhibitor, L-DOPA was used as substrate in this experiment. Therefore, the inhibitors used in this method are inhibitors of the diphenolase activity of tyrosinase (EC 1.14.18.1) and their effect on the enzyme was determined by spectrophotometry based on the formation of dopachrome at 475 nm. L-DOPA solution (0.87 ml, 4.5 mM) was mixed with 0.9 ml of 0.1 M phosphate buffer (pH 6.8) and incubated at 30 °C for 5 min. Then, 0.9 ml of sample solutions of various concentrations followed by 0.03 ml of aqueous fungal tyrosinase solution were added to the

mixture and the enzyme reaction was monitored for 25 min at 1 min intervals for changes in absorbance at 475 nm (at 30 °C, corresponding to dopachrome formation). The percentage inhibition of the enzyme was calculated and the IC₅₀ value will be determined.

2.4.5 Acetylcholinesterase (AChE) enzyme activity

AChE activities of the extracts were measured following the procedure described in the scientific studies with minor modifications. Acetylthiocholine iodide (AChI) was used as substrate, while 5,5-dithiobis (2-nitro-benzoic acid) (DTNB) was used for the determination of activity. Briefly, Tris/HCl buffer (100 ml, 1M, pH=8) and sample solution (10 ml) dissolved in ultrapure water at different concentrations and AChE solution (50 ml) were added and the mixture was incubated at 25 °C for 10 min. DTNB (50 ml, 0.5 mM) was added. AChI (50 ml, 10 mM) was added to initiate the reaction. Hydrolysis of the substrates was evaluated at 412 nm. IC₅₀ values were calculated using the graph of activity (%)-[Compound].

2.5 Insecticide activity studies

Insecticidal activity test studies were determined by contact toxicity studies on adult *Sitophilus granarius* and *Tribolium castaneum* cultures, which are warehouse pests, grown at Çankırı Karatekin University Food and Agriculture Vocational School, Department of Plant and Animal Production Laboratory (Alkan et al., 2024). To prepare concentrations, *K. bornmuelleri* plant extracts were diluted with enough acetone to make 10% (100µg ml⁻¹) plant extract/acetone by weight volume⁻¹ (w v⁻¹). The prepared plant extracts were applied ventral to the abdomen of the insect using a micro-applicator at the rate of 1 µl insect⁻¹ per insect. Acetone (1 µl insect⁻¹) was used as a blank control. In all treatments

including the control, 10 insects were used in each replicate. The treated insects were transferred to 90 mm plastic petri dishes containing 10 g of wheat and incubated at 27±2 °C. The results will be monitored at 24-hour intervals for 3 days and the number of dead individuals will be recorded. The experiments were arranged in a randomized block design and repeated 3 times on different days with 3 replicates. The data was subjected to variance analysis (ANOVA), followed by comparison with one another using the Tukey multiple comparison test with a p<0.05 significance level.

3. Results and Discussion

3.1 GC-MS analysis results of *Klasea bornmuelleri* plant extracts

Fatty acid esterification is a chemical reaction that produces an ester and water when a fatty acid and an alcohol combine, usually with the help of an acid or basic catalyst. This reaction is essential because it converts free fatty acids into more stable ester forms such as methyl esters or triglycerides. In this study, the results of GC-MS analysis of KBF-H and KBSL-H extracts are given in Table 1 and Table 2. According to the GC-MS analysis result of the KBF-H extracts, 9,12-octadecadienoic acid (10.45%) is the dominant compound that stands out with its potential role in anti-inflammatory and antioxidant reactions. Other important compounds such as heptacosane (7.38%) and nonacosane (6.79%) reveal the chemical diversity of the extract. The predominance of fatty acid methyl esters (e.g. Hexadecanoic acid, 4.82%) may indicate specific biological activities in terms of pharmacological or cosmetic applications. These results highlight the diversity of chemical constituents and their potential for antimicrobial or antioxidant activities.

Table 1. Fatty acid constituents of the flower part of *Klasea bornmuelleri* plant (KBF-H)

RT	Compound	% Area ($\mu\text{g g}^{-1}$ extract)
15.65	Dodecanoic acid methyl ester	0.22
18.50	Methyl tetradecanoate	0.29
19.67	Pentadecanoic acid methyl ester	0.12
19.82	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.30
19.91	Hexahydrofarneryl acetone	1.12
20.28	1,4-Eicosadiene	0.23
20.55	(Z)-9-Hexadecenoic acid methyl ester	1.12
20.83	Hexadecanoic acid methyl ester (Palmitic acid methyl ester)	4.82
21.27	n-Hexadecanoic acid	0.50
21.44	Hexadecanoic acid ethyl ester	0.63
21.75	Heptadecanoic acid methyl ester	0.22
22.54	(Z,Z)-9,12-Octadecadienoic acid methyl ester	10.45
22.62	Phytol	4.71
22.78	Stearic acid methyl ester	1.88
22.84	(Z,Z)-9,12-Octadecadienoic acid	0.27
22.89	7,10-Octadecadienoic acid methyl ester	0.81
23.06	9,12-Octadecadienoic acid ethyl ester	0.58
23.10	(Z)-9-Octadecenoic acid ethyl ester	0.38
23.28	6,9-Octadecadienoic acid methyl ester	0.63
23.58	Octadecanal	0.27
24.24	Tricosane	2.66
24.46	Eicosanoic acid methyl ester (Arachidic acid methyl ester)	0.90
24.70	cis-13-Eicosenoic acid	0.57
24.75	Eicosanoic acid (Arachidic acid)	0.26
25.01	Tetracosane	0.39
25.27	Methyl 14-methyl-eicosanoate	0.41
25.51	11-decyl-Docosane	0.22
25.85	Pentacosane	1.83
26.07	Behenic acid methyl ester	0.86
26.57	Hexacosane	0.63
26.84	Methyl 21-methyl-docosanoate	0.41
27.14	1-Heptacosanol	0.35
27.43	Heptacosane	7.38
27.61	Tetracosanoic acid methyl ester	0.93
27.97	Stearic acid allyl ester	0.24
28.07	Octacosane	1.86
28.32	2-cis-9-Octadecenyloxyethanol	0.22
28.48	9-n-Octylhexacosane	0.62
28.58	Octacosanol	0.94
28.84	Nonacosane	6.79
29.01	Hexacosanoic acid methyl ester	1.25
29.06	17-Pentatriacontane	0.36
29.14	Triacontane	1.01
29.41	Tetracontane	1.36
29.81	Stigmastan-3,5,22-triene	0.76
29.96	β -Sitosterol	0.26
30.12	Dotriacontane	3.96
30.19	Stigmastan-3,5-diene	0.66
30.31	Octacosanoic acid methyl ester	2.02
30.43	Heptatriacontane	0.75
30.74	Hexatriacontane	1.10
31.15	Campesterol	0.54
31.46	Stigmasterol	2.65
31.53	Docosane	0.99
31.85	Methyl melissate	0.79
31.97	γ -Sitosterol	3.62
32.29	β -Amyrin	0.55
32.45	Schottenol	0.80
32.50	Olean-12-en-3-one	0.57
32.74	α -Amyrin	0.67
33.24	Sitostenone	0.49

Table 2. Fatty acid constituents of the stem-leaf part of *Klasea bornmuelleri* plant (KBSL-H)

RT	Compound	% Area ($\mu\text{g g}^{-1}$ extract)
18.50	Methyl tetradecanoate	0.63
19.82	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2.31
19.89	Hexahydrofarneyl acetone	0.43
20.08	Phytol acetate	1.10
20.28	1,4-Eicosadiene	1.73
20.51	(Z)-9-Hexadecenoic acid methyl ester	0.20
20.78	Hexadecanoic acid methyl ester	4.97
20.96	Isophytol	0.17
21.21	Hexadecanoic acid	1.18
21.73	Heptadecanoic acid methyl ester	0.15
22.42	(Z,Z)-9,12-Octadecadienoic acid methyl ester	2.42
22.51	(Z,Z,Z)-9,12,15-Octadecatrienoic acid methyl ester	5.32
22.63	Phytol	4.89
22.70	Methyl stearate	1.28
22.75	Stearic acid methyl ester	0.74
22.84	9,12,15-Octadecatrienoic acid	0.67
23.02	Stearic acid	0.40
23.31	Heptacosane	0.14
23.57	Nonadecanoic acid methyl ester	0.10
23.87	cis-13-Eicosenoic acid	0.12
24.18	Tricosane	0.93
24.22	11-Eicosenoic acid methyl ester	0.22
24.44	Eicosanoic acid methyl ester (Arachidic acid methyl ester)	1.09
24.73	cis-13-Eicosenoic acid	0.13
24.76	Eicosanoic acid (Arachidic acid)	0.21
24.93	(Z)-13-Eicosenoic acid	0.11
25.01	Eicosane	0.18
25.21	Methyl 14-methyl-eicosanoate	0.91
25.75	1-Tricosanone	0.11
25.80	Pentacosane	1.20
25.92	2-Monopalmitin	0.13
26.09	Behenic acid methyl ester	4.99
26.33	β -Resorcylic acid	1.15
26.43	2-cis-9-Octadecenyl-oxyethanol	0.10
26.55	Hexacosane	0.23
26.80	Tricosanoic acid methyl ester	0.39
27.32	Octacosane	1.86
27.58	Tetracosanoic acid methyl ester	6.64
27.70	Terephthalic acid 2-ethylhexyl octyl ester	0.67
27.84	Isophytol acetate	0.36
28.00	Octadecane	0.23
28.25	Pentacosanoic acid methyl ester	0.21
28.61	Dihydro-3-oxo- β -ionol	0.38
28.71	Hexatriacontane	1.37
28.85	Nonacosane	0.17
28.97	Hexacosanoic acid methyl ester	4.01
29.17	Triacotane	0.11
29.36	Pentatriacontane	0.27
29.53	Stigmasterol acetate	0.12
29.60	Methyl 21-methyl-hexacosanoate	0.23
29.65	17-Pentatriacontane	0.20
29.76	(3 β ,22Z)-Stigmasta-5,22-dien-3-ol acetate	0.41
29.84	Cycloartenol acetate	0.17
29.91	Stigmast-5-en-3-ol oleate	0.17
30.01	β -Sitosterol	0.34

30.05	1-Cyclopentyleicosane	0.32
30.15	Stigmastan-3,5-diene	2.14
30.32	Octacosanoic acid methyl ester	5.30
30.36	α -Tocopherol	0.44
30.68	Latosterol	0.27
30.99	Melissic acid	0.16
31.16	Campesterol	0.29
31.46	Stigmasterol	2.98
31.86	Methyl melissate	0.64
31.97	γ -Sitosterol	6.48
32.01	Phylloquinone	1.55
32.43	Dihydrocondrilasterol	3.34
32.59	Cycloartenol acetate	0.95
32.74	α -Amyrin	0.33
33.13	β -Sitosterol acetate	0.59
33.24	α -1-Sitosterol	0.60
33.74	Lupeol	0.61
34.26	Phytol acetate (isomer)	4.23
36.92	Isopropyl linoleate	0.62

Klasea bornmuelleri, a plant from the Asteraceae family, has drawn significant scientific attention due to its phytochemical diversity and bioactive compounds. GC-MS analyses of extracts from the KBF and KBSL of *K. bornmuelleri* revealed the presence of various bioactive compounds. The KBF-H extract was particularly rich in fatty acid esters such as methyl linoleate (10.45%) and hydrocarbons like heptacosane (7.38%) and nonacosane (6.79%). Similarly, the KBSL extract exhibited high concentrations of sterols, including methyl tetracosanoate (6.64%), methyl linolenate (5.32%), and gamma-sitosterol (6.48%). While both extracts shared fatty acid esters, the proportions were notably higher in the KBF-H extracts. These findings align with studies on *Garcinia cowa*, which demonstrated that hexane extracts from its leaf, stems, and roots were also rich in fatty acid esters and hydrocarbons, indicating consistent therapeutic potential across species (Rahman et al., 2024). Previous studies on *K. bornmuelleri* have primarily focused on secondary metabolites such as flavonoids, lignans, and other phenolic compounds known for their antioxidant, anti-inflammatory, and antimicrobial properties. Although the plant shares similar scientific

interests with species like *Anisopus mannii* and *Melastoma malabathricum*, the specific compounds and their effects can vary due to ecological conditions and extraction methods. For instance, studies on *A. mannii*'s hexane leaf extracts highlighted fatty acid esters and hydrocarbons as significant contributors to its antimicrobial activity, suggesting a synergistic effect among these components (Musa et al., 2015). Similarly, phytochemical analyses of *M. malabathricum* hexane extracts identified 17 bioactive compounds, including sterols and fatty acids, associated with antioxidant and antimicrobial activities (Giri and Rajbhandari, 2018). Comparative phytochemical studies of *K. bornmuelleri* could further elucidate its bioactive components and potential synergies.

3.2 LC-MS/MS analysis results of *Klasea bornmuelleri* plant extracts

The results of LC-MS/MS analysis of the plant extracts contained various bioactive compounds identified on the basis of their retention times (RT), chemical formulas and calculated concentrations ($\mu\text{g g}^{-1}$) in the extract. Among the most abundant compounds for the KBF-MC extract were chlorogenic acid ($4536.22 \mu\text{g g}^{-1}$) and quinic acid ($2081.38 \mu\text{g g}^{-1}$), which have strong

antioxidant properties. Other bioactive compounds such as arbutin (11858.145 $\mu\text{g g}^{-1}$) and protocatechuic acid (51.656 $\mu\text{g g}^{-1}$) provide important contributions that may support potential pharmacological

applications of the extract. In addition, flavonoids such as luteoloside (276.18 $\mu\text{g g}^{-1}$) and rutin hydrate (1817.39 $\mu\text{g g}^{-1}$) stand out for their antimicrobial and antioxidant effects.

Table 3. LC-MS/MS analysis results of KBF-MC extract

No	RT	Compounds	% Area ($\mu\text{g g}^{-1}$ extract)	Formula
1	0.85	Quinic acid	2081.382	$\text{C}_7\text{H}_{12}\text{O}_6$
2	2.9	Arbutin	11858.146	$\text{C}_{12}\text{H}_{16}\text{O}_7$
3	6.22	Protocatechuic acid	51.656	$\text{C}_7\text{H}_6\text{O}_4$
4	7.49	Esculin hydrate	4.806	$\text{C}_{15}\text{H}_{16}\text{O}_9$
5	8.16	Chlorogenic acid	4536.216	$\text{C}_{16}\text{H}_{18}\text{O}_9$
6	8.57	Caffeic acid	62.38	$\text{C}_9\text{H}_8\text{O}_4$
7	8.78	Syringic acid	8.28	$\text{C}_9\text{H}_{10}\text{O}_5$
8	9.15	3-(4-Hydroxyphenyl) propionic acid	630.264	$\text{C}_9\text{H}_{10}\text{O}_3$
9	9.65	Coumaric acid	27.09	$\text{C}_9\text{H}_8\text{O}_3$
10	9.93	Ferulic acid	16.778	$\text{C}_{10}\text{H}_{10}\text{O}_4$
11	9.98	Sinapic acid	2.922	$\text{C}_{11}\text{H}_{12}\text{O}_5$
12	10.64	Luteolin 7-O- β -glucoside	276.182	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$
13	10.86	Rutin hydrate	1817.386	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$
14	10.89	Quercetin 3-O- β -glucoside	28.428	$\text{C}_{21}\text{H}_{20}\text{O}_{12}$
15	11.2	Apigenin 7-O-neohesperidoside	3.573	$\text{C}_{27}\text{H}_{30}\text{O}_{14}$
16	11.27	Ellagic acid	26.048	$\text{C}_{14}\text{H}_6\text{O}_8$
17	11.34	Apigenin 7-O- β -glucuronide	218.06	$\text{C}_{21}\text{H}_{18}\text{O}_{11}$
18	11.44	Quercetin 3-O- β -rutinoside 7-O- β -glucoside	52.919	$\text{C}_{33}\text{H}_{40}\text{O}_{20}$
19	11.44	Kaempferol 3-O- β -glucoside	51.46	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$
20	12.27	Quercetin	3.698	$\text{C}_{15}\text{H}_{10}\text{O}_7$
21	12.6	Luteolin	97.294	$\text{C}_{15}\text{H}_{10}\text{O}_6$
22	13.2	Apigenin	144.363	$\text{C}_{15}\text{H}_{10}\text{O}_5$
23	13.19	Quercetin 3'-methyl ether	3.285	$\text{C}_{16}\text{H}_{12}\text{O}_7$
24	13.34	Luteolin 4'-methyl ether	70.567	$\text{C}_{16}\text{H}_{12}\text{O}_6$

Among the most abundant compounds for KBSL-MC extract, chlorogenic acid (3815.43 $\mu\text{g g}^{-1}$) and quinic acid (3786.2 $\mu\text{g g}^{-1}$) with strong antioxidant and anti-inflammatory properties were identified. The high concentrations of these compounds can be considered as an important indicator of the biological activity of the extract. In addition,

polyphenols such as protocatechuic acid (70.13 $\mu\text{g g}^{-1}$) and caffeic acid (20.34 $\mu\text{g g}^{-1}$) stand out for their antimicrobial and antioxidant potential. The table provides critical data for evaluating the pharmacological potential of the extract and shows the chemical diversity of these bioactive compounds.

Table 4. LC-MS/MS analysis results of KBSL-MC extract

No	RT	Compounds	% Area ($\mu\text{g g}^{-1}$ extract)	Formula
1	0.87	Quinic acid	3786.19	$\text{C}_7\text{H}_{12}\text{O}_6$
2	2.95	Arbutin	171333.45	$\text{C}_{12}\text{H}_{16}\text{O}_7$
3	6.24	Protocatechuic acid	70.12	$\text{C}_7\text{H}_6\text{O}_4$
4	7.06	3,4-Dihydroxybenzaldehyde	75.14	$\text{C}_7\text{H}_6\text{O}_3$
5	7.49	Esculin hydrate	10.79	$\text{C}_{15}\text{H}_{16}\text{O}_9$
6	8.14	Chlorogenic acid	3815.43	$\text{C}_{16}\text{H}_{18}\text{O}_9$
7	8.59	Caffeic acid	20.32	$\text{C}_9\text{H}_8\text{O}_4$
8	9.66	Coumaric acid	84.07	$\text{C}_9\text{H}_8\text{O}_3$
9	9.93	Ferulic acid	1.61	$\text{C}_{10}\text{H}_{10}\text{O}_4$
10	10.86	Rutin hydrate	1817.27	$\text{C}_{27}\text{H}_{30}\text{O}_{16}$
11	10.6	Luteolin 7-O- β -glucoside	1892.81	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$
12	10.9	Quercetin 3-O- β -glucoside	48.90	$\text{C}_{21}\text{H}_{20}\text{O}_{12}$
13	11.21	Apigenin 7-O- β -glucoside	4.72	$\text{C}_{21}\text{H}_{20}\text{O}_{10}$
14	11.29	Ellagic acid	9.36	$\text{C}_{14}\text{H}_6\text{O}_8$
15	11.46	Kaempferol 3-O- β -glukozit	64.66	$\text{C}_{21}\text{H}_{20}\text{O}_{11}$
16	11.46	Quercetin 3-O- β -rutinoside 7-O- β -glucoside	61.84	$\text{C}_{33}\text{H}_{40}\text{O}_{20}$
17	12.6	Luteolin	9.94	$\text{C}_{15}\text{H}_{10}\text{O}_6$

The LC-MS/MS analysis of methanol:chloroform (MeOH:CHCl₃) extracts identified 24 compounds in the KBF-MC extract and 17 in the KBSL-MC extract, emphasizing the chemical diversity of these extracts. Phenolic acids and flavonoids were the dominant classes, with variations in their distribution. For instance, the quinic acid content in the KBSL-MC extract (3786.19 $\mu\text{g g}^{-1}$) was significantly higher than in the KBF-MC extract (2081.38 $\mu\text{g g}^{-1}$), whereas chlorogenic acid was more abundant in the KBF-MC extract (4536.22 $\mu\text{g g}^{-1}$) compared to the KBSL-MC extract (3815.44 $\mu\text{g g}^{-1}$). These results align with studies on *Scabiosa columbaria*, where high chlorogenic acid content was linked to strong antioxidant activities (Akar, 2021). Similarly, analyses of *Onobrychis argyrea* leaf extracts revealed a range of bioactive compounds, including quinic acid, with diverse biological properties (Yeniçeri et al., 2024). The observed chemical diversity and the prevalence of key bioactive compounds strongly support the therapeutic

potential of *K. bornmuelleri*. However, comprehensive in vitro and in vivo studies are required to fully understand the mechanisms and specific applications of these compounds in managing oxidative stress, metabolic disorders, and neurodegeneration. GC-MS and LC-MS/MS analyses contribute to the biological activity of specific metabolites by analysing the chemical composition of plant extracts. GC-MS analysis shows that volatile compounds (terpenoids, phenolic compounds) play a role in insecticidal activities, while LC-MS/MS reveals that high molecular weight compounds (flavonoids, alkaloids) are effective on enzyme inhibition.

3.4 Enzyme activity results of *Klasea bornmuelleri* plant extracts

In this study, the concentrations of substances causing 50% enzyme inhibition were calculated for each of the KBSL-H/KBF-H and KBSL-MC/KBF-MC extracts of *K. bornmuelleri* and given in Table 5.

Table 5. Enzyme inhibition (IC₅₀) activity results of *Klasea bornmuelleri* plant extracts

Enzimler	KBSL-H	KBF-MC	KBSL-MC	KBF-H
α-Glucosidase	105.34 ± 3.2	4620.98 ± 6.3	6931.47 ± 7.5	72.28 ± 1.6
Tyrosinase	-	1386.29 ± 4.9	1237.76 ± 3.9	1386.29 ± 6.4
Xanthine oxidase	1575.33 ± 5.8	468.34 ± 2.4	693.15 ± 4.7	1777.3 ± 6.9
AchE	4951.05 ± 7.5	686.28 ± 4.3	815.47 ± 4.8	3648.14 ± 7.2
α-amylase	949.52 ± 2.4	1359.11 ± 3.9	30136.83 ± 9.8	2310.49 ± 6.5

The results are shown as the mean ± SD.

The values indicated by the different superscripts within the same line are different according to Tukey's honestly significant difference post hoc test at a 5% significance level.

IC₅₀ (mg/mL), 50% inhibition concentration for all samples.

The effects of extracts obtained from the plant *K. bornmuelleri* were investigated for the α-glucosidase enzyme. Graphs showing inhibition values as a function of the concentration of extracts exhibiting enzymatic activity were plotted. The concentrations of substances causing 50% enzyme inhibition were calculated for each extract. The inhibition effects of extracts at different concentrations are presented in

Figure 1. The results indicate that hexane extracts exhibit a more pronounced inhibitory effect compared to MC extracts. While MC extracts require much higher concentrations to show a noticeable effect, the activity in the KBF-H extract decreases rapidly at relatively low concentrations, achieving significant inhibition (approximately 20% at 150 μg ml⁻¹).

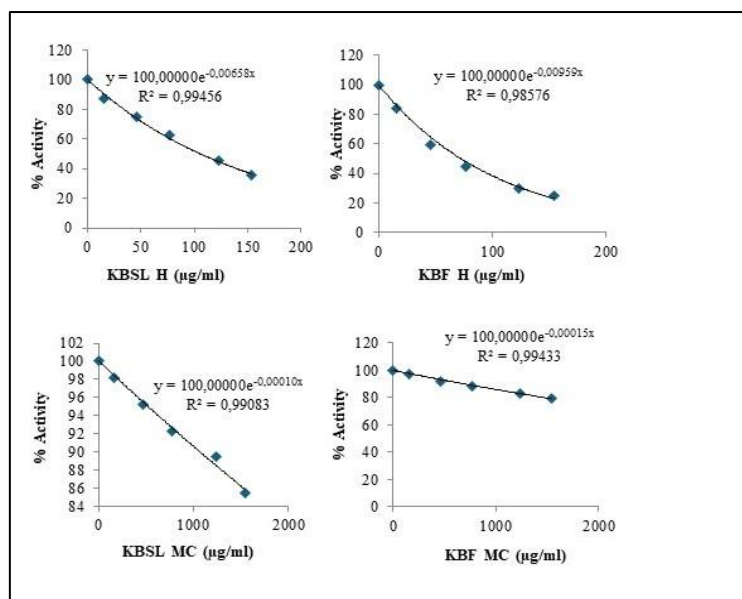


Figure 1. α-glucosidase enzymatic activity of plant extracts

Studies conducted on the tyrosinase enzyme reveal that the KBSL-H extract does not exhibit any inhibitory effect on the tyrosinase enzyme, as shown by the activity remaining constant at 100% (Figure 2).

While MC extracts demonstrate moderate inhibition, the KBF-H extract shows gradual inhibition of enzyme activity, reaching approximately 40-50% at higher concentrations.

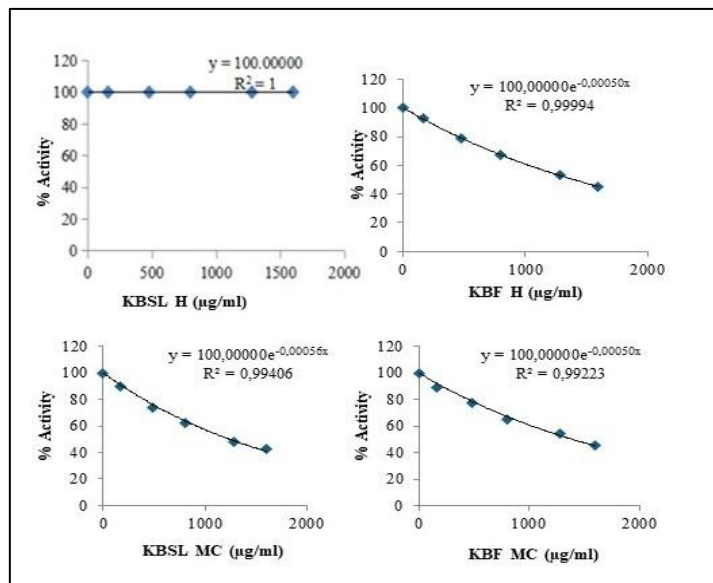


Figure 2. Tyrosinase enzymatic activity of plant extracts

The results of xanthine oxidase enzymatic activity indicate that the KBSL-H and KBF-H extracts exhibit a relatively moderate inhibitory effect, reaching approximately 50-60% of enzymatic activity at high concentrations (Figure 3). In

contrast, the KBF-MC extract showed strong inhibition with a rapid decrease in enzymatic activity. The inhibition becomes more pronounced at higher concentrations, reaching approximately 20-30% of activity.

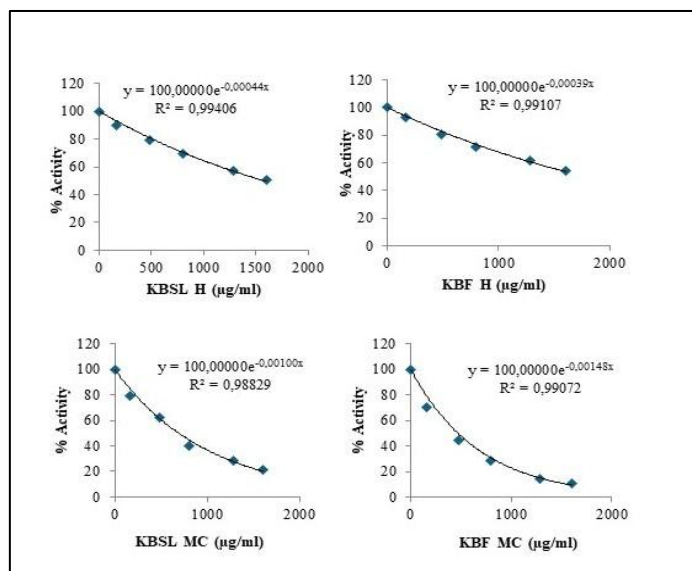


Figure 3. Xanthinoxidase enzymatic activity of plant extracts

The KBSL-H and KBF-H extracts demonstrated weaker inhibitory effects on the acetylcholinesterase enzyme, with slow progression (Figure 4). In contrast, the MC extracts showed more significant inhibition, with KBSL reaching approximately 30% of

enzyme activity. Meanwhile, KBF stood out as the most effective among the four extracts, exhibiting a notable inhibitory effect, reducing enzyme activity to around 20% at concentrations of 1500-2000 µg ml⁻¹.

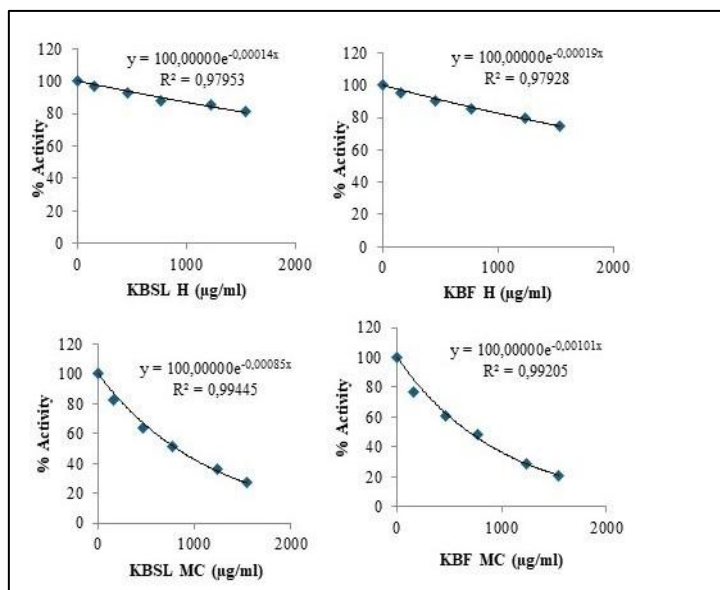


Figure 4. Acetylcholinesterase enzymatic activity of plant extracts.

For the α -amylase enzyme, the KBSL-H extract demonstrated the best inhibitory activity among the four extracts (Figure 5). Inhibition with the KBF-H extract was similar to KBSL-H, showing a gradual reduction in enzyme activity of up to

approximately 75% at high concentrations. For the MC extracts, KBSL exhibited a weak inhibitory effect, with enzyme activity remaining above 80%, while KBF showed moderate inhibition.

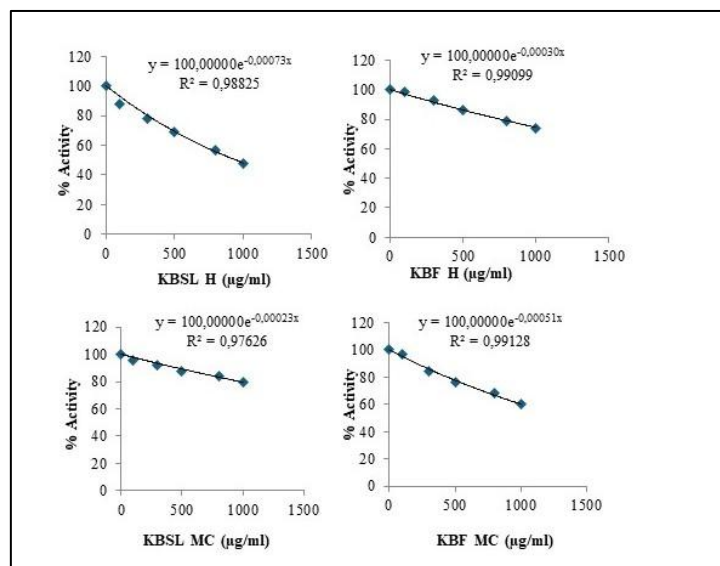


Figure 5. α -amylase enzymatic activity of plant extracts

The inhibitory effects of *K. bornmuelleri* extracts on five key enzymes (α -glucosidase, tyrosinase, xanthine oxidase, α -amylase, and acetylcholinesterase) were evaluated using polar (MC) and nonpolar

(H) solvents. The results indicated varying levels of inhibitory activity depending on the enzyme and the solvent type. MeOH:CHCl₃ extracts were particularly effective against tyrosinase, xanthine

oxidase, and acetylcholinesterase, achieving approximately 20-50% enzyme activity at concentrations between 1000-2000 $\mu\text{g ml}^{-1}$. Notably, the MC extract exhibited potent xanthine oxidase inhibition, with an IC_{50} value of 468.34 $\mu\text{g ml}^{-1}$. H extracts demonstrated moderate inhibitory activity, particularly against α -glucosidase and α -amylase, achieving 20-60% enzyme activity at concentrations around 150-1000 $\mu\text{g ml}^{-1}$. The hexane extracts were more effective against α -glucosidase, with low IC_{50} values (72-105 $\mu\text{g ml}^{-1}$). These findings are consistent with studies on *Cachrys crassiloba*, where hexane and ethanolic extracts exhibited moderate AChE inhibition and significant tyrosinase inhibition, with specific IC_{50} values observed for ethanolic extracts (Büyükyıldırım et al., 2024). Studies on *Polygonum hydropiper* also highlighted the superior α -amylase inhibitory activity of hexane leaf extracts compared to other solvents (Nasir et al., 2020). On the other hand, some *Klasea* species have been reported to have high enzyme activities. It was determined that the methanol extract of *Klasea serratuloides* has high enzyme activity against AChE and BChE enzymes (61.17-34.54 $\mu\text{g ml}^{-1}$) (Erugur et al., 2021). Hexane, being an apolar solvent, extracts lipophilic compounds (e.g. terpenoids) and these compounds are involved in enzyme

inhibition. Since the MeOH:CHCl₃ mixture is more polar, it offers a broader metabolite profile and contains biologically active compounds such as flavonoids, alkaloids, sesquiterpene lactones. These compounds make chemical bonds with enzymes, especially due to their polarity. This contributes to enzyme inhibition and thus insecticidal activity. Therefore, it is important to select the appropriate solvent to enhance biological activity.

3.5 Insecticidal activity results of *Klasea bornmuelleri* plant extracts

The insecticidal effects of different solvent extracts (H, MC) obtained from KBF and KBSL of *Klasea bornmuelleri* on *S. granarius* were comparatively investigated at 24, 48 and 72 hours intervals, but no activity was observed. Therefore, activity studies were carried out on a different storage pest species, *T. castaneum*. Insecticidal effects of different solvent extracts (H, MC) obtained from KBF and KBSL of *Klasea bornmuelleri* on *T. castaneum* were compared at 24, 48 and 72 hours intervals. While no activity was observed in KBF-H/KBSL-H extracts, MC extracts exhibited statistically significant mortality rates compared to the control group and H extracts at all time intervals ($p < 0.05$).

Table 6. Insecticidal effects of *K. bornmuelleri* plant extracts against *T. castaneum*

Application		% Death±Std.Deviation*		
		24 hours	48 hours	72 hours
Control	Aseton	0,00±0,00b	0,00±0,00b	0,00±0,00b
	KBF-H	0,00±0,00b	0,00±0,00b	0,00±0,00b
<i>Klasea bornmuelleri</i>	KBF-MC	26,07±7,40a	28,29±8,53a	28,29±8,53a
	KBSL-H	0,00±0,00b	0,00±0,00b	0,00±0,00b
	KBSL-MC	21,15±4,69a	21,15±4,69a	21,15±4,69a

a Standard deviation.

b Different small letter next to the results indicates that the mean results are significantly different from one another in each column (Anova $p < 0.05$, Tukey test).

The insecticidal effects of *K. bornmuelleri* extracts against *S. granarius* and *T. castaneum* were assessed. H and MC extracts from the KBF and KBSL were prepared. Results showed no insecticidal

activity against *S. granarius* across all extracts. However, MC extracts from KBF exhibited significant insecticidal effects on *T. castaneum*, with mortality rates stabilizing after 48 hours. In contrast, KBSL

extracts demonstrated lower mortality rates, albeit with notable insecticidal activity. These findings suggest that *K. bornmuelleri* possesses strong insecticidal properties, particularly in MC extracts from KBF. Previous studies on other plant species, such as *O. pimpinelloides*, demonstrated similar results, where methanol extracts showed superior insecticidal effects against *T. castaneum* compared to hexane extracts (Dal et al., 2023). The observed activity supports the potential of *K. bornmuelleri* as an environmentally friendly pesticide alternative, offering a sustainable solution for pest management. Future studies should focus on isolating and characterizing the responsible compounds and exploring possible synergies with other control methods to optimize the use of *K. bornmuelleri* extracts as biological pesticides. Many plant species in the Asteracea family have unlimited biological effects due to the secondary metabolites they contain. Among these, it is one of the leading families in terms of insect activity potential. The plant extract obtained from *K. sogdiana* species of the genus *Klasea* belonging to this family was powdered and used as an insecticide against red spider mites and cotton aphids in Central Asia (Belenovskaya et al., 1993). In another study, the flowering part of *Klasea pusilla* was traditionally used in Lebanon to treat insect bites (Baydoun et al., 2015).

4. Conclusion

This study provides a comprehensive analysis of the phytochemical composition, enzyme inhibitory activities, and insecticidal potential of *K. bornmuelleri*. The results underscore the plant's versatility in pharmacological and agricultural applications. Future research should aim at isolating active constituents, optimizing extraction techniques, and validating the findings through *in vivo* studies.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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