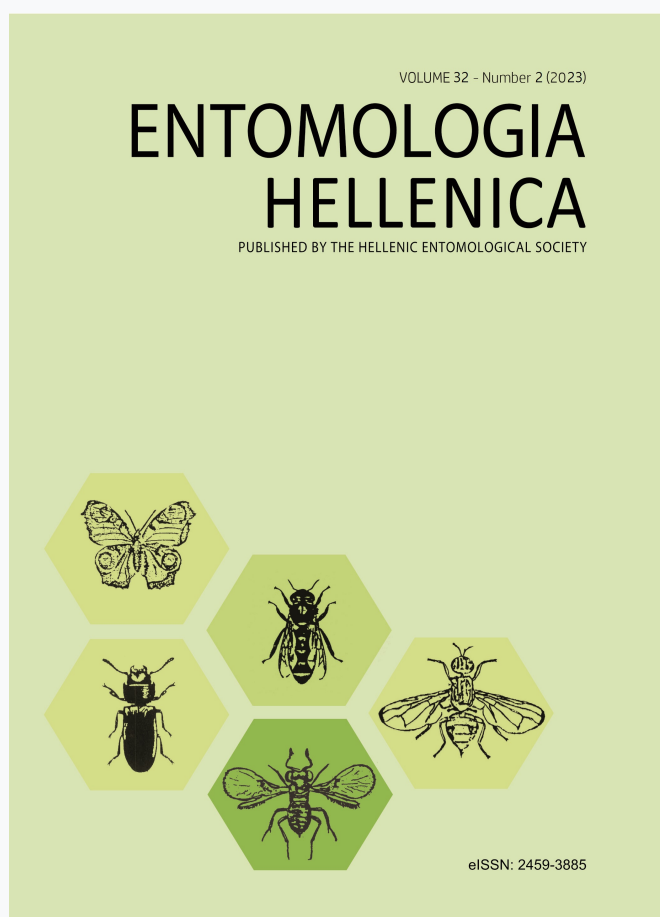


# ENTOMOLOGIA HELLENICA

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**Anti-obesity, Anti-diabetic and Anti-inflammatory activities of *Camponotus compressus* extract; in vitro and in silico studies**

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## Anti-obesity, anti-diabetic and anti-inflammatory activity of *Camponotus compressus* extract; *in vitro* and *in-silico* studies

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### ABSTRACT

**Background:** In recent years, there has been a growing interest in the potential health benefits of insects, mainly ants, due to the, in them, presence of potential bioactive substances that can play a major role in therapeutic research. In the present study, the therapeutic potential of *Camponotus compressus* extract was studied, focusing on its bioactive compounds and their diverse applications.

**Methods:** The anti-obesity, anti-diabetic, and anti-inflammatory activity of the *C. compressus* extract was assessed through various assays, i.e., its anti-obesity potential was determined by the pancreatic lipase inhibition assay, the anti-diabetic activity was analyzed using an  $\alpha$ -amylase inhibition assay, whereas the anti-inflammatory activity was studied using a heat induced hemolysis and albumin denaturation inhibition assay. The extract's mode of action was explored through *in-silico* studies, whereas ADMET properties were also evaluated.

**Results:** In line with previous findings, the GC-MS analysis revealed 32 bioactive compounds, including some major constituents, such as the 2,3-dihydroxypropyl elaidate and lycoxanthin. The extract exhibited concentration-dependent anti-obesity effects and  $\alpha$ -amylase inhibition, suggesting anti-diabetic potential. The anti-inflammatory properties of the extract were confirmed through heat-induced hemolysis and albumin denaturation inhibition assays. The *in-silico* studies revealed strong binding affinities with target proteins, whereas ADMET analysis supported drug-likeness.

**Conclusion:** Based on our results, the *C. compressus* extract holds therapeutic promise that could potentially be useful against obesity, diabetes, and inflammation. The study provides insights into its mode of action, supporting its potential as a novel drug candidate.

**KEY WORDS:** *Camponotus compressus*, bioactive compounds, anti-obesity, anti-diabetic, anti-inflammatory, *in-silico* study, ADMET analysis

### Introduction

Folk medicine across cultures has harnessed the healing potential of various insects, including bees, wasps, and ants. Such practices have involved the utilization of products derived from these insects, such as honey and venom, to treat a wide range of ailments. Ratcliffe et al. (2014) have noted that folk remedies have incorporated these insect-derived substances for the treatment of conditions such as wounds,

ulcers, inflammation, infections, pain, cancer, and allergies. This rich tradition of insect-based remedies is grounded in scientific observations that have uncovered their valuable properties. Research has established the presence of immunological, analgesic, antibacterial, diuretic, anaesthetic, and antirheumatic attributes within insect bodies (Costa-Neto, 2005). Notably, Costa-Neto (2005) discusses the documented usage of insect-derived products in both traditional and laboratory or clinical settings.

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Ants are omnipresent and diverse insects and have recently emerged as an interesting subject of scientific exploration due to the presence of bioactive compounds. While ants have long been recognized for their ecological importance, their therapeutic potential has gained prominence as researchers explore the multifaceted properties that these tiny creatures possess. Ants, as a specific subset of insects, have been employed in traditional medicine practices in various regions. For example, in Central Asia, ants have been utilized for the treatment of conditions like arthritis, owing to their believed anti-inflammatory properties (Abilov & Tursunbaev, 2018). Ancient European literature has also documented the use of ant extracts to address afflictions such as aching eyes, impaired vision and cataract (Rastogi, 2011). Studies elucidated a wide range of pharmacological effects attributed to ant extracts. These effects include anti-inflammatory, analgesic, anti-aging, stress-reduction, antitumor, hepatoprotective, blood sugar-regulating, gonadal function enhancing, and immunomodulatory properties (Kou et al., 2005).

Obesity, characterized by the excessive accumulation of body fat, transcends cosmetic concerns and poses a substantial global health challenge. It is intrinsically linked to several chronic diseases, including diabetes, heart disease, particular cancer types, sleep disturbances, and coronary artery disease (Kopelman, 2000). The relationship between obesity and fertility issues has also been documented, with obese women being at a threefold higher risk of infertility compared to those with a normal body mass index (Brewer & Balen, 2010).

Diabetes mellitus, on the other hand, is a chronic metabolic disorder marked by elevated blood sugar levels. This condition causes disorder on multiple end organs, including the retina, kidneys, nervous

system, heart, and blood vessels. According to the International Diabetes Federation (IDF), in 2011, diabetes affected 366 million individuals worldwide, with projections estimating a staggering rise to 552 million by 2030 (Alam et al., 2014).

Provided the use of ants and their extracts in traditional medicine and the growing interest in their bioactive properties, this study investigates the therapeutic potential of the ant species *Camponotus compressus*. In the present study, both in vitro and in silico approaches were used to examine its anti-obesity, anti-diabetic and anti-inflammatory activities. The potential discoveries hold significant promise in addressing two of the most pressing health challenges of the present time.

## Materials and Methods

**Sample collection & identification.** The site from which the ants were collected was Perumanallur, Tiruppur (Lat: 11.2064416666; Long: 77.3717833333) with the help of forceps in a collection tube, and consequently identified as species *Camponotus compressus*, which constitutes a relatively large ant species, with worker ants measuring between 6 to 12 mm in length. They exhibit variable coloration, ranging from reddish-brown to black, with a slightly darker head. The head is heart-shaped, broader than the thorax, and characterized by well-defined, convex posterior corners. Their mandibles are relatively large and armed with sharp teeth. Antennae are 12-segmented, with the first segment longer than the head width, and the last segment notably elongated. The thorax is robust, with a flat or slightly convex pronotum. The petiole and post-petiole segments are well-defined, with one or two nodes in the petiole and a single node in the post petiole. The gaster (abdomen) is oval, often bearing fine hairs or pubescence. Their legs are long and slender, equipped

with distinct spines and hairs. These ants are equipped with a stinger, although they are not highly aggressive and typically use their stingers as a last resort for defense (Fabricius, 1787).

**Extract preparation.** The collected ants (identified as *C. compressus*) were air-dried at room temperature (26°C) for 2 days (d) and ground to a uniform powder. Ant powder (1000g) was taken in a conical flask and 2L of distilled water were added. After 24h the solution was centrifuged at 5000 rpm for 10 min and the supernatant was collected (Yamuna & Raja, 2019).

**GC-MS analysis.** The vital compounds present in the aqueous extract of *C. compressus* were examined using GC-MS analysis. GC-MS analysis was performed using the Agilent technologies 6890 N JEOL GC-MS Mate II model instrument (IFGTC Coimbatore). The aqueous extract of *C. compressus* was injected into a HP-5 column (30 m X 0.25 mm i.d with 0.25 µm film thickness). Helium was used as a carrier gas at a flow rate of 1.0 ml/min. The injection port was maintained at 200°C and column oven temperature was programmed as 50-250°C at a rate of 10°C/min injection mode. Mass spectra were obtained at an ionization voltage of 70 eV with ion source temperature of 250°C and the interface temperature was maintained at 250°C. The scanning range was 1- 2540 mass units.

**Anti-obesity assay.** Sulfo Phospho Vanillin method was used to determine the anti-obesity activity. 200µl lipid, 500µl reagent and 500µl lipase were taken in all test tubes. To these mixture, different amounts of the ant extract (100, 200, 300, 400 and 500 µl) were mixed and incubated at 37°C for 1h. After incubation, absorbance was measured at 540 nm. Percentage of inhibition was calculated using the following formula:

$$PI = \frac{Ab_c - Ab_s}{Ab_c} \times 100$$

where:

PI - Percentage Inhibition

AbC - Absorbance of Control

AbS - Absorbance of Sample

**Anti-diabetic assay.** The α-amylase inhibition method was used to determine the antidiabetic activity (Abdel Ghani et al., 2023). The enzyme α-amylase was mixed with different amounts of the ant extract (10, 20, 30, 40, 50, 60, 70 and 80µl) and incubated at 37°C for 10 min. To this mixture, 500µl starch were added and incubated at 37°C for 20 min. The reaction was then stopped by adding 2mL of dinitro-salicylic acid (DNS), followed by heating the mixture in a boiling water bath for 15 min. After cooling, the absorbance was measured at 540 nm. Percentage of inhibition was calculated using the following formula:

$$PI = \frac{Ab_c - Ab_s}{Ab_c} \times 100$$

**Anti-inflammatory assay.** The anti-inflammatory activity of the extract was determined with two methods *i.e.*, heat induced hemolysis and albumin denaturation inhibition.

#### 1. Heat induced hemolysis

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500µg/ml) and 1ml of a 10% RBCs suspension. For the control, instead of the test sample, only saline was added to the test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min. At the end of the incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was measured at 560nm.

The experiment was performed in triplicates for all the test samples (Leelaprakash & Mohan, 2011).

The percentage inhibition of hemolysis was calculated using the formula:

$$\text{Percentage inhibition of hemolysis} = (1 - (\text{test/control})) * 100$$

where:

test, the absorbance of the supernatant from the sample tube and

control, the absorbance of the supernatant from the control tube.

## 2. Albumin denaturation inhibition assay

Test solution (1ml) containing different concentrations (50 - 250 µg/ml) of drug was mixed with 1 ml of egg albumin solution (1 mM) and incubated at  $27 \pm 1^\circ\text{C}$  for 15 min. Denaturation was induced by keeping the reaction mixture at  $70^\circ\text{C}$  in a water bath for 10 min. After cooling, the turbidity was measured spectrophotometrically at 660nm. Percentage inhibition of denaturation was calculated for each treatment and compared with the control, in which no drug was added. Each experiment was done in triplicate (Kumarappan et al., 2006).

***In-silico* anti-obesity activity.** The anti-inflammatory activity of the extract was determined with two methods *i.e.*, heat induced hemolysis and albumin denaturation inhibition.

## 1. Protein structure retrieval and Preparation:

The 3D protein structures of pancreatic lipase and leptin were downloaded from the Protein Data Bank. PDB ID of pancreatic lipase is 1LPB (Gandhi et al., 2019) and leptin is (1AX8). PDB file of protein was imported and prepared by using the Discovery Studios. Preparation of protein included adding missing hydrogen atoms and optimizing hydrogen bonds, correcting

ambiguous protonation states, and flipped residues and removing co-crystallized ligand.

## 2. Ligand retrieval and preparation

Major compounds present in *C. compressus* were selected for in silico studies against pancreatic lipase. The 3D structures of all the selected compounds were retrieved from Pub Chem database. The downloaded structures were subjected for energy minimization using PyRx Software. Universal force field (UFF) and first order steepest descent algorithm were used to obtain energetically stable conformations for the structures which give lower delta G values resembling biological systems.

## 3. Molecular docking analysis

To study ligand-enzyme interactions docking studies were performed using PyRx software. Receptor structure (PDB ID 1LPB) is treated as rigid structures during docking analysis irrespective of their conformation in biological system. All compounds are then docked into binding site of the protein. Visualization and analysis were done by Discovery Studios software. The docking score and interactions with important amino acids were also analyzed.

***In-silico* ADMET analysis and Drug likeness.** The two vital compounds viz, 2,3-dihydroxypropyl elaidate and lycoxanthin in *C. compressus* extract were subjected to ADMET analysis using admetSAR web tool. Selected compounds were assessed for blood brain barrier penetrability (BBB), Caco-2 cell permeability, human intestinal absorption, canine kidney cell permeability, plasma protein binding distribution and skin permeability using admetSAR software (Azad et al., 2019). Drug likenesses of selected compounds were analyzed by Lipinski rules.

Results

Identification of ant species

The collected ants were identified as *Camponotus compressus*.

Extract preparation

2000ml of the milky white extract of *C. compressus* were obtained.

GC-MS analysis of *C. compressus* extract

The GC-MS results for the whole-body extract of *C. compressus* showed the presence of 32 bioactive compounds (Fig. 1). These compounds and their chemical formulas are presented in Table 1. 2,3-dihydroxypropyl elaidate and Lycoxanthin have more area percentage. These results are in accordance with previously published results (Yamuna & Raja, 2019).

Concerning the essential oils, we noted that the corrected mortality rate of aphids was equal to or more than 50% for all

treatments except for the rate of 1000ppm (28.50 %), and the difference was not significant between treatments ( $p > 0.05$ ) according to ANOVA statistical analyses (Table 1). In addition, the estimated  $LC_{50}$  of the studied EOs was 349.33ppm.

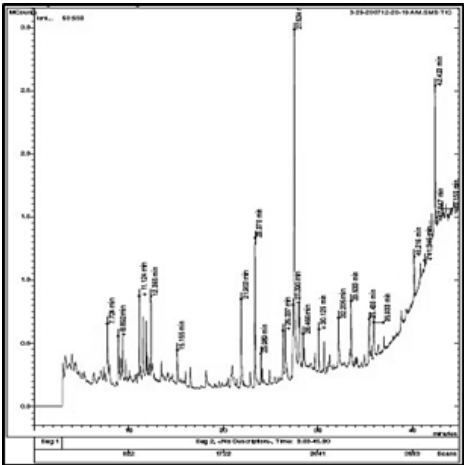


FIG. 1.: GC-MS micrograph of *C. compressus* extract

TABLE 1: Bioactive compounds identified from a *Camponotus compressus* extract

Compound	Structure	Retention time	Area	Percentage of total
5-Methyl-6-phenyltetrahydro – 1,3-oxazine-2-thione	C <sub>11</sub> H <sub>13</sub> NOS	7.734	2.357 e+6	3.419
Bicyclo[5.1.0]octane , 8-methylene	C <sub>24</sub> H <sub>2</sub> OO <sub>5</sub>	8.892	1.388 e+6	2.013
Pentadecane	C <sub>15</sub> H <sub>32</sub>	9.001	1.05e 4+6	1.529
7-Chloro-2,3-dihydro-3-(4nitrobenzylidene)-5-phenyl-1H-1,4-ben	C <sub>22</sub> H <sub>14</sub> ClN <sub>3</sub> O <sub>3</sub>	9.172	633188	0.918
Diphenyl r-2-methoxycarbonyl-2,c-5diphenylpyrrolidin-c-3,c-4-d	C <sub>32</sub> H <sub>27</sub> NO <sub>6</sub>	9.339	2.169e+6	3.146
Cyclohexene, 1-methyl-5-(1methylethenyl)-,(R)-	C <sub>25</sub> H <sub>28</sub> NO <sub>2</sub> P	9.668	424552	0.616
1-Heptanol, 2-propyl-	C <sub>10</sub> H <sub>22</sub> O	11.124	1.729e+6	2.507
Pyrroline, 1,2-dimethyl-	C <sub>6</sub> H <sub>11</sub> N	11.524	1.395	2.023
Decane	C <sub>10</sub> H <sub>22</sub>	11.832	966958	1.402

(TABLE 1 continues)

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(TABLE 1 continued)

Cyclopropane. -1-methyl-1-ethenyl-2-(2furyl)-	C <sub>10</sub> H <sub>12</sub> O	12.365	1.744e+6	2.529
Butyl 9-tetradecenoate	C <sub>18</sub> H <sub>34</sub>	15.155	858820	1.246
Benzenemethanol, α-[1-(ethylmethylamino)ethyl]-, [R-(R*,S*)]	C <sub>12</sub> H <sub>19</sub> NO	21.903	3.499e+6	2.529
Hexadecanoic acid (CAS)	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	23.370	5.536e+6	8.030
3-Hydroxymyristic acid	C <sub>14</sub> H <sub>28</sub> O <sub>3</sub>	23.989	921599	1.337
1-Dotriacontanol (CAS)	C <sub>32</sub> H <sub>66</sub> O	26.337	1.524e+6	2.210
Tritetracontane	C <sub>43</sub> H <sub>88</sub>	26.580	1.833e+6	2.658
Ethyl linoleate	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	27.390	2.532e+6	3.672
Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	27.974	2.205e+6	3.198
Octane	C <sub>8</sub> H <sub>18</sub>	28.062	3.087e+6	4.477
2,3-Dihydroxypropyl elaidate	C <sub>21</sub> H <sub>40</sub> O <sub>4</sub>	27.534	1.478e+7	21.439
5,8,10-Undecatrien-3-ol	C <sub>11</sub> H <sub>18</sub> O	28.466	809670	1.174
3-Undecen-1-yne	C <sub>11</sub> H <sub>18</sub>	30.125	1.231e+6	1.785
2-Pentacosanone	C <sub>25</sub> H <sub>50</sub> O	30.677	959982	1.392
Oleic anhydride	C <sub>36</sub> H <sub>66</sub> O <sub>3</sub>	32.205	2.046e+6	2.968
N'-(2-Methyl-4-hlorophenyl)-Ncyclohexyformamidine	C <sub>11</sub> H <sub>16</sub> N <sub>2</sub>	33.533	1.773e+6	2.571
Acetic acid, (triphenylphosphoranylidene)	C <sub>21</sub> H <sub>19</sub> O <sub>2</sub> P	35.430	1.296e+6	1.879
Cholesta-3,5-diene (CAS)	C <sub>27</sub> H <sub>44</sub>	35.933	1.472e+6	2.135
5HCyclopropa[3,4]benz[1,2-e]azulen-5-one,	C <sub>30</sub> H <sub>40</sub> O <sub>13</sub>	40.216	1.135e+6	1.646
Cholest-5-en-3-ol (3.beta.)-, 9octadecenoate,	C <sub>45</sub> H <sub>78</sub> O <sub>2</sub>	41.315	267233	0.388
Cholesta-4,6-dien-3-ol	C <sub>27</sub> H <sub>44</sub> O	41.839	712039	1.033
<b>Lyxoxanthin</b>	<b>C<sub>40</sub>H<sub>56</sub>O</b>	<b>42.423</b>	<b>5.438e+6</b>	<b>7.887</b>
Cholest-5-en-3-ol (3.beta.)-	C <sub>29</sub> H <sub>48</sub> O <sub>2</sub>	42.547	141821	0.206

### Anti-obesity activity

The extract showed excellent results for anti-obesity activity. The Sulfo Phospho Vanillin method was used to determine the anti-obesity activity. The amount of lipid

broken down was increased when the lipase was mixed with the ant extract. The amount of lipid which was not broken down binded with the reagent Sulfo Phospho Vanillin. This produced a colored solution. The

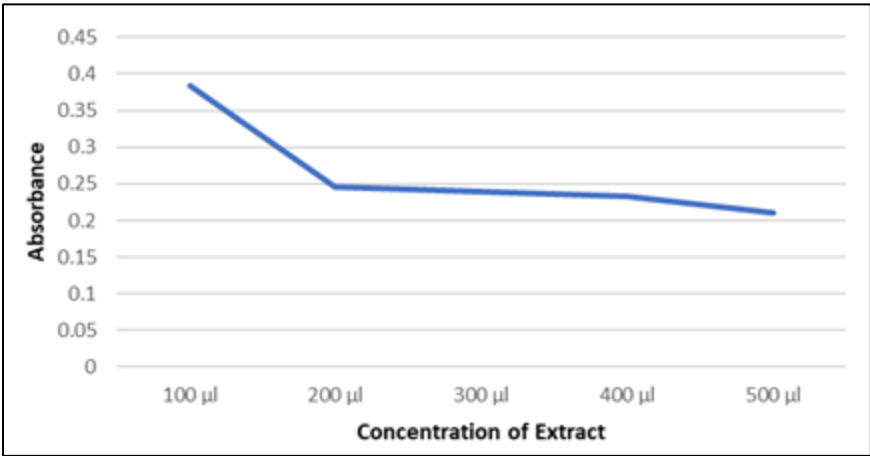


intensity of the color was measured spectrometrically at 540nm. When the concentration increased, the activity of the

extract also increased. The results of the anti-obesity activity are presented in the Table 2 and Figure 2.

**TABLE 2:** Anti-obesity activity of the *Camponotus compressus* extract

Sample	Extract concentration (μl)	Lipid (μl)	Reagent (μl)	Lipase (μl)	Absorbance at 540nm	% Inhibition
Blank	-	200	500	500	1.597	-
Sample I	100	200	500	500	0.384	75.94
Sample II	200	200	500	500	0.245	84.65
Sample III	300	200	500	500	0.239	85.03
Sample IV	400	200	500	500	0.233	85.41
Sample V	500	200	500	500	0.210	86.85



**FIG. 2.:** Anti-obesity activity of the *Camponotus compressus* extract

**Anti-diabetic activity**

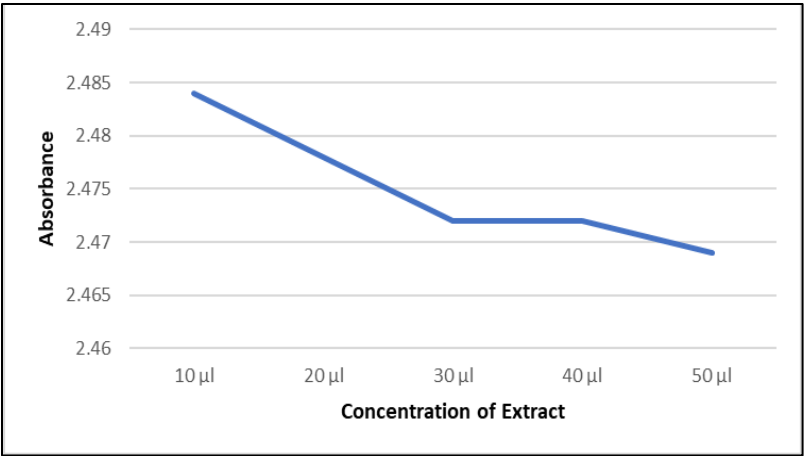
The potential of the extract to have anti-diabetic activity was confirmed by the results of the  $\alpha$ -amylase inhibition assay.  $\alpha$ -amylase is an important enzyme which helps in the breakdown of starch, which in turn releases glucose in blood. By inhibiting

the  $\alpha$ -amylase, the amount of glucose released into blood will be decreased. The compounds present in the extract inhibited the  $\alpha$ -amylase, providing some initial evidence for potential anti-diabetic activity. The absorbance measured at 540 nm are presented in Table 3 and Figure 3.



**TABLE 3:** Anti-diabetic activity of the *Camponotus compressus* extract.

Sample	Extract concentration (µl)	1% starch (µl)	α- amylase (µl)	DNS (ml)	Absorbance at 540nm	% inhibition
Blank	-	500	200	2	2.490	-
Sample I	10	500	200	2	2.484	0.24
Sample II	20	500	200	2	2.478	0.48
Sample III	30	500	200	2	2.472	0.72
Sample IV	40	500	200	2	2.472	0.72
Sample V	50	500	200	2	2.469	0.84



**FIG. 3.:** Anti-diabetic activity of *Camponotus compressus* extract

**Anti-inflammatory activity**

**1. Heat induced hemolysis**

The anti-inflammatory properties of the *C. compressus* extract were confirmed by the results of the heat induced hemolysis assay. In high temperatures, the RBCs start breaking down. The compounds present in the extract prevented the RBCs from lysis. Hemolysis was decreased when the extract concentration increased. At the concentration of 120 µl the percentage of inhibition of hemolysis was 54.68%. The absorbances measured during the heat

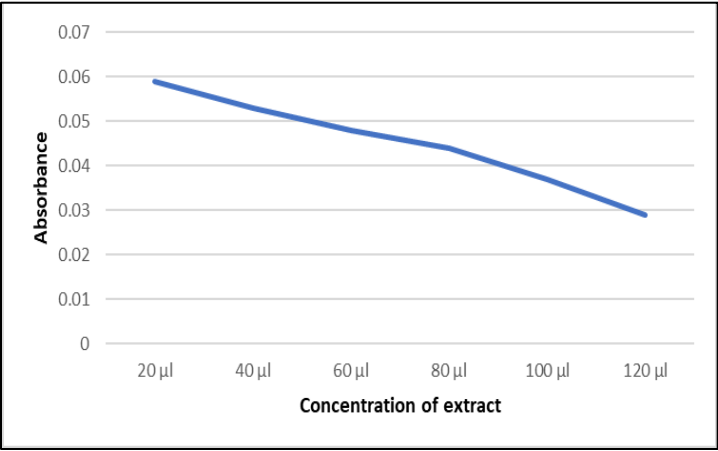
induced hemolysis assay are given in Table 4 and Figure 4.

**2. Albumin denaturation inhibition**

The results of albumin denaturation inhibition assay conform the anti-inflammatory properties of the whole-body extract of *C. compressus*. Albumin is a serum protein which has an important role in blood clotting. The compounds present in the extract preserved albumin from denaturation. This contributes to the potential of the extract to have anti-inflammatory activity. The results are presented in Table 5 and Figure 5.

**TABLE 4:** Anti-inflammatory activity of the *Camponotus compressus* extract

Sample	Extract concentration (μl)	Absorbance at 560 nm	% Inhibition
Blank	-	0.064	-
Sample I	20	0.059	7.81
Sample II	40	0.053	17.18
Sample III	60	0.048	25
Sample IV	80	0.044	31.25
Sample V	100	0.037	40.62
Sample VI	120	0.029	54.68



**FIG. 4.:** Anti-inflammatory activity of the *Camponotus compressus* extract

**TABLE 5:** Albumin denaturation inhibition assay

Sample	Extract concentration (μl)	Absorbance at 660 nm	% Inhibition
Blank	-	0.115	-
Sample I	20	0.112	2.6
Sample II	40	0.103	10.43
Sample III	60	0.099	13.91
Sample IV	80	0.084	26.95
Sample V	100	0.075	34.78
Sample VI	120	0.062	46.08

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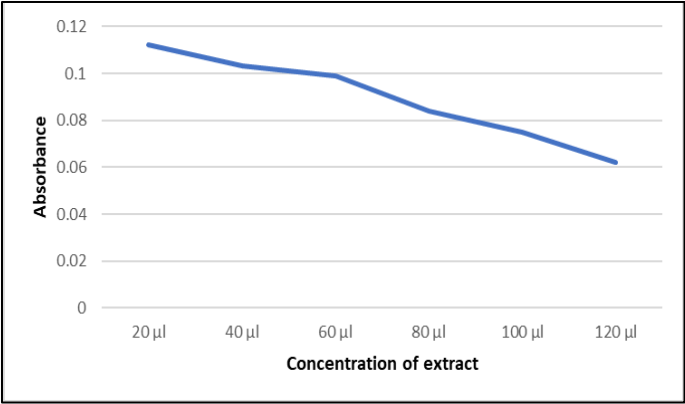


FIG. 5.: Albumin denaturation inhibition

**In silico anti-obesity activity**

The anti-obesity activity of the extract was also assessed with in silico studies to confirm the results obtained by the in vitro studies. The docking was done between ligands 2,3-dihydroxypropyl elaidate and lycoxanthin and receptors leptin and pancreatic lipase. The ligands showed excellent binding affinity with receptors.

The ligand 2,3-dihydroxypropyl elaidate binds with receptor leptin with the binding affinity of -4.8 and with receptor pancreatic lipase with the binding affinity of -6.1. Another ligand lycoxanthin binds with receptors leptin and pancreatic lipase with the binding affinity of -5.4 and -7.2, respectively. The binding affinity of ligands with receptors are presented in Table 6 and Figs. 6.1-6.4.

**TABLE 6:** *In silico* anti-obesity activity of the *Camponotus compressus* extract.

Ligand	Biding affinity with receptors	
	Leptin	Pancreatic lipase
2,3-Dihydroxypropyl elaidate	-4.8	-6.1
Lycoxanthin	-5.4	-7.2

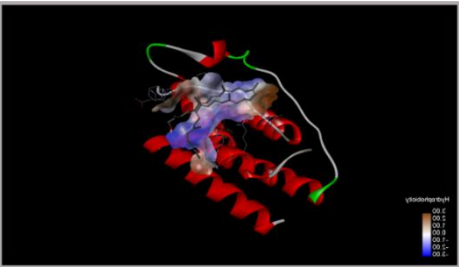


FIG. 6.1.: 3D structure leptin-2,3-dihydroxypropyl elaidate binding.

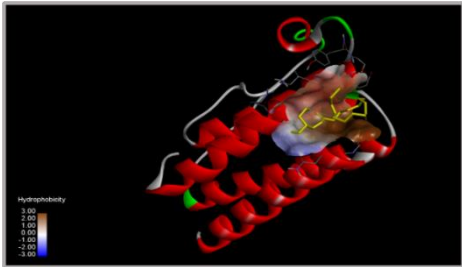


FIG. 6.2.: 3D structure leptin-lycoxanthin binding.

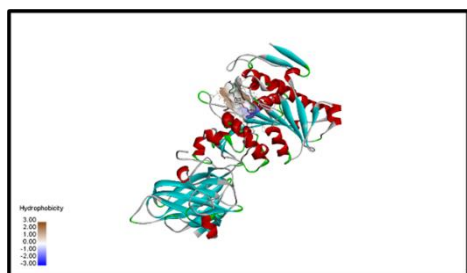


FIG. 6.3.: 3D structure of pancreatic lipase-2,3-dihydroxypropyl elaidate binding.

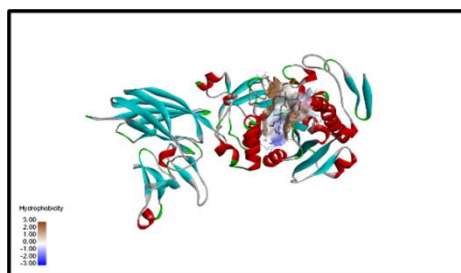


FIG. 6.4.: 3D structure of pancreatic lipase – lycoxanthin binding.

### ADMET analysis and drug likeness

The ADMET properties of the compounds present in the extract were analyzed using admetSAR webtool. The analysis exposed the characteristics of the compound's

absorption, distribution, metabolism, excretion and toxicity in human body. The compounds potential to be used as drug was confirmed by Lipinski's rule of five. The properties of the extract compounds are given in Table 7.

**TABLE 7:** ADMET analysis and drug likeness

ADMET Properties	lycoxanthin	2,3-dihydroxypropyl elaidate
Blood brain barrier	+	-
Caco-2	-	-
Human Intestinal Absorption	+	+
Respiratory toxicity	-	+
Drug likeness	Suitable	Suitable

### Discussion

The results of our study unveiled a promising therapeutic potential of the *C. compressus* extract with considerable anti-obesity, anti-diabetic, and anti-inflammatory activities, reinforcing their significance in the realm of natural remedies and medicinal research.

#### Anti-obesity activity

The assessment of the anti-obesity activity demonstrated compelling outcomes. The Sulfo Phospho Vanillin method enabled us to quantify the breakdown of lipids. It was observed that the presence of the ant extract led to an

increase in lipid degradation, consequently reducing the quantity of unmetabolized lipids that reacted with the Sulfo Phospho Vanillin reagent, generating a colored solution. The concentration-dependent relationship between the extract and its activity is evident, with higher concentrations resulting in more pronounced anti-obesity effects. This observation aligns with previous studies that have explored the anti-obesity properties of natural compounds and plant extracts (Kim et al., 2018; Song et al., 2022). The results suggest the presence of bioactive compounds in the *C. compressus* extracts with the potential to aid in weight

management and the prevention of obesity-related health issues.

### Anti-diabetic activity

In the realm of anti-diabetic activity, our study employed the  $\alpha$ -amylase inhibition assay to assess the extract's capacity to mitigate the release of glucose into the bloodstream by inhibiting the breakdown of starch. The results revealed that the compounds within the extract exhibit inhibitory effects against  $\alpha$ -amylase, leading to reduced glucose release. This aligns with established research on the anti-diabetic properties of various plant extracts and compounds (Sabiu et al., 2019). Our findings support the notion that *C. compressus* extract contain bioactive agents with potential applications in diabetes management by moderating blood glucose levels.

### Anti-inflammatory activity

The evaluation of anti-inflammatory activity unfolded through two distinctive assays: heat-induced hemolysis and albumin denaturation inhibition. The first assay demonstrated a concentration-dependent inhibition of hemolysis, emphasizing the extract's ability to shield red blood cells from lysis, particularly at higher concentrations. The second assay reinforced the presence of anti-inflammatory properties by preserving albumin from denaturation, with increasing concentrations of the extract corresponding to enhanced inhibitory effects. These findings coincide with research on the anti-inflammatory attributes of natural compounds and plant extracts (Soni et al., 2019), supporting the potential of *C. compressus* extracts in managing inflammation-related health conditions.

### In silico anti-obesity activity

The promising in vitro anti-obesity results prompted us to delve into in silico studies to further understand the molecular

interactions between the extract's compounds and key targets. The docking studies revealed that the ligands, 2,3-dihydroxypropyl elaidate and lycopanthin, exhibited strong binding affinities with Leptin and Pancreatic lipase, providing insight into the potential mechanisms underlying the extract's anti-obesity effects. This aligns with previous in silico studies on ligand-receptor interactions (Chen et al., 2018; 2020), indicating that *C. compressus* compounds may play a pivotal role in modulating pathways associated with obesity.

### ADMET analysis and drug likeness

The analysis of ADMET properties and drug likeness is crucial for assessing the safety, effectiveness, and pharmacokinetics of potential therapeutic agents. Our results indicate that the compounds in the extract display favorable characteristics in terms of blood-brain barrier penetration, human intestinal absorption, and drug likeness, making them promising candidates for further drug development. It's worth noting that the presence of drug-like compounds in natural extracts has been explored in prior research, underlining their potential as pharmaceutical agents (Ertl et al., 2010; Leeson & Springthorpe, 2007).

## Conclusion

In conclusion, the multifaceted potential of *C. compressus* extract, as revealed in our study, highlights its significance in natural medicine and pharmaceutical research. The anti-obesity, anti-diabetic and anti-inflammatory properties of the extract were also supported by the findings of the *in-silico* study and the favorable ADMET profiles, that suggest the possible development of novel therapeutic interventions. These findings open doors for further investigations into the isolation and characterization of bioactive compounds, paving the way for innovative healthcare and drug development solutions.

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## Δράση κατά της παχυσαρκίας, αντιδιαβητική δράση και αντιφλεγμονώδης δράση του εκχυλίσματος του *Camponotus compressus*. *In vitro* και *in-silico* μελέτες

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### ΠΕΡΙΛΗΨΗ

Ιστορικό: Τα τελευταία χρόνια, υπάρχει αυξανόμενο ενδιαφέρον για τα πιθανά οφέλη των εντόμων, κυρίως των μυρμηγκιών, για την ανθρώπινη υγεία, λόγω της παρουσίας σε αυτά πιθανών βιοδραστικών ουσιών που μπορούν να παίξουν σημαντικό ρόλο στη θεραπευτική έρευνα. Στην παρούσα μελέτη, μελετήθηκε το θεραπευτικό δυναμικό του εκχυλίσματος *Camponotus compressus*, εστιάζοντας στις βιοδραστικές του ενώσεις και τις ποικίλες εφαρμογές τους.

Μέθοδοι: Η κατά της παχυσαρκίας, αντιδιαβητική και αντιφλεγμονώδης δράση του εκχυλίσματος *C. compressus* αξιολογήθηκε μέσω διαφόρων μεθόδων, δηλαδή, το δυναμικό κατά της παχυσαρκίας προσδιορίστηκε με βάση την εκτίμηση αναστολής της παγκρεατικής λιπάσης, η αντιδιαβητική δράση αναλύθηκε χρησιμοποιώντας μια δοκιμασία αναστολής α-αμυλάσης, ενώ η αντιφλεγμονώδης δράση χρησιμοποιώντας δοκιμασίες αναστολής της επαγόμενης από θερμότητα αιμόλυσης και μετουσίωσης της αλβουμίνης. Ο τρόπος δράσης του εκχυλίσματος διερευνήθηκε μέσω *in-silico* μελετών, ενώ αξιολογήθηκαν οι ιδιότητες ADMET.

Αποτελέσματα: Σύμφωνα με προηγούμενα ευρήματα, η ανάλυση μέσω αέριας χρωματογραφίας-φασματομετρίας μάζας (GC-MS) αποκάλυψε 32 βιοδραστικές ενώσεις, συμπεριλαμβανομένων ορισμένων κομβικών μορίων, όπως το 2,3-διυδροξυπροπυλελιδικό και η λυκοξανθίνη. Το εκχύλισμα εμφάνισε δόσοεξαρτώμενη δράση κατά της παχυσαρκίας και αναστολή της α-αμυλάσης, υποδηλώνοντας αντιδιαβητικό δυναμικό. Οι αντιφλεγμονώδεις ιδιότητες του εκχυλίσματος επιβεβαιώθηκαν μέσω των δοκιμασιών επαγόμενης από θερμότητα αιμόλυσης και μετουσίωσης της αλβουμίνης. Οι μελέτες *in-silico* αποκάλυψαν ισχυρές συγγένειες δέσμευσης με πρωτεΐνες στόχους, ενώ η ανάλυση ADMET υποστήριξε την ομοιότητα με φάρμακα.

Συμπέρασμα: Με βάση τα αποτελέσματά μας, το εκχύλισμα *C. compressus* έχει θεραπευτικές δυνατότητες και θα μπορούσε ενδεχομένως να είναι χρήσιμο κατά της παχυσαρκίας, κατά του διαβήτη και κατά της φλεγμονής. Η μελέτη παρέχει πληροφορίες για τον τρόπο δράσης του συγκεκριμένου εκχυλίσματος, υποστηρίζοντας τις δυνατότητές του ως υποψήφιο νέο φάρμακο για ανθρώπινη χρήση.