INTERNATIONAL STUDIES AND EVALUATIONS IN THE FIELD OF

BIOLOGY

December 2024

EDITOR

PROF. DR. HASAN AKGÜL

SERÜVEN YAYINEVİ

Genel Yayın Yönetmeni / Editor in Chief • C. Cansın Selin Temana

Kapak & İç Tasarım / Cover & Interior Design • Serüven Yayınevi

Birinci Basım / First Edition • © Aralık 2024

ISBN • 978-625-5955-75-3

Serüven Yayınevi / Serüven Publishing
Türkiye Adres / Turkey Address: Kızılay Mah. Fevzi Çakmak 1. Sokak
Ümit Apt No: 22/A Çankaya/ANKARA
Telefon / Phone: 05437675765
web: www.seruvenyayinevi.com
e-mail: seruvenyayinevi@gmail.com

Baskı & Cilt / Printing & Volume
Sertifika / Certificate No: 47083

INTERNATIONAL STUDIES AND EVALUATIONS IN THE FIELD OF

BIOLOGY

DECEMBER 2024

EDITOR

PROF. DR. HASAN AKGÜL

CONTENTS

CHAPTER 1

ETHNOMEDICINAL USE, PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF RUTA GRAVEOLENS L.

Cennet ÖZAY[1]

1 Assist. Prof. Cennet ÖZAY, Izmir Katip Celebi University, Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Izmir, Türkiye. E-mail: cennet.ozay@ikcu.edu.tr, ORCID ID: 0000-0002-1120-6122

1. INTRODUCTION

Since the dawn of human history, plants have been used for medicinal purposes. When they discovered their healing properties, people began using herbs for health benefits thousands of years ago. After many years of use, folk remedy methods have endured in Anatolia, Türkiye, a region known for its folk medicine. Plants are also the source of many medications utilized in contemporary medicine (Faydaoğlu and Sürücüoğlu, 2011).

Herbal remedies are used differently in different countries based on their level of development. Eighty percent of people in developing nations use herbal items for medicinal purposes. This percentage increases to 95% in several Middle Eastern, Asian, and African nations. According to World Health Organization predictions, more people worldwide will receive medicinal plant treatments in the upcoming years (Hoenders et al., 2024).

The Rutaceae family, sometimes referred to as the rue or citrus family, is made up of about 1,500 species and 160 genera that are found all over the world, primarily in tropical and subtropical areas (Wei et al., 2015). Due to their antibacterial, antifungal, anti-leishmanial, and anti-plasmodial qualities, this family of natural products has been the subject of extensive research, which indicates their potential use in the treatment of a wide range of illnesses, including cancer, depression, and Alzheimer's disease (Adamska-Szewczyk et al., 2016). The *Ruta* genus is one of the Rutaceae family plants that have been investigated. *Ruta*, also known as rue, is the type genus of the Rutoideae subfamily and a member of the Ruteae tribe (Wei et al., 2015). *Ruta* is a strongly scented subshrub native to the Mediterranean region. Ten species of perennial shrubs belong to the genus *Ruta*, with *Ruta graveolens* being one of the most widely distributed (Hammami et al., 2015).

Ruta graveolens L. is a fascinating species in the pharmaceutical field, garnering considerable attention for its medicinal applications. Additionally, it serves as a source of various secondary metabolites that exhibit a wide range of biological activities (Parray et al., 2012). An overview of the botanical traits, traditional medical applications, phytochemical composition, therapeutic uses, and biological activities of *R. graveolens* is intended to be provided in this chapter.

2. ETYMOLOGY, BOTANICAL FEATURES AND GEOGRAPHICAL DISTRIBUTION OF *R. GRAVEOLENS*

The Common Rue (*Ruta graveolens*), also known as Herb-of-grace is one of the *Ruta* species cultivated as an ornamental plant and herb. It belongs to the Rutaceae family and is known as Turkish Sedefotu or kokar sedef (Akalin and Ertuğ, 2003). *R. graveolens* means strong-smelling. It has a peculiar, unpleasant odour. In ancient Greek, 'ruta' means 'helper and saviour' and

'graveolens' means 'strong-smelling'. It has been used for medicinal purposes since ancient times and was believed to have protective powers. Therefore, the word 'ruta' has been associated with such meanings. The pungent smell of *R. graveolens* causes animals such as snakes, martens, cats and mice to flee, while houseflies love this smell (Bennaoum et al., 2017).

R. graveolens is a small evergreen sub-shrub or semi-woody perennial, typically reaching 0.6 to 0.9 meters in height and spreading to a similar width. Its stems become woody near the base while remaining herbaceous towards the tips. The leaves, measuring 7.6 to 12.7 cm in length, are pinnately dissected into oblong or spoon-shaped segments. These somewhat fleshy leaves are often covered with a powdery bloom (Figure 1a). The sea-green foliage emits a strong, pungent, and rather unpleasant odor when bruised. In midsummer, small yellow flowers bloom in paniculate clusters, rising above the foliage and frequently covering much of the plant (Zargari, 1988; Kannan and Babu, 2012).

The herbaceous perennial *R. graveolens* is native to the Mediterranean region (Figure 1b). These days, it is grown in Asia, South America, and Europe for both medicinal and decorative purposes. Since ancient times, rue has been a staple plant in European pharmacopoeia (Mejri et al., 2010).

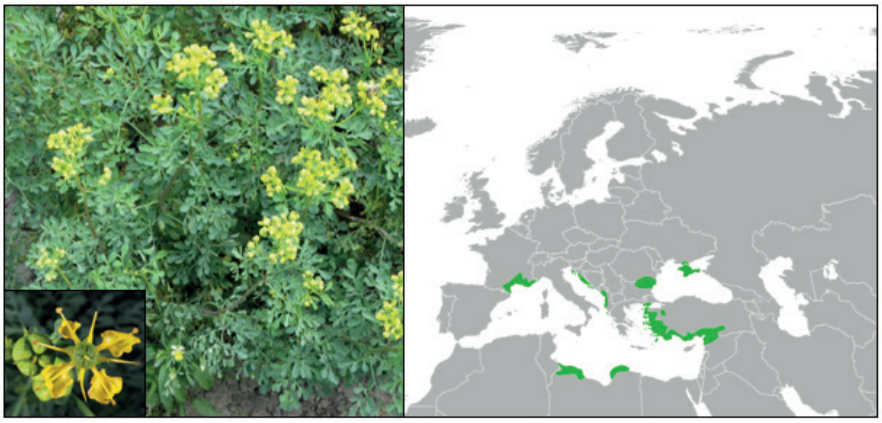

Figure 1. *Ruta graveolens (a) and its distribution (b) in the world*

3. PHYTOCHEMISTRY OF *R. GRAVEOLENS*

The plant *Ruta graveolens* has been utilized in various areas of the pharmaceutical industry. Extracts and essential oils of *R. graveolens* are important components in the development of drugs with numerous pharmacological activities in many countries. A number of chemical constituents such as alkaloids, coumarins, volatiles, terpenoids, flavonoids

and furoquinolines have been isolated from different parts of the plant (Kuzovkina et al., 2004). Many of these metabolites have attracted biological and pharmacological interest, demonstrating antifungal, phytotoxic, and antidotal activities (Sampaio et al., 2018). Figure 2 shows the wide variety of compounds that were described for *R. graveolens*.

Rutin, quercetin, psoralen, methoxypsoralen, rutacridone, rutacridone epoxide and gravacridondiol are the phytochemical compounds reported from this plant. α-Pinene, limonene and 1,8-cineole were identified as the main monoterpene constituents for *R. graveolens* essential oil (Hashemi et al., 2011).

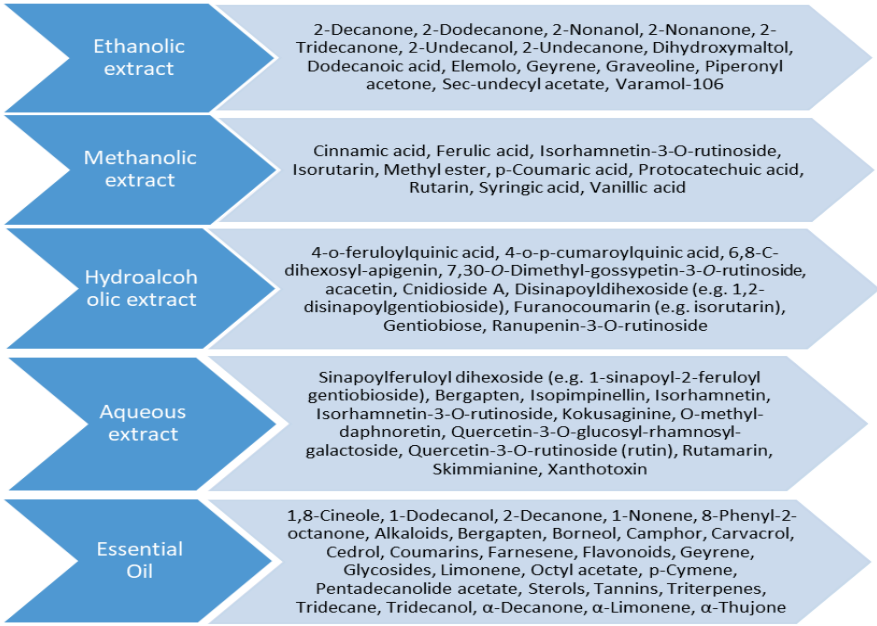

Figure 2. *Phytochemical composition of essential oil and different extracts of Ruta graveolens*

Plants produce numerous types and quantities of secondary metabolites, and these metabolites are crucial for environmental adaptation. In order to defend themselves against natural enemies including bacteria, viruses, fungus, and insects, plants have the ability to create secondary metabolites (Mammadov et al. 2017). Because of these compounds' pharmacological properties, they also serve as significant sources of pharmaceutical medications (Ozay et al. 2016). Chemical bioactive substances like terpenoids, phenolics, alkaloids, flavonoids, amino acids, saponins, glycosides, diterpenes, and triterpenes are abundant in medicinal plants (Alper and Ozay, 2022)

The widely known phytochemical compounds of *R. graveolens* are acridone alkaloids, coumarins, volatiles, terpenoids, flavonoids and furoquinolines. The presence of saponins, tannins and glycosides has also been proven (Hashemi et al., 2011). Rutin and quercetin are the main active flavonoids of *R. graveolens*. Rutin was first isolated from the leaves of R. graveolens (Pathak et al., 2003). *R. graveolens* essential oil contains high levels of aliphatic acids, alcohols and ketones (Ivanovaa et al., 2003). *R. graveolens* also produces high levels of linear furanocoumarins, mostly psoralen and methoxypsoralen (Gravot et al., 2004).

Furanoacridones and acridone alkaloids extracted from *R. graveolens* have been shown to have anticancer potential in vitro experiments carried out using human cell lines. *R. graveolens* has been rapidly cloned by the use of *in vitro* methods. It has been successfully found that applying hairy root culture is advantageous for increased synthesis of bioactive chemicals from this plant species. According to a survey of the literature, this plant species is intriguing to the pharmaceutical sector since it can have a variety of pharmacological effects (Malik et al. 2016).

Plant metabolisms are highly complex, involving intricate and lengthy chemical interactions that are frequently impractical to conduct in a lab. As a result, a large number of active raw ingredients used in the pharmaceutical industry come only from plant sources and are not produced by humans. Utilizing biotechnology, it is possible to extract bioactive molecules from plants that are unlikely to be produced in a lab. Using a variety of biotechnological techniques, it is also possible to increase the synthesis of secondary metabolites from plants (Malik et al. 2014).

4. ETHNOMEDICINAL USE OF *R. GRAVEOLENS*

Ruta species have been used in traditional medicine for a wide range of diseases. Their main therapeutic use is in the gynecological field, but the treatment of pain, fever, nausea, inflammation, infections, and nervous disorders, among others, are also described. *Ruta* species have been used to regulate fertility, as a fertility prevention agent, to control menstrual flow and bleeding, to induce abortion and as a contraceptive (Coimbra et al. 2020).

R. graveolens, or rue has a long history of ethnomedical use that dates back centuries, with numerous traditional medical systems documenting its benefits. Owing to its diverse therapeutic properties (Figure 3), this Mediterranean-native perennial herb has been used in folk medicine for a very long time (Malik et al. 2016).

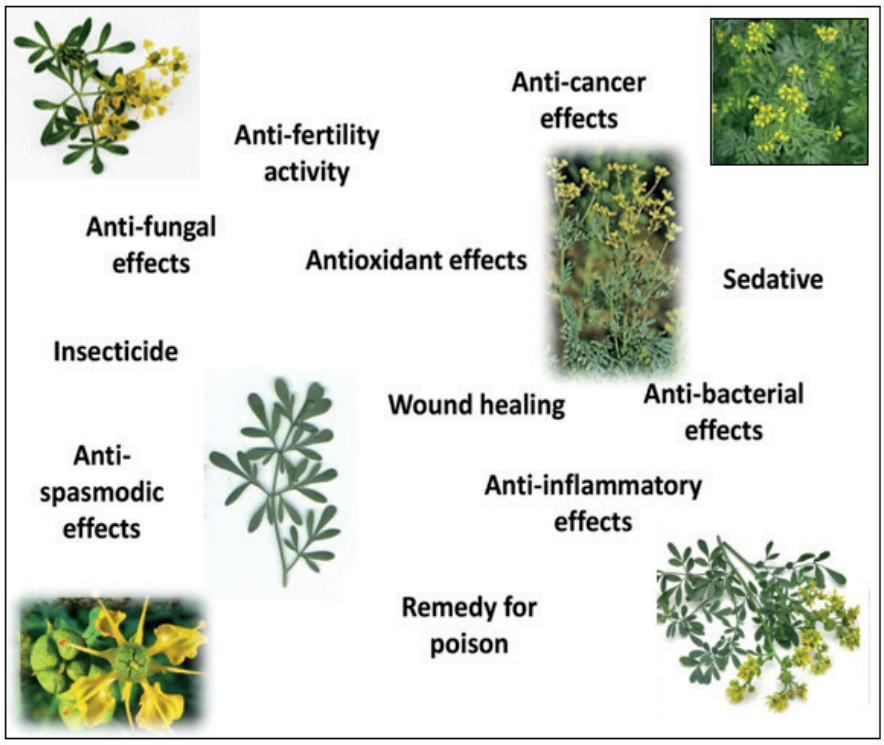

Figure 3. *Therapeutic properties of Ruta graveolens (Common Rue)*

In India, it is used in folk medicine to treat rheumatism, arthritis and other inflammatory conditions (Ratheesh et al., 2010). *R. graveolens* is also a plant used in magical rituals to treat supernatural folk diseases and against the evil eye or the influence of evil spirits (Cavender and Albán 2009; Quave and Pieroni, 2005). In the Amazon region, *R. graveolens* leaves have been used for headaches, dizziness, brain weakness, flu with cough, fever, hoarseness, paralysis, toothache, numbness after insect bites, and intestinal pain. It is also used for personal protection as a magical herb against bad energies (Coelho-Ferreira, 2009; Hale et al., 2004).

An ethnobotanical study in South Africa identified *R. graveolens* as the second most medicinal plant reported in the survey (Thring and Weitz, 2006). This description shows the importance of *R. graveolens* as a medicinal plant worldwide. Besides the medicinal purpose of this species, there are some reports of toxic effects on the liver and kidneys and some reports that it is abortive. *R. graveolens* can damage important organs of the body when taken in high doses (Prabhu et al., 2014). The leaves of this species can cause chemical irritation of the skin. Some dermatitis has been reported, especially in children (Cavender and Albán, 2009).

5. BIOLOGICAL ACTIVITIES OF *R. GRAVEOLENS*

R. graveolens is widely distributed and used for a variety of medical purposes throughout the world. This has led to the development of numerous studies assessing the biological activity of the plant to support its therapeutic use. *R. graveolens* has been investigated as a potential source for new therapeutic alternatives or supplements. This has involved the analysis of both isolated compounds and the whole phytocomplex to develop herbal medicines. Numerous studies have demonstrated various biological activities such as contraceptive, anti-inflammatory, antimicrobial and analgesic effects.

In the context of developing new contraceptive drugs, Guerra and Andrade (1978) conducted an in vivo study on pregnant albino primiparous female rats. They administered *R. graveolens* extract either intramuscularly or orally and observed a significant contraceptive effect, including the prevention of egg implantation. In the search for male contraceptive agents, Harat et al. (2008) conducted an in vitro study on human sperm using an aqueous extract of *R. graveolens*, which showed promising results. Oral administration of 5 g/kg of aqueous *R. graveolens* extract in male rats led to reduced sperm motility within an hour of administration, without affecting other sperm parameters, suggesting the plant's potential use in male contraception (Halvaei et al. 2012).

In a study investigating the anti-inflammatory effects of *R. graveolens* extract and rutin (a flavonoid found in the plant), Raghav et al. (2006) demonstrated that the extract exhibited stronger anti-inflammatory activity compared to rutin. This was shown using various models, including murine macrophage cells (J-774) stimulated with lipopolysaccharide (LPS), as well as the induction of inflammation through nitric oxide and other mediators. The isolated compound from methanolic extract of R. graveolens, identified as 3-(10-allyl-10-dimethyl)-6-hydroxy-7-methoxy-coumarin has been measured in iNOS,

COX-2 genes, and some cytokines pro-inflammatory. This compound revealed ability to inhibit protein and mRNA expression of iNOS and IL-1β in LPS challenged macrophages, showing also anti-oxidant activity (Raghav et al., 2007).

In vivo studies on the methanol extract of *R. graveolens* in hypercholesterolemic rats demonstrated a reduction in oxidative damage, inflammation, and aortic pathology. These findings suggest that this plant species has potential therapeutic applications for clinical conditions related to atherosclerosis (Ratheesh et al., 2011).

Kataki et al. (2014) reported the antioxidant and anti-inflammatory activities of the methanol extract from *R. graveolens* leaves using both in vitro and in vivo models, demonstrating strong inhibitory effects on the arachidonic

acid pathway. Additionally, the antioxidant activity of *R. graveolens* extract was evaluated in two models: free radical scavenging via the DPPH method and inhibition of lipid peroxidation using the ferric thiocyanate method (Mohammadi-Motamed et al., 2014).

The aqueous extract of *R. graveolens* leaves has been reported to exhibit antimicrobial activity against several pathogens, including *Fusarium solani*, *Pyrenochaeta lycopersici*, *Trichoderma viride*, *Penicillium* sp., *Thielaviopsis basicola*, and *Verticillium dahliae*, as well as bacteria such as *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes* (Alzoreky and Nakahara, 2003).

The effects of *R. graveolens* extract on lipid and glucose levels, as well as hematological parameters, were studied in rats with streptozotocin-induced diabetes. The findings revealed that administration of the extract led to a significant reduction in cholesterol and LDL-C levels, while no changes were observed in glucose, triglycerides, VLDL-C, or HDL levels (Toserkani et al. 2012).

In the search for new treatments for neurodegenerative diseases such as Parkinson's and Alzheimer's, in vivo models have been used to assess the inhibition of oxidative deamination of tyramine by monoamine oxidase (MAO) isolated from rat liver. The studies revealed that ethyl acetate extracts and oil derived from the leaves of *R. graveolens* possess a significant ability to inhibit this enzyme (Stafford et al. 2007).

An experimental study in goats indicated that oral administration of *R. graveolens* leaves (at doses of 1 and 5 g/kg) resulted in toxicity, causing pathological changes in various organs. The observed effects included alterations in serum aspartate and levels of copper, iron, zinc, manganese, calcium, and phosphorus, with some cases leading to the death of the animals (El Agraa et al. 2002).

6. CONCLUSION

Ruta graveolens L., commonly referred to as rue, is a versatile herb from the Rutaceae family. It is abundant in secondary metabolites such as coumarins, alkaloids, volatile oils, flavonoids, and phenolic acids. Due to its wide-ranging medicinal properties, it has been utilized extensively across the globe. Extracts and essential oils derived from this species exhibit numerous pharmacological activities, including contraceptive, anti-inflammatory, antimicrobial, antipyretic, antioxidant, and analgesic effects. Additionally, they demonstrate antihyperglycemic, free radical scavenging, hypotensive, antiviral, anticancer and antiplasmodial properties. Given its diverse therapeutic applications, the plant holds significant interest for the pharmaceutical industry, highlighting its potential to contribute to various pharmacological treatments.

REFERENCES

Adamska-Szewczyk, A., Glowniak, K., Baj, T. (2016). Furochinoline alkaloids in plants from Rutaceae family - a review. *Curr. Issues Pharm. Med. Sci*. 29, 33-38.

Akalin, E., Ertuğ, F. (2003). An Anatomical And Ethnobotanical Study On *Ruta* Species in Turkey. *J Fac Pharm Ist Uni*. 35, 121-132.

Alper, M., Ozay, C. (2022). Antioxidant Activity and Phenolic Composition of Ethanol Extracts of *Momordica charantia* and *Datura stramonium*. *KSU J. Agric. Nat.* 25, 1-9.

Alzoreky, N.S., Nakahara, K. (2003). Antibacterial activity of extracts from some edible plants commonly consumed in Asia. *Int J Food Microbiol.* 80, 223-230.

Bennaoum, Z., Benhassaini, H., Falconieri, D., Piras, A., Porcedda, S. (2017). Chemical variability in essential oils from *Ruta* species among seasons, and its taxonomic and ecological significance. *Nat Pro Res*, 31, 2329-2334.

Cavender AP, Albán M. (2009). The use of magical plants by curanderos in the Ecuador highlands. *J Ethnobiol Ethnomed.* 5: 1-20.

Coelho-Ferreira, M. (2009). Medicinal knowledge and plant utilization in an Amazonian coastal community of Marudá, Pará State (Brazil). *J Ethnopharmacol.* 103:261-275.

Coimbra, A. T., Ferreira, S., Duarte, A. P. (2020). Genus *Ruta*: A natural source of high value products with biological and pharmacological properties. *J Ethnopharm*. 260, 113076.

El Agraa, SEI., El Badwi, S.M.A., Adam, S.E. (2002). Preliminary observations on experimental *Ruta graveolens* toxicosis in nubian goats. *Trop Anim Health Prod*. 34, 271-281.

Faydaoğlu, E., Sürücüoğlu, M.S. (2011). Geçmişten Günümüze Tıbbi ve Aromatik Bitkilerin Kullanılması ve Ekonomik Önemi. *Kastamonu Uni J Forestry Fac*. 11, 52-67.

Gravot, A., Larbat, R., Hehn, A., Li_evre K, Gontier E, Goergen JL, Bourgauda FEE (2004). Cinnamic acid 4-hydroxylase mechanism-based inactivation by psoralen derivatives: cloning and characterization of a C4H from a psoralen producing plant-*Ruta graveolens*-exhibiting low sensitivity to psoralen inactivationq. Arch. Biochem. Biophys., 422: 71-80.

Guerra, M.O., Andrade, A.T.L. (1978). Contraceptive effects of native plants in rats. *Contraception* 18, 191-199.

Hale, A.L., Meepagala, K.M., Oliva, A., Aliotta, G. (2004). Duke SO Phytotoxins from the leaves of *Ruta graveolens*. *J Agric Food Chem*. 52, 3345-3349.

Halvaei, I., Roodsari, H.R.S., Harat, Z.N. (2012). Acute Effects of *Ruta graveolens* L. on sperm parameters and DNA integrity in rats. *J Reprod Infertil.* 13, 33-38.

Hammami, I., Smaoui, S., Hsouna, A. Ben, Hamdi, N., Triki, M.A. (2015). *Ruta montana* L. Leaf essential oil and extracts: characterization of bioactive compounds and supression of Crown Gall disease. *Excli J.* 14, 83-94.

Harat, Z.N., Sadeghi, M.R., Sadeghipour, H.R., Kamalinejad, M., Eshraghian, M.R. (2008). Immobilization effect of *Ruta graveolens* L. on human sperm: a new hope for male contraception. *J Ethnopharmacol*. 115, 36-41.

Hashemi, K.S.M., Sadeghpour, H.M., Gholampour, A.I., Mirzaei, J.H. (2011). Survey the antifungal effect of root ethanolic extract of *Ruta graveolens* on *Saprolegnia.* Spp. *Int. Con. Biotech. Environ. Manage*. 18, 19-23.

Hoenders, R., Ghelman, R., Portella, C., Simmons, S., Locke, A., Cramer, H., Gallego-Perez, D., Jong, M. (2024). A review of the WHO strategy on traditional, complementary, and integrative medicine from the perspective of academic consortia for integrative medicine and health. *Front. Med.* 11, 1395698.

Kannan, R., Babu, U.V. (2012). Identity and pharmacognosy of *Ruta graveolens* Linn. *Anc Sci Life* 32, 16-19.

Kataki, M.S., Kakotii B.B., Bhuyan, B., Rajkumari, A., Rajak, P. (2014). Garden rue inhibits the arachidonic acid pathway, scavenges free radicals, and elevates FRAP: role in inflammation. *Chin J Nat Med*. 12, 172-179.

Kuzovkina, I., Al-terman, I., Schneider, B. (2004). Specific accumulation and revised structures of acridone alkaloid glucosides in the tips of transformed roots of *Ruta graveolens*. *Phytochem.* 65, 1095-1100.

Malik, S., Moraes, D.F.C., do Amaral, F.M.M., Ribeiro, M.N.S. (2016). *Ruta graveolens*: Phytochemistry, Pharmacology, and Biotechnology. In: Jha, S. (eds) Transgenesis and Secondary Metabolism. Reference Series in Phytochemistry. Springer, Cham.

Malik, S., Bhushan, S., Sharma, M., Ahuja, P.S. (2014). Biotechnological approaches to the production of shikonins: a critical review with recent updates. *Crit Rev Biotechnol*. 16, 1-14.

Mammadov, R., Kaska, A., Özay, C. (2017). Phenolic composition, antioxidant and cytotoxic activities of *Prospero autumnale*. *Ind J Pharm Sci.* 79, 585-590.

Mejri, J., Abderrabba, M., Mejri, M. (2010). Chemical composition of the essential oil of *Ruta chalepensis* L.: influence of drying, hydro-distillation duration and plant parts. *Ind. Crop. Prod*. 32, 67-673.

Mohammadi-Motamed, S., Shahidi-Motlagh, S., Bagherzadeh, H., Forouz, S.A., Tafazoli, H. (2014) Evaluation of antioxidant activity of *Ruta graveolens* L. extract on inhibition of lipid peroxidation and DPPH radicals and the effects of some external factors on plant extract's potency. *Res J Pharmacogn* 1, 45-50.

Ozay, C., Kılıncarslan, O., Mammadov, R. (2016). Interactions Between Heavy Metals and Glucosinolates as Defense Mechanisms in Brassicaceae. *Turk J of Sci Rev.* 9, 12-22.

Parray, S.A., Bhat, J., Ahmad, G., Jahan, N., Sofi, G., Faisal Iqbal, S.M. (2012). *Ruta*

graveolens: from Traditional System of Medicine to Modern Pharmacology: an Overview. *Am. J. Pharm. Tech. Res*. 2, 239-252.

Pathak, S., Multani, A.S., Banerji, P., Banerji, P. (2003). Ruta 6 selectively induces cell death in brain cancer cells but proliferation in normal peripheral blood lymphocytes: A novel treatment for human brain cancer. *Int. J. Oncol*. 23, 975-982.

Prabhu, S., Vijayakumar, S., Morvin Yabesh, J.E., Ravichandran, K., Sakthivel, B. (2014). Documentation and quantitative analysis of the local knowledge on medicinal plants in Kalrayan hills of Villupuram district, Tamil Nadu, India. *J Ethnopharmacol*. 157, 7-20.

Quave, C.L., Pieroni, A. (2005). Ritual Healing in Arbëreshë Albanian and Italian Communities of Lucania, Southern, Italy. *J Folk Res*. 42, 57-95.

Raghav, S.K., Gupta, B., Agrawal, C., Goswami, K., Das, H.R. (2006). Anti-inflammatory effect of *Ruta graveolens* L. in murine macrophage cells. *J Ethnopharmacol.* 104, 234-236.

Raghav, S.K., Gupta, B., Shrivastava, A., Das, H. (2007). Inhibition of lipopolysaccharide-inducible nitric oxide synthase and IL-1β through suppression of NF-kB activation by 3-(10-10-dimethylallyl)-6-hydroxy-7-methoxy-coumarin isolated from *Ruta graveolens* L. *Eur J Pharmacol.* 560, 69-80.

Ratheesh, M., Shyni, G.L., Sindhu, G., Helen, A. (2011). Inhibitory effect of *Ruta graveolens* L. on oxidative damage, inflammation and aortic pathology in hypercholesteromic rats. *Exp Toxicol Pathol*. 63, 285-290.

Ratheesh, M., Shyni, G.L., Sindhu, G., Helen, A. (2010). Protective effects of isolated polyphenolic and alkaloid fractions of *Ruta graveolens* L. on acute and chronic models of inflammation. *Inflammation* 33, 18-24.

Sampaio, O.M., Vieira, L.C.C., Bellete, B.S., King-Diaz, B., Lotina-Hennsen, B., Da Silva, M.F. das G.F., Veiga, T.A.M. (2018). Evaluation of alkaloids isolated from *Ruta graveolens* as photosynthesis inhibitors. *Molecules* 23, 1-12.

Stafford, G.I., Pedersen, P.D., Jäger, A.K., Van Staden, J. (2007). Monoamine oxidase inhibition by southern African traditional medicinal plants. *S Afr J Bot.* 73, 384-390.

Thring, T.S.A., Weitz, F.M. (2006). Medicinal plant use in the Bredasdorp/Elim region of the Southern Overberg in the Western Cape Province of South Africa. *J Ethnopharmacol.* 103, 261-275.

Toserkani, A., Razi Jalali, M., Najafzaheh, H. (2012). Changes of lipid profiles, glucose, and hemogram after administration of *Ruta graveolens* extract in diabetic rats. *Comp Clin Pathol*. 21, 1587-1592.

Wei, L., Xiang, X.G., Wang, Y.Z., Li, Z.Y. (2015). Phylogenetic relationships and evolution of the androecia in Ruteae (Rutaceae). *PloS One*, 10, 10-14.

Zargari, A. (1988). Medicinal plants. Vol 2. Tehran University Press, Iran, p. 42.

CHAPTER 2

INVESTIGATION OF THE EFFICACY OF SCORPION VENOM (ANDROCTONUS TURKIYENSIS) IN THE BIOLOGICAL CONTROL OF THE PINE PROCESSIONARY MOTH (THAUMETOPOEA WILKINSONI), A FOREST PEST

Mehmet COLAK[1]

1 Asst. Prof. Mehmet COLAK, Department of Forest Entomology and Protection, Forest Engineering Division, Faculty of Forestry, Karabük, https://orcid.org/0000-0001-6879-6701

Introduction

Forest ecosystems are recognized as complex and dynamic systems that are critical to the sustainability of life on our planet. These ecosystems provide vital ecological services such as biodiversity conservation, carbon sequestration and climate regulation (Brockerhoff et al., 2017). Despite these ecological services, the intensification of anthropogenic activities and global climate change are putting increasing pressure on forest ecosystems (Seidl et al., 2017). In addition to this increasing pressure, insect pests are proving to be important factors that pose a serious threat to the health and sustainability of forests (Jactel et al., 2021). These threats from insect pests become even more critical in countries with rich forest resources, such as Türkiye.

These threats to Türkiye's forests are clear from the latest data from the General Directorate of Forestry (GDF). According to GDF reports, an average of 331,695 hectares of forest area are affected by insect damage annually in our country, resulting in an approximate annual timber loss of 351,844 m^3 (GDF, 2021). These recorded losses can exceed 1 million m^3 in years with high insect population densities, leading to significant impacts on forest ecosystem health and economic value (URL 1). To minimize these impacts, various approaches have been developed to control forest pests.

Traditional control methods include mechanical, chemical, biotechnical and biological methods (Bosch et al., 1982; Smith, 1919 Kenis et al., 2019). However, among these methods, the potential negative impact of chemical control methods on the ecosystem has been increasingly questioned in recent years with the growing importance of environmental sustainability (Desneux et al., 2007). As a result of the questioning of chemical control methods, the use of toxic chemicals has been banned in Türkiye since 2007 and the use of biological preparations, natural organic compounds and growth inhibitors has been promoted (GDF, 2021). This paradigm shift has led to biological control strategies becoming more important.

Biological control strategies aim to keep pest populations in ecological balance through the use of natural enemies and bioactive substances (Hajek and Eilenberg, 2018). With the emergence of these strategies, research on the use of arthropod venoms as biopesticides has begun to offer new and promising perspectives (Windley et al., 2012; King and Hardy, 2013). Among arthropod venoms, scorpion venom stands out as a complex cocktail of bioactive compounds perfected through evolutionary processes.

The strong neurotoxic effect and the various bioactive peptides contained in scorpion venom make it a potential biological control agent (Quintero-Hernández et al., 2013; Ortiz et al., 2015). The insect-selective peptides contained in the venom are considered valuable molecules that could be used in the control of forest and agricultural pests due to their potential for specific

effects on the target pests (Plessis et al., 2008; Schwartz et al., 2012; El-Aziz et al., 2022). This potential is particularly promising in the control of species that cause severe economic and ecological damage, such as the pine processionary moth (*Thaumetopoea wilkinsoni* Tams, 1924).

The pine processionary moth is an important pest species that causes great damage in the pine forests of Türkiye (Erkan, 2011). Considering the damage caused by this pest, the development of new and sustainable control strategies is crucial for the protection of forest ecosystems. In this context, the present research aims to investigate the potential biological control efficacy of the venom of *Androctonus turkiyensis* Yağmur, 2021, a scorpion species endemic to Türkiye, against the pine processionary moth (*T. wilkinsoni*).

The scope of the study includes the extraction of the venom of *A. turkiyensis*, its biochemical characterization and a detailed evaluation of its efficacy against the larvae of *T. wilkinsoni*.

Materials and Method

This study was conducted in the Forest Engineering Laboratory of the Faculty of Forestry at Karabük University. The study was conducted in two phases: Field and laboratory work.

The specimens of the pine processionary moth (*Thaumetopoea wilkinsoni*) were collected in the forests of Yenice in theWestern Black Sea region (41°15'0.81 N, 32°9'51.86 E, altitude: 150 m). Sampling took place between April 15 and May 15, 2022, i.e. during the period of increased insect population and larval density.

The larval nests were collected together with branches that were cut with telescopic scissors during the day. Some green needles were left on the branches to ensure the survival of the larvae during transportation. The samples were placed in ventilated plastic containers (62x40x26 cm) and brought to the laboratory (Figure 1).

Figure 1: Nest of the pine processionary moth (T. wilkinsoni) in the pine forest and larvae in the nest

Scorpions of the species *Androctonus turkiyensis* were collected between June 1 and 15, 2022 in their natural habitats in the Southeastern Anatolia Region, specifically in the provinces of Gaziantep (37° 5'27.67 N, 37°15'54.06 E, altitude: 850 m) and Şanlıurfa (37° 5'39.45 N, 38°47'25.22 E, altitude: 550 m). Sampling was conducted with the authorization of the Ministry of Agriculture and Forestry of the Republic of Türkiye, Directorate General of Nature Conservation and National Parks (date: 28.03.2022, number: E-21264211-288.04-5032056).

In accordance with their nocturnal activity pattern, scorpions were collected after 21:00. A 3W LED ultraviolet lamp with a wavelength of 395 nm was used during sampling and scorpions were captured using 30 cm long stainless steel forceps (Çolak, 2014). Ten adult scorpions were collected and placed in specially ventilated containers (Figure 2).

Figure 2. Androctonus turkiyensis scorpion

The scorpions brought to the laboratory were housed individually in plastic terrariums measuring 17x26x11 cm. The laboratory was kept at a temperature of 25 ± 2°C, a humidity of 60 ± 5% and a 12-hour lightand 12-hour dark phase. The bottom of the terrarium was sprinkled with sand and water was provided in a container. The scorpions were fed weekly with 1-2 medium sized mealworm larvae (*Tenebrio molitor*).

The scorpion venom was extracted using the electrical stimulation method proposed by Plessis (2005). For this procedure: The telson of the scorpion was positioned in a 1.5ml capped polypropylene Eppendorf tube. An electrical stimulator was used to apply a 22-volt current to the scorpion's tail for 2-3 seconds via two electrodes (Gupta et al., 2007). The extracted venom (average 0.5 mg/scorpion/extraction) was collected in polypropylene tubes and stored at -20°C. This procedure was repeated once every 30 days throughout the study period (Çalışkan et al., 2006).

The scorpion venom obtained was dissolved in pH 7.4, 0.1 M phosphate buffer (Sigma-Aldrich) at ratios of 1:50, 1:100 and 1:1000 (w/v).

For the application, 40 pine processionary moth larvae were divided into 4 groups of 10 animals each: Group 1: 1:50 venom ratio, Group 2: 1:100 venom ratio, Group 3: 1:1000 venom ratio and Group 4: control group (treated with phosphate buffer only).

Before application, the insects were kept at -4°C for 5 minutes to temporarily inactivate their movements. Subsequently, 1.00 µl, 3.00 µl, 5.00 µl and 10.00 µl of venom were applied topically to the dorsal area of the larvae using an Eppendorf Research® plus (0.5-10 µL) micropipette and spray application.

After application of the venom, the insects were observed for 72 hours. Observations were made every hour for the first 24 hours and every 6 hours for the following 48 hours. The following parameters were recorded: Mortality rates (mortality criterion: absence of movement and response for 5 minutes), effective dose of venom, percentage effectiveness of the dose in the population and mortality duration.

The IBM SPSS Statistics 26.0 program was used for data analysis. Descriptive statistics such as mean, standard deviation and frequency were calculated for normally distributed numerical data. One-way analysis of variance (ANOVA) was used for group comparisons. Probit analysis was used to analyze dose-response relationships. For all statistical analyzes, the significance level was set at $p<0.05$, and the results were evaluated within a 95% confidence interval.

Results

Field observations have shown that the pine processionary moth causes considerable damage, particularly to pine needles. The larvae inhibited tree growth and leaf development by consuming fresh tissue such as the meristem. It was found that prolonged infestation with the pest leads to the death of the trees. Trees infested with the pine processionary moth showed reduced height and diameter growth compared to uninfested trees (Figure 3). When pine processionary moth larvae were kept under laboratory conditions, it was observed that contact with the larvae caused allergic reactions in laboratory staff. Symptoms such as itching, redness and skin rashes were observed in the staff.

Figure 3: Nests formed by the larvae of the pine processionary moth (T. wilkinsoni)

The amount of venom obtained from *Androctonus turkiyensis* species was determined as follows: Maximum: 160 mg, minimum: 1 mg, and average: 5.7 mg. When the physical properties of the venom were examined, it was found to be of egg-white consistency, white in color and clear in appearance.

The mortality rates resulting from the application of scorpion venom at different concentrations are shown in Table 1.

Table 1 Mortality rate of the pine processionary moth

Group	Venom Concentration	Mortality (number of individuals)	Mortality Rate (%)
1	1:50	3	33.3
2	1:100	2	20
3	1:1000	2	20
4	Control	1	10

The highest mortality rate of 33.3% was observed in the 1:50 concentration (group 1), while the lowest rate of 10% was observed in the control group (group 4).

After application of the toxin, the larvae showed weakened locomotor and feeding activity, with partial loss of mobility (lasting about 10 minutes) observed especially when the toxin was sprayed on the abdomen. It was determined that the larval setae partially prevented penetration of the venom, with the venom being more effective in the abdominal regions where there was direct contact with the skin.

Statistical analysis of the mortality data was performed using a one-way analysis of variance (ANOVA) and Tukey's HSD post hoc test. The results of the ANOVA showed no statistically significant differences between the different concentrations of scorpion venom ($F = 1.0476$, $p = 0.3791$, $\alpha = 0.05$). While the highest mortality rate (33.3%) was observed in the group with the 1:50 concentration, the control group showed a mortality rate of 10. The results of the Tukey's HSD post-hoc test showed no significant differences in pairwise comparisons between the groups ($p > 0.05$). Analysis of standard deviation values showed similar variations in all groups. These results indicate that the tested concentrations of scorpion venom did not show statistically significant efficacy against pine processionary moth larvae. Further studies with higher concentrations and larger samples are recommended to better elucidate the potential insecticidal effect of the venom. The current statistical results require additional research to determine the optimal concentration for effective biological control.

The experimental setup used in the study is shown in Figure 4. This setup was used for the application of the venom and the observation of the larvae.

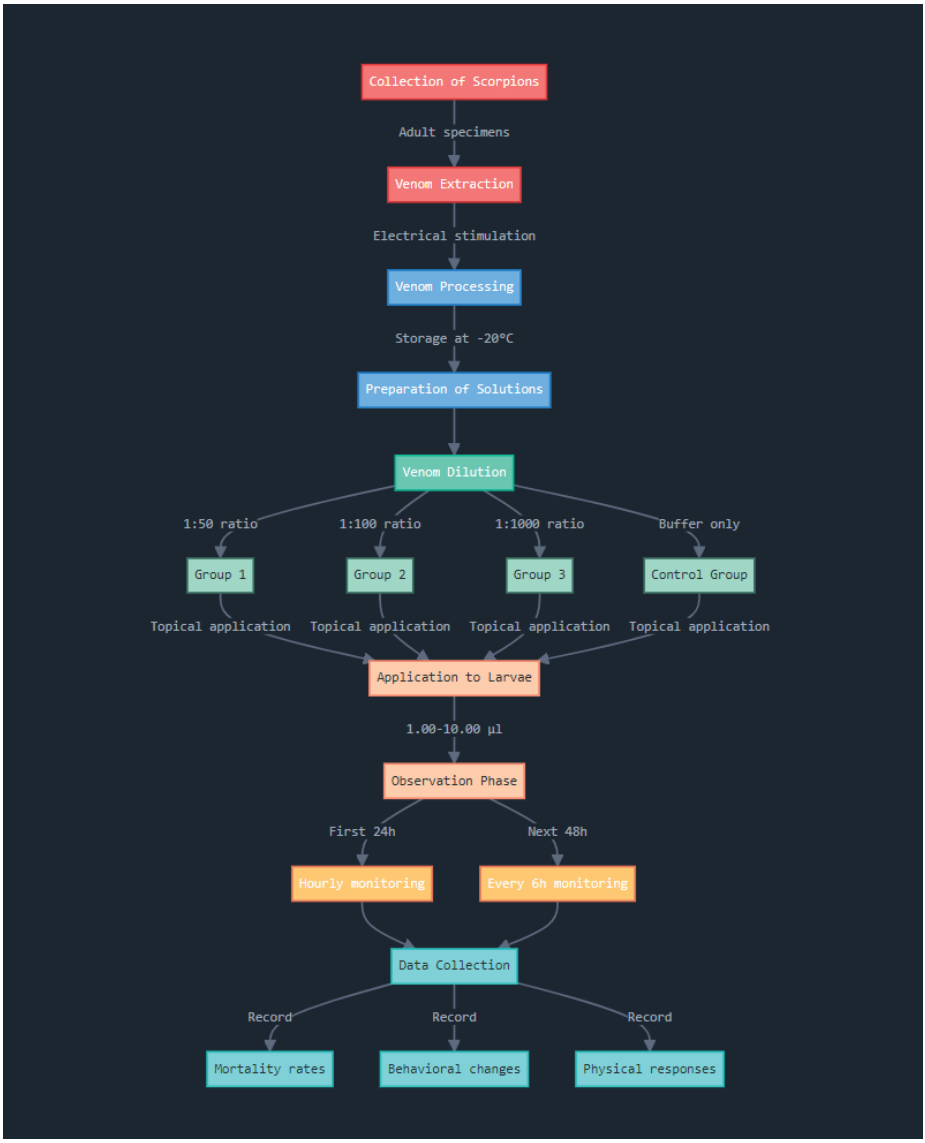

Figure 4: Experimental setup for the application of the poison and the monitoring of the larvae

Discussion and Conclusion

This study comprehensively investigated the biological control potential of the venom of *Androctonus turkiyensis*, an endemic scorpion species in Türkiye, against the pine processionary moth (*Thaumetopoea wilkinsoni*). The results showed that the scorpion venom has significant effects on the life cycle and physiology of the larvae of the pine processionary moth.

Insecticidal peptides and other components contained in scorpion venom have various biological effects and have the potential to play an important role in plant protection. Relevant literature indicates that peptides in scorpion venom have anticarcinogenic, antibacterial, antifungal and antiviral properties (Mikaelian et al., 2020; Wang et al., 2024; Uzair et al., 2018; Xia et al., 2023). These properties also offer potential applications for scorpion venom in various health interventions.

Considering the cost and environmental impact of current methods of controlling forest pests, the use of scorpion venom as a natural biological product represents an environmentally friendly alternative and sustainable solution. The resistance that insects have developed to existing chemicals and the undesirable effects of these chemicals on the environment increase the importance of research into natural-based insecticides.

The mortality rates observed in our study suggest that scorpion venom could be used as a potential bioinsecticide. Similar results were obtained in the study conducted by El-Zahraa et al. (2023) using Leiurus quinquestriatus venom against Spodoptera frugiperda. These results suggest that the venoms of different scorpion species may be effective against different insect pest species.

The study by Deng et al. (2019) on Hector insect toxin (AaIT) isolated from *Androctonus australis* sheds light on the mechanism of action of scorpion venom. The targeting of the voltage-gated sodium channels of insects by the AaIT toxin suggests that the venom of A. turkiyensis may act via a similar mechanism. This specific mode of action increases the potential of the scorpion venom as a selective bioinsecticide.

The transfer of insecticidal genes found in scorpions to plants is also promising. Wu et al. (2000) demonstrated that transgenic hybrid poplars (*Populus deltoides* × *P. simonii*) recombined with the AaIT gene showed significantly higher resistance to the larvae of the gypsy moth (*Lymantria dispar)* than wild-type poplars. This suggests that similar studies could be conducted with A. turkiyensis toxin to increase the natural resistance of forest trees to pests. This approach could increase the natural resistance of forest trees to pests and reduce the use of chemical pesticides. Future research could focus on utilizing the genetic components of the *A. turkiyensis* toxin in transgenic forestry applications.

The mortality rates observed in our study are comparable to those obtained in the study conducted by Yılmaz et al. (2013) using *Bacillus thuringiensis* strains. This underlines the potential of the A. turkiyensis toxin.

The study by Battisti et al. (2015) has shown that the urticarial protein thaumetopoein, which is contained in the larvae of the pine processionary

moth, causes allergic reactions in humans and animals. This should be taken into account both for the safety of the study and for the effective application of the poison. In our study, it was indeed observed that the antennae of the larvae partially hindered the penetration of the venom.

The LD50 of the scorpion venom of *A. turkiyensis* was determined to be 5.761 µg/mouse (Kanat et al., 2022). Greater efficacy was observed when the venom was sprayed on the abdominal region. This finding indicates the need to optimize application methods.

In conclusion, the biological control potential of the scorpion venom of *A. turkiyensis* against the pine processionary moth (*T. wilkinsoni*) is a significant alternative to reduce the negative environmental impact of conventional pesticides. Future studies should focus on the detailed analysis of the chemical components of the scorpion toxin, the investigation of its effect on different pests and the application in transgenic plants. In addition, the integration of existing control methods with new biological control strategies through integrated pest management practices could contribute to the development of sustainable forestry. More extensive research in this area will be of great benefit for effective resource utilization and environmental protection.

Acknowledgments

This study was supported by the Karabük University Scientific Research Projects Coordination Unit under project number KBÜBAP-22-KP-086. We would like to thank the Scientific Research Projects Coordination Unit of Karabük University for supporting this research.

REFERENCES

Battisti, A., Larsson, S., & Roques, A. (2015). Processionary moths and associated urtication risk: Global change driven effects. *Annual Review of Entomology, 60*, 337-355.

Bosch, R. van den, Messenger, R. P. S. and A. P. Gutierrez. 1982. "An introduction to biological control", New York: Plenum Press

Brockerhoff, E. G., Barbaro, L., Castagneyrol, B., Forrester, D. I., Gardiner, B., González-Olabarria, J. R., & Jactel, H. (2017). Forest biodiversity, ecosystem functioning and the provision of ecosystem services. *Biodiversity and Conservation, 26*(13), 3005-3035.

Çalışkan, F., Blanca, I., García, F., Coronas, V., & Cesar, B. (2006). Characterization of venom components from the scorpion *Androctonus crassicauda* of Turkey: Peptides and genes. *Toxicon, 48*, 12-22.

Çolak, M. 2014. "Türkiye'nin güneydoğu bölgesinin akrepleri (Arachnida: Scorpiones)", Doktora Tezi., Ankara Ünviersitesi, Ankara, 251s

Deng SQ, Chen JT, Li WW, Chen M, Peng HJ. Application of the Scorpion Neurotoxin AaIT against Insect Pests. Int J Mol Sci. 2019 Jul 15;20(14):3467. doi: 10.3390/ijms20143467. PMID: 31311095; PMCID: PMC6678123.

Desneux, N., Decourtye, A., & Delpuech, J. M. (2007). The sublethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*, 81-106.

El-Aziz, A., Eldeeb, S. M., Abdellah, N. Z., Shaltout, E. S., & Ebrahem, N. E. (2022). Influence of Scorpion Venom on Decomposition and Arthropod Succession. *Egyptian Academic Journal of Biological Sciences, B. Zoology*, *14*(1), 209-219.

El-Zahraa, F., Ahmed, M., & Hassan, R. (2023). Efficacy of *Leiurus quinquestriatus* scorpion venom against fall armyworm (*Spodoptera frugiperda*). *Journal of Pest Science, 96*(3), 1123-1135.

Erkan, N. (2011). Impact of pine processionary moth (Thaumetopoea wilkinsoni Tams) on growth of Turkish red pine (Pinus brutia Ten.). *African Journal of Agricultural Research*, *6*(21), 4983-4988.

GDF; Orman Genel müdürlüğü. "Sürdürülebilir Orman Yönetimi Kriter ve Göstergeleri 2019 Türkiye Raporu" https://www.ogm.gov.tr/tr/e-kutuphane-sitesi/SurdurulebilirOrmanYonetimi/2019%20SOY%20K.G%20T%C3%9CRK%C4%B0YE%20RAPORU.pdf Date of access: 26.12.2023.

Gupta, D. Debnath, A. Saha, A. Giri, B. Tripathi, G. Vedasiromoni, J.R. Gomes, A. and Gomes, A. 2007. "Indian black scorpion (Heterometrus bengalensis) Koch venom induced antiproliferative and apoptogenic activity against human leukemic cell lines U937 and K562S", Leukemia Research, 31, 817-825.

Hajek, A. E., & Eilenberg, J. (2018). Natural enemies: An introduction to biological control (2nd ed.). *Cambridge University Press*.

Jactel, H., Koricheva, J., & Castagneyrol, B. (2021). Responses of forest insect pests to climate change: Not so simple. *Current Opinion in Insect Science, 47*, 65-73.

Kanat, M. A., Altun, D., Kiliç, K., Namli Bozkurt, E. N., Turan, E., Cengiz, G., & Bozyiğit, İ. (2022). Evaluation of cross-protection of *Androctonus crassicauda* antivenom against *Leiurus abdullahbayrami* venom. *Turkish Bulletin of Hygiene and Experimental Biology, 79*(1), 47-58.

Kenis, M., Hurley, B. P., Hajek, A. E., & Cock, M. J. (2019). Classical biological control of insect pests of trees: Facts and figures. *Biological Invasions, 21*(11), 3351-3366.

King, G. F., & Hardy, M. C. (2013). Spider-venom peptides: Structure, pharmacology, and potential for control of insect pests. *Annual Review of Entomology, 58*, 475-496.

Mikaelian AG, Traboulay E, Zhang XM, Yeritsyan E, Pedersen PL, Ko YH, Matalka KZ. Pleiotropic Anticancer Properties of Scorpion Venom Peptides: *Rhopalurus princeps* Venom as an Anticancer Agent. Drug Des Devel Ther. 2020 Feb 27;14:881-893. doi: 10.2147/DDDT.S231008. PMID: 32161447; PMCID: PMC7051175.

Ortiz, E., Gurrola, G. B., Schwartz, E. F., & Possani, L. D. (2015). Scorpion venom components as potential candidates for drug development. *Toxicon, 93*, 125-135.

Plessis, J. L. (2005). Collection of venom from southern African scorpions. *Toxicon, 45*, 681-

Quintero-Hernández, V., Jiménez-Vargas, J. M., Gurrola, G. B., Valdivia, H. H., & Possani, L. (2013). Scorpion venom components that affect ion-channels function. *Toxicon, 76*, 328-342.

Schwartz, E. F., Mourao, C. B., Moreira, K. G., Camargos, T. S., & Mortari, M. R. (2012). Arthropod venoms: A vast arsenal of insecticidal neuropeptides. *Biopolymers, 98*(4), 385-405.

Seidl, R., Thom, D., Kautz, M., Martin-Benito, D., Peltoniemi, M., Vacchiano, G., ... & Reyer, C. P. (2017). Forest disturbances under climate change. *Nature Climate Change, 7*(6), 395-402.

Smith, H. S. 1919. "On some phase of insect control by the biological method", Journal of Economic Entomology, 12: 288-292

URL 1. Chamber of Forest Engineers; Orman Mühendisleri Odası. Orman Zararlıları ile Mücadele Eğitimi. https://ormuh.org.tr/uploads/docs/Orman%20zararlilari%20ve%20mucadelesi.pdf Date of access: 26.12.2021

Uzair B, Bint-E-Irshad S, Khan BA, Azad B, Mahmood T, Rehman MU, Braga VA. Scorpion Venom Peptides as a Potential Source for Human Drug Candidates. Protein Pept Lett. 2018;25(7):702-708. doi: 10.2174/0929866525666180614114307. PMID: 29921194.

Wang, K., Nguyen, T., Gao, Y., Guo, R., Fan, C., Liao, H., Li, J., Chai, J., Xu, X., Gong, Y., & Chen, X. (2024). Androcin 18−1, a novel scorpion-venom peptide, shows

a potent antitumor activity against human U87 cells via inducing mitochondrial dysfunction. *Insect Biochemistry and Molecular Biology, 170*. https://doi.org/10.1016/j.ibmb.2024.104137

Windley, M. J., Herzig, V., Dziemborowicz, S. A., Hardy, M. C., King, G. F., & Nicholson, G. M. (2012). Spider-venom peptides as bioinsecticides. *Toxins, 4*(3), 191-227.

Wu NF, Sun Q, Yao B, Fan YL, Rao HY, Huang MR, Wang MX. [Insect-resistant transgenic poplar expressing AaIT gene]. Sheng Wu Gong Cheng Xue Bao. 2000 Mar;16(2):129-33. Chinese. PMID: 10976310.

Xia, Z., He, D., Wu, Y., Kwok, H. F., & Cao, Z. (2023). Scorpion venom peptides: Molecular diversity, structural characteristics, and therapeutic use from channelopathies to viral infections and cancers. *Pharmacological Research, 197*, 106978. https://doi.org/10.1016/j.phrs.2023.106978

Yılmaz, S. Karabörklü, S., Azizoğlu, U., Ayvaz, A., Akbulut, M., and Yıldız, M. (2013) "Toxicity of native Bacillus thuringiensis isolates on the larval stages of pine processionary moth Thaumetopoea wilkinsoni at different temperatures," *Turkish Journal of Agriculture and Forestry*: Vol. 37: No. 2, Article 5. https://doi.org/10.3906/tar-1201-21

CHAPTER 3

SUSTAINABILITY AND SOLID WASTE MANAGEMENT

Kadriye URUÇ PARLAK[1]

1 Prof. Dr. Kadriye URUÇ PARLAK
ORCID İD: 0000-0002-1474-1868
Department of Molecular Biology and Genetics, Agri İbrahim Cecen University, Agri, Turkey 04200
Email: uruckadriye@gmail.com

Sustainability is commonly understood as the capacity to preserve the functionality, processes, and productivity of ecosystems for future generations (Chapin, Torn, & Tateno, 1996). It is increasingly recognized that human activities are pushing the Earth's resources and environment toward exhaustion (Turner, 2008). In light of this, sustainability can only be achieved if natural resources are utilized at a rate that allows for their natural replenishment. Social sustainability refers to the ability to fulfill the needs of the present without jeopardizing the ability of future generations to meet their own (United Nations, 2008). Economically, sustainability is often discussed in sustainable development, which focuses on transitioning to renewable resources in production and acknowledging the environmental costs of production practices.

However, sustainability is a multifaceted concept, not confined to these definitions alone. It applies to various essential areas with different interpretations depending on the context. Examples include the sustainability of forests and wetlands, sustainable cities, sustainable agriculture, and sustainable architecture, all of which contribute to the broader and more intricate discussion of sustainability (Yavuz, 2010). Environmental sustainability, as one of the three pillars of sustainability environmental, social, and economic aims to optimize the use of natural resources while ensuring the conservation of ecosystems. It seeks to balance economic, social, and environmental concerns to support long-term stability. This concept addresses the needs of current generations while working to secure the availability of resources for future generations (Turner, 2008). Its significance is profound, as it plays a crucial role in the long-term health of our planet.

1. **Protection of Ecosystems:** A key goal of sustainability is to protect ecosystems that offer vital services, such as clean water, air purification, climate regulation, and biodiversity. Ensuring these services remain sustainable is essential for fostering a healthy environment conducive to human life (Abubakar, Muniruzzaman, Dano, & Alshihri, 2022).

2. **Conservation of Natural Resources:** Sustainability acknowledges the limited nature of natural resources. Environmental sustainability emphasizes the responsible utilization of these resources to guarantee their availability for future generations (Mehta, Paliwa, Tege, & Sankhla, 2018).

3. **Addressing Climate Change:** Climate change is a growing global threat. Sustainability plays a role in mitigating this crisis by promoting practices like reducing reliance on fossil fuels, investing in renewable energy, and enhancing energy efficiency (Mehta et al., 2018).

4. **Public Health and Well-being:** Environmental sustainability contributes to public health by guaranteeing access to clean air, water, and a safe living environment. Reducing pollution-related illnesses creates healthier and more livable spaces (Abubakar et al., 2022).

5. **Protecting Future Generations' Rights:** Sustainability ensures that future generations inherit natural resources and a thriving environment. Today's actions are pivotal in securing a sustainable world for tomorrow's population (Mehta et al., 2018).

Environmental sustainability entails adopting strategies to address environmental challenges and conserve resources. Issues such as climate change, loss of biodiversity, water shortages, and -air and water-air and water pollution are significant threats to environmental stability. To address these, we need a variety of measures, including efficient resource usage, effective waste management, resource conservation, and sustainable transportation (Ozkan, Gültekin Subaşı, Kamiloglu, & Capanoglu, 2022).

As the global population grows, along with urbanization and industrialization, pollution continues to rise. This harms the environment, climate, wildlife, plants, and human health. Depending on where it comes from, solid waste can take many different forms, including waste products, municipal waste, sludge, plastics, industrial waste, e-waste, glass, and fly ash (Esparza et al., 2020; Zhang, Malik, Khan, & Ali, 2022) (Figure 1). Concerns regarding waste management have been highlighted by the increasing effect of solid waste, particularly electronic waste, or "e-waste." Electronic equipment including computers, cell phones, TVs, and DVD players that are no longer in use and are powered by electricity or batteries are referred to as e-waste. One of the worldwide pollution sources with the fastest rate of growth is e-waste (Nnorom & Odeyingbo, 2020).

Figure 1. Solid waste and its environmental problems (Esparza et al., 2020; Zhang et al., 2021)

Solid Waste Management: Challenges and Approaches

Solid waste provides a breeding ground for pests and disease vectors, facilitating their growth and transmission. Additionally, it emits foul odors that can render the surrounding air unpleasant and unhealthy. Various waste management strategies have been employed to address these issues, one of the most common being landfilling. This method involves burying waste in pits away from populated areas to reduce public inconvenience. However, landfilling has limitations, such as high costs and the slow decomposition rate of waste materials (Zhang, Malik, Khan, & Ali, 2022). Incineration is another frequently used waste management technique. While effective in reducing waste volume, incineration can contribute to global warming, making it less sustainable and reliable (Cunha et al., 2020). Another alternative is waste vitrification, which involves burning waste to create ash, flue gases, and heat as by-products. The resulting ash can be reused in construction and other applications (Zhang et al., 2022). Despite efforts to dispose of and segregate waste, recycling is more beneficial. Recycled waste materials can be processed physically or biologically, providing valuable resources. Reprocessed materials can generate energy, which can be used for electricity production, offering both environmental and economic advantages (Tulebayeva et al.,2020). The waste management process consists of several interconnected stages: collection, transportation, processing, recycling, and monitoring. Solid waste management remains a global challenge, linking ecological degradation with social and economic setbacks, ultimately threatening environmental sustainability. Numerous studies highlight that both developed and developing regions worldwide face inadequate hazardous waste management challenges.

Sources of Waste

The amount of solid waste generated is directly linked to urbanization and industrialization, with the industrial sector being the primary contributor. Factory emissions significantly raise pollution levels, while industries often discharge waste into rivers and streams, contaminating surface water. Key waste materials, such as plastics, glass, paints, pharmaceuticals, and chemicals, mainly come from industrial activities (Figure 2) (Vlachokostas et al., 2020). Developed nations are the major producers of e-waste. Countries like the United States, Europe, and Australia, characterized by high levels of industrialization and urbanization, rely heavily on electronic devices to improve efficiency and performance. This generates substantial amounts of e-waste, which is often exported to developing nations, such as Pakistan, India, China, Nepal, and Bangladesh. These regions offer vast spaces for waste disposal and have lower labor costs for recycling, which makes them attractive for e-waste processing (Nnorom & Odeyingbo, 2020). Commercial waste, including paper and plastics, contributes significantly to the overall

generated waste. Institutions such as universities, schools, offices, and cafeterias are primary sources of these materials. Household waste is another major contributor to the waste stream (Sartaj et al., 2020). Everyday activities like cleaning, cooking, and gardening generate large quantities of waste, such as food scraps, peels, and organic matter (Tomić & Schneider, 2020). The agricultural industry also contributes significantly, producing waste from weeds, husks, and livestock by-products. These various sources of waste are the primary drivers of waste generation (Zhang et al., 2022).

Figure 2. *Resources of solid waste (The diagram was created by the author of this study).*

Remembering that these waste sources are inevitable and essential to many facets of civilization is crucial. Although they are necessary for a prosperous living, residential, agricultural, and industrial operations invariably produce waste. Approximately 50–80% of waste is thought to originate from homes, with the remaining 10–30% coming from commercial sources such as enterprises, industries, and street waste (Gaustad, Williams, & Leader, 2020).Since they determine the waste's content and properties, the sources of solid waste are extremely important. Because waste items are often diverse, recycling or reusing them for particular purposes can be challenging (Stiborova et al., 2020). Common waste components include rubber, leather,

food scraps, plastics, inert materials, textiles, and batteries. Therefore, prior to treatment and disposal, these materials must be separated and categorized (Gaustad et al., 2020).The creation of e-waste has significantly increased as a result of the COVID-19 epidemic and technological advances. While physical waste transportation has reduced, e-waste production has grown due to the growing reliance on technology for online activities and the widespread usage of laptops, mobile phones, and computers during and beyond the lockdown hours. Effective e-waste management has thus emerged as a crucial issue (Shittu, Williams, & Shaw, 2020). E-waste recycling requires certain tools and procedures, frequently absent in underdeveloped nations. Nonetheless, some areas follow official recycling guidelines set forth by the government. In Germany, for example, only 17.4% of garbage is handled through official recycling facilities; the rest is recycled, exchanged, stored, or disposed of informally. Despite being a viable alternative for disposing of e-waste in some places, incineration is not commonly employed because of the health dangers involved, especially with regard to respiratory problems (Klugmann-Radziemska & Kuczyńska-Łażewska, 2020).

Environmental and Climatic Impact of Waste

Rising levels of pollution, whether in the form of soil, water, or air contamination, are directly caused by the increased demand for food, products, and services brought on by the world's population growth and rapid industrialization (Zhang, Malik, Khan, & Ali, 2022). Large amounts of obsolete products are finally disposed of as a result of increased production of necessities brought on by the growing population and rising resource needs. This waste has the potential to seriously harm the environment if improperly handled and disposed of. To prevent damage, waste placed in landfills must to be efficiently collected and sent to approved disposal locations (Esparza, Jiménez-Moreno, Bimbela, Ancín-Azpilicueta, & Gandía, 2020). There are major environmental consequences from improper waste management practices (Klugmann-Radziemska & Kuczyńska-Łażewska, 2020). Leachate from landfills is one of the major environmental concerns. This liquid waste can seep into the soil and contaminate groundwater. Additionally, animals and scavengers often disturb landfill sites, spreading waste and creating aesthetic and environmental problems (Shittu, Williams, & Shaw, 2020). Burning waste materials like plastics, rubber, and textiles releases harmful smoke and gases into the atmosphere, worsening air pollution. Moreover, the foul odors emitted from waste further contribute to air contamination. The large-scale generation of waste has a detrimental impact on human health (Tomić & Schneider, 2020). Landfills are home to various pests, such as rats and insects, which can spread diseases. Improper disposal of medical waste, such as pharmaceuticals from hospitals, can also contribute to the transmission of illnesses. Flies, for example, can contaminate food and water

sources, leading to diarrhea and dysentery (Esparza et al., 2020). Rats can transmit diseases like salmonellosis, plague, and trichinosis. Furthermore, contaminated wastewater is a breeding ground for diseases like jaundice, cholera, and hepatitis. Garbage-clogged sewers create ideal environments for mosquitoes, facilitating the spread of malaria and dengue fever (Jeswani & Azapagic, 2020). Additionally, colored plastics often contain toxic metals like cobalt, copper, lead, chromium, and mercury, which pose significant health risks due to their toxicity (Shooshtarian, Caldera, Maqsood, & Ryley, 2020). Excessive exposure to chemicals such as mercury, polychlorinated biphenyls (PCBs), and cyanide can even be fatal.

Environmental Impacts of Plastic-Based Waste

The breakdown of plastics using methods such as UV radiation or heat produces non-degradable by-products that negatively affect the environment particularly soil, water, and air. The types of by-products produced vary depending on the plastic material used. In an experiment where two different types of plastics were exposed to bacterial species (Zhang et al., 2022), it was observed that the bacteria interacted with the plastics in distinct ways, generating different by-products. The toxicity of these by-products was tested, revealing that most were either non-toxic or posed minimal threat to the environment. For example, when polyethylene terephthalate (PET) was incubated with *Streptomyces* sp., the produced by-products were mostly safe, except for o-xylene and ethylbenzene (Kang, Kang, Ilankoon, & Chong, 2020). Other research has explored the impact of mixed polymers like polystyrene, polyethylene, and PET on the environment. Using active sludge and soil, and employing colorimetric techniques to evaluate toxicity, it was found that the by-products from these polymers did not inhibit microbial growth or activity (Boehm, Wilde, Ver Ploeg, Costello, & Cash, 2018). The potential eco-toxicity of plastics, particularly when they degrade into micro or nanoparticles, is a growing concern. When these particles interact with other chemicals in soil or water, they could pose risks. While research on the toxicity of polymer micro and nanoparticles remains limited, some studies indicate negative effects, particularly when these particles accumulate in high concentrations in terrestrial and aquatic organisms (Mondal, Choudhury, Kundu, Dutta, & Samanta, 2023).

Environmental Impacts of Cereal-Based Waste

Waste generated from grain processing plants contains high levels of organic pollutants, which can contribute to soil and water pollution, posing serious health risks (Chen, Yu, Zhang, Wu, & Li, 2023). Typically, this waste appears yellowish or brown and emits a strong, unpleasant odor. The wastewater from these plants contains a high Chemical Oxygen Demand (COD), composed of substances such as phenol, lignin, cellulose, and others, causing

significant environmental problems (Hajam, Kumar, & Kumar, 2023). The main cause of excessive COD levels and algae development on water surfaces is wastewater. Many aquatic organisms have difficulties in their growth by the water's Biochemical Oxygen Demand (BOD). In particular, effluent from the corn sector can change the alkaline characteristics of adjoining soils and make nearby water bodies more acidic (Tulebayeva, Yergobek, Pestunova, Mottaeva, & Sapakova, 2020). In order to preserve and repair the ecosystem, recent studies have focused on enhancing the management of soil and water resources (Bello, Al-Ghouti, & Abu-Dieyeh, 2022).

Environmental Impacts of E-Waste

Hazardous metals including arsenic, copper, cobalt, lithium, chromium, cadmium, palladium, mercury, and others can be found in e-waste, which is a complex mixture of materials that includes plastics, glass, metals, and resins. It could also contain rare earth elements like indium, tantalum, and neodymium, as well as less dangerous metals like steel and iron. Additionally, a range of organic and inorganic materials included in e-waste have the potential to contaminate the environment (Klugmann-Radziemska & Kuczyńska-Łażewska, 2020). E-waste typically comprises 40% metals, 30% organic materials (such as fiberglass, polymers, and flame retardants), and 30% ceramics (such as alumina, mica, and silica). There are serious worries regarding the environmental impact of heavy metals in e-waste. For example, it has been stated that cathode ray tubes (CRTs) contain 468–732 mg/kg of copper and 429–9900 mg/kg of lead. (Boehm et al., 2018). Certain printing materials' copper and lead content can exceed permissible levels, ranging from 83,100 to 705,300 mg/kg. The burning of liquid crystals in LCDs also releases harmful polycyclic aromatic hydrocarbons (PAHs). The toxic substances in e-waste pose substantial environmental and human health risks (Vlachokostas, Achillas, Michailidou, Tsegas, & Moussiopoulos, 2020). Common health issues linked to e-waste exposure include respiratory problems, damage to the central nervous system, musculoskeletal disorders, skin conditions, cancer, and more. The environmental release of toxic components depends on the methods used for recycling and handling e-waste. Exposure to these substances can occur through inhalation, ingestion, or absorption through the skin, or indirectly through contaminated water, air, soil, or food. To assess the effects of e-waste on human health, evaluations can include exposure assessments, biomarker analysis, and disease control models. The release of harmful chemicals from e-waste also damages the atmosphere. Improper disposal of e-waste in landfills or through incineration contaminates surface and groundwater, posing significant health risks to marine life and humans. Burning e-waste to recover valuable metals, such as copper, is particularly hazardous, as it can lead to chronic diseases and even cancer (Tomić & Schneider, 2020).

Environmental Impacts of Food Waste

Food production, processing, and transportation require various resources, including water, energy, soil, pesticides, and fertilizers. These activities contribute to environmental degradation by generating pollutants. Carbon dioxide (CO_2), a greenhouse gas, is released during different food processing and production stages. CO_2 emissions primarily result from the transportation involved in the food supply chain, as vehicles burn gasoline and diesel (Cunha, Lima, Lima, da Silva, & Thue, 2020). Furthermore, food waste contributes significantly to CO_2 emissions, which in turn cause water bodies to become more acidic, hence harming marine ecosystems (Boehm et al., 2018). Food waste is also associated with nitrogen oxides (NOx), which combine with volatile organic substances to create ozone (O_3) and smoke. Additionally, soot and particulate matter are released into the environment during the manufacturing and transportation of food, posing serious health concerns to people (Zhang et al., 2022).

Solid Waste Management

Enhancing waste management techniques has become more crucial for creating a sustainable future as human activities continue to release different kinds of solid waste into the environment. Around 0.8 kg of solid waste are created per person worldwide, according to the World Bank's 2020 report, and by 2051, this quantity is predicted to rise by 75% (Chen et al., 2023). To address this, several waste management techniques have been put into place, emphasizing production, storage, collection, transportation, and disposal. The ecology is harmed by waste disposal techniques like burning outdoors or disposing of waste in the ocean. Aquatic life is threatened by waste that ends up into the oceans, especially from industries and tourisms. Oil spills are a serious problem because they prevent sunlight and oxygen from getting to the ocean, which puts marine life at further risk (Zhang et al., 2022; Pathak et al., 2023). The ozone layer is further weakened by the discharge of harmful substances into the atmosphere by open burning of waste (Klugmann-Radziemska & Kuczyńska-Łażewska, 2020). This method is commonly employed for plastic garbage, particularly in uncontrolled environments (Pathak et al., 2023).

The solid waste management process includes several key steps: i) minimizing waste at the source, ii) recycling, reusing, and composting, iii) recovering energy through incineration and other thermal methods. Various on-site and off-site waste disposal methods are utilized within a comprehensive waste management framework (Behrooznia et al., 2020).

Pits or Garbage Bins

Pits or garbage bins are designed for the hygienic disposal of waste at its point of origin, preventing environmental contamination. While these systems require further waste processing, accumulating waste in these bins can lead to leaks and microbial growth, posing health risks to humans (Zhang et al., 2022).

Landfills

Landfills offer a practical solution for waste disposal (Boehm et al., 2018). A large trench is dug into the ground, where waste is placed and covered with layers of soil (Pathak et al., 2023). The land used for landfills is typically flat or gently sloped to avoid flooding risks. Sanitary landfills are considered a safe method of waste disposal. However, improper or open waste disposal, which allows waste to mix with natural processes, can harm human life (Zhang et al., 2022; Janeeshma et al., 2024). Proper landfill management requires trained personnel, low groundwater levels, and appropriate protective materials for covering the waste.

Incineration

Combustible waste is burned in an aerobic environment during incineration (Zhang et al., 2022). Large volumes of solid waste are frequently thermally treated using this technique (Janeeshma et al., 2024). It uses less land space and is effective, odorless, and noiseless. Sludge is frequently managed by incineration rather than anaerobic digestion (Boehm et al., 2018). One viable strategy for managing waste and saving fossil fuels is the integration of waste incineration with coal-fired power plants (Shooshtarian et al., 2020). However, this process contributes to around 75% of the pollutants released, causing the environment to suffer from the discharge of heat, particulate matter, and hazardous chemicals (Behrooznia et al., 2020).

Gasification

Gasification involves the thermal decomposition of waste under limited oxygen, resulting in a mixture of combustible and non-combustible gases (Shooshtarian et al., 2020). This process can be used to generate energy or as a filler material for road construction (Shittu et al., 2020). The most efficient waste-to-energy process, plasma gasification, turns inorganic waste into inert vitrified glass and organic waste into fuel gas (Pathak et al., 2023). Sludge, biomass, and domestic waste may all be gasified to create synthesis gas, which can be used to produce hydrogen, fertilizer, energy, and as an alternative to natural gas. However, the process emits pollutants like dust, biomass ash, fly ash, and gas emissions, which can harm human health and the environment (Boehm et al., 2018).

Hydrothermal Carbonization

Hydrothermal carbonization involves producing hydrochar from solid waste at temperatures around 265°C in an aqueous environment. While this method is effective, its high pre-treatment costs are a significant limitation (Janeeshma et al., 2024).

Composting

Microorganisms turn organic waste into fertilizer through the environmentally friendly process of composting (Ozkan et al., 2022). Vermicomposting improves soil fertility and promotes sustainable agricultural

methods by using worms to turn biodegradable waste into nutrient-rich compost. Utilizing Black Soldier Fly larvae and Eudrilus Eugeniae to create compost of exceptional quality might further enhance the process. However, odor, dust, and lengthy processing periods are typical disadvantages (Janeeshma et al., 2024).

Biogas and Bioethanol Production

Known as bio-methanation, biogas production uses anaerobic conditions to produce energy from organic waste (Tulebayeva et al., 2020). When compressed, biogas produced by biogas digesters may be utilized to generate power with an efficiency of around 30%. Biogas can also be used to replace LPG or CNG. For soil enrichment, the process's byproducts make a great fertilizer (Abubakar et al., 2022). In 2016, the microorganism Cytophaga hutchsonni was used to create bioethanol from paper waste that had been processed with sulfuric acid (Byadgi & Kalburgi, 2016). The primary disadvantages are the method's high water use, demand for agricultural area, and labor expenditures.

Pyrolysis

Pyrolysis is solid waste's anaerobic chemical and thermal breakdown, particularly plastic (Shooshtarian et al., 2020). It creates carbonized charcoal to increase soil fertility, fuel gas for power production, and lubricants for diesel engines (Bello et al., 2022). Pyrolysis oil can be used as a liquid boiler fuel or an alternative to diesel (Tulebayeva et al., 2020). Pyrolysis has the potential to reduce carbon emissions, but it also runs the danger of emitting dioxins and producing inferior polymers as byproducts. The process's complexity and expensive setup are major disadvantages (Zhang et al., 2022).

Plowing Fields

Plowing fields helps incorporate organic waste, such as animal manure, into the soil, improving soil fertility and increasing crop yield (Pathak et al., 2023). This method reduces the need for chemical fertilizers but may not be fully environmentally friendly, as it requires skill to separate organic waste from inorganic or toxic pollutants (Boehm et al., 2018).

Pig Feeding

In some regions, food waste from kitchens and farms is used as pig feed, offering an effective recycling method for food waste (Tulebayeva et al., 2020). However, this practice poses health risks, including the spread of diseases like foot-and-mouth disease and African swine fever.

Bioremediation

Bioremediation utilizes naturally occurring microbes and neutralizes dangerous environmental contaminants (Tomić & Schneider, 2020). It is a low-cost, environmentally beneficial technique that guarantees the safe discharge of industrial waste with few risks (Janeeshma et al., 2024). However, bioremediation could not break down high-molecular-weight or chlorinated waste compounds (Tomić & Schneider, 2020).

Refuse-Derived Fuel (RDF)

Refuse-Derived Fuel (RDF) is created by burning dry solid waste that has a high calorific value but is not biologically degradable to produce high-energy products (Tulebayeva et al., 2020). Waste is compressed, dried, and shred in order to create pellets that may be used in industrial furnaces instead of coal. RDF is also advantageous because it can be transported and stored effectively over long distances (Boehm et al., 2018). This method's decreased energy efficiency when compared to the original solid waste products is a major disadvantage.

Recycling

Recycling involves recovering reusable solid waste materials compressed into blocks for safe transportation and recycling. This process helps reduce the amount of waste sent to landfills. Proper recycling can also transform waste into valuable products like geopolymer composites (Shooshtarian et al., 2020). Despite its benefits, the quality of second-generation products can sometimes be compromised, and the high cost of recycling specific solid waste materials is another limitation (Bello et al., 2022).

Conclusion

Traditional methods of solid waste management contribute to major climate changes and pose significant environmental and health risks, making the continuation of life on Earth more challenging. Therefore, it is essential to integrate green technologies into waste management systems. Embracing sustainable practices such as Reduce, Reuse, and Recycle will help protect and conserve resources, ensuring a cleaner and more sustainable future. By adopting these practices as part of daily life, we can preserve energy, reduce waste, and maintain a cleaner environment.

REFERENCES

Abubakar, I. R., Muniruzzaman, K. M., Dano, U. L., & Alshihri, F. S. (2022). Environmental sustainability impacts of solid waste management practices in the Global South. *International Journal of Environmental Research and Public Health, 19*(19), 12717.

Behrooznia, L., Sharifi, M., Hosseinzadeh-Bandbafha, H. (2020). Comparative life cycle environmental impacts of two scenarios for managing an organic fraction of municipal solid waste in Rasht, Iran. *Journal of Cleaner Production, 268*, 122217.

Bello, A. S., Al-Ghouti, M. A., & Abu-Dieyeh, M. H. (2022). Sustainable and long-term management of municipal solid waste: A review. *Bioresource Technology Reports, 18*, 101067.

Boehm, R., Wilde, P. E., Ver Ploeg, M., Costello, C., & Cash, S. B. (2018). A comprehensive life cycle assessment of greenhouse gas emissions from US household food choices. *Food Policy, 79*, 67–76.

Byadgi, S. A., & Kalburgi, P. B. (2016). Production of bioethanol from waste newspaper. *Procedia Environmental Sciences, 35*, 555–562. https://doi.org/10.1016/j.proenv.2016.07.040

Chapin, F. S., Torn, M. S., & Tateno, M. (1996). Principles of ecosystem sustainability. *American Naturalist, 148*(6), 1016-1037.

Chen, S., Yu, L., Zhang, C., Wu, Y., & Li, T. (2023). Environmental impact assessment of multi-source solid waste based on a life cycle assessment, principal component analysis, and random forest algorithm. *Journal of Environmental Management, 339*, 117942. https://doi.org/10.1016/j.jenvman.2023.117942

Cunha, M. R., Lima, E. C., Lima, D. R., da Silva, R. S., Thue, P. S., Seliem, M. K., Larsson, S. H. (2020). Removal of captopril pharmaceutical from synthetic pharmaceutical-industry wastewaters: Use of activated carbon derived from *Butia catarinensis*. *Journal of Environmental Chemistry and Engineering, 8*(6), 104506.

Esparza, I., Jiménez-Moreno, N., Bimbela, F., Ancín-Azpilicueta, C., & Gandía, L. M. (2020). Fruit and vegetable waste management: Conventional and emerging approaches. *Journal of Environmental Management, 265*, 110510.

Gaustad, G., Williams, E., & Leader, A. (2020). Rare earth metals from secondary sources: Review of potential supply from waste and byproducts. *Resources, Conservation & Recycling, 167*, 105213.

Hajam, Y. A., Kumar, R., & Kumar, A. (2023). Environmental waste management strategies and vermi transformation for sustainable development. *Environmental Challenges, 13*, 100747.

Janeeshma, E., Habeeb, H., Sinha, A., & Arora, P. (2024). Enzymes-mediated solid waste management: A sustainable practice for recycling. *Waste Management Bulletin, 1*, 104-113.

Jeswani, H. K., & Azapagic, A. (2020). Environmental impacts of healthcare and pharmaceutical products: Influence of product design and consumer behaviour. *Journal of Cleaner Production, 253*, 119860.

Kang, K. D., Kang, H., Ilankoon, I. M. S. K., & Chong, C. Y. (2020). Electronic waste collection systems using internet of things (IoT): Household electronic waste management in Malaysia. *Journal of Cleaner Production, 252*, 119801.

Klugmann-Radziemska, E., & Kuczyńska-Łażewska, A. (2020). The use of recycled semiconductor material in crystalline silicon photovoltaic modules production–A life cycle assessment of environmental impacts. *Solar Energy Materials and Solar Cells, 205*, 110259.

Mehta, D., Paliwa, D., Tege, S., & Sankhla, V. S. (2018). Sustainable waste management: An approach towards sustainability. *Journal of Emerging Technologies and Innovative Research, 5*(9).

Mondal, T., Choudhury, M., Kundu, D., Dutta, D., & Samanta, P. (2023). Landfill: An eclectic review on structure, reactions and remediation approach. *Waste Management, 164*, 127–142. https://doi.org/10.1016/j.wasman.2023.03.034

Nnorom, I. C., & Odeyingbo, O. A. (2020). Electronic waste management practices in Nigeria. In *Handbook of Electronic Waste Management* (pp. 323–354). Butterworth-Heinemann.

Ozkan, G., Gültekin Subaşı, B., Kamiloglu, S., & Capanoglu, E. (2022). Sürdürülebilir gıda ve tarımsal atık yönetimi. *Çevre, İklim ve Sürdürülebilirlik, 23*(2), 145-160.

Pathak, G., Nichter, M., Hardon, A., Moyer, E., Latkar, A., Simbaya, J., Pakasi, D., Taqueban, E., & Love, J. (2023). Plastic pollution and the open burning of plastic wastes. *Global Environmental Change, 80*, 102648. https://doi.org/10.1016/j.gloenvcha.2023.102648

Sartaj, S., Ali, N., Khan, A., Malik, S., Bilal, M., Khan, M., & Khan, S. (2020). Performance evaluation of photolytic and electrochemical oxidation processes for enhanced degradation of food dyes laden wastewater. *Water Science and Technology, 81*(5), 971–984.

Shittu, O. S., Williams, I. D., & Shaw, P. J. (2020). Global e-waste management: Can we make a difference? A review of e-waste trends, legislation, contemporary issues, and future challenges. *Waste Management, 120*, 549–563.

Shooshtarian, S., Caldera, S., Maqsood, T., & Ryley, T. (2020). Using recycled construction and demolition waste products: A review of stakeholders' perceptions, decisions, and motivations. *Recycling, 5*(4), 31.

Stiborova, H., Kronusova, O., Kastanek, P., Brazdova, L., Lovecka, P., Jiru, M., & Demnerova, K. (2020). Waste products from the poultry industry: A source of high-value dietary supplements. *Journal of Chemical Technology and Biotechnology, 95*(4), 985–992.

Taghavi, N., Singhal, N., Zhuang, W. Q., & Baroutian, S. (2021). Degradation of plastic waste using stimulated and naturally occurring microbial strains. *Chemosphere, 263*, 127975.

Tomić, T., & Schneider, D. R. (2020). Circular economy in waste management–Socio-economic effect of changes in waste management system structure. *Journal of Environmental Management, 267*, 110564.

Tulebayeva, N., Yergobek, D., Pestunova, G., Mottaeva, A., & Sapakova, Z. (2020). Green economy: Waste management and recycling methods. *E3S Web of Conferences, 159*, 01012.

Turner, G. M. (2008). A comparison of the limits to growth with 30 years of reality. *Global Environmental Change, 18*, 397-411.

United Nations. (2008). *Report of the World Commission on Environment and Development: Our Common Future*. Transmitted to the General Assembly as an Annex to document A/42/427. Retrieved from http://www.un-documents.net/ocf-ov.htm

Vlachokostas, C., Achillas, C., Michailidou, A. V., Tsegas, G., & Moussiopoulos, N. (2020). Externalities of energy sources: The operation of a municipal solid waste-to-energy incineration facility in the greater Thessaloniki area, Greece. *Waste Management, 113*, 351–358.

Yavuz, V. A. (2010). Sürdürülebilirlik kavramı ve işletmeler açısından sürdürülebilir üretim stratejileri. *Mustafa Kemal Üniversitesi Sosyal Bilimler Enstitüsü Dergisi, 7*(14), 63-86.

Zhang, Z., Malik, M. Z., Khan, A., & Ali, N. (2022). Environmental impacts of hazardous waste, and management strategies to reconcile circular economy and eco-sustainability. *Science of the Total Environment, 807*, 150856.

ilal ‘Environmental impacts of hazardous waste, and management strategies to reconcile circular economy and eco-sustainability

Science of The Total Environment

Volume 807, Part 2, 10 February 2022, 150856).

CHAPTER 4

MOLECULAR DOCKING STUDIES OF PLANT EXTRACTS AND DRUG DISCOVERY

Selim Can KAMALAK[1]
Ela Nur ŞİMŞEK SEZER[2]

1 Selim Can KAMALAK (0009-0000-2751-9070),
2 Assoc. Prof. Dr. Ela Nur ŞİMŞEK SEZER (0000-0003-2805-7204)

1. Importance of Plant Extracts in Drug Discovery

1.1. The Usage of Plant Extracts in Traditional Medicine and Drug Discovery

The use and popularity of plant extracts have increased in recent years with the treatment of several diseases and drug discovery efforts. The main reason for the use of plant extracts is the significant side effects associated with many drugs. This has led to increased therapeutic studies on natural products, which are believed to have fewer side effects and higher therapeutic efficacy. In developing countries and many regions, medicinal plants are widely used in traditional medicine (R. Singh, 2015). In China, India and other Asian, Middle Eastern and South American countries, plants are still used to treat health problems such as gastrointestinal disorders, hormonal imbalances, fertility problems, headaches and sleep disorders, skin infections and neurological disorders (Da-Yuan, Dong-Lu, & Xi-Can, 1996). The choice of medicinal plants can vary depending on their traditional, ethnobotanical and historical uses. In the 20th and 21st centuries, research on medicinal plants has intensified and their use for minor health problems and drug development has been adopted by doctors and pharmacologists in clinical practice. Studies provide data that traditional medicine can be improved, and further studies with plant extracts aim to conduct drug discovery research more comprehensively as targeted by the research (Gilani, 2005). Extracts used in drug discovery studies are usually obtained from aerial and belowground parts of plants and may have different effects on important pathways (Veiga, Costa, Silva, & Pintado, 2020). Therefore, it is essential to evaluate the extracts through *in vitro* and *in vivo* studies as well as *in silico* analysis.

1.2. Extraction and Purification of Medicinal Plants

The extraction of medicinal plants is essential for their use in therapeutic applications and for the investigation of their drug-like properties. Common extraction methods include Soxhlet extraction, ultrasonic extraction, maceration, microwave-assisted extraction and supercritical fluid extraction. Among them, ultrasonic extraction and microwave-assisted extraction are particularly favoured in modern studies due to their ability to deliver high yields in a shorter time frame. Besides extraction methods, the selection of suitable solvents is equally critical. Solvents commonly used in extraction procedures include ethanol, methanol, water, acetone and hexane. The choice of solvent significantly affects both the efficiency of the extraction process and the purity of the resulting compounds. For example, polar solvents are ideal for the extraction of hydrophilic compounds, while non-polar solvents are more effective for lipophilic compounds. Therefore, careful selection of both extraction methods and appropriate solvents is crucial to improve the overall success and efficiency of extraction studies (Azwanida, 2015). Purification of

secondary metabolites from plants is of great importance for the investigation of their bioactive effects. Procedures tailored to physical charge, molecular weight and various chemical properties can be applied in purification studies. Thin layer chromatography (TLC) D. Singh, Verma, and Shyam (2024), ion exchange chromatography (IEC) Acikara (2013), ultrafiltration and nanofiltration X. Wang et al. (2021) are among the most preferred purification methods. The purity of chemical compounds from plant extracts can be assessed using NMR (Nuclear Magnetic Resonance) (Pieri, Belancic, Morales, & Stuppner, 2011).

1.3. Secondary Metabolites and Chemical Characterization

Chemical profiling and characterization studies are necessary to obtain plant extracts and use them in drug discovery research (Ramakrishna et al., 2024). Plants produce highly unique compounds synthesized in a distinctive manner, known as secondary metabolites. Secondary metabolites are classified into different groups according to their structure, such as alkaloids, terpenes, phenolic and flavanoid compounds. Secondary metabolites play important roles in important mechanisms in plants such as environmental adaptation, defense against stress factors, pollination and signal transduction (Wink, 2010). In addition to the mechanisms by which they function in plants, studies have shown that they also serve as drug-like structures and highly important bioactive compounds for human health. Their small molecular structures and highly unique chemical profiles explain their drug-like properties and emphasize their importance for drug discovery studies (Seca & Pinto, 2019).

In the characterization studies of secondary metabolites from plant extracts, analyses are conducted using LC-MS (liquid chromatography-mass spectrometry), GC-MS (gas chromatography-mass spectrometry), HPLC (high-performance liquid chromatography), supercritical fluid extraction and other well-known analytical methods. It is known that each extraction has its unique advantages. In the profiling of secondary metabolites, two or more different methods are often combined to develop techniques, enabling more precise analyses. LC-MS/MS (Liquid Chromatography - Tandem Mass Spectrometry), UPHLC (Ultra High-Performance Liquid Chromatography), HPLC-UV, and GC-MS/MS (Gas Chromatography-Tandem Mass Spectrometry) are among the best examples of precise analytical techniques (Ramakrishna et al., 2024). The importance of the precision of these methods lies in the varying molecular weights of secondary metabolites and their presence in different concentrations within plant extracts. For this reason, conducting broad-range chemical profiling is a highly challenging task.

Secondary metabolites are important for human health such as camptothecin, capsaicin, nicotine, berberine, vinblastine, reserpine and piperine have been studied for their bioactive roles as alkaloids and their

derivatives. These compounds classified as alkaloids and their derivatives are known to have various effects such as anticancer, analgesic, anti-inflammatory, neurostimulant, antibacterial and hypoglycemic activities (Gutiérrez-Grijalva, López-Martínez, Contreras-Angulo, Elizalde-Romero, & Heredia, 2020). Phenolic compounds such as resveratrol, curcumin, epigallocatechin, genistein, coumarin and p-coumarin, ellagic acid, gallic acid, daidzein, eugenol, thymol and syringic acid are known to have bioactive effects. These include antioxidant, cardioprotective, anticancer, anticoagulant, anti-inflammatory, hormonal balance, phytoestrogenic, and antimicrobial effects. Another group of terpenes and their derivatives are paclitaxel, artemisinin, limonene, saponins, and menthol. In addition to anticancer research, researchers have conducted studies on antimalarial effects, immune system disorders, and analgesic properties. The best-known and most studied flavonoid compounds include quercetin, rutin, hesperidin, anthocyanidins, fisetin, apigenin, kaempferol, naringenin, luteolin, and myricetin. Studies have frequently investigated the various effects of flavonoids, such as antioxidant, anticancer, neuroprotective, anti-inflammatory, and antiatherogenic effects, and significant findings have been reported on their potential (Kabera, Semana, Mussa, & He, 2014).

Secondary metabolites have an important place in human health, especially in the context of cancer drug discovery. Although the methods used in cancer treatment and FDA (Food and Drug Administration) approved chemotherapy drugs have significant therapeutic effects, they also cause serious side effects on patient health. Therefore, interest in natural products with minimized side effects and increased targeted anticancer effects has increased significantly. Secondary metabolites have been studied in cell lines and experimental animals to investigate their cytotoxic effects, inhibition of enzyme groups involved in carcinogenesis mechanisms and induction of apoptosis mechanisms. However, the synergistic effects of secondary metabolites in plant extracts, the difficulties in purifying each metabolite, and the potential paucity of *in vitro* and *in vivo* data require the support of cancer drug discovery studies (Seca & Pinto, 2018).

2. *In Silico* Approaches in Drug Discovery Studies

In drug discovery studies, *in silico* methods have been developed to support experimental studies, and these methods allow discovery steps to be performed more efficiently and in shorter time frames. These methods are known as Computer-Aided Drug Design (CADD) and have an important position in drug discovery due to their widespread applications. One of the biggest advantages of CADD methods is the creation of chemical libraries (Veselovsky & Ivanov, 2003). These libraries, created to access the chemical profiles of both natural products and approved drugs, are invaluable for researchers in the field of CADD. The best-known libraries include databases such as PubChem, DrugBank, ZINC, ChemSpider and others (Ghani, 2020). The chemical profiles of most secondary metabolites and their derivatives can

be accessed through these databases and can be used in the field of de novo drug design by following synthetic pathways. In addition, the presence of 3D structures in these databases enables their use in structure-based studies in the field of CADD.

CADD is divided into two approaches: ligand-based drug discovery (LBDD) and structure-based drug discovery (SBDD). Ligand-based approaches include unique areas such as pharmacophore modelling, SAR, and QSAR studies. These studies are usually conducted to create and access large ligand libraries (Krovat, Frühwirth, & Langer, 2005). Structure-based approaches, on the other hand, specialize in studies that require 3D structures of both the ligand and its target. 3D structures aim to investigate the effects of the ligand on the target structure and to optimize its structure in the desired direction. De novo drug discovery, molecular modelling (MM), molecular dynamics simulations (MD), and molecular docking are known as the most commonly used structure-based drug discovery approaches (Anderson, 2003). Molecular docking has a special place among these approaches and provides researchers with important insights into the drug-like properties of plant extracts.

2.1. Molecular Docking in Drug Discovery Studies

Molecular docking is a structure-based CADD method that operates by predicting the poses of small molecules (ligands) with known 3D structures when docked to a target macromolecular structure with a known 3D conformation and scoring these poses to identify the best fit (Meng, Zhang, Mezei, & Cui, 2011). In drug discovery studies, the macromolecular target is typically a protein structure, and research focuses on how the docking of small molecules at binding sites on the protein alters its structural conformation and metabolic activity. In molecular docking, where the best poses are searched and scored, the scores are obtained in two different types: binding affinity scores and RMSD scores. In addition to these scoring methods, docking scores are optimized using compatibility scores, consensus scores, ligand efficiency scores, and similar metrics, while erroneous calculations are eliminated through different software tools. Binding affinity scores are directly related to the free energy change (ΔG) and indicate the strength of the interaction between the target protein and the ligand. A negative increase in this score reflects better results. The reason is that a decrease in the free energy change (ΔG) indicates stronger interactions between the target protein and the ligand, suggesting a higher likelihood of binding. Although positive scores are rare, they indicate that interactions between the target protein and the ligand are thermodynamically unfavourable and that binding will not occur (Wang, Lu, & Wang, 2003).

Target proteins are considered key proteins involved in metabolism in drug discovery studies. Target protein structures must be well understood in

terms of their mechanisms, purification and characterization, classification, chemical properties, and structural mutations (Nehete, Bhambar, Narkhede, & Gawali, 2013). In molecular docking and other structure-based approaches, the 3D structure of the target protein and other macromolecular structures must be obtained. There are three different methods used to obtain the 3D structures of proteins, commonly known as crystallization studies. These methods are NMR, X-ray crystallography, and cryogenic electron microscopy (Cryo-EM). Through crystallization studies, various properties of target protein structures can be determined, including their molecular weights, number of amino acids, symmetric and asymmetric structures, side chains and backbone structures, different protein chain compositions, surface features, and number of atoms. The resolution of the protein crystal structure is also determined similarly and is highly important for studies. Research conducted with high-resolution structures is more likely to produce scores that are closer to real systems. The resolution of a protein crystal structure is expressed in Å (angstroms), and a resolution value of 2.0 Å or lower indicates very high resolution for the protein backbone and side chain structures (Lanci et al., 2012). Crystal structures of target proteins for molecular docking studies can be accessed through the RCSB PDB (Protein Data Bank). Currently, the PDB, which contains numerous experimentally determined crystal protein structures and modelled 3D crystal structures, provides significant convenience for researchers in the field of CADD (Berman et al., 2000).

The most frequently used target protein structures in molecular docking studies are usually enzymes. For example, among the most studied structures is the cytochrome P450 family, which plays critical roles in drug metabolism, steroid hormone synthesis catalysis, cell signalling and inflammatory responses. Understanding drug metabolism makes the cytochrome P450 enzyme family highly valuable for designing new drug candidates, investigating the drug-like properties of secondary metabolites and understanding the effects of toxic metabolites (Manikandan & Nagini, 2018). Another example is the oncogenic proteins Ras and P53, which are crucial in cancer development mechanisms. Ras proteins are a family of proteins involved in extracellular signalling and play a regulatory role in cell growth and differentiation. Normally, GTP-bound forms are active and GDP-bound forms are inactive; however, structural mutations cause them to remain permanently active, leading to uncontrolled cell growth and cancer development. P53 oncoprotein, known as the 'guardian of the genome', is a tumour suppressor protein that functions in cell cycle regulation, DNA repair mechanisms and as an inducer of apoptosis (Baran, Kiraz, & Ulu, 2018).

Protein-ligand interactions occur at binding sites on proteins. These regions, which specifically recognize ligands, undergo conformational changes to accommodate the size, shape, polarity, and other chemical properties of the ligands. Their fundamental characteristics include a flexible structure, high

ligand-binding affinity, and specificity. They are shaped by amino acids, the protein backbone, and side chain structures, gaining their unique properties. Chemically, they can possess hydrophobic or hydrophilic properties, which significantly influence ligand binding (Mattos & Ringe, 1996). Binding sites are functionally divided into two categories: active binding sites and allosteric binding sites. Active binding sites are known as the primary binding regions where proteins exhibit catalytic effects. Ligand binding at these sites can have catalytic effects on the ligand and is responsible for its metabolism. These regions, which specifically bind ligands, undergo conformational changes to accommodate ligand binding. Additionally, they can enable the ligand to adopt constrained conformations for compatibility. These regions are highly significant in drug discovery, particularly for *de novo* synthesis, and targeted therapies against diseases are often designed around them. Allosteric binding sites are known as regions involved in the mechanisms of protein activation and inhibition. Ligand binding at allosteric sites induces conformational changes in the protein's active binding sites (Avashthi, Srivastava, & Singh, 2020). Targeting allosteric binding sites in drug discovery is crucial for the metabolism of drugs. In cancer drug discovery, studies focusing on allosteric binding sites are conducted to inhibit mutated protein structures.

2.1.1. Identification of Binding Sites in Docking Studies

Another important aspect of molecular docking studies is the identification and targeting of binding sites on the target protein structure in a manner suitable for the studies. The characteristics and chemical properties of binding sites, their physical charge states, the amino acid residues shaping their structure, and the properties of side chains must be well understood. Binding site selection is made in two different scenarios. The first scenario is targeted/site-specific docking, where the localization and properties of the binding sites on the target protein are well understood. Today, the binding site localizations of most target protein crystal structures are available in the PDB, and their functions and properties are well understood. The second scenario occurs when the localizations and properties of the binding sites on the target protein structure are unknown. In such cases, blind docking studies are conducted to determine the binding site localizations (Hetényi & Spoel, 2011). The identified binding sites are analyzed by searching for regions with the best scores, and their properties are determined. To achieve better scores, subsequent docking studies are focused on the preferred region, similar to targeted/site-specific docking.

2.1.2. Flexibility in Molecular Docking

The essential aspects of molecular docking involve determining the target protein and ligand structures, followed by selecting the software to perform the docking process. During the software selection phase, it is essential to determine

which flexibility procedure the docking will follow, and the scoring and search algorithms of the software must be understood (Pagadala, Syed, & Tuszynski, 2017). Three different flexibility procedures are available: rigid docking, semi-flexible docking, and flexible docking. The rigid docking procedure is based on fixing the target protein and ligand structures in a single conformation. It is not sufficient for obtaining the best scores or the most efficient poses; however, its ability to provide quick results offers a significant advantage that can guide studies. It is not effective in producing good docking scores for a large number of ligands but allows the elimination of ligands that are unlikely to interact well, enabling higher-scoring ligands to be used in advanced studies (Meng et al., 2011). For example, among a large number of secondary metabolites characterized from plant extracts, the one with the best binding score can be selected to provide a preliminary estimation for studies, and further research can be conducted on the identified secondary metabolite structure in advanced studies.

More advanced studies involve semi-flexible and flexible docking. Semi-flexible docking studies are conducted in scenarios where the target protein structure is kept in a fixed conformation, while ligand structures are allowed to undergo potential conformational changes. Compared to rigid docking studies, it provides better scores and binding poses. The calculation of conformations based solely on the flexibility of the ligand structure and the ease of these calculations make it the most popular flexibility approach. It provides sufficient information about the movements of the ligand on the binding site. As shown in Figure 1, AutoDock Vina, one of the most popular applications, operates based on a semi-flexible procedure (Trott & Olson, 2010).

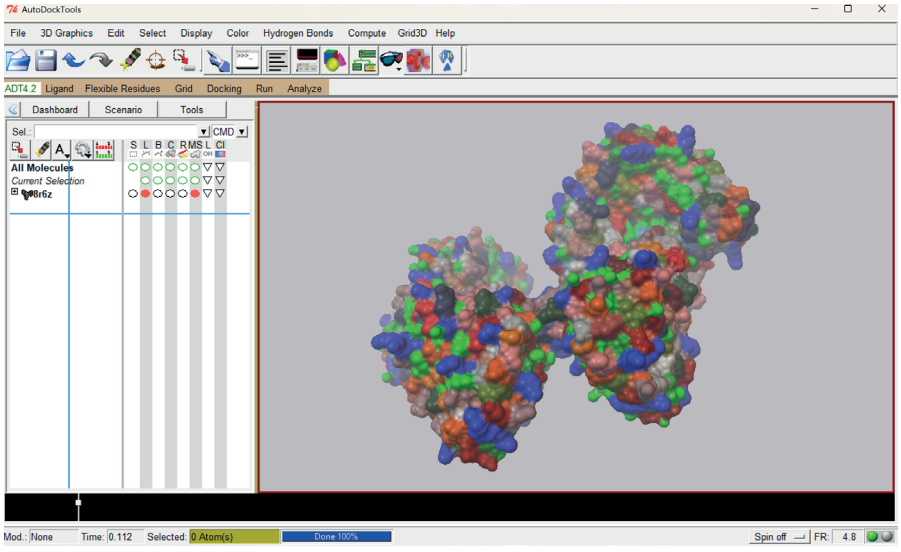

Figure 1: *The interface of AutoDock Tools 1.5.7 software. AutoDock Vina operates through the AutoDock Tools interface.*

Flexible docking is known as the approach where both the target protein and ligand structures are allowed to undergo all possible conformational changes. The flexible docking procedure, which has the significant advantage of producing the best scores and poses, provides docking poses that are closest to real systems in cellular mechanisms. These studies are typically conducted in combination with MD simulations. The mobility of amino acid residues and side chains in the receptor's binding site generates a large number of conformations of the target protein structure (Meng et al., 2011). However, this situation leads to target protein-ligand interactions occurring with high degrees of freedom, making the calculations of the flexible docking procedure highly challenging and costly. Another disadvantage is that the calculations can take extended periods to complete. Therefore, in multi-system studies or when screening ligand libraries, following a stepwise approach—rigid docking → semi-flexible docking → flexible docking—will enable more effective molecular docking studies.

2.1.3. Scores and Poses in Molecular Docking

In molecular docking studies, the acquisition of scores and poses are achieved through two types of algorithms. These algorithms consist of the search algorithm, which identifies the best poses, and the scoring function, which evaluates poses to obtain the one with the highest score. Algorithms are automatically utilized by software and form the fundamental working principle of their operation. Search algorithms can be categorized into two main subtypes: systematic search and stochastic search algorithms. Systematic search involves systematically evaluating poses based on the target protein-ligand structure, considering different rotatable bonds, rotations, and translations. Stochastic search, on the other hand, relies on randomness and genetic algorithms to optimize energy and identify the best poses. Protein populations based on genetic evolution, crossovers, and parameters derived from simulated annealing and thermodynamic principles are utilized to search for the optimal poses. Scoring functions, on the other hand, are algorithms that numerically represent interactions and generated poses, searching for the best scores through mathematical energy calculations. They utilize energy-based, pharmacophore-based, and free-energy calculations. They also include scenarios where molecular mechanics (MM), experimental data, knowledge-based approaches, physicochemical properties of binding sites and ligands, or a combination of these are employed as a consensus scoring function (Yadava, 2018).

2.1.4. Preparation of Target Protein and Ligand Structures in Molecular Docking Studies

2.1.4.1. Preparation of Target Protein Structure

In molecular docking studies, the target protein structure must be prepared for docking. Proper and complete preparation steps are crucial to enhance the quality and accuracy of the scores. The protein preparation process consists of several steps, the first of which is verifying the protein structure through homology modelling. In homology modelling, the accuracy of the target protein model is assessed by analysing allowed positions and torsion angles using a Ramachandran Plot. Additionally, energy distributions are evaluated using Z-scores to determine global quality, and the TM Score (Template Modelling Score) is used to assess the folding pattern. A value above 90% indicates that the modelling accuracy is very high (Wang et al., 2003). Protein sequences are also critical for structural validation. The structure can be validated by aligning the protein sequence with the target protein crystal structure. If there are missing amino acid residues in the target protein structure as a result of homology modelling, they should be added to complete the structure. Homology modelling can be performed using online servers or software. Swiss-Model is a frequently preferred online server among researchers as an example (Waterhouse et al., 2018). Missing amino acid residues are often located on the side chains of the target protein and can alter the protein conformation. In crystallization studies, the target protein structure may also lack hydrogen atoms. During the protein preparation stage in the software interface, H atoms are added to complete the structure. This increases the surface area of the binding sites, ensuring more stable interactions.

In the target protein crystal structure, ligands that may be bound during crystallization studies and free water molecules present in the surrounding environment can also crystallize along with the structure. These ligands are typically the protein's specific ligands and are known as co-crystallized ligands. Co-crystallized ligands and free water molecules should be removed from the structure as they occupy binding sites and may negatively impact docking scores. Software tools refer to co-crystallized ligands as heteroatoms. The localizations of heteroatoms can provide potential binding site information in cases where the binding sites are unknown and may be used in docking studies. All changes in the target protein structure alter its initial charge calculated during crystallization. In this case, reassigning charges to the structure becomes necessary. This process is called energy minimization and is essential for achieving a more stable protein structure. Different software tools may offer various charge assignment options in their interfaces (Madhavi Sastry, Adzhigirey, Day, Annabhimoju, & Sherman, 2013). By assigning charges, the target protein structure is prepared for molecular docking.

2.1.4.2. Ligand Structure Preparation Steps

Like protein structures, ligand structures also require preparation. To use the characterized and profiled secondary metabolites as ligand structures in molecular docking studies, their 3D structures must be obtained. NMR provides information about the 3D structures of secondary metabolites through their magnetic field interaction maps. These structures can typically be obtained from databases such as PubChem, ChemSpider, ZINC, and DrugBank, where chemical profiles of numerous secondary metabolites are readily available today (Madhavi Sastry et al., 2013). Secondary metabolites with 3D structures must be prepared and validated in a suitable format for use by the software. Optimizing ligand parameters to achieve the best scores is highly important. These parameters include protonation, structural optimization, energy minimization, tautomerization, and the verification of isomer/stereoisomer states.

The investigation of drug-like properties of secondary metabolites can be conducted using online software tools. These studies are known as ADME (Absorption-Distribution-Metabolism-Excretion) studies and predict the absorption, distribution, metabolism, and elimination of secondary metabolites and other drug molecules in metabolic processes. Absorption operates based on predicting the molecule's uptake through intestinal pathways and membranes. Molecules with a high LogP value can pass through membrane structures more easily, leading to higher absorption. Distribution is a tool used to evaluate the dispersion of secondary metabolites across different tissues. Factors such as molecular weight, the ability to cross the blood-brain barrier, and the number of hydrogen bonds formed influence distribution. Metabolism aims to understand the metabolic processing of secondary metabolites by investigating their interactions with metabolic enzymes and different classes of protein families. Finally, the excretion tool aims to provide and predict information about the excretion of metabolites from the body. It focuses on how long a molecule remains in the body after intake and its excretion following its role in metabolic pathways. Lipophilic molecules tend to stay in the body for longer periods, while hydrophilic molecules are excreted in shorter times. In research based on ADME studies, the drug-like properties of secondary metabolites are investigated, and Christopher Lipinski developed the rules known as Lipinski's Rule of Five (Pollastri, 2010). According to these rules, a secondary metabolite or drug candidate molecule must meet the following criteria: its molecular weight should be less than 500 daltons, the maximum number of hydrogen bond donors should be 5, the maximum number of hydrogen bond acceptors should be 10, its LogP value should be less than 5, and its breakpoint values should range between 4 and 130. ADME studies can be easily performed using the SwissADME online software, developed by the Swiss Institute of Bioinformatics (SIB). This free-access software is widely

used by researchers in pharmaceutical research and development processes to predict the drug-likeness and ADME properties of secondary metabolites and in drug discovery studies. It provides quick compliance information for Lipinski's Rule of Five and other drug-likeness filters (e.g., Pfizer's 3/75 Rule, Ghose Filter, Veber Rule) and their assessments regarding the molecule (Daina, Michielin, & Zoete, 2017). Another important feature is its accessible interface, the BOILED-Egg model shown in Figure 2 provides an accessible interface that illustrates the ability of molecules to cross the blood-brain barrier and their gastrointestinal absorption properties (Daina & Zoete, 2016).

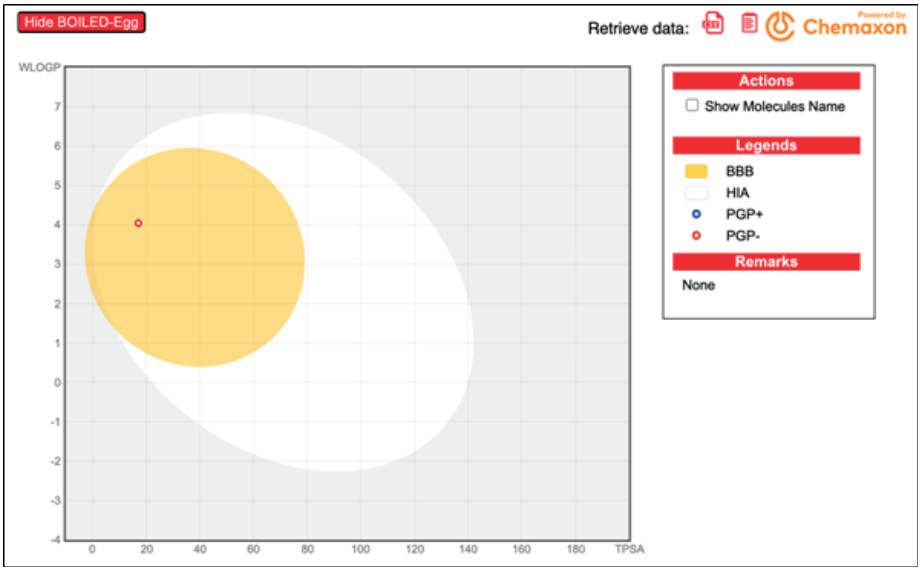

Figure 2: *In the SwissADME online tool interface, the BOILED-Egg graph shows that the molecule, located in the yellow region, has the potential to cross the blood-brain barrier (BBB), while being outside the white region suggests low gastrointestinal absorption (Flachsenberg, Ehrt, Gutermuth, & Rarey), and the red dot (PGP-) indicates that it is not a P-glycoprotein substrate (Daina & Zoete, 2016).*

2.1.5. Grid Box Parameter in Molecular Docking

The grid box parameter is crucial in molecular docking studies that define the region where the target protein and ligand will interact. It is typically described as a grid box that encloses the desired binding site, with adjustable localization on the X-Y-Z coordinates of the target protein and modifiable dimensions along the X-Y-Z axes. Precise adjustment of this parameter will positively influence the accuracy of molecular docking scores.

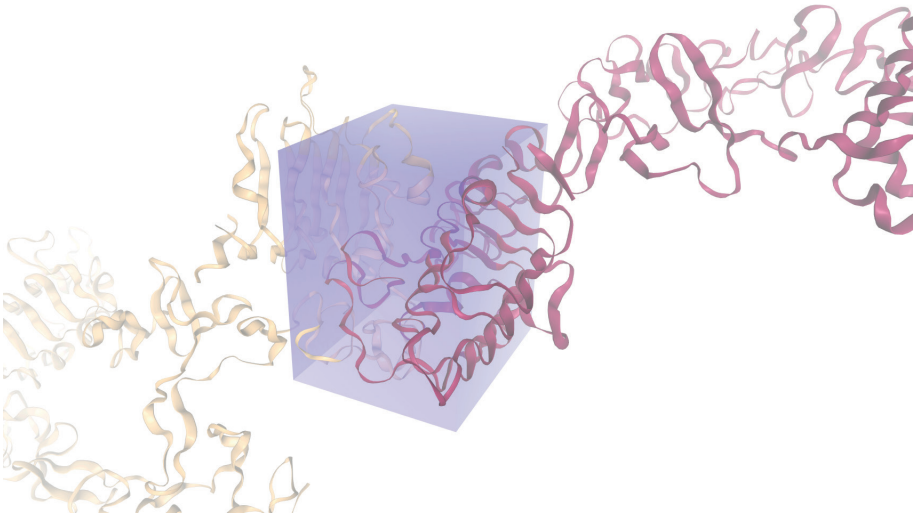

Figure 3: *Grid box parameter displayed on the 3D structure of the protein in an example molecular docking study conducted using the SwissDock online software (Grosdidier, Zoete, & Michielin, 2011).*

Grid box parameter data is used as input in a configuration file for the software to process docking scores. In some software, this process requires manual editing in a text file format, while in others, it is automatically generated by the software itself. After this step, which is the final stage of molecular docking, poses and scores are generated by the software using search algorithms and scoring functions for researchers to evaluate (Meng et al., 2011). To ensure the accuracy of scores, additional steps are taken to validate and verify their reliability, which is crucial for the credibility of the studies. Typically, re-docking is performed using the same parameters to repeat the docking process from the beginning, or the scores can be validated and assessed through more advanced methods, such as molecular dynamics simulations (Flachsenberg et al., 2023). Molecular docking scores can be evaluated in several different ways. These evaluations may include the analysis of binding affinity scores, assessment of the error margin in scores obtained through RMSD values, examination of 2D interaction profiles, visualization of binding site charges and poses, investigation of electrostatic and hydrophobic interactions, and assessment of ligand efficiency.

3. Molecular Docking and Secondary Metabolites

Secondary metabolites frequently used in molecular docking studies and with well-documented interactions are listed below. These secondary metabolites include:

3.1. Curcumin

Curcumin, a secondary metabolite belonging to the polyphenol group with hydrophobic characteristics, is derived from the *Curcuma longa* L. plant, which is shown in Figure 4a, while curcumin itself is shown in Figure 4b. Preclinical and clinical studies have found that curcumin and its derivative compounds inhibit proliferation in MCF7 breast cancer cells, concluding that this effect is due to the inhibition of cyclin-dependent kinase 2 (CDK2) activation. With these results, molecular docking studies were conducted between curcumin and its derivatives and the CDK protein family, demonstrating an inhibitory effect at the binding site where it interacts with a high binding affinity score (Sumirtanurdin, Sungkar, Hisprastin, Sidharta, & Nurhikmah, 2020).

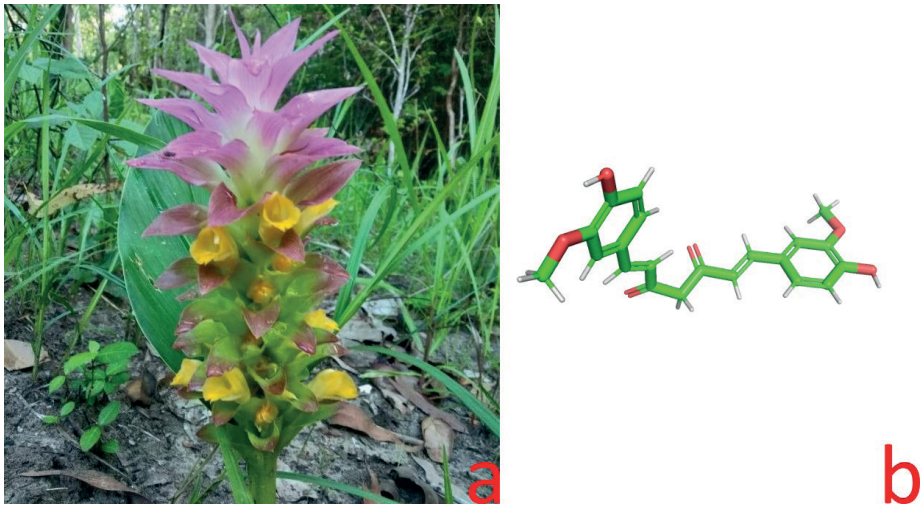

Figure 4: *Curcuma longa (a) Joly et al. (2016), and the 3D structure of the curcumin compound visualized using PyMOL software (b) (Yuan, Chan, & Hu, 2017).*

3.2. Resveratrol

Resveratrol, a polyphenol, is known as a secondary metabolite frequently found in the Liliaceae and Vitaceae families. Various bioactivity studies indicate that it is an important compound in terms of its pharmacological effects. In particular, studies have been conducted in the fields of cancer drug discovery, anti-inflammatory effects, and apoptosis induction. In a study, molecular docking experiments were conducted with trypsin, a digestive enzyme, and the resveratrol compound. As a result, it was observed that resveratrol caused significant conformational changes in trypsin. The results of the molecular docking studies concluded that resveratrol interacts with the active binding site of the trypsin enzyme, regulating its catalytic activity and that an increase in concentration would inhibit trypsin activity (Ren et al., 2019).

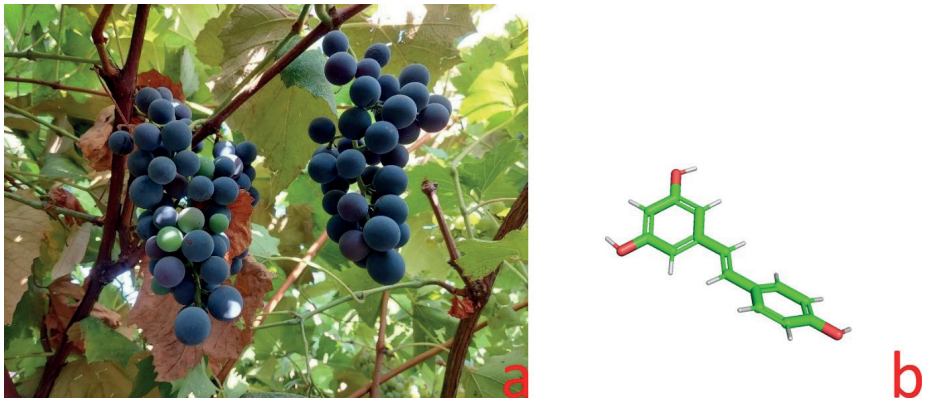

Figure 5: *The fruits of Vitis vinifera L., which are the parts containing the highest amount of resveratrol (a) Joly et al. (2016), and the 3D structure of the resveratrol compound visualized using PyMOL software (b) (Yuan et al., 2017).*

3.3. Epigallocatechin Gallate

Epigallocatechin gallate (Figure 6b), a flavonoid derived from *Camellia sinensis* L. shown in Figure 6a, along with its derivatives, has been shown in several studies to be a potential drug candidate. Among these effects, inhibition of HIV-reverse transcriptase and cellular polymerase activities has been demonstrated. It plays a role in antioxidant, antimicrobial, anticancer, and antifungal effects. The molecular docking study focused on the interactions of selected ligands with SARS-CoV-2 spike proteins and the human ACE2 protein, aiming to inhibit the spike protein-ACE2 interaction. This inhibition of spike protein interaction represents a significant study demonstrating that viral infection could be prevented using flavonoid-based compounds (Maiti & Banerjee, 2021).

Figure 6: *The leaf parts of the Camellia sinensis plant (a) Joly et al. (2016), and the 3D structure of the epigallocatechin gallate compound visualized using PyMOL software (b) (Yuan et al., 2017).*

3.4. Quercetin

Quercetin, shown in Figure 7b, is a flavonoid that plays a key role in scavenging free radicals and is generally found in its glycoside form. Observed in the plant *Allium cepa* L. (Figure 7a), it influences inflammation and carcinogenesis processes by regulating the signaling pathways such as MEK/ERK and Nrf2/Keap1. Molecular docking studies conducted with quercetin and its derivatives are frequently used in anti-inflammatory research, cancer drug discovery, and neurodegenerative disorders. In a study conducted by Murakami, Ashida, and Terao (2008), inducible nitric oxide synthase (iNOS) enzyme was targeted as a quercetin target, aiming to prevent cancer formation in cells by inhibiting the enzyme, thereby blocking NO production and suppressing p53 protein overexpression. High binding affinity scores suggest that inhibiting iNOS, which is associated with cancer, and reducing excessive NO production could be effective in preventing tumour development.

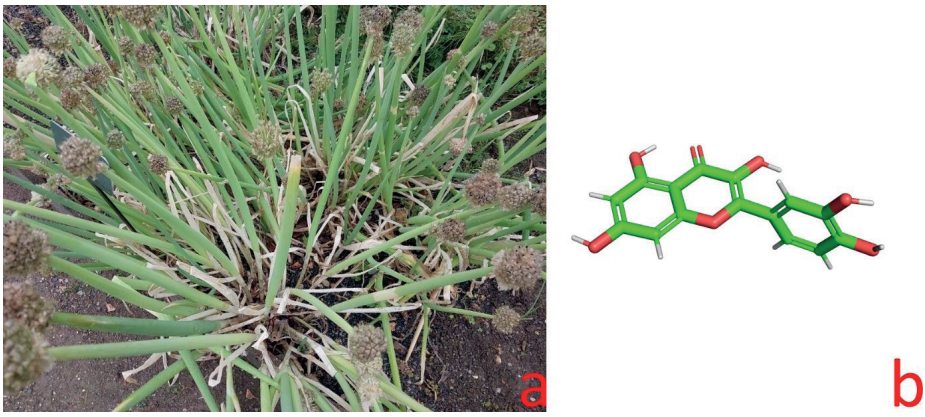

Figure 7: *The aerial parts of Allium cepa (a) Joly et al. (2016), and the 3D structure of the quercetin compound visualized using PyMOL software (b) (Yuan et al., 2017).*

3.5. Apigenin and Luteolin

Apigenin and luteolin, shown in Figure 8, which are secondary metabolites frequently found in the leaves, fruits, and fruit peels of *Apium graveolens* L., *Petroselinum crispum* Mill., *Thymus vulgaris* L., *Citrus sinensis* L., *Glycine max* L., and *Salvia officinalis* L., have been evaluated *in vitro* studies for their antimicrobial, anticancer, cardioprotective, neuroprotective, and anti-inflammatory effects. Molecular docking studies are conducted to evaluate the mechanisms underlying these effects (Sahraei & Sahraei, 2024).

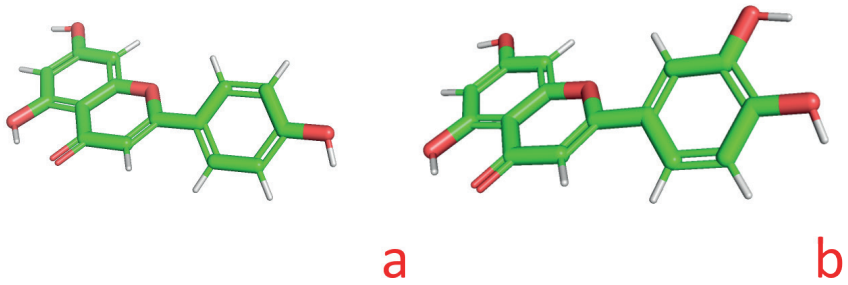

Figure 8: *The 3D structures of apigenin (a) and luteolin (b) compounds visualized using PyMOL software (Yuan et al., 2017).*

Plants and their secondary metabolites have become a focal point in drug discovery studies, thanks to advancements in technology and analytical methods. The ultimate goal is to eliminate most known human diseases through natural product-based drug discovery efforts. Studies evaluating the drug-like properties of secondary metabolites and developing their derivatives for the treatment of cancer and other diseases are highly promising. The use of *in silico* methods in drug discovery continues to gain popularity over time, leading to larger-scale studies and research teams, while interest *in silico* approaches grows day by day.

References

Acikara, Ö. B. (2013). Ion-exchange chromatography and its applications. *Column chromatography*, 10, 32-58.

Anderson, A. C. (2003). The process of structure-based drug design. *Chemistry & biology, 10*(9), 787-797.

Avashthi, H., Srivastava, A., & Singh, D. B. (2020). Cavity/Binding Site Prediction Approaches and Their Applications. *Computer-Aided Drug Design, 1*(1), 49-69.

Azwanida, N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med aromat plants, 4*(196), 2167-0412.

Baran, Y., Kiraz, Y., & Ulu, G. (2018). Kanser Moleküler Biyolojisi. *Kısayol Yayıncılık, Ankara, 536s, 2*, 582.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., . . . Bourne, P. E. (2000). The protein data bank. *Nucleic acids research, 28*(1), 235-242.

Da-Yuan, Z., Dong-Lu, B., & Xi-Can, T. (1996). Recent studies on traditional Chinese medicinal plants. *Drug Development Research, 39*(2), 147-157.

Daina, A., Michielin, O., & Zoete, V. (2017). SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Scientific reports, 7*(1), 42717.

Daina, A., & Zoete, V. (2016). A boiled-egg to predict gastrointestinal absorption and brain penetration of small molecules. *ChemMedChem, 11*(11), 1117-1121.

Flachsenberg, F., Ehrt, C., Gutermuth, T., & Rarey, M. (2023). Redocking the PDB. *Journal of chemical information and modeling, 64*(1), 219-237.

Ghani, S. S. (2020). A comprehensive review of database resources in chemistry. *Eclética Química, 45*(3), 57-68.

Gilani, A. (2005). Role of medicinal plants in modern medicine. *Malaysian Journal of Science, 24*(1), 1-5.

Grosdidier, A., Zoete, V., & Michielin, O. (2011). SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic acids research, 39*(2), W270-W277.

Gutiérrez-Grijalva, E. P., López-Martínez, L. X., Contreras-Angulo, L. A., Elizalde-Romero, C. A., & Heredia, J. B. (2020). Plant alkaloids: Structures and bioactive properties. *Plant-derived bioactives: chemistry and mode of action*, 85-117.

Hetényi, C., & Spoel, D. v. d. (2011). Toward prediction of functional protein pockets using blind docking and pocket search algorithms. *Protein Science, 20*(5), 880-893.

Joly, A., Bonnet, P., Goëau, H., Barbe, J., Selmi, S., Champ, J., . . . Molino, J.-F. (2016). A look inside the Pl@ ntNet experience: The good, the bias and the hope. *Multimedia Systems, 22*, 751-766.

Kabera, J. N., Semana, E., Mussa, A. R., & He, X. (2014). Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J. Pharm.*

Pharmacol, 2(7), 377-392.

Krovat, E. M., Frühwirth, K. H., & Langer, T. (2005). Pharmacophore identification, in silico screening, and virtual library design for inhibitors of the human factor Xa. *Journal of chemical information and modeling, 45*(1), 146-159.

Lanci, C. J., MacDermaid, C. M., Kang, S.-g., Acharya, R., North, B., Yang, X., . . . Saven, J. G. (2012). Computational design of a protein crystal. *Proceedings of the National Academy of Sciences, 109*(19), 7304-7309.

Madhavi Sastry, G., Adzhigirey, M., Day, T., Annabhimoju, R., & Sherman, W. (2013). Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments. *Journal of computer-aided molecular design, 27*, 221-234.

Maiti, S., & Banerjee, A. (2021). Epigallocatechin gallate and theaflavin gallate interaction in SARS-CoV-2 spike-protein central channel with reference to the hydroxychloroquine interaction: bioinformatics and molecular docking study. *Drug Development Research, 82*(1), 86-96.

Manikandan, P., & Nagini, S. (2018). Cytochrome P450 structure, function and clinical significance: a review. *Current drug targets, 19*(1), 38-54.

Mattos, C., & Ringe, D. (1996). Locating and characterizing binding sites on proteins. *Nature biotechnology, 14*(5), 595-599.

Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). Molecular docking: a powerful approach for structure-based drug discovery. *Current computer-aided drug design, 7*(2), 146-157.

Murakami, A., Ashida, H., & Terao, J. (2008). Multitargeted cancer prevention by quercetin. *Cancer letters, 269*(2), 315-325.

Nehete, J. Y., Bhambar, R. S., Narkhede, M. R., & Gawali, S. R. (2013). Natural proteins: Sources, isolation, characterization and applications. *Pharmacognosy reviews, 7*(14), 107.

Pagadala, N. S., Syed, K., & Tuszynski, J. (2017). Software for molecular docking: a review. *Biophysical reviews, 9*, 91-102.

Pieri, V., Belancic, A., Morales, S., & Stuppner, H. (2011). Identification and quantification of major steviol glycosides in Stevia rebaudiana purified extracts by 1H NMR spectroscopy. *Journal of agricultural and food chemistry, 59*(9), 4378-4384.

Pollastri, M. P. (2010). Overview on the Rule of Five. *Current protocols in pharmacology, 49*(1), 9.12. 11-19.12. 18.

Ramakrishna, K., Chakravarthi, G., Rai, S., Singh, P., Mishra, S., Mishra, D., . . . Tiwari, N. (2024). Biochemical characterization of plant secondary metabolites. In *Secondary Metabolites and Biotherapeutics* (Vol. 1, pp. 39-61): Elsevier.

Ren, G., Sun, H., Guo, J., Fan, J., Li, G., & Xu, S. (2019). Molecular mechanism of the interaction between resveratrol and trypsin via spectroscopy and molecular docking. *Food & function, 10*(6), 3291-3302.

Sahraei, A., & Sahraei, R. (2024). Revealing binding mechanism of β-casein to chrysin, apigenin, and luteolin and locating its binding pockets by molecular docking and molecular dynamics. *Biochemical and Biophysical Research Communications, 733*, 150438.

Seca, A. M., & Pinto, D. C. (2018). Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application. *International journal of molecular sciences, 19*(1), 263.

Seca, A. M., & Pinto, D. C. (2019). Biological potential and medical use of secondary metabolites. In (Vol. 6, pp. 66): MDPI.

Singh, D., Verma, S. K., & Shyam, P. (2024). Identification and purification of plant secondary metabolite as medicinal raw materials. In *Secondary Metabolites and Biotherapeutics* (Vol. 1, pp. 9-38): Elsevier.

Singh, R. (2015). Medicinal plants: A review. *Journal of plant sciences, 8*(2), 50-55.

Sumirtanurdin, R., Sungkar, S., Hisprastin, Y., Sidharta, K. D., & Nurhikmah, D. D. (2020). Molecular docking simulation studies of curcumin and its derivatives as cyclin-dependent kinase 2 inhibitors. *Turkish Journal of Pharmaceutical Sciences, 17*(4), 417.

Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry, 31*(2), 455-461.

Veiga, M., Costa, E. M., Silva, S., & Pintado, M. (2020). Impact of plant extracts upon human health: A review. *Critical reviews in food science and nutrition, 60*(5), 873-886.

Veselovsky, A., & Ivanov, A. (2003). Strategy of computer-aided drug design. *Current Drug Targets-Infectious Disorders, 3*(1), 33-40.

Wang, R., Lu, Y., & Wang, S. (2003). Comparative evaluation of 11 scoring functions for molecular docking. *Journal of medicinal chemistry, 46*(12), 2287-2303.

Wang, X., Feng, H., Muhetaer, H., Peng, Z., Qiu, P., Li, W., & Li, Z. (2021). Studies on the separation and purification of the Caulis sinomenii extract solution using microfiltration and ultrafiltration. *Separations, 8*(10), 185.

Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., . . . Bordoli, L. (2018). SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic acids research, 46*(W1), W296-W303.

Wink, M. (2010). Introduction: biochemistry, physiology and ecological functions of secondary metabolites. *Annual plant reviews volume 40: Biochemistry of plant secondary metabolism, 40*(2), 1-19.

Yadava, U. (2018). Search algorithms and scoring methods in protein-ligand docking. *Endocrinol Int J, 6*(6), 359-367.

Yuan, S., Chan, H. S., & Hu, Z. (2017). Using PyMOL as a platform for computational drug design. *Wiley Interdisciplinary Reviews: Computational Molecular Science, 7*(2), e1298.

CHAPTER 5

BIOLOGICAL ACTIVITIES AND USES OF GENUS *Terfezia*

Hatice ÜSTÜNER[1]

1 Akdeniz University, Faculty of Science, Department of Biology, Antalya, Türkiye, Corr. Autor: h.ustuner1977@gmail.com

Introduction

Mushrooms, which are now used for the prevention of many diseases, have historically been a medically useful therapeutic food (Barros et al., 2007). Rich in plant vitamins, proteins and minerals but low in fats and essential fatty acids, mushrooms are healthy foods with low calories. They contain vitamins such as niacin, riboflavin and folates, various minerals and trace elements such as copper and potassium (Cheung, 2010). Especially edible wild mushrooms are highly consumed due to their high nutritional value and delicious flavour. In addition, their pharmacological properties have made mushrooms increasingly important (Akyüz, 2013). In addition to their nutritional properties, many mushroom species have been reported to have many biological activities such as anticancer, antimicrobial, antiallergic, DNA protective, antioxidant, hepatoprotective, and antiaging (Akgül et al., 2016a; Akgül et al., 2016b; Akgül et al., 2021; Bal et al., 2019; Bal et al., 2022; Eraslan et al., 2021; Gürgen, et al., 2020; Korkmaz et al., 2021; Korkmaz et al., 2023; Sevindik et al., 2015; Sevindik et al., 2016; Sevindik et al., 2017a; Sevindik et al., 2017b; Sevindik et al., 2017c; Sevindik et al., 2018a; Sevindik et al., 2018b; Sevindik et al., 2018c; Sevindik et al., 2021a; Sevindik et al., 2021b).

Truffles, mycorrhizal fungi representing the symbiotic relationship of *Terfezia* fungi with the roots of Helianthemum species as hosts, have become important for culinary use due to their flavour and high nutritional content. They are a complex family including the genera Picoa, Tirmania, Tuber and *Terfezia* (Trappe and Sundberg, 1977). Besides being edible, truffles have been reported to have different biological uses including antioxidant, anti-inflammatory, antiviral, antimicrobial, anti-mutagenic, hepatoprotective activities (Veeraraghavan et al., 2022).

Species of the *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul. are medically important mycorrhizal fungi as they contain various polyphenols besides their gastronomic importance (Gücin and Dülger, 1997; Norman and Egger, 1999; Tagnamas et al., 2020; Morte et al., 2021). The genus, which is generally referred to as the Desert Deruff, is known locally in different regions as 'tirfas', 'terfase', 'terfez', 'fagga', 'faga', 'el faga', 'faqah', 'dombal', It is known by names such as 'kame', 'kamaa' and 'al-kamah', 'keme', 'kumi', 'dümbelek', 'dolaman', 'domalan', 'topalak', 'soil mushroom' (Bokhary, 1987; Mandeel and AlLaith, 2007; Akyüz et al. , 2012; Neggaz et al., 2015; Atila and Kazankaya, 2022).

The members of the genus, whose tuber-shaped fruits are formed under the soil, are classified in the desert fungi group since they are distributed in arid and semi-arid regions (Morte et al., 2021; Atila and Kazankaya, 2022). Different species of the genus, reported from every continent except Australia and Antarctica, appear after rainfall in March and May (Alsheikh, 1994).

Usage Areas

Terfezia genus, which are widely used as food due to their different aromas, odours, low calorie nutrient content, high fibre, protein, vitamin and mineral richness (Kıvrak, 2015), have been used in the treatment of various diseases throughout history with their rich bioactive contents (Shavit and Shavit, 2014). It is an important source for the production of therapeutic compounds in terms of antioxidant, anti-inflammatory, antimicrobial, immunosuppressor, antimutagenic, anticarcinogenic properties (Murcia et al., 2002).

Biological activities

Thanks to the therapeutic potential of the bioactive compounds they produce, they have many medical activities such as antioxidant, anti-tumour, antiviral, anti-inflammatory, anti-urease, immunomodulatory activity, cytotoxic and apoptosis inducing effect, positive effects on cholesterol, hypertension and diabetes (Smith et al., 2002). In this study, the biological activities of the species belonging to the genus *Terfezia* reported in the literature were reviewed. In the in vivo and in vitro biological activity studies on the species belonging to the genus, it was observed that extractions such as acetone, butanol, methanol, ethanol, chloroform distilled water, n-hexane, dichloromethane, ethyl acetate were used (Table 1).

Table 1. *Biological activity of genus Terfezia*

Plant species	**Biological activities**	**Extraction**	**Geographic regions**	**References**
Terfizia claveryi Chatin	Antimikrobiyal activity	Acetone	Kastamonu, Türkiye	Bekçi et al., 2011
Terfezia boudieri Chatin	Flavonoid content, radical scavenging activity	Soxhlet extraction	Elazığ, Türkiye	(Akyüz, 2013)
Terfezia claveryi Chatin	Antioxidant, antibacterial and antifungal activities	Methanol	Tiaret, Algeria	(Neggaz et al., 2015)
Terfezia claveryi Chatin	Antioxidant, Antimicrobial activity	Distilled water	Elazığ, Türkiye	(İnci and Kırbağ, 2018)
Terfezia arenaria (Moris)Trappe	Antimicrobial activity	Maceration with methanol and Soxhlet with dichloromethane	Algerian Saharan soils	(Harir et al., 2019)
Terfezia clavery Chatin	**Antioxidant and phenolic activity; fatty acid composition and mineral contents**	Methanol	Konya, Türkiye	(Şahin et al., 2020)

Terfezia clavery Chatin	**Total phenolics, antioxidative and antimicrobial activity**	Methanol, ethanol	Niğde, Türkiye	(Canpolat et al., 2021)
Terfezia claveryi Chatin	**Antimicrobial activity**	Methanol, ethyl acetate, and distilled water	Saudi Arabia	Khojah et al., 2022)
Terfezia boudieri Chatin, *Terfezia claveryi* Chatin	**Antioxidant activity, metal content, and essential oil composition**	Aqueous extracts, acetone and butanol	Syrian	(Sonji et al., 2022)
Terfezia boudieri Chatin	**Antioxidant activity**	n-hexane, dichloromethane, and methanol	Arar,Saudi Arabia	(Guetat et al., 2024)
Terfezia claveryi Chatin	**Mineral contents, antioxidant and antimicrobial activity**	Different solvents (water, ethyl acetate, ethanol, methanol, acetone and chloroform)	Algerian	(Hadjira et al., 2024)
Terfezia claveryi Chatin	Antibacterial and antifungal activity	Organic solvents	Tindouf, Algeria	(Mohamed-Benkada et al., 2024)

Antioxidant activity

In the studies, it has been reported that the antioxidant activity is due to the fact that it is a strong inhibitor of lipid peroxidation occurring at high concentrations of mushroom extracts (Cheung and Cheung, 2005) and many mushroom species have high antioxidant activity (Wang et al., 2014).

Dündar et al. (2012) investigated the chemical composition, nutritional value and antioxidant activity potentials of *Terfezia boudieri* Chatin collected from different regions of Southeastern Anatolia Region and found that *T. boudieri* showed excellent antioxidant activity compared to standard compounds.

Taskin et al. (2018), In addition to the antioxidant effects of methanol extracts of *Terfezia claveryi*, anti-urease activities and genotoxic effects on human lymphocyte cells were studied. They found that *T. claveryi* methanol extracts caused genotoxicity on lymphocytes and the extracts showed low antioxidant activity compared to standards.

Fidan et al. (2022) investigated the antioxidant activities of *Terfezia claveryi, Terfezia boudieri, Terfezia olbiensis* with acetone and methanol extracts. In addition, ion chelating effects, antimicrobial activities, cytotoxic and protective effects of the species were studied. Both methanol and acetone extracts of T. boudieri were found to have the highest FRAP and DPPH scavenging abilities.

Other activity

Antimicrobial activity of medicinal mushrooms has a wide spectrum against aerobic-anaerobic bacteria, Gram positive-Gram negative bacteria. Species of the genus *Terfezia* are abundant in active compounds with anticancer, antioxidant, anti-inflammatory, antimicrobial and therapeutic properties compared to other genera (Janakat et al., 2005).

Gouzi et al. (2011) studied the in vitro effects of aqueous extracts of the Algerian desert truffle *Terfezia claveryi* on the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* using agar well diffusion and kinetic bacterial growth curves methods. They found that aqueous extracts of the species have very strong antibacterial activity against both *P. aeruginosa* and *S. aureus* using agar well diffusion. Kadhila et al. (2013) investigated the anti-plasmodial effects of aqueous extracts from *Terfeziya pfeilii* using cellular infection models of *Plasmodium falciparum* 3D7A. They found that aqueous extracts of *T. pfeilii* showed antiplasmodial activity at concentrations in the range of 5-50 µg/ml. *Terfezia claveryi* organik ekstraksiyonunun in vitro antimikrobiyal aktivitesini araştıran Schillaci vd. (2015), heksan ve aseton ekstraktlarının, 5 mg/ml tarama konsantrasyonunda *Pseudomonas aeruginosa*'nın büyümesinin engellenmesinde aktif sonuç verdiğini belirlemişlerdir.

Al Obaydi et al. (2020) investigated the anticancer and immunomodulatory effects of different extracts of *Terfezia boudieri* species. They found that the most effective extract in stimulating lymphocyte proliferation was the ethyl acetate extract of the species and all other extracts showed moderate effects in stimulating phagocytosis. They explained that aqueous/methanol extract was the most effective extract; ethyl acetate extract was the most effective extract in stimulating pinocytosis. N-hexane, ethyl acetate and aqueous/methanol extracts showed anticancer activities and were potent stimulants of innate and acquired immunity.

Nouiri et al. (2021) investigated the protective and therapeutic effect of *Terfezia boudieri* aqueous extract against paracetamol (PCM) induced liver and kidney damage in rats. They explained that *Terfezia boudieri* aqueous extract promotes the ultimate protective and curative drug against acute toxicity.

Saleh et al. (2022) investigated the cytotoxic and apoptosis inducing effects of aqueous extracts obtained from *Terfezia claveryi, Terfezia boudieri, Terfezia olbiensis* species on pancreatic cancer cell line PANC-1 cells. They found that aqueous extracts of the three species reduced the progression of PANC-1 cells by inducing apoptosis, indicated by up-regulation of the proapoptotic genes BAX, CDKN1A and TP53 and down-regulation of the antiapoptotic gene BCL2, and stated that all three species can be considered as a functional and therapeutic food.

Abu-Odeh et al. (2022) studied the total phenolic content of *Terfezia claveryi* and investigated the blood glucose lowering potential of different aqueous extracts of the species using in vitro and in vivo models. At the end of their study, they found that the species showed antidiabetic activity both in vitro and in vivo and supported its traditional use as a natural hypoglycaemic.

Dawood et al. (2023), investigated the anti-inflammatory activities of *Terfezia boudieri* and *Terfezia claveryi* and the mechanisms associated with their anti-inflammatory activities in lipopolysaccharide/interferon-gamma (LPS/IFN-γ)-stimulated RAW 264.7 macrophages. They found that both species exhibited anti-inflammatory activities by suppressing multiple inflammatory mediators and cytokines and indicated that they may be potential anti-inflammatory agents.

Sawaya et al. (2023) carried out their study by treating colon cancer cell lines HCT-116 and Caco-2 with water or ethanol extract of Terfezia boudieri. They found that *T. boudieri* water extract showed cytotoxic effect on colon cancer cells.

Conclusion

In this study, the biological activities of members of the genus *Terfezia* reported in the literature were reviewed. Since different *Terfezia* species have different pharmacological effects, their biological activities were examined in a wide range. In addition to antioxidant activities, anti-inflammatory, antitumoural, antibacterial and antifungal activities have also been reported in the literature. It is thought that *Terfezia* species, which are widely used in public health, can be used in different designs as a pharmacologically important natural material.

REFERENCES

Abu-Odeh, A., Shehadeh, M., Suaifan, G. A., Karameh, N., Abdel Rahman, D., & Kandil, Y. (2022). In vitro and in vivo antidiabetic activity, phenolic content and microscopical characterization of *Terfezia claveryi*. Molecules, 27(15), 4843.

Akgül, H., Aslan, A., Akata, I., Günal, S., Bal, C., & Baba, H. (2021). Phenolic content and biological activities of Trametes hirsuta. Fresenius Environmental Bulletin, 30, 4130-4135.

Akgül, H., Nur, A. D., Sevindik, M., & Doğan, M. (2016a). Determination of some biological activities of Tricholoma terreum and Coprinus micaceus. Artvin Coruh University Journal of Forestry Faculty.17(2), 158-162.

Akgül, H., Sevindik, M., Akata, I., Altuntaş, D., Bal, C., & Doğan, M. (2016b). Determination of heavy metal content and oxidative stress status of Macrolepiota procera (Scop.) Singer. mushroom.

Akyüz, M. (2013). Nutritive value, flavonoid content and radical scavenging activity of the truffle (*Terfezia boudieri* Chatin). Journal of soil science and plant nutrition, 13(1), 143-151.

Akyüz, M., Kirbağ, S., ve Kurşat, M. (2012). Ecological aspects of the arid and semi-arid truffle in Turkey: evaluation of soil characteristics, morphology, distribution, and mycorrhizal relationships. Turk. J. Botany, 36(4), 386-391.

Al Obaydi, M. F., Hamed, W. M., Al Kury, L. T., & Talib, W. H. (2020). *Terfezia boudieri*: a desert truffle with anticancer and immunomodulatory activities. Frontiers in Nutrition, 7, 38.

Alsheikh, A. M. (1994). Taxomony and mycorrhizal ecology of the desert truffles in the genus *Terfezia*. Doktoral Thesis, Corvallis, Oregon State University, p. 239

Atila, F., & Kazankaya, A. (2022). Terfezia Species Natural Distributed in Türkiye and Their Ecologies. The Journal of Fungus, 13(3), 119-130.

Bal, C., Akgül, H., & Sevindik, M. (2019). The Antioxidant potential of ethanolic extract of edible mushroom Lycoperdon molle Pers.(Agaricomycetes). Eurasian Journal of Forest Science, 7(3), 277-283.

Bal, C., Baba, H., Akata, I., Sevindik, M., Selamoglu, Z., & Akgül, H. (2022). Biological activities of wild poisonous mushroom Entoloma sinuatum (Bull.) P. Kumm (Boletales). KSU J. Agric. Nat, 25(1), 83-87.

Barros, L., Baptista, P., Ferreira, I.C. (2007). Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food and chemical Toxicology, 45(9), 1731-1737.

Bekçi, H., Altınsoy, B., Sarıkaya, S., Onbasılı, D., & Çelik, G. Y. (2011). Kastamonu yöresinden toplanan bazı makrofungusların antimikrobiyal aktivitesi. Kastamonu University Journal of Forestry Faculty, 11(2), 187-190.

Bokhary HA (1987). Desert truffl es 'al-kamah' of the Kingdom of Saudi Arabia. I.

Occurrence identifi cation and distribution. Arab Gulf Journal of Scientifi c Research 5: 245-255.

Canpolat, Ş., Akata, I., İşlek, Y., Canpolat, E., & İşlek, C. (2021). Total phenolics, antioxidative and antimicrobial activities of some edible ascomycota collected from Niğde. Turkish Journal of Agriculture-Food Science and Technology, 9(10), 1915-1920.

Cheung, L.M., Cheung, P.C. (2005). Mushroom extracts with antioxidant activity against lipid peroxidation. Food Chemistry, 89(3), 403-409.

Cheung, P.C.K. (2010). Nutritional and health benefits of mushrooms. Nutrition Bulletin 35, 292-299.

Dawood, A. S., Sedeek, M. S., Farag, M. A., & Abdelnaser, A. (2023). *Terfezia boudieri* and *Terfezia claveryi* inhibit the LPS/IFN-γ-mediated inflammation in RAW 264.7 macrophages through an Nrf2-independent mechanism. Scientific Reports, 13(1), 10106.

Eraslan, E. C., Çırçırlı, B., Özkan, A., & Akgül, H. (2021). Anticancer Mechanisms of Action of Macrofungus Extracts. Eurasian Journal of Medical and Biological Sciences, 1(2), 58-69.

Fidan, M., Ali, M. M., Erez, M. E., Cigerci, I. H., Ozdemir, S., & Sen, F. (2022). Antioxidant, antimicrobial, cytotoxic and protective effects of truffles. Analytical Biochemistry, 641, 114566.

Gouzi, H., Belyagoubi, L., Abdelali, K. N., & Khelifi, A. (2011). In vitro antibacterial activities of aqueous extracts from Algerian desert truffles (*Terfezia* and *Tirmania*, Ascomycetes) against Pseudomonas aeruginosa and Staphylococcus aureus. International journal of medicinal mushrooms, 13(6).

Guetat, A., Alzahrani, A. K., Oueslati, M. H., Abd Ealrhman, M. E., Bouajila, J., & Shahhat, I. M. (2024). Chemical Composition, Fatty Acids, Total Phenolics and Antioxidant Activity of the Desert Truffle Terfezia Boudieri Chatin in the Northern Region of Saudi Arabia. Engineering, Technology & Applied Science Research, 14(4), 15016-15021.

Gücin, F., Dülger, B. (1997) The researches on the keme truffle-*Terfezia boudieri* Chatin that is edible and showing antimicrobial activities. J Environ 23: 27- 33.

Gürgen, A., Sevindik, M., Yildiz, S., & Akgul, H. (2020). Determination of antioxidant and oxidant potentials of Pleurotus citrinopileatus mushroom cultivated on various substrates. KSU Tarım Ve Doga Dergısı-Ksu Journal Of Agrıculture And Nature, 23(3).

Hadjira, G., Bakchiche, B., Erenler, R., Yildiz, İ., Mohamed, A. S., & El-shazly, M. A. (2024). Mineral contents, Antioxidant and Antimicrobial Activities of Algerian *Terfezia claveryi* extracts. Fabad Journal of Pharmaceutical Sciences, 49(1), 19-36.

Harir, M., Bendif, H., Yahiaoui, M., Bellahcene, M., Zohra, F., & Rodríguez-Couto, S. (2019). Evaluation of antimicrobial activity of *Terfezia arenaria* extracts colle-

cted from Saharan desert against bacteria and filamentous fungi. 3 Biotech, 9, 1-7.

İnci, Ş., & Kırbağ, S. (2018). *Terfezia claveryi* Chatin'in besinsel içeriği, antioksidan ve antimikrobiyal aktivitesi. Artvin Coruh University Journal of Forestry Faculty. 19(2), 138-143.

Janakat S, Al-Fakhiri S, Sallal AK. (2005). Evaluation of antibacterial activity of aqueous and methanolic extracts of the truffle *Terfezia claveryi* against Pseudomonas aeruginosa. Saudi Medical Journal. 26:952-955.

Kadhila-Muandingi, N. P., Nametso, O., Du Preez, I., & Mumbengegwi, D. R. (2013). Antiplasmodial activity of indigenous *Ganoderma lucidum* and *Terfezia pfeilii* Namibian mushrooms. Journal of Parasitology Photon, 104, 186-193.

Khojah, H. M., Abdelhalim, O. B., Mostafa, M. A., & Habib, E. S. E. (2022). Antimicrobial efficacy of extracts of Saudi Arabian desert *Terfezia claveryi* truffles. Saudi Journal of Biological Sciences, 29(11), 103462.

Kıvrak, İ. (2015). Analytical Methods Applied to Assess Chemical Composition, Nutritional Value and In Vitro Bioactivities of *Terfezia olbiensis* and *Terfezia claveryi* from Turkey. Food Anal. Methods. 8, 1279–1293.

Korkmaz, A. I., Bal, C., Eraslan, E. C., Sevindik, M., & Akgul, H. (2023). Biological activities of Agrocybe praecox (spring fieldcap mushroom). Prospects in Pharmaceutical Sciences, 21(4), 33-39.

Korkmaz, A. İ., Eraslan, E. C., Uysal, I., & Akgül, H. (2021). Functional food Agaricus arvensis: antioxidant and antimicrobial potentials. Eurasian Journal of Medical and Biological Sciences, 1(2), 86-91.

Mandeel, Q.A., Al-Laith, A.A. (2007). Ethnomycological aspects of the desert truffle among native Bahraini and non-Bahraini peoples of the Kingdom of Bahrain. J. Ethnopharmacol. 110, 118-129

Mohamed-Benkada, M., Achira, D. Y., & Ghareeb, M. A. (2024). Assessment of antimicrobial activity and mycelium insights of *Terfezia claveryi* truffle harvested from Tindouf desert. Journal of Applied Pharmaceutical Science, 14(2), 161-173.

Morte, A., Kagan-Zur, V., Navarro-Ródenas, A., ve Sitrit, Y. (2021). Cultivation of desert truffles—a crop suitable for arid and semi-arid zones. Agronomy, 11(8), 1462.

Murcia, M.A., Martınez-Tome, M., Jimenez, A.M., Vera, A.M., Honrubia, M., Parras, P. 2002. "Antioxidant activity of edible fungi (truffles and mushrooms): losses during industrial processing ", Journal of Food Protection, 65(10), 1614- 1622.

Neggaz, S., Fortas, Z., Chenni, M., El Abed, D., Ramli, B., & Kambouche, N. (2015). In vitro evaluation of antioxidant, antibacterial and antifungal activities of *Terfezia claveryi* Chatin. Phytothérapie, 16(1), 20-26.

Norman JE, Egger KN (1999) Molecular phylogenetic analysis of Peziza and related genera. Mycologia 91 (5) : 820–9

Nouiri, E., Ben Ali, R., Ghali, R., Araoud, M., Véronique El May, M., & Hedhili, A. (2021). Protective and Curative Effects of Aqueous Extract of *Terfezia boudieri* (Edible Desert Truffle Specie) against Paracetamol Acute Toxicity in the Rat. Nutrition and Cancer, 73(1), 113-123.

Saleh, K. K., Kirbag, S., & Dalkılıç, S. (2022). Inhibitory effects of *Terfezia* (Ascomycota) desert truffles on PANC-1 cell growth via upregulation of proapoptotic genes TP53, CDKN1A, and BAX and downregulation of antiapoptotic gene BCL2. International Journal of Medicinal Mushrooms, 24(8).oooo

Sawaya, K., Abou Najem, S., Khawaja, G., & Khalil, M. (2023). Proapoptotic and Antiproliferative Effects of the Desert Truffle Terfezia boudieri on Colon Cancer Cell Lines. Evidence-Baseod Complementary and Alternative Medicine, 2023(1), 1693332.

Schillaci, D., Arizza, V., Davino, S., Gargano, M. L., & Venturella, G. (2015). Antimicrobial Activity of the Desert Truffles "*Tirmania pinoyi*" and "*Terfezia claveryi*" Against Human Pathogens. In The 8th International Medicinal Mushrooms Conference." Medicinal Mushrooms: Entrepreneurial opportunities for young people and Healthcare trends in the 21st Century, 169-169.

Sevindik, M., Akgul, H., Akata, I., & Selamoglu, Z. (2017a). Geastrum pectinatum as an alternative antioxidant source with some biochemical analysis. Medical Mycology: Open Access, 3(2), 1-4.

Sevindik, M., Akgul, H., Bal, C., Altuntas, D., Korkmaz, A. I., & Dogan, M. (2018a). Oxidative stress and heavy metal levels of pholiota limonella mushroom collected from different regions. Current Chemical Biology, 12(2), 169-172.

Sevindik, M., Akgul, H., Selamoglu, Z., & Braidy, N. (2021a). Antioxidant, antimicrobial and neuroprotective effects of Octaviania asterosperma in vitro. Mycology, 12(2), 128-138.

Sevindik, M., Akgül, H., & Bal, C. (2017b). Determination of oxidative stress status of Ompholatus olearius gathered from Adana and Antalya provinces in Turkey. Sakarya University Journal of Science, 21(3), 324-327.

Sevindik, M., Akgül, H., & Bal, C. (2017c). Determination of oxidative stress status of Ompholatus olearius gathered from Adana and Antalya provinces in Turkey. Sakarya University Journal of Science, 21(3), 324-327.

Sevindik, M., Akgül, H., Günal, S., & Doğan, M. (2016). Pleurotus ostreatus' un doğal ve kültür formlarının antimikrobiyal aktiviteleri ve mineral madde içeriklerinin belirlenmesi. Kastamonu University Journal of Forestry Faculty, 16(1).

Sevindik, M., Baba, H., Bal, C., Colak, O., & Akgul, H. (2018c). Antioxidant, oxidant and antimicrobial capacities of Physarum album (Bull.) Chevall. JBMOA, 6(6), 317-320.

Sevindik, M., Bal, C., & Akgül, H. (2018b). Comparison of antioxidant potentials of the wild and cultivated forms of edible Pleurotus ostreatus and Agaricus bisporus mushrooms. Turkish Journal of Life Sciences, 3(2), 263-266.

Sevindik, M., Eraslan, C. E., & Akgül, H. (2015). Bazı makrofungus türlerinin ağır metal içeriklerinin belirlenmesi. Ormancılık Dergisi, 11(2), 48-53.

Sevindik, M., Özdemir, B., Braidy, N., Akgül, H., Akata, I., & Selamoğlu, Z. (2021b). Potential cardiogenic effects of poisonous mushrooms. Mantar Dergisi, 12(1), 80-86.

Shavit, E., ve Shavit, E. (2014). The medicinal value of desert truffles. In Desert Truffles (pp. 323-340). Springer, Berlin, Heidelberg.

Smith, J., Rowan, N., Sullivan, R. (2002). Medicinal mushrooms: their therapeutic properties and current medical usage with special emphasis on cancer treatments, 256. London: Cancer Research UK.

Sonji, G. M., Assi, M., Sonji, N. M., Boukhary, R., & Rahal, M. (2022). Antioxidant activity, metal content, and essential oil composition of two desert truffles species: *Terfezia boudieri* and *Terfezia claveryi*. Journal of Pharmacognosy and Phytochemistry, 11(4), 10-19.

Şahin, A., Kaşık, G., Alkan, S., & Özcan, M. M. (2020). Total phenol, antioxidant activity, fatty acid composition and mineral contents of the species of fungi known as domalan. Journal of Agroalimentary Processes Technologies, 26, 149-154.

Tagnamas, Z., Bahammou, Y., Kouhila, M., Hilali, S., Idlimam, A., & Lamharrar, A. (2020). Conservation of Moroccan truffle (*Terfezia boudieri*) using solar drying method. Renewable Energy, 146, 16-24.

Taşkın, T., Devecioglu, T. Y., & Şenkardeş, İ. (2018). Antioxidant, anti-urease activities and genotoxic effects of *Terfezia claveryi* methanol extracts on human lymphocytes. Clinical and Experimental Health Sciences, 8(3), 190-195.

Trappe JM, Sandberg WJ, 1977. *Terfezia gigantea* (Tuberales) in North America. Mycologia 69: 433–437.

Veeraraghavan, V. P., Hussain, S., Papayya Balakrishna, J., Dhawale, L., Kullappan, M., Mallavarapu Ambrose, J., & Krishna Mohan, S. (2022). A comprehensive and critical review on ethnopharmacological importance of desert truffles: *Terfezia claveryi*, *Terfezia boudieri*, and *Tirmania nivea*. Food Reviews International, 38(sup1), 846-865.

Wang, X.M., Zhang, J., Wu, L.H., Zhao, Y.L., Li, T., Li, J.Q., Liu, H.G. (2014). A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. Food chemistry, 151, 279-285.

CHAPTER 6

INSECTS IN COMPLEMENTARY AND ALTERNATIVE MEDICINE: CURRENT STATUS AND FUTURE SCOPE

Meena Yadav[1]

Ahmad Pervez[2]

Hakan Bozdoğan[3]

1 Department of Zoology, Maitreyi College, University of Delhi, New Delhi, India
2 Biocontrol Laboratory, Department of Zoology, Pt. L.M.S. Campus, Sri Dev Suman Uttarakhand University, Rishikesh, Uttarakhand, India
3 Ahi Evran University, Vocation School of Technical Sciences, Department of Plant and Animal Production, Kırşehir, Turkey

1. INTRODUCTION

Insects represent the largest number of species on earth. They are an important component of various food webs and necessary for a healthy ecosystem. Lately, profound interest has arisen in insects for their medicinal properties as they have been used in traditional medicinal practices in many cultures since times immemorial globally. Also, lack of sufficient healthcare facilities for people in developing and/or poor countries, lack of healthcare professionals, side effects of allopathic treatments, high cost of treatments, and non-availability of medicine are some other factors that have contributed to this interest. The use of invertebrates, especially insects, for treating human diseases forms part of a branch of medicine known as *complementary and alternative medicine* (CAM) and insects have huge potential. The use of insects and insect-based products in medicine is known as entomotherapy. Entomotherapy has been traditionally practiced in different regions of the world, such as India, China, Mexico, Korea, Argentina, Brazil, Spain, and other African and American countries, for treating several human diseases (Hinman, 1933; Morge, 1973; Seabrooks and Hu, 2017; Siddiqui et al., 2023; Zhang et al., 2023). These practices have been passed down from generation to generation (Costa-Neto, 2005). Among insects having healing properties, honey bees and their products need special mention (Banerjee et al., 2003; Gupta & Stangaciu, 2014). Various products of honeybees have been found more usable in countries with poor healthcare systems (Gupta & Stangaciu, 2014). Maggots are utilized in wound cleaning as they make the wound alkaline and have antibacterial properties (Baer, 1931; Thomas et al., 1999). Maggots remove the dead tissues and promote healing. Their usage is increasing due to the safety, ease, and efficacy of the treatment (Sherman et al., 2000). There are many more traditional uses of insects for medicinal purposes in different regions of the world and several review papers are available on these uses (Costa-Neto, 2005; Seabrooks and Hu, 2017; Devi et al., 2023; Siddiqui et al., 2023). More than 235 insect species have been used in folk medicine in China, India, Africa, and Latin America. The order Hymenoptera has the most species (62) that have medicinal properties followed by Coleoptera (47), Orthoptera (28), Lepidoptera (23), and Blattodea (21) (Siddiqui et al., 2023).

There is a great deal of information available on the use of insects and insect products in traditional medicine, so in this chapter, we focus on recent developments in the identification of novel chemicals from insects and their potential applications in CAM.

2. Use of Insects for Medicinal Purposes

2.1. Bees (Apidae)

Honey bees produce several products and almost all have some medicinal properties. The use of honeybees and their products for healing practices is

known as apitherapy. Honey is antibacterial, antidepressant, anticonvulsant, and has anti-anxiety and wound healing properties, while bee pollen has anti-aging and antiallergic properties (Simon et al., 2005). Propolis from honey bees has been used to treat ulcers, burns, bedsores, wounds that are difficult to heal, and also as mouth disinfectant (Kędzia and Kędzia, 2013). It has some other properties also such as antioxidant, anticancer, anti-hepatotoxic, antibiotic, and antifungal (Lee and Bae, 2016). The health benefits of bees are due to the different metabolites they contain such as biotin, folic acid, niacin, thiamine, phytosterols, polyphenols, enzymes, and coenzymes (Siddiqui et al., 2023).

Honey is the most widely used product from honey bees, which has nutritional as well as medicinal properties. Honey produced by *Melipona marginata* Lepeletier (endangered stingless bee from Brazil) contains 11 phenolic compounds including caffeic acid and kaempferol. When applied topically it reduced skin inflammation in mice, probably by reducing edema, producing reactive oxygen species (ROS), and promoting leukocyte migration (Borsato et al., 2014). In the Northeastern part of Brazil, there is another native stingless bee *Melipona subnitida* Ducke whose honey is widely used by local people, however, it is not included in the international standards for honey and neither is it controlled by the food control authorities as there is little knowledge about it. The honey collected from various *M. subnitida* sources showed high antioxidant activity owing to its high phenolic content. Other chemicals in this honey included flavonoids (quercetin, naringenin and isorhamnetin), gallic acid, vanillic acid, cumaric acid, and 3,4-dihydroxybenzoic acid (Silva et al., 2013).

Bee venom or apitoxin is a complex mixture of several components such as enzymes (like hyaluronidase, phospholipase-A2), proteins, peptides (like apamin, melittin, adolapin, mast cell degranulating (MCD) peptide), amino acids (histamine), carbohydrates, catecholamines, and lipids (Kolayli and Keskin, 2020). Studies have assessed the therapeutic potential of several components of bee venom to treat inflammatory and central nervous system (CNS) diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amylotrophic lateral sclerosis (ALS) (Moreno and Giralt, 2015). Melittin is one of the major constituents of bee venom and is primarily obtained from *Apis mellifera* Linnaeus. It shows toxicity towards cancer cells as well as healthy cells. Hence, if it is to be used as an anticancer chemical, it must be conjugated with a suitable delivery vehicle such that only target cancer cells respond to melittin (Gajski and Garaj-Vrhovac, 2013). Moreover, the monomeric form of melittin was found to be more toxic to gastric cancer cells at low concentrations (1-5μM) than the dimeric form, while at high concentrations (10μM), both forms showed comparable toxicity (Jamasbi et al., 2018). Apamin in bee venom is the smallest neurotoxin, as it is an 18 amino acid peptide and readily crosses the blood-brain barrier (Son et al., 2007). It blocks calcium-activated K^+ channels

and inhibits vascular smooth muscle proliferation and migration via Erk and Akt signaling pathways. So, it has the potential to be used for atherosclerosis therapy (Kim et al., 2015). MCD causes release of histamine from mast cells, inhibits K^+ channels, and reduces blood pressure in rats. It is an epileptogenic neurotoxin, as well as a powerful anti-inflammatory agent (Wehbe et al., 2019). Adolapin inhibits prostaglandin synthesis, and cyclooxygenase (COX) activity. It can also inhibit lipooxygenase activity in human platelets and show antipyretic effect (Jung et al., 2015; Cherniack and Govorushko, 2018). Bee venom has shown therapeutic actions in animal models for neurodegenerative disorders such as PD, AD, and ALS (Wehbe et al., 2019).

Crude venom extracts from honey bees such as *Apis dorsata* Fabricius, *A. cerana* Fabricius and *A. florea* Fabricius show antimicrobial activity against *Salmonella typhimurium, Escherichia coli,* and *Xanthomonas subtilis* (Surendra et al., 2011). In addition to antibacterial activity, bee venom also shows antiviral activity, although the exact mechanism is not clear yet. Uddin et al. (2016) showed that bee venom and melittin show antiviral activity against enveloped (such as influenza A virus, herpes simplex virus, vesicular stomatitis virus) and non-enveloped (such as enterovirus-71, coxsackie virus) viruses under in vitro conditions. Bee venom therapy has been practiced to treat symptoms such as discomfort, muscle weakness, lack of coordination, and inflammatory conditions such as tendinitis, herpes, zoster, arthritis and bursitis (Adjare, 1990; Uddin et al., 2016).

Propolis from *A. mellifera* showed antimicrobial activity against *Pseudomonas aeruginosa*, *E. coli, Staphylococcus aureus,* and *Candida albicans* (Silva et al., 2012). The ethanol extract of red propolis (EEP) (produced by the Brazilian honey bee *A. mellifera*) contains aurones, catechins, chalcones, flavonones, flavonols, guttiferones, pentacyclic triterpenoids, phlobaphene tannins, and xanthones. EEP has antioxidant properties and showed intense cytotoxicity against tumor cell lines. Thus, it has the potential to be developed as an anticancer drug (de Mendonça et al., 2015). Similarly, geopropolis from *Scaptotrigona postica* (Latreille) (Brazilian stingless bee) contains pyrrolizidine alkaloids and C-glycosyl flavones, and was found to be effective against antiherpes simplex virus-1 (HSV-1) (Coelho et al., 2015). Propolis from another stingless bee *Tetragonisca fiebrigi* (Schwarz), which majorly was composed of benzoic and kaurenoic acids, showed antimicrobial activity against *S. aureus, Staphylococcus epidermidis, Enterococcus faecalis, Proteus mirabilis, Klebsiella pneumonia,* and *Pseudomonas aeruginosa*. EEP of *T. fiebrigi* also inhibited hyaluronidase enzyme showing its anti-inflammatory activity (Campos et al., 2015). Propolis from Indian stingless bee *Trigona* sp. contained 24 compounds (15 were novel compounds) and it showed antimicrobial activity against common pathogenic bacteria such as *E. coli, S. aureus, S. typhimurium, K. pneumoniae, A. baumannii* and *C. glabrata* (yeast) (Choudhari et al., 2012).

Nigerian honey bee propolis increased the survivorship of mice infected with the malarial parasite *Plasmodium berghei* suggesting that it could be developed as a potential antimalarial drug (Olayemi, 2014).

2.2. Beetles (Coleoptera)

Coleoptera (beetles) is the largest insect order with 350,000-386,000 species. Beetles are present on land, in freshwater, and sea (Seabrooks and Hu, 2017). Beetles employ chemical defense to protect themselves and their progeny from predators. Their hemolymph contains toxic and repelling chemical substances, primarily alkaloids. Several alkaloids from plants and animals have been used for therapeutics or as insecticidal agents that target ion channels including nicotinic acetylcholine receptors (nAChRs) which are targets for analgesic drugs (Umana et al., 2013). In traditional medicine, ladybirds have been used as an analgesic to relieve toothache (Majerus, 2016) acting via nAChRs. Coccinellines, the alkaloids that are major constituents of ladybird venom, isolated from several ladybird species have been found to inhibit piscean nAChRs through non-competitive mechanisms (Leong et al., 2015). There have been approaches to synthesize azaphenalene alkaloids through enantioselective mechanisms (Alujas-Burgos et al., 2018). Alkaloid extract from *Harmonia axyridis* (Pallas) contains 90% harmonine and has been shown to inhibit insect and mammalian nAChRs, showing preference for the former (Patel et al., 2020). Similarly, in another study, alkaloid extract was isolated from *Adalia bipunctata* (L.) and *Adalia decempunctata* (L). The extract was majorly composed of (−)-adaline with strong selectivity for α3β4 nAChRs in vertebrates which could be explored as a potent therapeutic compound (Richards et al., 2022).

Blister beetle *Berberomeloe majalis* (L.) (Coleoptera: Meloidae) is the source of the potent molecule cantharidine (CTD), a terpenoid, which has amazing antitumor activity. CTD is produced by these beetles as a chemical defense against their predators. It is effective against parasites such as protozoans, ticks, nematodes, and insects (Whitman et al., 2019).

The Chinese beetle *Blaps japanensis* has been used to treat diseases such as fever, rheumatism, cough, cancer, and inflammatory disorders (Zheng et al., 2018). This species contains many chemicals. Four new compounds-Blapsols A-D, and five known N-acetyldopamine dimers have been isolated from the beetle, which act by inhibiting cyclooxygenases - COX-1 and COX-2 (Yan et al., 2015). Two novel compounds - pipajiains F and G, and four known compounds L-tryptophan, ginsenine, 3-indolealdehyde, and anoectochine were isolated from *B. japanensis* by Zheng et al. (2018), and only 3-indolealdehyde was found to inhibit COX-2, JAK3 kinases and ROCK1/2. Cyclooxygenases convert arachidonic acid to prostaglandins, the latter being associated with pain, inflammation and fever (Narsinghani &

Sharma, 2014), hence by inhibiting COX, these molecules hold potential to be developed as antiinflammatory, analgesic and antipyretic drugs. Further, Yan et al. (2023) isolated 15 new compounds from the whole body of *B. japanensis*: Blapspirooxindoles A–C (1-3) (novel spiroindole alkaloids; with a unique spiro[chromane-4,3′-indoline]-2,2′-dione motif), blapcumaranons A-B (4,5), blapoxindoles A–J (6-15). Compounds (+)-4 and (–)-4 were toxic toward human cancer cells (A549, Huh-7, and K562) and inhibited ROCK1 kinase. Compounds (–)-1, (+)-1, (+)-4, (–)-4, (+)-5, and (–)-5 showed strong inhibitory activity against JAK3 kinase, and most of the compounds inhibited COX-2. Also, compounds (+)-8, (–)-8, (+)-10, (–)-10, and 12 showed anti-renal fibrosis activity. These compounds, thus, have potential to be developed as anti-tumor, anti-inflammatory, and renal-protective agents.

Three new N-acetyldopamine dimers, *viz*. molossusamide A–C (1-3) and two known compounds (4,5) were isolated from the dung beetle *Catharsius molossus* Linnaeus. Compound 4 (cis-2-(3′,4′-dihydroxyphenyl)-3-acetylamino-7-(N-acetyl-2″-amino-ethylene)-1,4-benzodioxane) was found to inhibit COX1 and COX2 activity (Lu et al., 2015).

2.3. Ants (Formicidae)

Ants and their by-products have been traditionally used in many cultures to relieve/treat several health issues such as scabies, boils, wounds, toothache, cold, flu, severe cough, stopping hemorrhage during miscarriages, removal of any aftermath from uterus after childbirth, restoration of uterus after childbirth, treating dizziness, and stimulating heartbeat and pulse. There are many more traditional uses of different species of ants that have been reviewed by Seabrooks and Hu (2017). The chemical constitution of several medically important ant species has been found out, and many of the chemicals are promising to be developed as potential drugs. For example, a tetraponerine (T7) isolated from the iron ant *Tetraponera rufonigra* (Jerdon) has shown anticancer activity against breast cancer cell line (MCF-7) and colorectal cancer (HT29) cells (Rouchaud and Braekman, 2009; Bosque et al., 2014), suggesting its potential for developing anticancer drugs**.**

(±)-polyrhadopamine A, (+)-polyrhadopamine B and trolline isolated from ethanol extract of Chinese black ant *Polyrhachis dives* Smith inhibited ROCK1 and ROCK2 (isoforms of Rho-associated protein kinase), both of which are known targets for treatment of neurological, cardiovascular and renal diseases. (–)-polyrhadopamine B promoted neural stem cell proliferation, showing potential to treat CNS disorders. Further, polyrhadopamine C, a dopamine derivative, showed anti-proliferation effects on T-lymphocytes, thereby showing immunosuppressive and antiinflammatory activity, which could have potential to treat rheumatoid arthritis (Tang et al., 2014a). Two more compounds- 5-(3-indolylmethyl)-nicotinsaureamide and β-carboline-3-

carboxamide isolated from *P. dives* also inhibited T-lymphocyte proliferation. 5-(3-indolylmethyl)-nicotinsaureamide and 3-hydroxypyridine reduced the overproduction of fibronectin, collagen IV, and interleukin 6 (IL-6), and due to this they could possibly be used to treat kidney problems (Tang et al., 2015).

2.4. Wasps (Hymenoptera: Vespidae), Flies and Mosquitoes (Diptera)

The venom from Stenogastrinae (hover wasps), Polistinae (Brazialian social wasp - *Synoeca cyanea* (Olivier), and *Vespa orientalis* L.) have shown antimicrobial activities against *E. coli, E. faecalis, S. aureus, Klesiella pneumonia, Bacillus subtilis* and *Saccharomyces cerevisiae* (Seabrooks and Hu, 2017). Polybioside isolated from the wasp *Polybia paulista* Ihering is a neuroactive substance in different parts of the brain and causes convulsions in rats when applied topically (Saidemberg et al., 2010). Chitosan is isolated from the housefly *Musca domestica* L. (Muscidae) and blowfly *Chrysomya megacephala* (Fabricius) (Calliphoridae) larvae. It is produced by alkaline de-acetylation of chitin and is used for wound healing, and as a hemostatic and antimicrobial agent. Chitosan from *M. domestica* showed strong antifungal activity against *Rhizopus stolonifer* (Ai et al., 2012).

2.5. Cockroaches and Termites (Blattodea)

Cockroaches have been used in different forms in traditional medicinal practices. The chemical profiling of the American cockroach *Periplaneta americana* (L.) (Blattidae) revealed presence of four new compounds namely, isocoumarins periplatins A-D, and four known compounds. These compounds showed anticancer activity against human liver (HepG2) and breast cancer (MCF-7) cell lines (Zhang et al., 2013). The mother tincture of *Blatta orientalis* L. (Blattidae) showed anti-asthamatic and anti-anaphylactic activity in guinea pigs. The anti-anaphylactic activity might be due to suppression of IgE, mast cells, and eosinophil cell count (Nimgulkar et al., 2011).

The extracts of the Chinese cockroach *Eupolyphaga sinensis* Walker (Corydiidae) are used in preparing several Chinese traditional medicines, including Shu-Mai Tang, which is used to treat ischemic heart disease as it acts against inflammation-induced myocardial fibrosis (Yin et al., 2008). Extracts of *E. sinensis* showed anticancer activity against several cell lines as tested by Jiang et al. (2012). Similarly, whole body extract of *Polyphaga plancyi* (Bolivar) showed presence of five new compounds, *viz*. Plancyamides A(1)-B(3), plancypyrazine A(2), plancyols A(4) and B(5), and three known compounds. Compounds 2 and 4 inhibited JAK3 kinase, while compound 4 also inhibited DDR1 kinase. Thus, compounds 2 and 4 can be explored as potential drug targets for cancer (Zhu et al., 2016).

2.6. Other Insects

The cochineal (scale insect), *Dactylopius coccus* Costa (Dactylopiidae), is used for treating nasal congestion and ear pain. The major chemical present in its extract is carminic acid which is a powerful free radical scavenger (González et al., 2010). Shellolic acid F isolated from the shell of the lac insect *Kerria lacca* (Kerr) (Kerriidae) showed antimicrobial activity against *B. subtilis* (Lu et al., 2014). An oxazole and three known N-acetyldopamine (NADA) derivatives were isolated from the Chinese stink bug *Aspongopus chinensis* Dallas (Pentatomidae) by Luo et al. (2012). These compounds showed anticancer activity against several lines. Another novel trimer of NADA known as (±)-aspongamide A was isolated from *A. chinensis*, which may be explored as a potential drug to treat chronic kidney disease. It acts via TGF-B/Smad signaling pathways (Yan et al., 2014). Other potential compounds with renal-protective roles include Aspongopusamide A, Aspongopusamide B, and asponguanine B (Di et al., 2015).

Two species of grasshoppers, *Calliptamus barbarous* (Costa) (Acrididae) and *Oedaleus decorus* (Germar) (Acrididae), are sources of chitosan which has antimicrobial activity against various pathogens that infect fish, clinical samples and food (Kaya et al., 2015).

The crude extract of *Bombyx mori* L. cocoon showed a significant impact in the case of hypercholesterolemia and atherosclerosis. The chemical profiling of the cocoon showed presence of flavonoids such as kaempferol 7-O-β-D-glucopyranoside, quercetin 7-O-β- D-glucoside, 9,12-dihydroxy stearic acid and 2-hydroxy-nonadecanoic acid, and these flavonoids were responsible for the cardioprotective properties of the cocoon (Khan et al., 2014).

2.5. Maggots

Using maggots to heal wounds is known as maggot therapy or larval therapy. Different types of wounds such as necrotic, infected, sloughy, and chronic, can be healed using maggots (Rafter, 2012). The green bottle fly, *Lucilia sericata* (Meigen), larvae are the most commonly used larvae in maggot therapy. They are aseptically bred and are used in clinical treatments under medical supervision (Hancock et al., 2012). Maggots remove the necrotic tissue, and disinfect the wound leading to quick healing, as their excretions/secretions contain the enzyme DNA whose activity depends on Na^{+}, Mg^{2+} and Ca^{2+}. The enzyme helps in removing extracellular DNA from the tissue and biofilm, thereby helping in clinical wound debridement (Brown et al., 2012). The Food and Drug Administration (FDA) approved the use of medicinal maggots for wound healing in 2004 (Malekian et al., 2019).

3. Pharmacologically Important Chemicals from Insects

In addition to the chemicals listed above, many more important chemicals have been isolated from crude extracts of larvae and adult insects. Among the isolated chemicals, some have antimicrobial properties, while others have healing properties such as antitumor, hepatoprotective, antioxidant, and renal-protective.

3.1. Antimicrobial Peptides

Antimicrobial peptides (AMPs), obtained from insects, are effective against microbes such as bacteria, fungi, viruses, and parasites. Recent research has suggested that chemicals derived from insects inhibit at least 30 species of bacteria, 13 species of fungus, 5 species of viruses, and 10 parasites. The derivatives include AMPs, secretions, venom, associated microbiota, different types of whole body extracts such as ethyl acetate extract and water extract (adult and/or larvae), honey, propolis, hemolymph polypeptides, polysaccharides, and dinoponeratoxin peptides (Siddiqui et al., 2023). The major group of AMPs is defensins. Defensins are not insect specific, and more than 300 defensins have been identified. They show antibacterial activity against Gram-positive bacteria, including *S. aureus* (Hetru et al., 2003). Defensin from rabbit neutrophils possesses potent antibacterial activity against multi-drug-resistant (MDR) strains of *P. aeruginosa* (Zhao et al., 2005).

Cecropins (made up of around 35 amino acid residues) are another group of peptides found in insects, and are effective against Gram-positive and Gram-negative bacteria. They were first isolated from the giant silk moth *Hyalophora cecropia* L., hence named so. Cecropin A and B were isolated from the moth, of which cecropin A was found to be specifically bactericidal (Steiner et al., 1981), while cecropin B was not only a potent antibacterial compound but also attenuated the motility of the female nematode *Brugia pahangi* in adult female *Aedes aegypti* (L.), and reduces the number of developing larvae (Chalk et al., 1995).

Attacins are proteins rich in glycine and were first discovered in the moth *Hyalophora cecropia* (L.) (Hultmark et al., 1983). Attacins A-F have been isolated and have been found to be bactericidal for Gram-negative bacteria, including *E. coli*. *H. cecropia* also contains cecropins and lysozymes. Attacin and related proteins have been isolated from tse tse fly (*Glossina morsitans* Westwood), *B. mori, Heliothis virescens* (Fabr.), wild silkmoth (*Samia cynthia ricini* (Drury)), *Trichoplusia ni* (Hubner), *M. domestica* and *Drosophila melanogaster* Meigen (Wu et al., 2018).

Drosophila melanogaster produces an antibacterial peptide known as metchnikowin which has antibacterial and antifungal activity. The peptide interacts with an important cell wall synthesizing enzyme of fungi i.e., β(1,3)-

glucanosyltransferase Gel1 (FgBGT), and interferes with cell wall synthesis. It also inhibits succinate dehydrogenase (SDH) activity of mitochondrial succinate-coenzyme Q reductase (SQR) in *Fusarium graminearum*, SQR being the primary target of many antifungal agents. Thus, metchnikowin may provide a sustainable alternative to chemical fungicides (Moghaddam et al., 2017). The antibacterial activity of bee venom is due to its two components - melittin and phospholipase A2 (PLA2). They induce pores in the bacterial cell wall leading to their lysis. Due to this property, melittin also shows hemolytic, anti-fungal and antitumor activities (Wehbe et al., 2019). Melittin, when used with other drugs against MDR bacteria such as *P. aeruginosa* and *Acinetobacter baumannii*, shows highly synergistic effects (Akbari et al., 2019). Melittin derived peptides (MDP1 and MDP2) show potent antibacterial properties against *E. coli, P. aeruginosa* and *S. aureus* (Akbari et al., 2018). Melittin also shows anti-inflammatory, anti-nociceptive and anti-arthrtitic effects (Lee and Bae, 2016; Wehbe et al., 2019).

Some more AMPs from insects, with potential for developing antimicrobial/antiparasitic compounds, have been listed in Table 1.

Table 1: Chemicals Isolated from Insects Showing Antimicrobial/antiparasitic Properties

Insect Source	Compounds	Target Microbe	Reference
Acrocinus longimanus (L.) (Harlequin beetle) (Coleoptera)	Alopeptides (isolated from hemolymph)	Antifungal	Barbault et al. (2003)
Acromyrmex octospinosus (Reich) (Hymenoptera)	Candicidin (isolated from Streptomyces associated with integument)	Antifungal	Barke et al. (2010)
Apis mellifera (European honey bee) (Hymenoptera)	Melittin (Isolated from venom)	*Listeria monocytogenes, Borrelia burgdorferi, P. aeruginosa*, and *S. aureus*	Chen et al. (2016); Jamasbi et al. (2018)

A. mellifera	- Abaecin peptide (honey) - Flavonoids-Galangin and Pinocembrin; Ferulic and caffeic acid and derivatives of benzoic acid; (Propolis) - Secapin (bee venom)	- Antibacterial - Antimicrobial - Antibacterial and antifungal	- Luiz et al. (2017) - Velasquez and Montenegro (2017) - Lee et al. (2016)
A. mellifera (Royal jelly)	Apisimin	Stimulates secretion of TGF-α from monocytes	Bılıková et al. (2002); Gannabathula et al. (2015)
	Jelleines (I-IV)	Jelleines I-III show antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi, yeast	Romanelli et al. (2011); Jia et al. (2018);
	Royalisin	Paenibacillus larvae (causes American foulbrood)	Bíliková et al. (2001)
Apoica pallens (Fabr.) (Central American paper wasp) (Hymenoptera)	Protein c-type lectin 6	Antimicrobial	Mendonça et al. (2019)
Antheraea mylitta (L.) (Tasar silkworm) (Lepidoptera)	Antimicrobial Peptides	*Bacillus pumilus*	Dutta et al. (2016)
Berberomeloe majalis (L.) (Coleoptera)	CTD	Protozoa (*Trichomonas vaginalis*), ticks (*Hyalomma lusitanicum*), insects (*Myzus persicae* and *Rhopalosiphum padi*), nematods (*Meloidogyne javanica*)	Whitman et al. (2019)

Bombyx mori L. (Lepidoptera)	• Lebocins • Antimicrobial Peptides • Cecropins, Gloverin A2, Defensins (hemolymph); • Lysozymes, attacins and moricin peptides	• *E. coli* • *Micrococcus luteus* • Antibacterial and antifungal • Antibacterial	• Hara and Yamakawa, (1995) • Mastore et al. (2021) • Buhroo et al. (2018) • Islam et al. (2016); Buhroo et al. (2018)
Byasa polyeuctes termessa (Fruhstorfer) (Doubleday) (Taiwan butterfly) (Lepidoptera)	Papilistatin (isolated from adult)	Gram-positive bacteria: *Enterococcus faecalis, Micrococcus luteus, Staphylococcus aureus, Streptococcus pneumoniae*; Gram-negative bacterium: *Neisseria gonorrhoeae*	Pettit et al. (2010)
Chalcophora mariana (L.) (European jewel beetle) (Coleoptera)	Buprestin H	*Schistosoma mansoni* (parasite)	Gallinger et al. (2022)
Sarcophaga peregrina (Robineau-Desvoidy) , *Phormia terranovae* (Robineau-Desvoidy), *D. melanogaster, Mayetiola destructor* (Say) (Diptera)	Diptericin A-C (Glycine rich antibacterial peptide)	Few Gram-negative bacteria (*E. coli* K12, *Erwinia carotovora* 113, *Erwinia hericola* T)	Dimarcq et al. (1988); Reichhart et al. (1992); Ishikawa et al. (1992);
Dinoponera quadriceps (Kempf) (Hymenoptera)	Dinoponeratoxin peptides	*Trypanosoma cruzi*	Lima et al. (2018)
Drosophila melanogaster (Diptera)	Drosocin	*E. coli* and fungi	Imler and Bulet (2005)
	Metchnikowin (Proline-rich peptide)	Gram-positive bacteria and fungi	Levashina et al. (1995)
	Antimicrobial Peptides	*Plasmodium falciparum*	Tonk et al. (2019)

Forficula auricularia L. (European earwig) (Dermaptera)	2-Methyl-1,4-benzoquinone and 2-ethyl-1,4-benzoquinone (secretions from adult and larvae)	Antibacterial against Gram-positive and Gram-negative bacteria	Gasch and Vilcinskas (2014)
Hermetia illucens (L.) (Black soldier fly) (larvae) (Diptera)	α-pyrone, diketopiperazine	α-pyrone shows antibacterial activity against *Staphylococcus aureus*	Mudalungu et al. (2021)
Hermetia illucens (L.) (Black soldier fly) (Diptera)	Cecropins, sarcotoxin and stomoxyn peptides (hemolymph)	Antibacterial and antifungal	Elhag et al. (2017)
Holotrichia diomphalia (Scarabaeidae) (Coleoptera)	Tricin, eicosane, cholesterol and palmitinic acid (Isolated from larvae)	*Pyricularia oryzae* (fungus)	Dong et al. (2008)
Hyalophora cecropia L. (giant silk moth) (Lepidoptera)	Cecropin B	*Candida albicans*	Andrä et al. (2001)
Hymenoptera, Hemiptera, Coleoptera, Diptera, Trichoptera and Odonata	Defensins	Gram-positive bacteria	Hoffmann & Hetru (1992); Hetru et al. (2003)
Lucilia sericata (Meigen) (Common green bottle fly) (Diptera)	• Attacins, cecropins, diptericins and proline rich peptides; Lucimycin; Lucifensin; sarcotoxins, proline-rich peptides • Sarcotoxin and stomoxyn peptides	• Antibacterial (*Enterococcus faecalis; Proteus vulgaris*) and antifungal • Antimicrobial	• Pöppel et al. (2015) • Hirsch et al. (2019)

Musca domestica L. (House fly) (Diptera)	• Cecropins, Attacins, defensins, diptericins peptides, lysozymes • Domesticin and muscin peptides • Novel antimicrobial protein • Defensin • Chitosan	• Antibacterial • Antibacterial against *Serratia marcescens* and *Micrococcus luteus* • Antifungal against *Candida albicans* • Strong antibacterial activity against Gram-positive bacteria • Antifungal and antiviral	• Tang et al. (2014a) • Tang et al. (2014b) • Guo et al. (2017) • Dang et al. (2010) • Ai et al. (2012)
Pachycondyla goeldii (predatory ant; isolated from venom) (Hymenoptera)	Ponericins G, W and L	Antibacterial; hemolytic; insecticidal against cricket larvae	Orivel et al. (2001)
Perga affinis (Kirby) (*Pergidae* sp.) (Australian sawfly; Isolated from methanolic extracts) (Hymenoptera)	Macrocarpal M (Novel) and Grandinol (isolated from larvae)	*Bacillus subtilis*	Yin et al. (2013)
Periplaneta americana (L.) (American cockroach) (Blattodea)	• Isoflavone compounds • 3-Acetylbenzamide • Periplanetasin-2 peptide	• Antibacterial against *B. subtilis* • Antifungal • Antifungal	• Gao et al. (2016) • Fang et al. (2018) • Yun et al., (2017)
Podalia sp. and *Megalopyge albicolis* (Walker) (Lepidoptera: Megalopigydae)	Antiviral proteins in hemolymph	Antiviral against influenza, measles, picornavirus and herpes simplex virus.	Carvalho et al. (2022)
Pyrrhocoris apterus (L.) (sap sucking bug; isolated from hemolymph) (Hemiptera)	Pyrrhocoricin (Proline rich peptide)	Antibacterial	Cociancich et al. (1994)
Rhodnius prolixus Stål (Hemiptera)	Prolixicin	Antibacterial	Ursic-Bedoya et al. (2011)

Spodoptera frugiperda (Smith) (fall armyworm) (Lepidoptera)	Classical lysozymes- *Sf*-Lys1, *Sf*-Lys2, *Sf*-Lys3; Lysozyme-like proteins - *Sf*-LLP1, *Sf*-LLP2	Antibacterial against *Micrococcus luteus, E. coli, Photorhabdus luminescens*	Chapelle et al. (2009)
Streptomyces davaonensis YH01 (Actinomycete) isolated from the surface of the termite queen *Odontotermes formosanus* (Shiraki) (Blattodea)	Roseoflavin (Isolated from the actinomycete)	Roseoflavin showed antibacterial activity against *Bacillus subtilis, S. aureus* and methicillin-resistant *S. aureus* (MRSA) strains	Zhou et al. (2021)
Synoeca surinama (L.) (wasp) (Hymeoptera)	Antimicrobial peptides like synoeca-MP (Mastoparan) (Isolated from venom)	Antibacterial against MRSA, *E. coli*, vancomycin-resistant *E. faecalis* (VREF), *P. aeruginosa*, *Klebsiella pneumoniae*	Dantas et al. (2019); Freire et al. (2020)
Tetramorium bicarinatum (Nylander) (Hymenoptera)	Antimicrobial Peptides	*Staphylococcus xylosus*	Rifflet et al. (2012)
Tetramorium spp. (Red ants) (Hymeoptera)	Alkaloid: N-(2-hydroxyethyl)-benzamide	*Bacillus subtilis*	Song et al. (2012)

3.2. Insect Chemicals with Antitumor Properties

Insect derivatives have been proven to be effective against various cancer types, such as breast cancer, leukemia, liver cancer, pancreatic cancer, esophageal cancer, bladder cancer, lung cancer, colorectal cancer, colon cancer, ovarian cancer, cervical cancer, murine melanoma, and ascites cancers (Siddiqui et al., 2023). Several antitumor peptides have been identified in various insect species. For example, chartergellus-CP1 peptide isolated from the venom of the wasp, *Chartergellus communis* Richards, showed potent antitumor effects on breast cancer cell lines (HR+ and triple-negative) where it killed only the tumor cells leaving healthy cells unaffected (Soares et al., 2022). Two compounds were isolated from *Bombyx mori* L. infected with the fungus *Cordyceps militaris* i.e., 3′-deoxyinosine and cordycepin. Both these compounds showed cytotoxic activity against A549, PANC-1, and MCF-7 cancer cells (Qiu et al., 2017). The peptide, cecropin A isolated from *B. mori*

has shown two activities- antimicrobial and antitumor (esophageal cancer cells) (Ramos-Martín et al., 2022).

Cantharidine (CTD) is a terpenoid and gifted by male green blister beetle *B. majalis* to female as a pre-copulatory gift, which the latter use to protect their eggs (Sharma and Singh, 2018). The molecule itself and its derivatives have anti-cancer properties (Ratcliffe, 2006) due to which CTD has been used in clinical practices (Zhang et al., 2017). CTD induced severe apoptosis in Ehrlich ascites carcinoma (EAC) cells in mice *via* the mitochondrial intrinsic pathway. It inhibited lactate dehydrogenase activity leading to short supply of NAD+ and thereby cutting the energy supply to the EAC cells causing their apoptosis (Verma and Prasad, 2013). Cantharidine has also been clinically tested to treat warts as it healed blistering skin patches (Maglio et al., 2003). FDA approved cantharidine to treat warts and to prevent cancers such as kidney, ovary and urogenital tracts (Ratcliffe et al., 2011). The diacid metabolite form of CTD, a synthetic derivative known as noncantharidine (DM-NCTD), obtained from Chinese blister beetle *Mylabris* spp., has been developed and is widely used in anti-tumor treatments in modern medicine (Liu et al., 2020). The defence compounds released by the Argentinian darkling beetle *Ulomoides dermestoides* (Fairmaire) (Coleoptera, Tenebrionidae) include methyl-1,4-benzoquinones, ethyl-1,4-benzoquinones and 1-pentadecene. These compounds inhibited the growth of tumor cells (A549 cells) and damaged their DNA (Crespo et al., 2011), showing potential to be developed as anticancer compounds. Deng et al. (2022) explored mechanism of action of blister beetle's active components in treating lung adenocarcinoma (LUAD) and found that 7 active compounds showed anti-tumor effects via 8 targets (upregulation of CRABP2, KAT2A, BIRC5, ABCC3, PLK1, IGHG1, EPCAM; downregulation of FABP4) in 32 pathways, and BIRC5 and PLK1 showed significant survival.

Chitosan derivatives from scarab beetles (Scarabaeidae) were found to show anticancer properties against lung (A549) and colorectal (HCT-116) cancer cell lines (Abdel Wahid et al., 2018). Similarly, carboxymethyl derivatives of chitosan (CM-Ch) extracted from two dipteran larvae species- *Sarcophaga aegyptiaca* (Salem) and *Chrysomya albeceps* (Wiedemann), showed anticancer activity against hepatocellular carcinoma cells (Abdel Rahman et al., 2020).

A polysaccharide isolated from the wingless cockroach *Eupolyphaga sinensis* Walker, known as ESPS, showed anticancer activity against liver cancer cells by stimulating lymphocyte proliferation and activity, especially natural killer (NK) cells. It has the potential to be developed as an immunotherapy chemical (Xie et al., 2020).

3.3. Chemicals with other medicinal properties

The extracts of *B. mori* contain active molecules such as flavonoids, and free amino acids, (Wang et al., 2012a) while active molecules in propolis obtained from *A. mellifera* are epicatechin and p-coumaric (Cauich-Kumul et al., 2020). All of these molecules regulate blood sugar levels and thus, have the potential to be used to manage diabetes.

A bradykinin-related peptide, Polisteskinin R, was isolated from the venom of the social wasp *P. paulista*. This peptide showed potent anxiolytic effects (dos Anjos et al., 2016). Syrup prepared from *B. mori* cocoons is rich in proteins and was found to be helpful in management of anxiety and depression in patients suffering from mild to moderate mixed anxiety-depressive disorder (MADD) (Zeinalpour et al., 2021).

Certain insect molecules show anti-thrombotic activity, for example, 105 serine proteases were identified from the ground beetle *Eupolyphaga sinensis* Walker (Wang et al., 2012b). Sericin from *B. mori* L. cocoon is a good candidate to treat cardiac mitochondrial abnormalities (Rujimongkon et al., 2022). Four molecules having antithrombotic activity were isolated from the edible grasshopper *Oxya chinensis sinuosa* Mishchenko, of which N-acetyldopamine dimers 1-2 were novel molecules. These molecules inhibited blood coagulation via FXa, and also inhibited platelet-aggregation. Thus, they can be explored as potential pharmacological drugs for treating/preventing vascular diseases involving blood coagulation (Lee et al., 2017).

The aqueous extract of the darkling beetle *U. dermestoides* has several antioxidant compounds such as superoxide dismutase (SOD), vitellogenin-like protein, phenolic compounds, ethyl p-hydroquinone, methyl esters of widespread octadecenoic (oleic) and hexadecanoic fatty acids (Ushakova et al., 2021). Some more insect chemicals having medicinal properties are presented in Table 2.

Table 2: Potential therapeutic chemicals from insects

Insect Source	Chemical/s	Activity/Mode of action	Reference
Allomyrina dichotoma L. (Rhinoceros beetle)	Allomyrinasin (AMP)	Anti-inflammatory	Lee et al. (2019)
	Larval extract	• Hepatoprotective and anticancer activity; antioxidant • Maintains gut homeostasis by upregulating E-cadherin • Reduces hyperlipidemia by inhibiting hepatic lipogenesis via AMPK signaling pathway (can be used to treat hepatic insulin resistance in Type II diabetes • Prevents lipotoxic beta-cell death (prevents onset of diabetes) • Suppressed adipogenesis and lipogenesis in 3T3-L1 cells (anti-obesity effect)	• Lee et al. (2015) • Lee et al. (2021) • Kim et al. (2019) • Kim et al. (2021a) • Chung et al. (2014)
	AF-13 (major component of larval extract)	Anti-apoptotic and anti-inflammatory effects	Kim et al. (2021b)
Apis mellifera	• Abaecin peptide (honey) • Flavonoids-Galangin and Pinocembrin (Propolis)	• Recombinant abaecin (rAbaecin) peptides showed antibacterial activity against *E. coli* • Antibacterial	• Luiz et al. (2017) • Dimov et al (1992)
Byasa polyeuctes	Papilistatin (isolated from adult)	Against six human cell lines: breast MCF-7, P388 leukemia, colon KM20L-2, pancreatic BXPC-3, CNS-28, lung NCI-H460, prostate DU-145	Pettit et al. (2010)
Catharsius molossus (dung beetle)	Water extract	Water extract added as a supplement to submerged fermentation of *Ganoderma lucidum* (medicinal mushroom) enhances the anticancer activity of the latter	Liu et al. (2009)
Holotrichia parallela Motschulsky (Edible beetle)	• Ethanol extract: majorly contains Catechin • Water extract: rich in proteins	• Metal chelator and inhibited lipid peroxidation • Metal chelator and antioxidant (scavenged DPPH radicals) Used for treating tetanus, gout, superficial infections and erysipelas	Liu et al. (2012)

Holotrichia diomphalia Bates	• Grub extract • Crude larvae extract	• Inhibits tumor growth (in vitro in HeLa cells) and in vivo in mice; induces apoptosis • Anticoagulant	• Song et al. (2014) • Xu et al. (2016)
Orancistrocerus drewseni drewseni Saussure (Wasp)	Eumenine mastoparan-OD; Orancis-Protonectin	More potent hemolytic activity than mastoparan peptides	Murata et al. (2009)
Palembus ocularis Casey (Beetle)	Crude extract contains quinones such as hydriquinone, 2-ethylhydroquinone and 2-methylhydroquinone	Anti-inflammatory (hence, used to treat asthma)	Wahrendorf and Wink (2006)
Polyphaga plancyi (Bolivar) (Chinese sand roach)	Plancyamide A Plancypyrazine A Plancyol A Plancypyrazine B	Anticancer	Zhu et al. (2016)
Pseudomyrmex triplarinus (Weddell) (Ant)	Myrmexin I-VI (venom)	Anti-inflammatory analgesic activity	Pan and Hink (2000); Mans et al. (2016);

Conclusion

Insects are a rich source of plethora of chemicals many of which have pharmacological properties. Scientists have been able to profile the chemicals from several insects, and tested their properties wherein some were found to have antimicrobial and antitumor properties, while others had anti-inflammatory, hepato-protective, antioxidant, and renal-protective properties to name a few. The traditional use of insects for medical purposes has enthused the quest for more chemicals from insects that have medicinal properties. However, further work is needed to understand their exact mode of action so that they can be used in CAM without side effects.

References

1. Abdel Rahman, R. M., Ghaffar, H. A., Alkhuriji, A. F., Khalil, M. I., Amaly, N., El-Kott, A. F., ... & Capasso, R. (2020). Dipteran carboxymethyl chitosan as an inexhaustible derivative with a potential antiproliferative activity in hepatocellular carcinoma cells. Evidence-Based Complementary and Alternative Medicine, 2020, 1-14. https://doi.org/10.1155/2020/4396305

2. Abdel Wahid, A. A., Maged, M., & Mohamed, A. F. (2018). Evaluation of *Scarabaeus sacer* derived-chitosan, anti cancer potentials and related changes: in vitro study. Journal of the Egyptian Society of Parasitology, 48(2), 443-448. https://doi.org/10.12816/0050452

4. Adjare M. 1990. Beekeeping in Africa. FAO Agricultural Series, Bulletin 68/6, Rome, Italy. pp. 130-135

5. Ai, H., Wang, F., Xia, Y., Chen, X., & Lei, C. (2012). Antioxidant, antifungal and antiviral activities of chitosan from the larvae of housefly, *Musca domestica* L. Food chemistry, 132(1), 493-498. https://doi.org/10.1016/j.foodchem.2011.11.033

6. Akbari, R., Hakemi Vala, M., Hashemi, A., Aghazadeh, H., Sabatier, J. M., & Pooshang Bagheri, K. (2018). Action mechanism of melittin-derived antimicrobial peptides, MDP1 and MDP2, de novo designed against multidrug resistant bacteria. Amino acids, 50, 1231-1243. https://doi.org/10.1007/s00726-018-2596-5

7. Akbari, R., Hakemi-Vala, M., Pashaie, F., Bevalian, P., Hashemi, A., & Pooshang Bagheri, K. (2019). Highly synergistic effects of melittin with conventional antibiotics against multidrug-resistant isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. Microbial Drug Resistance, 25(2), 193-202. https://doi.org/10.1089/mdr.2018.0016

8. Alujas-Burgos, S., Bayón, P., & Figueredo, M. (2018). Recent advances in the synthesis of azaphenalene alkaloids: First enantioselective approaches. Organic & biomolecular chemistry, 16(37), 8218-8229. https://doi.org/10.1039/C8OB01443D

9. Andrä, J., Berninghausen, O., & Leippe, M. (2001). Cecropins, antibacterial peptides from insects and mammals, are potently fungicidal against *Candida albicans*. Medical Microbiology and Immunology, 189, 169-173. https://doi.org/10.1007/s430-001-8025-x

10. Baer, W. S. (1931). The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). The Journal of Bone & Joint Surgery, 13(3), 438-475.

11. Banerjee, P., Sahoo, K. N., Biswas, T. K., Basu, S. K., Chatterjee, J., Hui, A. K., ... & Debnath, P. K. (2003). Bees make medicine for mankind. Indian Journal of Traditional Knowledge. 2003;2(1):22–6.

12. Barbault, F., Landon, C., Guenneugues, M., Meyer, J. P., Schott, V., Dimarcq,

J. L., & Vovelle, F. (2003). Solution structure of Alo-3, a new knottin-type antifungal peptide from the insect *Acrocinus longimanus*. Biochemistry, 42(49), 14434–14442. https://doi.org/10.1021/bi035400o

13. Barke, J., Seipke, R. F., Grüschow, S., Heavens, D., Drou, N., Bibb, M. J., Goss, R. J. M., Yu, D. W., & Hutchings, M. I. (2010). A mixed community of actinomycetes produce multiple antibiotics for the fungus farming ant *Acromyrmex octospinosus*. BMC Biology. https://doi.org/10.1186/1741-7007-8-109

14. Bıliková, K., Hanes, J., Nordhoff, E., Saenger, W., Klaudiny, J., & Šimúth, J. (2002). Apisimin, a new serine–valine-rich peptide from honeybee (*Apis mellifera* L.) royal jelly: purification and molecular characterization. FEBS letters, 528(1-3), 125-129. https://doi.org/10.1016/S0014-5793(02)03272-6

15. Bíliková, K., Wu, G., & Šimúth, J. (2001). Isolation of a peptide fraction from honeybee royal jelly as a potential antifoulbrood factor. Apidologie, 32(3), 275-283. https://doi.org/10.1051/apido:2001129

16. Borsato, D. M., Prudente, A. S., Doell-Boscardin, P. M., Borsato, A. V., Luz, C. F., Maia, B. H., ... & Miguel, O. G. (2014). Topical anti-inflammatory activity of a monofloral honey of *Mimosa scabrella* provided by *Melipona marginata* during winter in Southern Brazil. Journal of Medicinal Food, 17(7), 817-825. https://doi.org/10.1089/jmf.2013.0024

17. Bosque, I., Gonzalez-Gomez, J. C., Loza, M. I., & Brea, J. (2014). Natural tetraponerines: A general synthesis and antiproliferative activity. The Journal of Organic Chemistry, 79(9), 3982-3991. https://doi.org/10.1021/jo500446f

18. Brown, A., Horobin, A., Blount, D. G., Hill, P. J., English, J., Rich, A., ... & Pritchard, D. I. (2012). Blow fly *Lucilia sericata* nuclease digests DNA associated with wound slough/eschar and with *Pseudomonas aeruginosa* biofilm. Medical and Veterinary Entomology, 26(4), 432-439.

19. Buhroo, Z. I., Bhat, M. A., Bali, G. K., Kamili, A. S., & Ganai, N. A. (2018). Antimicrobial peptides from insects with special reference to silkworm Bombyx mori L.: A review. Journal of Entomology and Zoology Studies, 6(4), 752–759.

20. Campos, J. F., Santos, U. P. D., Rocha, P. D. S. D., Damião, M. J., Balestieri, J. B. P., Cardoso, C. A. L., ... & Santos, E. L. D. (2015). Antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of propolis from the stingless bee *Tetragonisca fiebrigi* (Jataí). Evidence-Based Complementary and Alternative Medicine. https://doi.org/10.1155/2015/296186

21. Carvalho, N. D., Curti, S. P., Oliveira, M. I., Rofatto, H. K., Figueiredo, C. A., Senna de Villar, K., ... & Mendonça, R. Z. (2022). Antiviral activity of the hemolymph of *Podalia* sp and *M. albicolis* (Lepidoptera: Megalopigydae). Journal of Infectious Diseases and Therapy, S4:005.

22. Cauich-Kumul, R., Sauri-Duch, E., Toledo-López, V., Acevedo-Fernández, J. J., & Segura-Campos, M. (2020). Propolis of *Apis mellifera* from Yucatán, México: Study of biological properties. Ecosistemas y recursos agropecuarios, 7(3), 1-11. https://doi.org/10.19136/era.a7n3.2604

23. Chalk, R., Townson, H., & Ham, P. J. (1995). *Brugia pahangi*: the effects of cecropins on microfilariae *in vitro* and in *Aedes aegypti*. Experimental Parasitology, 80(3), 401-406.

24. Chapelle, M., Girard, P. A., Cousserans, F., Volkoff, N. A., & Duvic, B. (2009). Lysozymes and lysozyme-like proteins from the fall armyworm, *Spodoptera Frugiperda*. Molecular Immunology, 47(2–3), 261–269. https://doi.org/10.1016/j.molimm.2009.09.028

25. Chen, J., Guan, S. M., Sun, W., & Fu, H. (2016). Melittin, the major pain-producing substance of bee venom. Neuroscience Bulletin, 32, 265-272. https://doi.org/10.1007/s12264-016-0024-y

26. Cherniack, E. P., & Govorushko, S. (2018). To bee or not to bee: The potential efficacy and safety of bee venom acupuncture in humans. Toxicon, 154, 74-78. https://doi.org/10.1016/j.toxicon.2018.09.013

27. Choudhari, M. K., Punekar, S. A., Ranade, R. V., & Paknikar, K. M. (2012). Antimicrobial activity of stingless bee (*Trigona* sp.) propolis used in the folk medicine of Western Maharashtra, India. Journal of Ethnopharmacology, 141(1), 363-367. https://doi.org/10.1016/j.jep.2012.02.047

28. Chung, M. Y., Yoon, Y. I., Hwang, J. S., Goo, T. W., & Yun, E. Y. (2014). Anti-obesity effect of *Allomyrina dichotoma* (Arthropoda: Insecta) larvae ethanol extract on 3T3-L1 adipocyte differentiation. Entomological Research, 44(1), 9-16. https://doi.org/10.1111/1748-5967.12044

29. Cociancich, S., Dupont, A., Hegy, G., Lanot, R., Holder, F., Hetru, C., ... & Bulet, P. (1994). Novel inducible antibacterial peptides from a hemipteran insect, the sap-sucking bug Pyrrhocoris apterus. Biochemical Journal, 300(2), 567-575.

30. Coelho, G. R., Mendonça, R. Z., Vilar, K. D. S., Figueiredo, C. A., Badari, J. C., Taniwaki, N., ... & Negri, G. (2015). Antiviral action of hydromethanolic extract of geopropolis from *Scaptotrigona postica* against antiherpes simplex virus (HSV-1). Evidence-Based Complementary and Alternative Medicine, 2015. https://doi.org/10.1155/2015/296086

31. Costa-Neto E M. (2005). Entomotherapy or the medicinal use of insects. Journal of Ethnobiology, 25, 93-114.

32. Crespo, R., Villaverde, M. L., Girotti, J. R., Güerci, A., Juárez, M. P., & de Bravo, M. G. (2011). Cytotoxic and genotoxic effects of defence secretion of *Ulomoides dermestoides* on A549 cells. Journal of Ethnopharmacology, 136(1), 204-209. https://doi.org/10.1016/j.jep.2011.04.056

33. Dang, X. L., Wang, Y. S., Huang, Y. D., Yu, X. Q., & Zhang, W. Q. (2010). Purification and characterization of an antimicrobial peptide, insect defensin, from immunized house fly (Diptera, Muscidae). Journal of Medical Entomology, 47(6), 1141–1145. https://doi.org/10.1603/me10016

34. Dantas, E. M. G. L., Lima, S. M. F., Cantuária, A. P. C., Amorim, I. A., Almeida, J. A., Cunha, T. F., Franco, O. L., & Rezende, T. M. B. (2019). Synergistic activity of chlorhexidine and synoeca-MP peptide against *Pseudomonas aeruginosa*.

Journal of Cellular Physiology, 234(9), 16068–16079. https://doi.org/10.1002/jcp.28265

35. de Mendonça, I. C. G., Porto, I. C. C. D. M., do Nascimento, T. G., de Souza, N. S., Oliveira, J. M. D. S., Arruda, R. E. D. S., ... & Barreto, F. S. (2015). Brazilian red propolis: phytochemical screening, antioxidant activity and effect against cancer cells. BMC Complementary and Alternative Medicine, 15(1), 1-12. https://doi.org/10.1186/s12906-015-0888-9

36. Deng, S., Mao, Y., Li, H., & Li, G. (2022). Network Pharmacology Integrated Molecular Docking to Explore the Mechanism of Blister Beetle Therapy for Lung Adenocarcinoma. Contrast Media & Molecular Imaging, 2022. https://doi.org/10.1155%2F2022%2F1892384

37. Devi, W. D., Bonysana, R., Kapesa, K., Mukherjee, P. K., & Rajashekar, Y. (2023). Edible insects: as traditional medicine for human wellness. Future Foods, 100219. https://doi.org/10.1016/j.fufo.2023.100219

38. Di, L., Shi, Y. N., Yan, Y. M., Jiang, L. P., Hou, B., Wang, X. L., ... & Cheng, Y. X. (2015). Nonpeptide small molecules from the insect *Aspongopus chinensis* and their neural stem cell proliferation stimulating properties. RSC Advances, 5(87), 70985-70991.

39. DIMARCQ, J. L., Keppi, E., Dunbar, B., Lambert, J., Reichhart, J. M., Hoffmann, D., ... & Hoffmann, J. A. (1988). Insect immunity: purification and characterization of a family of novel inducible antibacterial proteins from immunized larvae of the dipteran *Phormia terranovae* and complete amino-acid sequence of the predominant member, diptericin A. European Journal of Biochemistry, 171(1-2), 17-22.

40. Dimov, V., Ivanovska, N., Bankova, V., & Popov, S. (1992). Immunomodulatory action of propolis: IV. Prophylactic activity against Gram-negative infections and adjuvant effect of the water-soluble derivative. Vaccine, 10(12), 817-823.

41. Dong, Q. F., Wang, J. L., Zhang, S. F., Wang, Z., Zhang, C. X., Gao, H., ... & Zhang, L. (2008). Antifungal activity of crude extracts and fat-soluble constituents of *Holotrichia diomphalia* larvae. Bioresource Technology, 99(17), 8521-8523. https://doi.org/10.1016/j.biortech.2008.03.010

42. dos Anjos, L. C., Gomes, F. M. M., do Couto, L. L., Mourão, C. A., Moreira, K. G., Silva, L. P., & Mortari, M. R. (2016). Anxiolytic activity and evaluation of potentially adverse effects of a bradykinin-related peptide isolated from a social wasp venom. Life Sciences, 149, 153-159. https://doi.org/10.1016/j.lfs.2016.02.063

43. Dutta, S. R., Gauri, S. S., Mondal, B., Vemula, A., Haider, S. K., Mondal, K. C., & Ghosh, A. K. (2016). Screening of antimicrobial peptides from hemolymph extract of tasar silkworm *Antheraea mylitta* against urinary tract and wound infecting multidrug-resistant bacteria. Acta Biologica Szegediensis, 60(1), 49-55.

44. Elhag, O., Zhou, D., Song, Q., Soomro, A. A., Cai, M., Zheng, L., Yu, Z., &

Zhang, J. (2017). Screening, expression, purification and functional characterization of novel antimicrobial peptide genes from *Hermetia illucens* (L.). PLoS ONE, 12(1), 1–15. https://doi.org/10.1371/journal.pone.0169582

45. Fang, X., Shen, J., Wang, J., Chen, Z., Lin, P., Chen, Z., Liu, L., Zeng, Hu., & Jin, X. (2018). Antifungal activity of 3-acetylbenzamide produced by actinomycete WA23-4-4 from the intestinal tract of *Periplaneta americana*. Journal of Microbiology, 56(7), 516–523. https://doi.org/10.1007/s12275-018-7510-z

46. Freire, D. O., Cunha, N. B., Leite, M. L., Kostopoulos, A. G. C., Silva, S. N. B., Souza, A. C. B., Nolasco, D. O., Franco, O. L., Mortari, M. R., & Dias, S. C. (2020). Wasp venom peptide, synoeca-MP, from *Synoeca surinama* shows antimicrobial activity against human and animal pathogenic microorganisms. Peptide Science, 112(3), 1–6. https://doi.org/10.1002/pep2.24141

47. Gajski, G., & Garaj-Vrhovac, V. (2013). Melittin: a lytic peptide with anticancer properties. Environmental Toxicology and Pharmacology, 36(2), 697-705. https://doi.org/10.1016/j.etap.2013.06.009

48. Gallinger, T. L., Aboagye, S. Y., Obermann, W., Weiss, M., Grünweller, A., Unverzagt, C., ... & Haeberlein, S. (2022). First in silico screening of insect molecules for identification of novel anti-parasitic compounds. Pharmaceuticals, 15(2), 119. https://doi.org/10.3390/ph15020119

49. Gannabathula, S., Krissansen, G. W., Skinner, M., Steinhorn, G., & Schlothauer, R. (2015). Honeybee apisimin and plant arabinogalactans in honey costimulate monocytes. Food Chemistry, 168, 34-40. https://doi.org/10.1016/j.foodchem.2014.07.007

50. Gao, J. Y., Jiang, Y. L., Niu, L. L., Li, H. D., & Yin, W. P. (2016). Novel isoflavone from the cockroach *Periplaneta americana*. Chemistry of Natural Compounds, 52(3), 362–364. https://doi.org/10.1007/s10600-016-1661-0

51. Gasch, T., & Vilcinskas, A. (2014). The chemical defense in larvae of the earwig *Forficula auricularia*. Journal of Insect Physiology, 67, 1–8. https://doi.org/10.1016/j.jinsphys.2014.05.019

52. González, E. A., García, E. M., & Nazareno, M. A. (2010). Free radical scavenging capacity and antioxidant activity of cochineal (Dactylopius coccus C.) extracts. Food chemistry, 119(1), 358-362. https://doi.org/10.1016/j.foodchem.2009.06.030

53. Guo, G., Tao, R., Li, Y., Ma, H., Xiu, J., Fu, P., & Wu, J. (2017). Identification and characterization of a novel antimicrobial protein from the housefly *Musca domestica*. Biochemical and Biophysical Research Communications, 490(3), 746–752. https://doi.org/10.1016/j.bbrc.2017.06.112

54. Gupta, R. K., & Stangaciu, S. (2014). Apitherapy: holistic healing through the honeybee and bee products in countries with poor healthcare system. In Beekeeping for poverty alleviation and livelihood security (pp. 413-446). Springer, Dordrecht.

55. Hancock R E, Nijnik A, Philpott D J. 2012. Modulating immunity as a therapy

for bacterial infections. Nature Reviews Microbiology, 10, 243-254.

56. Hara, S., & Yamakawa, M. (1995). A novel antibacterial peptide family isolated from the silkworm, *Bombyx mori*. Biochemical Journal, 310(2), 651-656.

57. Hetru, C., Troxler, L., & Hoffmann, J. A. (2003). *Drosophila melanogaster* antimicrobial defense. The Journal of infectious diseases, 187(Supplement_2), S327-S334. https://doi.org/10.1086/374758

58. Hinman, E. H. (1933). The Use of Insects and other Arthropods in Medicine. Journal of Tropical Medicine and Hygiene, 36(9).

59. Hirsch, R., Wiesner, J., Marker, A., Pfeifer, Y., Bauer, A., Hammann, P. E., & Vilcinskas, A. (2019). Profiling antimicrobial peptides from the medical maggot *Lucilia sericata* as potential antibiotics for MDR Gram-negative bacteria. Journal of Antimicrobial Chemotherapy, 74(1), 96–107. https://doi.org/10.1093/jac/dky386

60. Hoffmann, J. A., & Hetru, C. (1992). Insect defensins: inducible antibacterial peptides. Immunology Today, 13(10), 411-415. https://doi.org/10.1016/0167-5699(92)90092-L

61. Hultmark, D., Engström, A., Andersson, K., Steiner, H., Bennich, H., & Boman, H. G. (1983). Insect immunity. Attacins, a family of antibacterial proteins from *Hyalophora cecropia*. The EMBO Journal, 2(4), 571-576.

62. Imler, J. L., & Bulet, P. (2005). Antimicrobial peptides in *Drosophila*: structures, activities and gene regulation. Mechanisms of Epithelial Defense, 86, 1-21.

63. Ishikawa, M., Kubo, T., & Natori, S. (1992). Purification and characterization of a diptericin homologue from *Sarcophaga peregrina* (flesh fly). Biochemical Journal, 287(2), 573-578.

64. Islam, S., Bezbaruah, S., & Kalita, J. (2016). A review on antimicrobial peptides from *Bombyx mori* L. and their application in plant and animal disease control. Journal of Advances in Biology & Biotechnology, 9(3), 1–15. https://doi.org/10.9734/jabb/2016/27539

65. Jamasbi, E., Lucky, S. S., Li, W., Hossain, M. A., Gopalakrishnakone, P., & Separovic, F. (2018). Effect of dimerized melittin on gastric cancer cells and antibacterial activity. Amino Acids, 50, 1101-1110. https://doi.org/10.1007/s00726-018-2587-6

66. Jia, F., Wang, J., Peng, J., Zhao, P., Kong, Z., Wang, K., ... & Wang, R. (2018). The in vitro, in vivo antifungal activity and the action mode of Jelleine-I against Candida species. Amino Acids, 50, 229-239. https://doi.org/10.1007/s00726-017-2507-1

67. Jiang, H. L., Luo, X. H., Wang, X. Z., Yang, J. L., Yao, X. J., Crews, P., ... & Wu, Q. X. (2012). New isocoumarins and alkaloid from Chinese insect medicine, *Eupolyphaga sinensis* Walker. Fitoterapia, 83(7), 1275-1280.

68. Jung, S. Y., Lee, K. W., Choi, S. M., & Yang, E. J. (2015). Bee venom protects against rotenone-induced cell death in NSC34 motor neuron cells. Toxins, 7(9),

3715-3726. https://doi.org/10.3390/toxins7093715

69. Kaya, M., Baran, T., Asan-Ozusaglam, M., Cakmak, Y. S., Tozak, K. O., Mol, A., ... & Sezen, G. (2015). Extraction and characterization of chitin and chitosan with antimicrobial and antioxidant activities from cosmopolitan Orthoptera species (Insecta). Biotechnology and Bioprocess Engineering, 20, 168-179. https://doi.org/10.1007/s12257-014-0391-z

70. Kędzia B, Kędzia H E. 2013. The antibiotic activity of native and European propolis. Postępy Fitoterapii, 2, 97-101

71. Khan, M. S., Singh, M., Khan, M. A., Arya, D. S., & Ahmad, S. (2014). Scientific validation of cardioprotective attribute by standardized extract of *Bombyx mori* against doxorubicin-induced cardiotoxicity in murine model. EXCLI Journal, 13, 1043.

72. Kim, J. Y., Kim, K. H., Lee, W. R., An, H. J., Lee, S. J., Han, S. M., ... & Park, K. K. (2015). Apamin inhibits PDGF-BB-induced vascular smooth muscle cell proliferation and migration through suppressions of activated Akt and Erk signaling pathway. Vascular Pharmacology, 70, 8-14. https://doi.org/10.1016/j.vph.2014.12.004

73. Kim, K., Bae, G. D., Lee, M., Park, E. Y., Baek, D. J., Kim, C. Y., ... & Oh, Y. S. (2019). Allomyrina dichotoma larva extract ameliorates the hepatic insulin resistance of high-fat diet-induced diabetic mice. Nutrients, 11(7), 1522. https://doi.org/10.3390%2Fnu11071522

74. Kim, K., Kwak, M. K., Bae, G. D., Park, E. Y., Baek, D. J., Kim, C. Y., ... & Oh, Y. S. (2021a). Allomyrina dichotoma larva extract attenuates free fatty acid-induced lipotoxicity in pancreatic beta cells. Nutrition Research and Practice, 15(3), 294. https://doi.org/10.4162%2Fnrp.2021.15.3.294

75. Kim, K., Park, E. Y., Baek, D. J., Kim, C. Y., & Oh, Y. S. (2021b). Anti-inflammatory activity of AF-13, an antioxidant compound isolated from the polar fraction of *Allomyrina dichotoma* larva, in palmitate-induced INS-1 cells. Life, 11(6), 470. https://doi.org/10.3390/life11060470

76. Kolayli, S., & Keskin, M. (2020). Natural bee products and their apitherapeutic applications. Studies in Natural Products Chemistry, 66, 175-196. https://doi.org/10.1016/B978-0-12-817907-9.00007-6

77. Lee, G., & Bae, H. (2016). Anti-inflammatory applications of melittin, a major component of bee venom: Detailed mechanism of action and adverse effects. Molecules, 21(5), 616. https://doi.org/10.3390/molecules21050616

78. Lee, J. E., Jo, D. E., Lee, A. J., Park, H. K., Youn, K., Yun, E. Y., ... & Kang, B. H. (2015). Hepatoprotective and anticancer activities of *Allomyrina dichotoma* larvae. Journal of Life Science, 25(3), 307-316.

79. Lee, J. H., Seo, M., Lee, H. J., Baek, M., Kim, I. W., Kim, S. Y., ... & Hwang, J. S. (2019). Anti-inflammatory activity of antimicrobial peptide allomyrinasin derived from the dynastid beetle, *Allomyrina dichotoma*. Journal of Microbiology and Biotechnology, 29(5), 687-695. https://doi.org/10.4014/jmb.1809.09031

80. Lee, K. S., Kim, B. Y., Yoon, H. J., Choi, Y. S., & Jin, B. R. (2016). Secapin, a bee venom peptide, exhibits anti-fibrinolytic, anti-elastolytic, and anti-microbial activities. Developmental and Comparative Immunology, 63, 27–35. https://doi.org/10.1016/j.dci.2016.05.011

81. Lee, S. H., Goo, T. W., & Yun, E. Y. (2021). *Allomyrina dichotoma* larval extract has protective effects against gut permeability of dextran sulfate sodium-fed Drosophila by E-cadherin and armadillo. Journal of Ethnopharmacology, 279, 113786. https://doi.org/10.1016/j.jep.2021.113786

82. Lee, W., Lee, H., Kim, M. A., Choi, J., Kim, K. M., Hwang, J. S., ... & Bae, J. S. (2017). Evaluation of novel factor Xa inhibitors from *Oxya chinensis sinuosa* with anti-platelet aggregation activity. Scientific Reports, 7(1), 7934. https://doi.org/10.1038/s41598-017-08330-1

83. Leong, R. L., Xing, H., Braekman, J. C., & Kem, W. R. (2015). Non-competitive inhibition of nicotinic acetylcholine receptors by ladybird beetle alkaloids. Neurochemical Research, 40, 2078-2086. https://doi.org/10.1007/s11064-014-1466-0

84. Levashina, E. A., Ohresser, S., Bulet, P., Reichhart, J. M., Hetru, C., & Hoffmann, J. A. (1995). Metchnikowin, a novel immune-inducible proline-rich peptide from *Drosophila* with antibacterial and antifungal properties. European Journal of Biochemistry, 233(2), 694-700.

85. Lima, D. B., Mello, C. P., Bandeira, I. C. J., Pessoa Bezerra de Menezes, R. R. P., Sampaio, T. L., Falcao, C. B., ... & Martins, A. M. C. (2018). The dinoponeratoxin peptides from the giant ant Dinoponera quadriceps display in vitro antitrypanosomal activity. Biological Chemistry, 399(2), 187-196. https://doi.org/10.1515/hsz-2017-0198

86. Liu, G., Ding, C., Zhang, K., & Wang, X. (2009). Effects of medicinal insect *Catharsius molossus* on submerged fermentation and in vivo anti-hepatoma activity of Ganoderma lucidum. Sheng Wu Gong Cheng xue bao (Chinese Journal of Biotechnology), 25(6), 880-886.

87. Liu, M., Tu, J., Feng, Y., Zhang, J., & Wu, J. (2020). Synergistic co-delivery of diacid metabolite of norcantharidin and ABT-737 based on folate-modified lipid bilayer-coated mesoporous silica nanoparticle against hepatic carcinoma. Journal of Nanobiotechnology, 18(1), 1-8. https://doi.org/10.1186/s12951-020-00677-4

88. Liu, S., Sun, J., Yu, L., Zhang, C., Bi, J., Zhu, F., ... & Yang, Q. (2012). Antioxidant activity and phenolic compounds of *Holotrichia parallela* Motschulsky extracts. Food Chemistry, 134(4), 1885-1891. https://doi.org/10.1016/j.foodchem.2012.03.091

89. Lu, J., Sun, Q., Tu, Z. C., Lv, Q., Shui, P. X., & Cheng, Y. X. (2015). Identification of N-acetyldopamine dimers from the dung beetle *Catharsius molossus* and their COX-1 and COX-2 inhibitory activities. Molecules, 20(9), 15589-15596. https://doi.org/10.3390/molecules200915589

90. Lu, J., Wang, H., Huang, J., Li, G., Wang, Q., Xu, W., ... & Wang, J. (2014). Sesquiterpene acids from Shellac and their bioactivities evaluation. Fitoterapia, 97, 64-70. https://doi.org/10.1016/j.fitote.2014.05.014

91. Luiz, D. P., Almeida, J. F., Goulart, L. R., Nicolau-Junior, N., & Ueira-Vieira, C. (2017). Heterologous expression of abaecin peptide from *Apis mellifera* in Pichia pastoris. Microbial Cell Factories, 16(1), 76. https://doi.org/10.1186/s12934-017-0689-6

92. Luo, X. H., Wang, X. Z., Jiang, H. L., Yang, J. L., Crews, P., Valeriote, F. A., & Wu, Q. X. (2012). The biosynthetic products of Chinese insect medicine, *Aspongopus chinensis*. Fitoterapia, 83(4), 754-758. https://doi.org/10.1016/j.fitote.2012.03.002

93. Maglio, D., Teng, R., Thyrum, P. T., Nightingale, C. H., & Nicolau, D. P. (2003). Pharmacokinetic profile of meropenem, administered at 500 milligrams every 8 hours, in plasma and cantharidin-induced skin blister fluid. Antimicrobial Agents and Chemotherapy, 47, 1771-1773.

94. Majerus, M. E. (2016). A natural history of ladybird beetles. Cambridge University Press.

95. Malekian, A., Djavid, G. E., Akbarzadeh, K., Soltandallal, M., Rassi, Y., Rafinejad, J., Totonchi, M. (2019). Efficacy of maggot therapy on *Staphylococcus aureus* and *Pseudomonas aeruginosa* in diabetic foot ulcers: a randomized controlled trial. The Journal of Wound, Ostomy and Continence Nursing 46: 25-29

96. Mans, D. R. A., Sairras, S., Ganga, D., & Kartopawiro, J. (2016). Exploring the global animal biodiversity in the search for new drugs–insects. Journal of Translational Science, 3(1), 371–386. https://doi.org/10.15761/JTS.1000164

97. Mastore, M., Quadroni, S., Caramella, S., & Brivio, M. F. (2021). The silkworm as a source of natural antimicrobial preparations: efficacy on various bacterial strains. Antibiotics, 10(11), 1339. https://doi.org/10.3390/antibiotics10111339

98. Mendonça, A., Bernardi Marchiotti, R. C., Firmino, E. L. B., Santos, P. P., Sguarizi Antonio, D., Serrão, J. E., Cardoso, C. A. L., & Antonialli, W. F. J. (2019). Proteomic analysis of the venom of the social wasp *Apoica pallens* (Hymenoptera, Vespidae). Revista Brasileira De Entomologia, 63(4), 322–330. https://doi.org/10.1016/j.rbe.2019.10.001

99. Moghaddam, M. R. B., Gross, T., Becker, A., Vilcinskas, A., & Rahnamaeian, M. (2017). The selective antifungal activity of *Drosophila melanogaster* metchnikowin reflects the species-dependent inhibition of succinate–coenzyme Q reductase. Scientific reports, 7(1), 8192. https://doi.org/10.1038/s41598-017-08407-x

100. Moreno, M., & Giralt, E. (2015). Three valuable peptides from bee and wasp venoms for therapeutic and biotechnological use: melittin, apamin and mastoparan. Toxins, 7(4), 1126-1150. https://doi.org/10.3390/toxins7041126

101. Morge G. (1973). Entomology in the western world in antiquity and in medieval times. In: Smith RF, editor. History of entomology. Palo Alto: Annual Reviews, Inc; 1973. p. 37–80.

102. Mudalungu, C. M., Tanga, C. M., Kelemu, S., & Torto, B. (2021). An overview of antimicrobial compounds from African edible insects and their associated microbiota. Antibiotics, 10(6), 621. https://doi.org/10.3390/antibiotics10060621

103. Murata, K., Shinada, T., Ohfune, Y., Hisada, M., Yasuda, A., Naoki, H., & Nakajima, T. (2009). Novel mastoparan and protonectin analogs isolated from a solitary wasp, *Orancistrocerus drewseni drewseni*. Amino Acids, 37(2), 389–394. https://doi.org/10.1007/s00726-008-0166-y

104. Narsinghani, T., & Sharma, R. (2014). Lead optimization on conventional non-steroidal anti-inflammatory drugs: an approach to reduce gastrointestinal toxicity. Chemical Biology & Drug Design, 84(1), 1-23. https://doi.org/10.1111/cbdd.12292

105. Nimgulkar, C. C., Patil, S. D., & Kumar, B. D. (2011). Anti-asthmatic and anti-anaphylactic activities of *Blatta orientalis* mother tincture. Homeopathy, 100(03), 138-143.

106. Olayemi, K. I. (2014). Therapeutic potentials of Nigerian insect-propolis against the malarial parasite, Plasmodium berghei (Haemosporida: Plasmodidae). American Journal of Drug Discovery and Development, 4(4):241-247.

107. Orivel, J., Redeker, V., Le Caer, J. P., Krier, F., Revol-Junelles, A. M., Longeon, A., ... & Rossier, J. (2001). Ponericins, new antibacterial and insecticidal peptides from the venom of the ant Pachycondyla goeldii. Journal of Biological Chemistry, 276(21), 17823-17829.

108. Pan, J., & Hink, W. F. (2000). Isolation and characterization of myrmexins, six isoforms of venom proteins with anti- inflammatory activity from the tropical ant, *Pseudomyrmex triplarinus*. Toxicon, 38, 1403–1413.

109. Patel, R. N., Richards, D. P., Duce, I. R., Birkett, M. A., Sattelle, D. B., & Mellor, I. R. (2020). Actions on mammalian and insect nicotinic acetylcholine receptors of harmonine-containing alkaloid extracts from the harlequin ladybird *Harmonia axyridis*. Pesticide Biochemistry and Physiology, 166, 104561. https://doi.org/10.1016/j.pestbp.2020.104561

110. Pettit, G. R., Ye, Q., Herald, D. L., Hogan, F., & Pettit, R. K. (2010). Antineoplastic agents. 573. Isolation and structure of papilistatin from the papilionid butterfly *Byasa polyeuctes* termessa. Journal of Natural Products, 73(2), 164-166. https://doi.org/10.1021%2Fnp9004689

111. Pöppel, A. K., Vogel, H., Wiesner, J., & Vilcinskas, A. (2015). Antimicrobial peptides expressed in medicinal maggots of the blow fly *Lucilia sericata* show combinatorial activity against bacteria. Antimicrobial Agents and Chemotherapy, 59(5), 2508–2514. https://doi.org/10.1128/AAC.05180-14

112. Qiu, W., Wu, J., Choi, J. H., Hirai, H., Nishida, H., & Kawagishi, H. (2017). Cytotoxic compounds against cancer cells from *Bombyx mori* inoculated with *Cordyceps militaris*. Bioscience, Biotechnology, and Biochemistry, 81(6), 1224-1226. https://doi.org/10.1080/09168451.2017.1289075

113. Rafter, L. (2012). Debridement of a traumatic haematoma using larval therapy.

Wounds UK: 81-88

114. Ramos-Martín, F., Herrera-León, C., & d'Amelio, N. (2022). Molecular basis of the anticancer, apoptotic and antibacterial activities of *Bombyx mori* Cecropin A. Archives of Biochemistry and Biophysics, 715, 109095. https://doi.org/10.1016/j.abb.2021.109095

115. Ratcliffe, B. C. (2006). Scarab beetles in human culture. Coleopterists Bulletin, 60, 85-101

116. Ratcliffe, N. A., Mello, C. B., Garcia, E. S., Butt, T. M., Azambuja, P. (2011). Insect natural products and processes: New treatments for human disease. Insect Biochemistry and Molecular Biology, 41, 747-769. https://doi.org/10.1016/j.ibmb.2011.05.007

117. Reichhart, J. M., Meister, M., Dimarcq, J. L., Zachary, D., Hoffmann, D., Ruiz, C., ... & Hoffmann, J. A. (1992). Insect immunity: developmental and inducible activity of the Drosophila diptericin promoter. The EMBO Journal, 11(4), 1469-1477.

118. Richards, D. P., Patel, R. N., Duce, I. R., Khambay, B. P., Birkett, M. A., Pickett, J. A., & Mellor, I. R. (2022). (−)-Adaline from the *Adalia* Genus of ladybirds is a potent antagonist of insect and specific mammalian nicotinic acetylcholine receptors. Molecules, 27(20), 7074. https://doi.org/10.3390%2Fmolecules27207074

119. Rifflet, A., Gavalda, S., Téné, N., Orivel, J., Leprince, J., Guilhaudis, L., ... & Treilhou, M. (2012). Identification and characterization of a novel antimicrobial peptide from the venom of the ant *Tetramorium bicarinatum*. Peptides, 38(2), 363-370. https://doi.org/10.1016/j.peptides.2012.08.018

120. Romanelli, A., Moggio, L., Montella, R. C., Campiglia, P., Iannaccone, M., Capuano, F., ... & Capparelli, R. (2011). Peptides from Royal Jelly: studies on the antimicrobial activity of jelleins, jelleins analogs and synergy with temporins. Journal of Peptide Science, 17(5), 348-352. https://doi.org/10.1002/psc.1316

121. Rouchaud, A., & Braekman, J. C. (2009). Synthesis of new analogues of the tetraponerines. European Journal of Organic Chemistry, 2009(16):2666-2674. https://doi.org/10.1002/ejoc.200900064

122. Rujimongkon, K., Ampawong, S., Isarangkul, D., Reamtong, O., & Aramwit, P. (2022). Sericin-mediated improvement of dysmorphic cardiac mitochondria from hypercholesterolaemia is associated with maintaining mitochondrial dynamics, energy production, and mitochondrial structure. Pharmaceutical Biology, 60(1), 708-721. https://doi.org/10.1080/13880209.2022.2055088

123. Saidemberg, D. M., da Silva-Filho, L. C., Tognoli, L. M. C., Tormena, C. F., & Palma, M. S. (2010). Polybioside, a neuroactive compound from the venom of the social wasp *Polybia paulista*. Journal of Natural Products, 73(4), 527-531. https://doi.org/10.1021/np900424t

124. Seabrooks, L., & Hu, L. (2017). Insects: an underrepresented resource for the discovery of biologically active natural products. Acta Pharmaceutica Sinica B,

7(4), 409-426. https://doi.org/10.1016%2Fj.apsb.2017.05.001

125. Sharma R K, Singh S. 2018. Host range and abundance of blister beetle [*Mylabris pustulata* (thunberg)] in sub-mountainous Punjab. Agricultural Research, 55, 696-700.

126. Sherman, R. A., Hall, M. J. R., & Thomas, S. (2000). Medicinal maggots: an ancient remedy for some contemporary afflictions. Annual Review of Entomology, 45(1), 55-81.

127. Siddiqui, S. A., Li, C., Aidoo, O. F., Fernando, I., Haddad, M. A., Pereira, J. A., ... & Câmara, J. S. (2023). Unravelling the potential of insects for medicinal purposes–A comprehensive review. Heliyon, 9, e15938. https://doi.org/10.1016/j.heliyon.2023.e15938

128. Silva, J. C., Rodrigues, S., Feás, X., & Estevinho, L. M. (2012). Antimicrobial activity, phenolic profile and role in the inflammation of propolis. Food and Chemical Toxicology, 50(5), 1790-1795. https://doi.org/10.1016/j.fct.2012.02.097

129. Silva, T. M. S., dos Santos, F. P., Evangelista-Rodrigues, A., da Silva, E. M. S., da Silva, G. S., de Novais, J. S., ... & Camara, C. A. (2013). Phenolic compounds, melissopalynological, physicochemical analysis and antioxidant activity of jandaíra (*Melipona subnitida*) honey. Journal of Food Composition and Analysis, 29(1), 10-18. https://doi.org/10.1016/j.jfca.2012.08.010

130. Simon A, Traynor K, Santos K, Blaser G, Bode U, Molan P. 2005. Medical honey for wound care-still the 'latest resort'? Ecam, 6, 165-173.

131. Soares, S., Lopes, K. S., Mortari, M., Oliveira, H., & Bastos, V. (2022). Antitumoral potential of Chartergellus-CP1 peptide from *Chartergellus communis* wasp venom in two different breast cancer cell lines (HR+ and triple-negative). Toxicon, 216, 148-156. https://doi.org/10.1016/j.toxicon.2022.07.004

132. Son, D. J., Lee, J. W., Lee, Y. H., Song, H. S., Lee, C. K., & Hong, J. T. (2007). Therapeutic application of anti-arthritis, pain-releasing, and anti-cancer effects of bee venom and its constituent compounds. Pharmacology & Therapeutics, 115(2), 246-270. https://doi.org/10.1016/j.pharmthera.2007.04.004

133. Song, L., Sun, S., Jin, L., Xue, L., & Fu, Y. (2014). The extracts of *Holotrichia diomphalia* larvae inhibit proliferation and induce apoptosis of cancer cells in vitro and in vivo. Molecular & Cellular Toxicology, 10, 251-259. https://doi.org/10.1007/s13273-014-0028-5

134. Song, Z. W., Liu, P., Yin, W. P., Jiang, Y. L., & Ren, Y. L. (2012). Isolation and identification of antibacterial neo-compounds from the red ants of ChangBai Mountain, *Tetramorium* sp. Bioorganic & Medicinal Chemistry Letters, 22(6), 2175-2181. https://doi.org/10.1016/j.bmcl.2012.01.112

135. Steiner, H., Hultmark, D., Engström, Å., Bennich, H., & Boman, H. G. (1981). Sequence and specificity of two antibacterial proteins involved in insect immunity. Nature, 292(5820), 246-248. https://doi.org/10.1038/292246a0

136. Surendra, N. S., Jayaram, G. N., & Reddy, M. S. (2011). Antimicrobial activity

of crude venom extracts in honeybees (*Apis cerana, Apis dorsata, Apis florea*) tested against selected pathogens. African Journal of Microbiology Research, 5(18), 2765-2772.

137. Tang, J. J., Fang, P., Xia, H. L., Tu, Z. C., Hou, B. Y., Yan, Y. M., ... & Cheng, Y. X. (2015). Constituents from the edible Chinese black ants (*Polyrhachis dives*) showing protective effect on rat mesangial cells and anti-inflammatory activity. Food Research International, 67, 163-168. https://doi.org/10.1016/j.foodres.2014.11.022

138. Tang, J. J., Zhang, L., Jiang, L. P., Di, L., Yan, Y. M., Tu, Z. C., ... & Cheng, Y. X. (2014a). Dopamine derivatives from the insect *Polyrhachis dives* as inhibitors of ROCK1/2 and stimulators of neural stem cell proliferation. Tetrahedron, 70(46), 8852-8857. https://doi.org/10.1016/j.tet.2014.09.095

139. Tang, T., Li, X., Yang, X., Yu, X., Wang, J., Liu, F., & Huang, D. (2014b). Transcriptional response of *Musca domestica* larvae to bacterial infection. PLoS ONE. https://doi.org/10.1371/journal.pone.0104867

140. Thomas, S., Andrews, A. M., Hay, N. P., & Bourgoise, S. (1999). The anti-microbial activity of maggot secretions: results of a preliminary study. Journal of Tissue Viability, 9(4), 127-132. https://doi.org/10.1016/S0965-206X(99)80032-1

141. Tonk, M., Pierrot, C., Cabezas-Cruz, A., Rahnamaeian, M., Khalife, J., & Vilcinskas, A. (2019). The Drosophila melanogaster antimicrobial peptides Mtk-1 and Mtk-2 are active against the malarial parasite *Plasmodium falciparum*. Parasitology Research, 118, 1993-1998.https://doi.org/10.1007/s00436-019-06305-x

142. Uddin, M. B., Lee, B. H., Nikapitiya, C., Kim, J. H., Kim, T. H., Lee, H. C., ... & Kim, C. J. (2016). Inhibitory effects of bee venom and its components against viruses in vitro and in vivo. Journal of Microbiology, 54, 853-866. https://doi.org/10.1007/s12275-016-6376-1

143. Umana, I. C., Daniele, C. A., & McGehee, D. S. (2013). Neuronal nicotinic receptors as analgesic targets: it's a winding road. Biochemical Pharmacology, 86(8), 1208-1214. https://doi.org/10.1016/j.bcp.2013.08.001

144. Ursic-Bedoya, R., Buchhop, J., Joy, J. B., Durvasula, R., & Lowenberger, C. (2011). Prolixicin: a novel antimicrobial peptide isolated from *Rhodnius prolixus* with differential activity against bacteria and Trypanosoma cruzi. Insect Molecular Biology, 20(6), 775-786.

145. Ushakova, N. A., Brodsky, E. S., Tikhonova, O. V., Dontsov, A. E., Marsova, M. V., Shelepchikov, A. A., & Bastrakov, A. I. (2021). Novel extract from beetle *Ulomoides dermestoides*: a study of composition and antioxidant activity. Antioxidants, 10(7), 1055. https://doi.org/10.3390/antiox10071055

146. Velásquez, B. D., & Montenegro Gómez, S. P. (2017). Actividad antimicrobiana de extractos etanólicos de propóleos obtenidos de abejas *Apis mellifera*. Revista de Investigación Agraria y Ambiental, 8(1), 185–193. https://doi.org/10.22490/21456453.1848

147. Verma, A. K., & Prasad, S. B. (2013). Antitumor effect of blister beetles: An ethno-medicinal practice in Karbi community and its experimental evaluation against a murine malignant tumor model. Journal of Ethnopharmacology, 148(3), 869-879. https://doi.org/10.1016/j.jep.2013.05.032

148. Wahrendorf, M. S., & Wink, M. (2006). Pharmacologically active natural products in the defence secretion of *Palembus ocularis* (Tenebrionidae, Coleoptera). Journal of Ethnopharmacology, 106(1), 51-56. https://doi.org/10.1016/j.jep.2005.12.007

149. Wang, H. Y., Wang, Y. J., Zhou, L. X., Zhu, L., & Zhang, Y. Q. (2012a). Isolation and bioactivities of a non-sericin component from cocoon shell silk sericin of the silkworm *Bombyx mori*. Food & Function, 3(2), 150-158. https://doi.org/10.1039/C1FO10148J

150. Wang, Y., Yan, H., Wang, Y., Yang, H., Wei, L., Xiao, Y., ... & Liu, R. (2012b). Proteomics and transcriptome analysis coupled with pharmacological test reveals the diversity of anti-thrombosis proteins from the medicinal insect, *Eupolyphaga sinensis*. Insect Biochemistry and Molecular Biology, 42(8), 537-544. https://doi.org/10.1016/j.ibmb.2012.04.001

151. Wehbe, R., Frangieh, J., Rima, M., El Obeid, D., Sabatier, J. M., & Fajloun, Z. (2019). Bee venom: Overview of main compounds and bioactivities for therapeutic interests. Molecules, 24(16), 2997. https://doi.org/10.3390/molecules24162997

152. Whitman, D. W., Andrés, M. F., Martínez-Díaz, R. A., Ibáñez-Escribano, A., Olmeda, A. S., & González-Coloma, A. (2019). Antiparasitic properties of cantharidin and the blister beetle *Berberomeloe majalis* (Coleoptera: Meloidae). Toxins, 11(4), 234. https://doi.org/10.3390/toxins11040234

153. Wu, Q., Patočka, J., & Kuča, K. (2018). Insect antimicrobial peptides, a mini review. Toxins, 10(11), 461. https://doi.org/10.3390%2Ftoxins10110461

154. Xie, X., Shen, W., Zhou, Y., Ma, L., Xu, D., Ding, J., ... & Zhou, C. (2020). Characterization of a polysaccharide from *Eupolyphaga sinensis* Walker and its effective antitumor activity via lymphocyte activation. International Journal of Biological Macromolecules, 162, 31-42. https://doi.org/10.1016/j.ijbiomac.2020.06.120

155. Xu, X., Liu, W., Li, W., & Liu, S. (2016). Anticoagulant activity of crude extract of *Holotrichia diomphalia* larvae. Journal of Ethnopharmacology, 177, 28-34. https://doi.org/10.1016/j.jep.2015.11.015

156. Yan, Y. M., Ai, J., Shi, Y. N., Zuo, Z. L., Hou, B., Luo, J., & Cheng, Y. X. (2014). (±)-Aspongamide A, an N-acetyldopamine trimer isolated from the insect *Aspongopus chinensis*, is an inhibitor of p-Smad3. Organic letters, 16(2), 532-535. https://doi.org/10.1021/ol403409v

157. Yan, Y. M., Li, L. J., Qin, X. C., Lu, Q., Tu, Z. C., & Cheng, Y. X. (2015). Compounds from the insect Blaps japanensis with COX-1 and COX-2 inhibitory activities. Bioorganic & Medicinal Chemistry Letters, 25(12), 2469-2472. htt-

ps://doi.org/10.1016/j.bmcl.2015.04.085

158. Yan, Y. M., Luo, Q., Li, J. J., Tu, Z. C., & Cheng, Y. X. (2023). Novel spirooxindole alkaloid derivatives from the medicinal insect *Blaps japanensis* and their biological evaluation. Bioorganic Chemistry, 141, 106845. https://doi.org/10.1016/j.bioorg.2023.106845

159. Yin, H. Q., Wang, B., Zhang, J. D., Lin, H. Q., Qiao, Y., Wang, R., & Liu, F. Y. (2008). Effect of traditional Chinese medicine Shu-Mai-Tang on attenuating TNFα-induced myocardial fibrosis in myocardial ischemia rats. Journal of Ethnopharmacology, 118(1), 133-139. https://doi.org/10.1016/j.jep.2008.03.022

160. Yin, W., Zhao, C., Liao, C., Trowell, S., & Rickards, R. W. (2013). Preparative isolation of novel antimicrobial compounds from Pergidae sp. by reversed-phase high-performance liquid chromatography. Chemistry of Natural Compounds, 49, 41-45. https://doi.org/10.1007/s10600-013-0501-8

161. Yun, J., Hwang, J. S., & Lee, D. G. (2017). The antifungal activity of the peptide, periplanetasin-2, derived from American cockroach *Periplaneta americana*. Biochemical Journal, 474(17), 3027–3043. https://doi.org/10.1042/BCJ20170461

162. Zeinalpour, Y., Kabiri, M., Rahimi, R., Khalilzadeh, R., & Karimi, M. (2021). Effect of medicinal syrup made from silkworm cocoon on mixed anxiety-depression disorder: a triple-blind randomized clinical trial. Iranian Red Crescent Medical Journal, 23(12). https://doi.org/10.32592/ircmj.2021.23.12.1345

163. Zhang X, Lin C C, Chan W K N, Liu K L, Yang Z J and Zhang H Q. (2017). Augmented anticancer effects of Cantharidine with liposomal encapsulation: In vitro and in vivo evaluation. Molecules, 22, 1052-1058.

164. Zhang, E., Ji, X., Ouyang, F., Lei, Y., Deng, S., Rong, H., ... & Shen, H. (2023). A minireview of the medicinal and edible insects from the traditional Chinese medicine (TCM). Frontiers in Pharmacology, 14, 1125600. https://doi.org/10.3389/fphar.2023.1125600

165. Zhang, H., Wei, L., Zhang, Z., Liu, S., Zhao, G., Zhang, J., & Hu, Y. (2013). Protective effect of *Periplaneta americana* extract on intestinal mucosal barrier function in patients with sepsis. Journal of Traditional Chinese Medicine, 33(1), 70-73. https://doi.org/10.1016/S0254-6272(13)60103-X

166. Zhao, W. Y., Dong, B. R., & Zhou, Y. (2005). In vitro antimicrobial activity of defensins from rabbit neutrophils against *Pseudomonas aeruginosa* and its multiple-drug-resistance strains. Sichuan da xue xue bao. Yi xue ban= Journal of Sichuan University. Medical Science Edition, 36(1), 83-85.

167. Zheng, T., Ming, Y. Y., Tu, Z. C., Xu, F. R., & Cheng, Y. X. (2018). Two new compounds from medicinal insect *Blaps japanensis* and their biological evaluation. Natural Product Communications, 13(2), 1934578X1801300210.

168. Zhou, L. F., Wu, J., Li, S., Li, Q., Jin, L. P., Yin, C. P., & Zhang, Y. L. (2021). Antibacterial potential of termite-associated Streptomyces spp. ACS omega, 6(6), 4329-4334. https://doi.org/10.1021/acsomega.0c05580

169. Zhu, H. J., Yan, Y. M., Tu, Z. C., Luo, J. F., Liang, R., Yang, T. H., ... & Wang, S. M. (2016). Compounds from *Polyphaga plancyi* and their inhibitory activities against JAK3 and DDR1 kinases. Fitoterapia, 114, 163-167. https://doi.org/10.1016/j.fitote.2016.09.005