International Journal for Multidisciplinary Research (IJFMR)



E-ISSN: 2582-2160 • Website: <u>www.ijfmr.com</u> • Email: editor@ijfmr.com

# **Comprehensive Insights into the Versatile Benefits of Hop Extracts**

# Narendra Kumar S<sup>1</sup>, Anushri Acharya<sup>2</sup>, Riddhi Daga<sup>3</sup>, Sashwathi V<sup>4</sup>, Siddhi Daga<sup>5</sup>, Varshini Ganesan Selvi<sup>6</sup>

<sup>1</sup>Assistant Professor, Department of Biotechnology, RVCE <sup>2,3,4,5,6</sup>B.E. Students, Department of Biotechnology, RVCE

### Abstract:

Humulus lupulus L., commonly known as hops, is a plant widely recognized for its use in brewing beer. This paper explores the phylogenetic origins of hops, its evolution, and the global trends in hop production. The female inflorescences, or cones, of the hop plant contain biologically active compounds such as phloroglucinol derivatives, polyphenolic compounds, and essential oils, which contribute to the bitter flavour, characteristic aroma, and antimicrobial properties of beer. These compounds also exhibit antioxidant, antimicrobial, antifungal, anti-inflammatory, anti-allergic, and cognitive-enhancing properties. Notably, xanthohumol, a prenylated flavonoid found in hops, has shown anticancer effects against various types of cancer. Hops extracts have been used as natural preservatives in food products, and their application extends to cosmetics and medicine. Overall, hop extracts offer a promising source of bioactive compounds with potential applications across various industries.

**Keywords:** Hops, beer, antimicrobial, antioxidant, antifungal, xanthohumol, anticancer, antiinflammatory, antiallergic, cognitive

#### Introduction:

*Humulus lupulus L.* belongs to the Cannabaceae family and is a widely grown perennial. The flowers are commonly known as hops and are mainly used as a key ingredient in brewing beer[1,2]. It has been used for medicinal and industrial purposes[13]. Phylogenetic analysis has suggested that the hop species migrated to Europe from its probable origin China and then drifted to North America. There is a widespread acknowledgment that the evolution of hop lineages from Europe to North America has taken a million years, likely diverged from their common ancestor Central Asia [9,10]. The hop production worldwide has seen a positive trend since the 1970s. Recent statistical reports suggest that the top producing countries of hops are the United States of America, Germany and Ethiopia[14].

*H.lupulus* is dioecious i.e., it has male and female reproductive organs in separate plants [1]. The bitter flavor, characteristic hoppy aroma, antimicrobial property against Gram-positive bacterial species such as Lactobacillus, Streptococcus, Staphylococcus, Micrococcus, Bacillus, and Pediococcus and its antioxidant activity of the beer is due to the female inflorescences (cones). This is the result of cones having lupulin glands rich in phloroglucinol derivatives, polyphenolic compounds and essential oils[2,3,5,11]. These biologically active secondary metabolites also contribute to preserving and extending the shelf life of the beer[3].



In addition to its antimicrobial properties, the hop extract can be used as an antifungal agent for bread making [6] and can be used as a biofungicide against damaging pathogens[4]. It has been identified that the essential acids found in the resin of hops, humulones ( $\alpha$ -acids), and lupulones( $\beta$ -acids), contribute to the antibacterial, antioxidant, and multiple antimicrobial properties of hop extracts [7,11,15]. Experimental results suggested that the hop seed extract showed remarkable antioxidant and antimicrobial activity highlighting the prospect of hop seeds being utilized in the food and pharma sectors[2]. Studies suggest that the antioxidant property of hop-leaf extracts varies depending on the concentration of secondary metabolites[8]. Findings of a study suggested that the prenylated flavonoid derivatives of hop plants have therapeutic potential in fighting cancer, highlighting its potential as an anti-cancer agent[12].

### **Properties:**

### Anticancer

Xanthohumol is the most prevalent prenylated flavonoid in hops. It exhibits anticancer properties [20]. This essential flavonoid is found in the hop plant's female inflorescences [21]. Given its higher lipophilicity compared to other beer polyphenols, xanthohumol is suggested to have greater bioactivity [22]. Cancer is a deadly and abnormal disease. Chemopreventive options for the treatment of cancer encompass a wide range of agents that hinder the inception, development, and advanced stages of carcinogenesis [22]. Xanthohumol has been proven to prevent both metastasis and carcinogenesis in several types of cancer [20].

# **Respiratory System Cancers-**

#### Non-small Cell Lung Cancer-

Lung cancer ranks as the primary cause of global cancer occurrences and mortality [26]. Two main lung cancer categories are Small and Non-small cell lung cancer [23]. Nonetheless, approximately 85% of lung cancer patients each year are NSCLC patients. Squamous Cell Carcinoma and Adenocarcinoma are the two subtypes of NSCLC [23]. According to research studies, Xanthohumol (Xn) has demonstrated anti-cancer properties against NSCLC adenocarcinoma, specifically A549 cells. The extracellular-signal-regulated kinase (ERK1/2) pathway is a key signalling pathway involved in the proliferation and differentiation of cells [20]. Xn interferes with the Ras/Raf/MEK/ERK1/2 pathway, inducing apoptosis and hence inhibiting cell growth. Furthermore, by suppressing the activity of mitochondrial complex I, Xn stimulates the overproduction of ROS (reactive oxygen species) ultimately causing death in A549 cells. Further, in vivo studies are necessary to validate these findings and explore any possible therapeutic implications, even if these results point to possible pathways for Xn's anti-cancer effects in NSCLC. [20].

# Hepatocellular Carcinoma-

The third most common cause of cancer-related fatalities worldwide is hepatocellular carcinoma. Around 70% of patients have advanced illness when they first arrive, frequently with concurrent cirrhosis. As a result, 50–70% of these individuals will survive for five years [24]. Liver transplantation can be an option but surgery might not be as effective [24]. In pancreatic cells, following Xanthohumol treatment, cell multiplication is reduced. This occurs when Xn blocks the Notch1 signalling pathway, which in turn causes increased cell death. Notch1 is essential for the survival of cancer cells, but XN lowers its levels as well as those of other proteins that promote cancer cell growth. Additionally, XN also lessens the activity of the promoter of Notch1, which functions as a switch to activate Notch1. Notch1 decrease is



essential for Xn's action in pancreatic cancer cells, as evidenced by the fact that overexpression of active Notch1 reverses Xanthohumol-induced growth suppression. The results emphasise Xn's potential as a targeted treatment for pancreatic cancer [25].

# Antimicrobial

Hops extracts are weak acids which inhibit the growth of bacteria in dissociated forms. They do not exhibit any toxic effect on the human body. Proteus mirabilis, Escherichia coli, Yersinia enterocolitica, Salmonella enteritidis, S. typhimurium, Klebsiella oxytoca Enterobacteriaceae, Enterococci, and anaerobic bacteria are only a few of the pathogens against which hop essential oil has been demonstrated to have antibacterial action. Its impact on oral microbiota, both by itself and in conjunction with other antimicrobials, has, however, only been the subject of a small number of investigations.

The ethanol extract from hop infructescence has been shown to have strong antibacterial activity against S. aureus (MSSA and MRSA), S. gordonii, S. mutans, S. sobrinus, and S. salivarius. It also shows a synergistic effect with several conventional antimicrobials.

Hop extracts that include  $\beta$ -acid have demonstrated strong antibacterial action against Gram-positive bacteria both in vitro and in a practical application for food preservation.

To enhance the safety and shelf life of fresh products, antimicrobial hop extracts can be employed as natural preservatives in food applications.

According to reports, the growth of bacteria such as Salmonella enteritidis, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus was hindered by acids from a supercritical CO2 hop extract. This resulted in an inhibition of the active transport of sugars and amino acids, which in turn caused disruptions to cellular respiration, bacterial replication, transcription, and translation [17].

#### Antifungal

Hops have garnered a lot of attention because of its potential use in food and crop protection, either as a food preservative or as a defence against pest insects and diseases. Hop metabolites or essential oil have been linked to toxic or repellant properties against a variety of pests, including Drosophila suzukii, Sitophilus granarius, and Varroa destructor. Studies on hop's antifungal or anti-oomycete properties are becoming more and more prevalent. It was discovered that cone essential oil was effective against Zymoseptoria tritici, a wheat disease. Hop extracts have also been shown to effectively suppress the mycelial growth and spore germination of a variety of diseases, such as Aspergillus species, Penicillium species, Fusarium species, Botrytis cinerea, and Phytophthora infestans.

Hop cones and their metabolites, which are currently extensively employed in the brewing of beer, are typically credited with these biological characteristics [18].

In an investigation into the antifungal properties of hop bitter resins and related compounds, we discovered that Trichophyton spp., a pathogenic fungus that affects humans, as well as other microorganisms, were inhibited in their growth by humulones (a acids), lupulones (f3 acids), and related compounds with distinct side chains [19].

#### Antioxidant

An antioxidant is a compound that slows down or prevents the oxidation of a substance, thereby reducing oxidative stress[27]. Beer's antioxidant activity is greatly enhanced by phenolic acids and are known to be potent antioxidants, metal chelators, and free radical scavengers[35]. These are typically categorised into



enzymatic types (such as superoxide dismutase, glutathione reductase, catalase, peroxidase, etc.) and nonenzymatic types (such as glutathione, vitamins)[29]. The primary contributors to the antioxidant benefits include specific chemicals like xanthohumol, isoxanthohumol, and the resin produced by lupulin glands, which contain bitter acids. Recent studies have uncovered an upregulation of transcription factors and structural genes within lupulin glands after leaf development, leading to an increase in the levels of bitter acids and prenylflavonoids in these glands[27].

In one of the researchers, scientists had employed 14 hop genotypes and isolated the essential hop components (xanthohumol, beta-acids, and alpha-acids) in order to use comparative analysis to assess the antioxidant activity[<u>31</u>].

Three antioxidant assays—FRAP, ORAC, and ImmunoAssay—were used for a thorough analysis.

- FRAP (ferric reducing antioxidant power) assay: This method is used in the detection of antioxidant capacity of nutrient supplements or food(ex.hops) containing polyphenols [30].
- ORAC (Oxygen Radical Absorbance Capacity): The antioxidant capacity and reactive oxygen species(ROS) of a substance is determined[32].
- ImmunoAssay: In a cellular environment that is mimicked, this assay is utilised to evaluate the antioxidant potential[35].

The results portrayed the antioxidant activity of different hop genotypes varied significantly, and this variation might be linked to the levels of alpha-acids, beta-acids, and other bioactive compounds present in each genotype, suggesting that certain genotypes could be more valuable sources of natural antioxidants for various industries [31]. Based on parameters under a study by(Codina-Torrella et al.), the BSG(Brewer's spent grain) and BL(Beer Lees) samples are clearly separated by PCA(Principal component analysis)analysis. These findings imply that the types of malts and hops used during brewing have a significant impact on the TPC, antioxidant activity, and individual phenolic content of the various samples. Incorporating various hop cultivars produces varying antioxidant activity[36].

Cold methanol extraction showed higher reducing activity than hot water extraction for all hop varieties tested. Saaz hops had the highest activity, while hybrid varieties had lower activity. Methanol extracts had consistent reducing activity across hop types, with higher polyphenol content but lower anthocyanogen levels compared to water extracts. Antioxidant activity is closely correlated with polyphenol content in both extract types.[33].

(Codina-Torrella et al.) examined the antioxidant properties of Brewer's spent grain (BSG) and brewer's spent hops (BSH) extracts, key solid by-products in brewing. Various extraction techniques were assessed, revealing that using an alkaline solvent (NaOH) at 1.45% concentration and 80°C temperature yielded optimal results. Higher total polyphenol content (TPC) corresponded with increased antioxidant activity, as evaluated through TEAC and ORAC methods using ABTS and APPH radicals. Industrial and artisanal BSG extracts displayed the highest TPC values compared to BSH extracts. Ferulic acid emerged as a significant polyphenol in BSG extracts, positively correlating with TPC and radical scavenging activity. Emulsions containing 10% BSG extract exhibited a 97% reduction in oxidation products over 14 days, while PLA films with 1% BSG extract reduced oxidation by 35%, indicating their potential as antioxidants in food preservation. In summary, the research underscores the potential of BSG and BSH extracts as natural antioxidants with promising applications in the food industry.[34].

#### Anti-inflammatory

Osteoarthritis is a degenerative joint disease characterised by inflammation. This is due to cartilage de-



gradation caused by ageing, hereditary or by trauma where current treatments offer limited relief and can have side effects. But scientists were able to detect that Hop extracts contain compounds with known anti-inflammatory properties, offering a potential natural treatment option for Osteoarthritis[28,68].

Research has shown that liposomes offer a promising avenue for delivering medications to chondrocytes, the cells composing cartilage. Consequently, investigations were conducted to assess whether hop extract enclosed within nanoliposomes could serve as a therapeutic agent to reduce inflammation in human chondrocytes, particularly targeting osteoarthritis or joint inflammation. The study utilised a methanol-based method to extract hop leaf components, isolating soluble molecules which were subsequently synthesised alone into nanoliposomes. Different dosages of these formulations were tested on chondrocytes, revealing that all except the highest doses were safe for the cells. Notably, both the hop extract and empty nanoliposomes reduced the production of inflammatory compounds, with the combination within nanoliposomes exhibiting an even stronger effect. This demonstrates that encapsulating the extract enhances its anti-inflammatory properties, suggesting potential for future therapies targeting joint inflammation.[28].

In addition, Xanthohumol showed inhibitory effects against a number of viral infections, such as SARS-CoV-2 and other serious illnesses brought on by alpha- or beta-coronaviruses. It was anticipated that Xanthohumol could improve clinical progression and prognosis in severely ill COVID-19 patients requiring mechanical ventilation because of its antiviral and anti-inflammatory properties[45].

These findings highlight the therapeutic potential of hop-derived compounds in both joint inflammation and viral infections.

# Anti-allergic

Research suggesting anti-allergic effects of *Humulus Iupulus* are quite limited, more studies are needed to establish a conclusive understanding of hops' role in allergy management. Hops contain various compounds, such as polyphenols and flavonoids, which possess antioxidant and anti-inflammatory properties. These properties may contribute to potential anti-allergic effects. Allergies often involve an inflammatory response, and substances with anti-inflammatory properties could potentially help alleviate symptoms.

Several studies have explored the potential anti-allergic properties of hops extract, revealing inhibitory effects on allergic reactions. For instance, compounds within hops have demonstrated the ability to hinder histamine release, a critical mediator in allergic responses. One study focused on the impact of hop extract on histamine release from rat peritoneal mast cells and human basophilic KU812 cells. Both hop water extract (HWE) and the XAD-4 50% methanol fraction of HWE (MFH) showed inhibitory effects on histamine release from rat mast cells induced by compound 48/80, with concentrations of 100 and 10  $\mu$ g/ml, respectively. Additionally, the study assessed the effects of hop extracts on antigen-induced nasal rubbing and sneezing in sensitized BALB/c mice. HWE significantly inhibited nasal rubbing and sneezing is a dose of 500 mg/kg, and the effects of both extracts were more pronounced with repeated administration [37].

Another similar study investigated the anti-allergic effect of HWE on human basophilic KU812 cells induced by calcium ionophore A23187. The 50% methanol-eluted fraction from HWE, known as MFH, demonstrated a stronger inhibitory effect compared to HWE. MFH contained quercetin glycosides and kaempferol glycosides, with quercetin glycosides identified as key contributors to the inhibition of histamine release. In HWE, most quercetin existed in glycoside form, with a content of approximately 200



 $\mu$ g/g determined through acid hydrolysis. Both HWE and MFH significantly inhibited protein kinase C, a crucial player in the degranulation of chemical mediators, suggesting their potential to inhibit type-I allergic reactions [38].

Furthermore, the anti-allergic properties of HWE were explored in another study, evaluating Evans blue leakage induced by compound 48/80 stimulation in ICR mice and histamine release in ovalbumin (OVA)-sensitized BALB/c mice. Oral administration of HWE effectively inhibited vascular permeability and histamine release. Notably, HWE did not impact total or antigen-specific immunoglobulin E (IgE) production in OVA-sensitized mice. These findings suggest that HWE exerts an anti-allergic effect by specifically inhibiting the release of chemical mediators from mast cells and basophils [39].

# Cognitive

Several studies indicate potential cognitive benefits associated with extracts derived from hops. In a specific investigation targeting matured hop bitter acids (MHBAs), the study aimed to explore their impact on mental fatigue, mood states, and human cognition. The findings suggested that individuals consuming MHBAs at a dosage of 35 mg per day exhibited enhanced verbal memory retrieval in the Verbal Fluency Test (VFT) and improved inhibition of executive functions in the Stroop test when compared to the placebo group. Moreover, MHBAs consumption was associated with improvements in mental fatigue and mood states in comparison to the placebo group [40].

A recent review delves into the current findings regarding the effects and underlying mechanisms of bitter acids derived from hops, particularly those found in beer. The main bitter components, Iso- $\alpha$ -acids (IAAs), have been identified to enhance hippocampus-dependent memory and cognitive function associated with the prefrontal cortex through the activation of dopamine neurotransmission. Another category, Matured hop bitter acids (MHBAs), characterised by oxidised components with  $\beta$ -carbonyl moieties originating from aged hops, has also demonstrated the ability to improve memory functions via mechanisms mediated by norepinephrine neurotransmission. Notably, both IAAs and MHBAs exhibit effects that are diminished by vagotomy, indicating that these bitter acids enhance cognitive function through vagus nerve stimulation. Additionally, supplementation with IAAs has shown promise in mitigating neuroinflammation and cognitive impairments in various rodent models of neurodegenerative conditions, such as Alzheimer's disease. The review suggests that daily supplementation with hop-derived bitter acids, for instance, 35 mg/day of MHBAs, could be considered a safe and effective strategy for stimulating the vagus nerve and thereby enhancing cognitive function[41]. A separate study illustrated that the intake of Iso- $\alpha$ -acids (IAA), the hop-derived compound responsible for the bitter taste in beer, diminishes microglial activation and mitigates age-related memory decline in elderly mice. Inflammation within the brain is linked to several neurological disorders, including dementia, depression, and chronic fatigue. The consumption of IAA could potentially aid in the management or even reversal of inflammation-related conditions such as cognitive deterioration[42].

# Methods of isolation and characterization of chemical compounds found in hops:

Traditionally, distillation has been employed to extract essential oils from hops. One approach involved passing steam through finely ground hops, followed by the extraction of the oil from the condensate using ether. This distillation process required approximately 4 hours to extract essential oils from a 100g sample of coarsely ground hops in 3 litres of water. The impact of shortening the duration of the typical steam



distillation process is being investigated. The steam distilled oils were analysed using gas-liquid chromatography[3,43].

In 1969, Freiherr Von Horst and Kellner were granted US Patent 3436319 [44] A for their innovation titled "Thin Layer Steam Distillation of Hop Oil Extract." This patented process is characterised as continuous and was designed for the extraction of essential oil from hop preparations. The key highlight of this method lies in its ability to fully recover hop oil from the steam distillate while concurrently generating a residual hop extract containing other components of the extract in a substantially unaltered state[3].

Liquid carbon dioxide (LCO2) has been utilised in hop extraction, with temperature and pressure ranges reported in 1966 for this process. However, the stability of the resulting extract was found to be insufficient, undergoing chemical changes during storage. An alternative method introduced in 1977 involved supercritical CO2 extraction at higher temperatures and pressures, yielding a high-quality hop extract. Variations included extraction below the critical temperature, with added ethanol as a solvent to LCO2, providing hop extracts free of hard resins and polyphenolic materials. Supercritical fluid extraction, specifically using CO2, has become the preferred industrial method for producing brewery ingredients, offering the advantage of being solvent-free. While liquid-liquid and solid-liquid extractions remain commonly used isolation procedures, solid phase microextraction is applied for characterising aromatic properties, and solid-phase extraction is employed for the isolation of nonvolatile compounds. Accelerated solvent extraction is also utilised for bitter acid extraction from hops. Despite the efficiency of supercritical fluid extraction in obtaining bitter acid extracts for brewing, a drawback is the retention of a group of biologically active prenylflavonoids (Several studies have focused on prenylflavonoids as potential cancer preventing compounds) in the plant material under the extraction conditions. A novel method developed by Mertens and Pascal involves the extraction and dissolution of hop acids, including  $\alpha$ -acids, iso- $\alpha$ -acids,  $\beta$ -acids, and their derivatives in aqueous media. This method forms quaternary ammonium salts of hop acids with quaternary ammonium compounds, enhancing their solubility in acidic aqueous mediums compared to the free acid form. This innovative approach not only improves the utilisation of hop acids but also finds application in the beer brewing process, contributing to enhanced flavour profiles and bitterness in the final product[3].



Figure 1. Structures of phenolic compounds and bitter acids[67]



Ultrasonic extraction can also be performed in order to obtain hops extract. One such study reveals that the extraction process was performed using mixtures of acetone or methanol and water. The hop strobilus along with extraction mixture is left in an ultrasonic bath at room temperature for 30 min. This was then subjected to filtration and stored in dark[67].

GC-MS technique has been put forth as a valuable analytical tool to provide precise and accurate qualitative and quantitative data of various constituents present in a liquid mixture. Beer has been analysed for various phenolic compounds utilising different detection methods, including coulometric, electrochemical, and photodiode arrays, as well as ultraviolet–visible spectrophotometry and low-resolution mass spectrometry. Over two hundred compounds, including essential oils, prenylflavonoids, and bitter acids commonly categorised as  $\alpha$ -acids and  $\beta$ -acids, can be identified and subsequently isolated through such detection and separation processes. Main components of hop essential oils such as sesquiterpenes and monoterpenes comprising humulene, caryophyllene, farnesene, bisabolene and elemene skeletons are commonly determined by the evaluation of the total volatile content by GC–MS[3,46].

The  $\alpha$ -,  $\beta$ -, and iso- $\alpha$ -acids exhibit significant oxidising potential. It is crucial to develop direct preparative techniques (excluding other hop constituents like polyphenols, lipids, waxes, and polysaccharides) to isolate fractions of oxidised bitter acids. This is essential not only for their impact on beer properties but also considering their potential health benefits. The quantitative analysis of matured hop bitter acids (MHBA), predominantly comprising oxides derived from  $\alpha$ -acids, is conducted using a high-performance liquid chromatography (HPLC) method frequently coupled with atmospheric pressure ionisation tandem mass spectrometry (APCI-MS-MS) or negative electrospray ionisation mass spectrometry[3,47]. Utilising a LTQ-Orbitrap high-resolution mass spectrometer, a range of phenolic compounds, including hexosides, caffeic acid-O-hexoside, kaempferol-O-hexoside, sinapic acid-O-hexoside, catechin-O-dihexoside, and apigenin-C-hexoside, were efficiently identified in beer extracts[3,48].

Techniques like capillary electrophoresis are also employed in determination of bioactive compounds present in hops extract. The separation is facilitated by a fused silica capillary[67].

The antioxidative activities of the hops extracts are assessed using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) scavenging assay, while the total phenolics are measured through the reduction of phosphotungstic acid and phosphomolybdic  $acid[\underline{3},49]$ . Other antioxidant assays used to identify the activity include the hydrogen peroxide scavenging (HPS) assay, the linoleic acid (LA) assay, oxygen radical absorbance capacity (ORAC) assay etc[3,50].

Hops extract are also known for their potential antimicrobial activity. They have proven to inhibit the growth of certain gram positive bacteria. Bioactive compounds present in hops extract such as lupulone, humulone, isohumulone, and humulinic acid have shown high antimicrobial activity against bacteria like *Bacillus subtilis* [3,51]. A recent study reveals that seven flavonoids, including two natural compounds ( $\alpha$ , $\beta$ -dihydro xanthohumol and 8-prenylnaringenin), exhibited noteworthy activity against both methicillin-sensitive and resistant strains of Staphylococcus aureus, as well as Staphylococcus epidermidis[3,52]. The most extensively used techniques to investigate the antibacterial activity of natural substances and plant extracts are diffusion methods which are based on the use of discs or holes as reservoirs containing solutions of substances to be examined according to Brantner and Grein[3,53].



#### **Conclusion:**

Based on the paper's discussion of hop extracts' characteristics, it can be said that there may be a wide range of uses for them in different industries. Research indicates that hop extracts are a great way to extend the shelf life of numerous products, including fresh meat, by preventing oxidative and microbiological deterioration [54,55]. Several papers highlight its importance in food preservation industries [<u>16</u>,58,].

Their application in the bread-making industry and its long-term stability is attributed to its antifungal activity [6]. Due to its antifungal properties, hop extract is utilised in hair cosmetic products as well. It makes hair less brittle, enriches it, makes it stronger, and stops hair loss [57]. When scientists created shower gels using supercritical CO2 samples from hop cones, the outcomes demonstrated that the biologically active compounds in the extracts improved the shower gels' ability to moisturise the skin [56,57]. Studies have reported the application of  $\alpha$ -acids and  $\beta$ -acids as potential drugs against bone diseases such as osteoporosis [65, 66].

Additionally, the tea industry is breeding and testing hops. Due to its decreased bitterness and growing popularity as medicinal teas with antifungal and anti-inflammatory properties, reduced alpha-acid variants are popular in the herbal tea industry [59]. The essential oils present in hops have also been researched as affordable, environmentally beneficial insecticides and repellents [59,60,61].

Additional bioactive substances are being investigated for their potential uses in medicine and health, such as improving insulin-signalling channels, reducing obesity, minimising the risk of metabolic syndrome and multiple forms of cancer, alleviation of some menopausal symptoms [59, 62, 63, 64, 65].

The paper provides a comprehensive overview of the various properties and applications of hop extracts, highlighting their potential in multiple industries, including food preservation, cosmetics, and medicine. The key findings include the antimicrobial, antifungal, antioxidant, anti-inflammatory, anti-allergic, and cognitive-enhancing properties of hop extracts, particularly focusing on the bioactive compounds such as xanthohumol, humulones, and lupulones. The antimicrobial properties of hop extracts make them effective natural preservatives in food products, while their antifungal properties are beneficial in bread making and hair care products. The antioxidant effects of hop extracts are attributed to compounds like xanthohumol, which can scavenge reactive oxygen species, potentially aiding in preventing oxidative damage in cells and tissues. Additionally, the anti-inflammatory properties of hop extracts show promise in managing conditions like osteoarthritis and neuroinflammation.

Moreover, the paper discusses the cognitive benefits of hop extracts, particularly their potential in improving memory and cognitive function, as well as their application in managing neurodegenerative conditions. The anti-allergic effects of hop extracts are also explored, indicating their potential in alleviating allergic reactions. In conclusion, hop extracts represent a valuable source of bioactive compounds with diverse applications across various industries.

#### **References:**

- Keskin Ş, Şirin Y, Çakir HE, Keskin M. An investigation of Humulus lupulus L.: Phenolic composition, antioxidant capacity and inhibition properties of clinically important enzymes. South African Journal of Botany [Internet]. 2019 Jan [cited 2019 Oct 14];120:170–4.
- 2. Available from: https://www.sciencedirect.com/science/article/pii/S0254629918305830
- 3. Alonso-Esteban, J. I., Pinela, J., Barros, L., Ćirić, A., Sokóvić, M., Calhelha, R. C., Torija-Isasa, E., De Cortes Sánchez-Mata, M., & Ferreira, I. C. (2019). Phenolic composition and antioxidant,



antimicrobial and cytotoxic properties of hop (Humulus lupulus L.) Seeds. Industrial Crops and Products, 134, 154–159.

- Knez Hrnčič M, Španinger E, Košir IJ, Knez Ž, Bren U. Hop Compounds: Extraction Techniques, Chemical Analyses, Antioxidative, Antimicrobial, and Anticarcinogenic Effects. Nutrients. 2019 Jan 24;11(2):257.
- 5. Bocquet L, Rivière C, Dermont C, Samaillie J, Hilbert JL, Halama P, et al. Antifungal activity of hop extracts and compounds against the wheat pathogen Zymoseptoria tritici. Industrial Crops and Products. 2018 Oct;122:290–7.
- 6. Kramer B, Thielmann J, Hickisch A, Muranyi P, Wunderlich J, Hauser C. Antimicrobial activity of hop extracts against foodborne pathogens for meat applications. Journal of Applied Microbiology. 2015 Jan 9;118(3):648–57.
- Nionelli L, Pontonio E, Gobbetti M, Rizzello CG. Use of hop extract as antifungal ingredient for bread making and selection of autochthonous resistant starters for sourdough fermentation. International Journal of Food Microbiology. 2018 Feb;266:173–82.
- 8. Li Y, Dalabasmaz S, Gensberger-Reigl S, Heymich ML, Krofta K, Pischetsrieder M. Identification of colupulone and lupulone as the main contributors to the antibacterial activity of hop extracts using activity-guided fractionation and metabolome analysis. Food Res Int. 2023 Jul;169:11283-2.
- Ceh B, Kac M, Košir I, Abram V. Relationships between Xanthohumol and Polyphenol Content in Hop Leaves and Hop Cones with Regard to Water Supply and Cultivar. International Journal of Molecular Sciences. 2007 Sep 12;8(9):989–1000.
- 10. Murakami A, Darby P, Javornik B, Pais MSS, Seigner E, Lutz A, et al. Molecular phylogeny of wild Hops, Humulus lupulus L. Heredity [Internet]. 2006 Jul 1 [cited 2023 Mar 24];97(1):66–74.
- McCallum JL, Nabuurs MH, Gallant ST, Kirby CW, Mills AAS. Phytochemical Characterization of Wild Hops (Humulus lupulus ssp. lupuloides) Germplasm Resources From the Maritimes Region of Canada. Frontiers in Plant Science. 2019 Dec 11;10.
- 12. Karabín M, Hudcová T, Jelínek L, Dostálek P. Biologically Active Compounds from Hops and Prospects for Their Use. Comprehensive Reviews in Food Science and Food Safety. 2016 Mar 1;15(3):542–67.
- 13. Miranda CL, Stevens JF, Helmrich A, Henderson MC, Rodriguez RJ, Yang YH ., et al. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (Humulus lupulus) in human cancer cell lines. Food and Chemical Toxicology. 1999 Apr;37(4):271–85.
- 14. Rossini F, Virga G, Loreti P, Iacuzzi N, Ruggeri R, Provenzano ME. Hops (Humulus lupulus L.) as a Novel Multipurpose Crop for the Mediterranean Region of Europe: Challenges and Opportunities of Their Cultivation. Agriculture. 2021 May 24;11(6):484.
- 15. FAOSTAT. Available online: <u>http://www.fao.org/faostat/en/#data/QC/visualize</u>
- Bassolé IHN, Juliani HR. Essential Oils in Combination and Their Antimicrobial Properties. Molecules. 2012 Apr 2;17(4):3989–4006.
- Kramer B, Thielmann J, Hickisch A, Muranyi P, Wunderlich J, Hauser C. Antimicrobial activity of hop extracts against foodborne pathogens for meat applications. Journal of Applied Microbiology. 2015 Jan 9;118(3):648–57
- Noreña I, Jéssica López, Salazar FN. Antimicrobial activity of compounds from hop (Humulus lupulus L.) following supercritical fluid extraction: An overview. Chilean Journal of Agricultural Research. 2023 Aug 1;83(4):499–509.



- Moureu S, Jacquin J, Samaillie J, Deweer C, Rivière C, Muchembled J. Antifungal Activity of Hop Leaf Extracts and Xanthohumol on Two Strains of Venturia inaequalis with Different Sensitivities to Triazoles. Microorganisms [Internet]. 2023 Jun 1 [cited 2024 Mar 19];11(6):1605. Available from: https://www.mdpi.com/2076-2607/11/6/1605.
- 20. Mizobuchi S, Sato Y. Antifungal Activities of Hop Bitter Resins and Related Compounds. Agricultural and Biological Chemistry. 1985 Feb;49(2):399–403.
- 21. 20. Guo Y, Pan W, Liu S, Shen Z, Xu Y, Hu L. ERK/MAPK signalling pathway and tumorigenesis (Review). Experimental and Therapeutic Medicine [Internet]. 2020 Jan 15;19(3).
- 22. 21. Chen Q, Fu M, Chen M, Liu J, Liu X, He G, et al. Preparative isolation and purification of xanthohumol from hops (Humulus lupulus L.) by high-speed counter-current chromatography. Food Chemistry. 2012 May;132(1):619–23.
- 23. 22. Liu M, Hansen P, Wang G, Qiu L, Dong J, Yin H, et al. Pharmacological Profile of Xanthohumol, a Prenylated Flavonoid from Hops (Humulus lupulus). Molecules. 2015 Jan 7;20(1):754–79.
- 24. Kuribayashi K, Funaguchi N, Nakano T. Chemotherapy for advanced non-small cell lung cancer with a focus on squamous cell carcinoma. Journal of Cancer Research and Therapeutics. 2016;12(2):528.
- 25. Kunnimalaiyaan S, Sokolowski KM, Balamurugan M, Gamblin TC, Kunnimalaiyaan M. Xanthohumol Inhibits Notch Signaling and Induces Apoptosis in Hepatocellular Carcinoma. Calvisi D, editor. PLOS ONE. 2015 May 26;10(5):e0127464.
- 26. Kunnimalaiyaan S, Trevino J, Tsai S, Gamblin TC, Kunnimalaiyaan M. Xanthohumol-Mediated Suppression of Notch1 Signaling Is Associated with Antitumor Activity in Human Pancreatic Cancer Cells. Molecular Cancer Therapeutics. 2015 Jun 1;14(6):1395–403.
- Thandra KC, Barsouk A, Saginala K, Aluru JS, Barsouk A. Epidemiology of lung cancer. Contemp Oncol (Pozn). 2021;25(1):45-52. doi: 10.5114/wo.2021.103829. Epub 2021 Feb 23. PMID: 33911981; PMCID: PMC8063897.
- 28. Vazquez-Cervantes GI, Ortega DR, Blanco Ayala T, Pérez de la Cruz V, Esquivel DFG, Salazar A, Pineda B. Redox and Anti-Inflammatory Properties from Hop Components in Beer-Related to Neuroprotection. Nutrients. 2021 Jun 10;13(6):2000. doi: 10.3390/nu13062000. PMID: 34200665; PMCID: PMC8226943.
- 29. Velot É, Ducrocq F, Girardeau L, Hehn A, Piutti S, Kahn C, Linder M, Bianchi A, Arab-Tehrany E. Hop Extract Anti-Inflammatory Effect on Human Chondrocytes Is Potentiated When Encapsulated in Rapeseed Lecithin Nanoliposomes. Int J Mol Sci. 2022 Oct 17;23(20):12423. doi: 10.3390/ijms232012423. PMID: 36293278; PMCID: PMC9603919.
- 30. Zugravu CA, Bohiltea RE, Salmen T, Pogurschi E, Otelea MR. Antioxidants in Hops: Bioavailability, Health Effects and Perspectives for New Products. Antioxidants (Basel). 2022 Jan 27;11(2):241. doi: 10.3390/antiox11020241. PMID: 35204124; PMCID: PMC8868281.
- Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996 Jul 15;239(1):70-6. doi: 10.1006/abio.1996.0292. PMID: 8660627.
- 32. Kolenc Z, Hribernik T, Langerholc T, Pintarič M, Prevolnik Povše M, Bren U. Antioxidant Activity of Different Hop (*Humulus lupulus* L.) Genotypes. *Plants*. 2023; 12(19):3436. https://doi.org/10.3390/plants12193436
- Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem. 2005 Mar 23;53(6):1841-56. doi: 10.1021/jf030723c. PMID: 15769103.



- 34. Mikyska, A., Haskova, D., & Krofta, K. (2008). Evaluation of antioxidant properties of raw hop and hop products. *Acta horticulturae*, 778, 97.
- 35. Codina-Torrella I, Rodero L, Almajano MP. Brewing By-Products as a Source of Natural Antioxidants for Food Preservation. *Antioxidants*. 2021; 10(10):1512. https://doi.org/10.3390/antiox10101512
- 36. Silva, S.; Oliveira, A.I.; Cruz, A.; Oliveira, R.F.; Almeida, R.; Pinho, C. Physicochemical Properties and Antioxidant Activity of Portuguese Craft Beers and Raw Materials. Molecules 2022, 27, 8007.
- 37. Petrón, M. J., Andrés, A. I., Esteban, G., & Timón, M. L. (2021). Study of antioxidant activity and phenolic compounds of extracts obtained from different craft beer by-products. *Journal of cereal science*, *98*, 103162.
- Takubo M, Inoue T, Jiang S, Tsumuro T, Ueda Y, Yatsuzuka R, Segawa S, Watari J, Kamei C. Effects of hop extracts on nasal rubbing and sneezing in BALB/c mice. Biol Pharm Bull. 2006 Apr;29(4):689-92. doi: 10.1248/bpb.29.689. PMID: 16595900.
- 39. Segawa S, Yasui K, Takata Y, Kurihara T, Kaneda H, Watari J. Flavonoid glycosides extracted from hop (Humulus lupulus L.) as inhibitors of chemical mediator release from human basophilic KU812 cells. Biosci Biotechnol Biochem. 2006 Dec;70(12):2990-7. doi: 10.1271/bbb.60384. Epub 2006 Dec 7. PMID: 17151464.
- 40. Segawa S, Takata Y, Kaneda H, Watari J. Effects of a hop water extract on the compound 48/80stimulated vascular permeability in ICR mice and histamine release from OVA-sensitized BALB/c mice. Biosci Biotechnol Biochem. 2007 Jun;71(6):1577-81. doi: 10.1271/bbb.70047. PMID: 17587695.
- 41. Fukuda, T., Obara, K., Saito, J., Umeda, S., & Ano, Y. (2020). Effects of Hop Bitter Acids, Bitter Components in Beer, on Cognition in Healthy Adults: A Randomized Controlled Trial. Journal of Agricultural and Food Chemistry, 68(1), 206-212. <u>https://doi.org/10.1021/acs.jafc.9b06660</u>
- 42. Ayabe T, Fukuda T, Ano Y. Improving Effects of Hop-Derived Bitter Acids in Beer on Cognitive Functions: A New Strategy for Vagus Nerve Stimulation. *Biomolecules*. 2020; 10(1):131. https://doi.org/10.3390/biom10010131
- 43. Ano, Y., Ohya, R., Kondo, K., & Nakayama, H. (2019). Iso-α-acids, Hop-Derived Bitter Components of Beer, Attenuate Age-Related Inflammation and Cognitive Decline. *Frontiers in Aging Neuroscience*, 11, Article 16. https://doi.org/10.3389/fnagi.2019.00016
- 44. Green, C. P., & Osborne, P. (1993). RAPID METHODS FOR OBTAINING ESSENTIAL OIL FROM HOPS. Journal of the Institute of Brewing, 99(4), 335–339. doi:10.1002/j.2050-0416.1993.tb01172.x
- 45. Von Horst, L.A.F.; Kellner, M. Thin Layer Steam Distillation of Hop Oil Extract. U.S. Patent No. 3436319A, 1969.
- 46. Dabrowski W, Gagos M, Siwicka-Gieroba D, Piechota M, Siwiec J, Bielacz M, Kotfis K, Stepulak A, Grzycka-Kowalczyk L, Jaroszynski A, Malbrain ML. *Humulus lupus* extract rich in xanthohumol improves the clinical course in critically ill COVID-19 patients. Biomed Pharmacother. 2023 Feb;158:114082. doi: 10.1016/j.biopha.2022.114082. Epub 2022 Dec 9. PMID: 36508996; PMCID: PMC9732508.
- 47. Ligor, M.; Stankevičius, M.; Wenda-Piesik, A.; Obelevičius, K.; Ragažinskienė, O.; Stanius, Ž.; Maruška, A.; Buszewski, B. Comparative Gas Chromatographic–Mass Spectrometric Evaluation of Hop (*Humulus lupulus* L.) Essential Oils and Extracts Obtained Using Different Sample Preparation Methods. *Food Anal. Methods* 2014, 7, 1433–1442.



- 48. Taniguchi, Y.; Matsukura, Y.; Taniguchi, H.; Koizumi, H.; Katayama, M. Development of preparative and analytical methods of the hop bitter acid oxide fraction and chemical properties of its components. *Biosci. Biotechnol. Biochem.* 2015, *79*, 1684–1694.
- 49. Quifer-Rada, P.; Vallverdú-Queralt, A.; Martínez-Huélamo, M.; Chiva-Blanch, G.; Jáuregui, O.; Estruch, R.; Lamuela-Raventós, R. A comprehensive characterisation of beer polyphenols by high resolution mass spectrometry (LC–ESI-LTQ-Orbitrap-MS). *Food Chem.* 2015, *169*, 336–343.
- 50. Abram, V.; Čeh, B.; Vidmar, M.; Hercezi, M.; Lazić, N.; Bucik, V.; Smole Mozina, S.; Kosir, I.J.; Kac, M.; Demšar, L.; et al. A comparison of antioxidant and antimicrobial activity between hop leaves and hop cones. *Ind. Crop. Prod.* 2015, *64*, 124–134.
- 51. Barbosa-Pereira, L.; Bilbao, A.; Vilches, P.; Angulo, I.; Lluis, J.; Fité, B.; Paseiro-Losada, P.; Cruz, J.M. Brewery waste as a potential source of phenolic compounds: Optimisation of the extraction process and evaluation of antioxidant and antimicrobial activities. *Food Chem.* 2014, *145*, 191–197.
- 52. Teuber, M.; Schmalreck, A.F. Membrane Leakage in Bacillus subtilis 168 induced by the hop constituents lupulone, humulone, isohumulone and humulinic acid. *Arch. Microbiol.* 1973, *94*, 159–171.
- 53. Bartmańska, A.; Wałecka-Zacharska, E.; Tronina, T.; Popłoński, J.; Sordon, S.; Brzezowska, E.; Bania, J.; Huszcza, E. Antimicrobial Properties of Spent Hops Extracts, Flavonoids Isolated Therefrom, and Their Derivatives. *Molecules* 2018, *23*, 2059.
- 54. Brantner, A.; Grein, E. Antibacterial activity of plant extracts used externally in traditional medicine. *J. Ethnopharmacol.* **1994**, *44*, 35–40.
- 55. Astray G, Gullón P, Gullón B, Munekata PES, Lorenzo JM. *Humulus lupulus* L. as a Natural Source of Functional Biomolecules. *Applied Sciences*. 2020; 10(15):5074. https://doi.org/10.3390/app10155074.
- 56. Fernandes RPP, Trindade MA, Lorenzo JM, de Melo MP. Assessment of the stability of sheep sausages with the addition of different concentrations of Origanum vulgare extract during storage. Meat Sci. 2018 Mar;137:244-257. doi: 10.1016/j.meatsci.2017.11.018. Epub 2017 Nov 15. PMID: 29223559.
- 57. Nagybákay NE, Syrpas M, Vilimaitė V, Tamkutė L, Pukalskas A, Venskutonis PR, Kitrytė V. Optimized Supercritical CO<sub>2</sub> Extraction Enhances the Recovery of Valuable Lipophilic Antioxidants and Other Constituents from Dual-Purpose Hop (*Humulus lupulus* L.) Variety *Ella*. Antioxidants (Basel). 2021 Jun 6;10(6):918. doi: 10.3390/antiox10060918. PMID: 34204047; PMCID: PMC8228826.
- 58. Vogt, O., Sikora, E., & Ogonowski, J. (2014). The effect of selected supercritical CO plant extract addition on user properties of shower gels. *Polish Journal of Chemical Technology*, *16*(4), 51-54.
- 59. Rodrigues Arruda, T., Fontes Pinheiro, P., Ibrahim Silva, P., & Campos Bernardes, P. (2021). Exclusive raw material for beer production? Addressing greener extraction techniques, the relevance, and prospects of hops (Humulus lupulus L.) for the food industry. *Food and Bioprocess Technology*, 1-31.
- 60. Teghtmeyer, S. (2018). Hops. Journal of Agricultural & Food Information, 19(1), 9-20.
- 61. Aydin, T., Bayrak, N., Baran, E., & Cakir, A. (2017). Insecticidal effects of extracts of Humulus lupulus L. (hops) cones and its principal component, xanthohumol. Bul- letin of Entomological Research, 107, 543–549. <u>http://doi.org/10.1017/S0007485317000256</u>.



- 62. Bedini, S., Flamini, G., Girardi, J., Cosci, F., & Conti, B. (2015). Not just for beer: Evaluation of spent hops (Humulus lupulus L.) as a source of eco-friendly repellents for insect pests of stored foods. Journal of Pest Science, 88, 583–592. http://doi.org/10.1007/s10340-015-0647-1doi:10.1007/s10340-015-0647-1.
- 63. Bland, J. S., Minich, D., Lerman, R., Darland, G., Lamb, J., Tripp, M., & Grayson, N. (2015). Isohumulones from hops (Humulus lupulus) and their potential role in medical nutrition therapy. PharmaNutrition, 3, 46–52. <u>http://doi.org/10.1016/j.phanu.2015.03.001</u> doi:10.1016/j.phanu.2015.03.001
- 64. Hamm, A. K., D. K. Manter, J. S. Kirkwood, L. M. Wolfe, K. Cox–York, and T. L. Weir. 2019. The effect of hops (*Humulus lupulus* L.) extract supplementation on weight gain, adiposity and intestinal function in ovariectomized mice. Nutrients 11:3004.
- 65. Zanoli, P.; Zavatti, M. Pharmacognostic and pharmacological profile of Humulus lupulus L. J. Ethnopharmacol. 2008, 116, 383–396. [Google Scholar] [CrossRef] [PubMed]
- 66. Bolton, J. L., T. L. Dunlap, A. Hajirahkimkhan, O. Mbachu, S–N. Chen, L. Chadwick, D. Dejan Nikolic, R. B. van Breemen, G. F. Pauli, and B. M. Dietz. 2019. The multiple biological targets of hops and bioactive compounds. Chemical Research in Toxicology 32:222–233.
- 67. Korpelainen, H., Pietiläinen, M. Hop (*Humulus lupulus* L.): Traditional and Present Use, and Future Potential. *Econ Bot* **75**, 302–322 (2021). <u>https://doi.org/10.1007/s12231-021-09528-1</u>
- 68. Helmja, K., Vaher, M., Püssa, T., Kamsol, K., Orav, A., & Kaljurand, M. (2007). *Bioactive* components of the hop strobilus: Comparison of different extraction methods by capillary electrophoretic and chromatographic methods. Journal of Chromatography A, 1155(2), 222–229. doi:10.1016/j.chroma.2006.12.067
- 69. Branch, Niams Science Communications And Outreach. "NIAMS Health Information on Osteoarthritis." National Institute of Arthritis and Musculoskeletal and Skin Diseases, 15 Dec. 2023, www.niams.nih.gov/health-topics/osteoarthritis.