American Journal of Applied Sciences and Engineering | *ISSN 2766-7596*
Published by AIR JOURNALS | *https://airjournal.org/ajase*
12011 WestBrae Pkwy, Houston, TX 77031, United States
airjournals@gmail.com; enquiry@airjournal.org

Research Article

STUDIES ON PHYTOCHEMICAL AND ANTIMICROBIAL PROFILE OF HOP (*Humulus lupulus* L.) EXTRACTS ON FOOD PATHOGENS (*Escherichia coli* AND *Salmonella* sp.)

Iloputaife, E. J.[1] & Ogunbodede, T. T.[2]

[12]*Department of Applied Microbiology and Brewing, Enugu State University of Science and Technology (ESUT) Enugu, Nigeria*

Abstract

In addition to their importance in beer brewing, hops have been used in traditional herbal medicines as antimicrobial agents against pathogens. This study is aimed at investigating the phytochemical and antimicrobial profile of hop (Humulus lupulus L.) extracts on food pathogens (Escherichia coli and Salmonella species). The bacterial food pathogens were isolated and identified from soymilk by phenotypic characterization. Phytochemical screening of the extracts was carried out using standard methods. The antibacterial assay was performed using agar well diffusion method. The pathogenic isolates recovered from soymilk were Escherichia coli and Salmonella spp. The preliminary phytochemical screening revealed that flavonoid and saponin were abundantly present; alkaloid, steroids, tannin and glycosides were moderately present while terpenoids and phenol were slightly present in the hop (Humulus lupulus L.) extract. The results of antibacterial assay showed minimum inhibitory concentration (MIC) for Escherichia coli at 200mg/ml concentration with zone of inhibition 2.00mm while minimum inhibitory concentration (MIC) for Salmonella spp. at 100mg/ml concentration with zone of inhibition 1.00mm. The extract obtained from hops flowers showed little antimicrobial activity against the tested food pathogens (Escherichia coli and Salmonella spp.). The results indicated that this plant considered mainly as raw material in brewing industry has antibacterial and antioxidant potential.

Keywords	*hop (Humulus lupulus L.); Escherichia coli; Salmonella spp; Antimicrobial Profile; Food Pathogen*
Citation	Iloputaife, E. J. & Ogunbodede, T. T. (2023). Studies on phytochemical and antimicrobial profile of hop (humu lupulus l.) Extracts on food pathogens (Escherichia coli AND Salmonella sp.). *American Journal of Applied Sciences and Engineering, 4(2) 25-31.* *https://doi.org/10.5281/zenodo.8329795*

Introduction

Plant extracts were used in folk medicine in the form of tinctures, infusions and decoctions or essential oils, as a cure and treatment of diseases all over the world (Biendl, 2012). The most common studied plants for their therapeutic potential are considered the spices and medicinal herbs (Jaskula *et al.,* 2018). Plant antibacterial substances provide an opportunity to develop potential disease controlling agents despite being extensively used for the design and development of novel medications for use in human medicine (Korpelainen and Pietilainen, 2021).

The use of plant products as antimicrobial agents is an ancient idea, but the researches in the area are gaining attention lately (Kolenc *et al.,* 2023). As a response to the acquired pathogens resistance to antibiotics existing on the market, new alternatives should be designed for the treatment of infectious diseases. It is therefore desirable to explore the potential of plant extracts for the development and design of new antimicrobial agents, as this could be a solution for both medical and phytopharmaceutical industry (Overk *et al.,* 2018). Plants contain natural bioactive compounds such as secondary metabolites and antioxidants. The medicinal plants used as traditional medicine all over the world are rich in secondary metabolites (Flythe, 2009).

Hop (*Humulus lupulus*) is an industrial plant massively used in the brewing industry. Lupulin glands present in the female hop inflorescences (cones or strobiles) contain a variety of substances, including resins, bitter acids, essential oils, and flavonoids that are crucial to the brewing process. These compounds improve the preservation of beer and provide bitterness (Frolich *et al.,* 2015). Bitter acids are phloroglucinol prenylated derivatives and are generally classified as α-acids and β-acids; the most important are humulone and lupulone, respectively (Zanoli and Zavatti, 2018). Hop contains a variety of flavonoids, including proanthocyanidins, flavan-3-ols, flavonols, flavanones, and prenylflavonoids, which have prenyl or geranyl chains attached to the flavonoid structure. The most significant prenylflavonoid is xanthohumol, a chalcone that, as a result of brewing conditions, can be transformed to the flavanone isoxanthohumol. Isoxanthohumol is therefore the main prenylflavonoid present in beer (Fukuda *et al.,* 2020).

In traditional medicine, hop cones have been used to treat conditions such as anxiety, spasms, coughs, fevers, toothaches, and inflammation in addition to the brewing business. Hop resins have also been used as a moderate sedative (Butterweck *et al.,* 2017). In fact, the health-promoting effects and bioactivities of hop flavonoids are numerous and the most outstanding are the cardioprotective effect and the antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial activities (Franco *et al.,* 2019). Because of this, there is a growing interest in the possible use of the hop plant for non-brewing purposes, especially in the pharmaceutical and nutraceutical industries.

Numerous hops extracts and specific hops chemicals are recognized for their antibacterial properties. Humulones and lupulones, which are bitter compounds found in hops, have been proven to have antibacterial effects against Gram-positive bacteria such *Lactobacillus, Streptococcus, Staphylococcus, Listeria, Clostridium* and *Bacillus* species (Rossini *et al.,* 2021), gram-negative bacteria i.e. *Helicobacter pylori* and *Brucella* species and some fungi i.e. *Candida*, *Trichophyton*, *Fusarium* and *Mucor* species (Niknejad *et al.,* 2014). This action has been attributed to the interference of a prenyl group of hop acids with the bacterial cell plasma membrane (Milligan *et al.,* 2018).

A wide variety of phytochemicals, some uniquely found in hops, have previously been reported, with a particular focus on prenylflavonoids, alpha and beta acids (soft resins), and volatile terpenoids. In addition to their importance in beer brewing, hops have been used in traditional herbal medicines while modern biomedical research has focused on various components in the development of new therapeutic agents. Although the antimicrobial activity of hops extracts have been previously examined, none of these studies investigated the antimicrobial effects of different hop extract on food pathogens, hence this study. The aim of this study is to investigate the phytochemical and antimicrobial profile of hop (*Humulus lupulus* L.) extracts on food pathogens (*Escherichia coli* and *Salmonella* species).

Materials and Method

Sources of Materials

Soymilk sample was gotten from a local vendor within ESUT campus, Agbani in Nkanu-West Local Government Area of Enugu State. The hop (*Humulus lupulus* L.) extracts were supplied by Prof. E.O. Ogu of the Department of Applied Microbiology and Brewing. All reagents, media, chemicals and equipment used for this study were supplied by the Laboratory section of the Department of Applied Microbiology and Brewing, ESUT.

Identification of the Isolates from Soymilk

The isolates were identified according to the methods of Cheesbrough (2000). One millilitre (1 ml) of the soymilk sample was added into a test tube containing 9ml of sterile distilled water (This was used as stock). Serial dilution of up to 10^{-5} of the homogenates was made in sterile test tubes. Using a sterilized wire loop, one millilitre of the sample from the 10^{-3} dilution was inoculated into already prepared solidified Nutrient agar plates to propagate the microorganisms. The media were aerobically incubated overnight at 37^0C for 24 hours. Thereafter, suspected colonies of *Escherichia coli* and *Salmonella* spp. were sub-cultured into already prepared solidified MacConkey and Salmonella Shigella agar plates, respectively. The media plates were incubated at 37^0C for 24 hours. The cultures were examined periodically for microbial growths and recorded accordingly. *Escherichia coli* and *Salmonella* isolates were characterized on basis of their Gram-stain reaction and biochemical test (catalase, citrate, oxidase, indole and sugar fermentation tests) and the identification was according to Bergey's Manual of Determinative Bacteriology. Purified isolates were stocked in nutrient agar slants for further studies.

Phytochemical Analysis

Phytochemical analysis was done to ascertain the presence of bioactive component present in the hop (*Humulus lupulus* L.) extract. This was carried out as illustrated by Bocquet *et al.* (2019). The assayed bioactive components included alkaloid, flavonoid, glycoside, saponins, steroid, tannin, terpenoids and resins.

Preparation of Inoculum

A loopful of isolated colonies of each bacterial strain (*Escherichia coli* and *Salmonella* sp.) stored on nutrient agar slant was taken and inoculated into 4ml of peptone water and then incubated at 37°C for 4 h. The concentration of the bacterial suspension was adjusted with peptone water to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate ($BaCl_2.2H_2O$) with 99.5 ml of 1% (v/v) sulfuric acid (H_2SO_4). This turbidity is equivalent to approximately $1 - 2 \times 10^8$ colony forming units per ml (CFU/ml). This activated culture was then used as inoculum for further testing.

Preparation of Different concentrations of Isomerized Hop Extract

This was done according to the method of Di Lodovico *et al.* (2020). A total of 4g of each of the hop (*Humulus lupulus* L.) extract was dissolved in 8ml of dimethylsulfoxide (DMSO) to give a concentration of 400mg/ml (stock extract) which was serially diluted to give different concentrations of 200gm/ml, 100mg/ml, 50mg/ml and 25mg/ml respectively. The tubes containing the different concentration of the isomerized hop extracts were labelled accordingly and the concentrations were used for the sensitivity test while a gentamycin antibiotic was used as the standard drug control.

Antibacterial Evaluation

The antibacterial activity of the hop (*Humulus lupulus* L.) extracts on the test organisms was determined using agar well diffusion method according to Di Lodovico *et al.* (2020). The test organisms (*Escherichia coli* and *Salmonella* spp.) in the broth cultures were inoculated differently under aseptic conditions into different Mueller Hilton agar plates and appropriately spread with a sterile swab stick. After spreading the culture, 6 holes of 6mm in diameters were bored on the plates by using a sterile cork borer. The wells were open with the help of sterile forceps. Using a sterile micropipette and under sterile conditions, the hop (*Humulus lupulus* L.) extracts were pipetted and dispensed 1ml each into five (5) wells made in the different plates containing the test organisms (*Escherichia coli* and *Salmonella* spp.) while the standard drug gentamycin (40mg/ml) was introduced into the sixth hole for each plate. It was left to

stand so that the hop (*Humulus lupulus* L.) extracts and control poured into the holes can diffuse into the agar culture appropriately after which it was incubated at 37^0C for 24–72hours. The plates were later examined for zones of inhibitions from 24hours of incubation. The diameter of inhibition zones was measured in millimetres with the aid of ruler.

Results

Escherichia coli and *Salmonella* spp. were isolated and identified from soymilk sample sold at ESUT campus, Agbani. Isolates identified as *Escherichia coli* showed bright pink, smooth, mucoid and medium sized colonies on MacConkey agar while *Salmonella* spp. showed smooth and opaque black-centered colonies on SSA based on cultural and morphological characteristics. Both *Escherichia coli* and *Salmonella* spp. were gram negative (Table 1).

Table 1: Identification Scheme of the Bacterial Isolates

Isolates	Colonial Appearance on Media	Gram reaction	Possible organisms
A	Bright pink, smooth, mucoid and medium sized colonies on MacConkey agar	Gram negative short rod in pairs coloured pink	*Escherichia coli*
B	Smooth and opaque black-centered colonies on SSA	Gram negative short rod in singles coloured pink	*Salmonella* spp.

From the Table 2 of results, it is revealed that flavonoid and saponin were abundantly present; alkaloid, steroids, tannin and glycosides were moderately present while terpenoids and phenol were slightly present in the hop (Humulus lupulus L.) extract.

Table 2: Phytochemical Analysis of the Hop (*Humulus lupulus* L.) Extract

Phytochemical Components	Isomerized Hop (*Humulus lupulus* L.) Extract
Alkaloids	++
Flavonoids	+++
Saponin	+++
Phenol	+
Steroid	++
Tannin	++
Terpenoids	+
Glycosides	++

Key: + = slightly present, ++ = moderately present, +++ = Abundant, present - = absent

The hop (Humulus lupulus L.) extract exhibited antimicrobial activity against the test organisms (Escherichia coli and Salmonella spp). The results showed minimum inhibitory concentration (MIC) for Escherichia coli at 200mg/ml concentration with zone of inhibition 2.00mm while minimum inhibitory concentration (MIC) for Salmonella spp. at 100mg/ml concentration with zone of inhibition 1.00mm (Table 3).

Table 3: Antibacterial Activities of Hop (Humulus lupulus L.) Extracts on the Isolates

Hop (*Humulus lupulus* L.) Extracts at different Concentrations (mg/ml)	Zone of Inhibition (mm)	
	Escherichia coli	*Salmonella* spp.
400	3.00	4.00
200	2.00	3.00
100	0.00	1.00
50	0.00	0.00
25	0.00	0.00
Gentamycin (drug control)	14.00	15.00

Discussion

Correct information and knowledge about the chemical components in medicinal plants are necessary since these compounds can have adverse effects on plants' health (Monte *et al.,* 2014). Numerous research have recently focused on hops' (*Humulus lupulus* L.) capacity to combat microorganisms. Compounds in hops have antibacterial properties against infections (Chadwick *et al.,* 2016). Hops (*Humulus lupulus* L.) extract were examined using a qualitative phytochemical analysis to assess the presence and concentration of bioactive chemicals. On a few pathogens from soymilk, the antibacterial activity of hops was assessed using the agar well diffusion method.

The microorganisms isolated from soymilk in this project work (Table 2) tally with already known organisms isolated from previous studies on microbiological quality of local soymilk (Adeleke *et al.,* 2013). The isolates identified in this study included *Escherichia coli* and *Salmonella* sp. These microorganisms were identified on the basis of their morphological, microscopic and biochemical activities. This study opted for *Escherichia coli* and *Salmonella* sp. for the following reasons: First, these bacteria produce a lot of virulence factors that are crucial for pathogenesis. Second, they are on the list of alarming multi-resistant pathogens. Third, their ability to build biofilms is a significant medical issue. Biofilm-associated infections are exceptionally challenging to treat because bacteria in biofilms are very resistant to antimicrobial treatments, especially when the causing organism is multidrug resistant.

A wide variety of bioactive compounds have been reported to be present in hop extract of female flowers. According to the result obtained in this present study, flavonoid and saponin were abundantly present; alkaloid, steroids, tannin and glycosides were moderately present while terpenoids and phenol were slightly present in the hop (*Humulus lupulus* L.) extract.

The antimicrobial activity of extracts obtained from vegetal material is generally attributed to the content of biologically active compounds present in the plant. It is widely considered that the antibacterial activity of plant extracts is related to the polyphenol content (Zanoli and Zavatti, 2018). Therefore, besides their role as antioxidants, polyphenols (especially flavonoids) have antimicrobial properties. Hops (*Humulus lupulus* L.) are source of various bioactive compounds. Result of qualitative phytochemical studies of hops extract presented in Table 1 showed variability of the presence of phytochemicals in hop extracts. Flavonoid and saponin were abundantly present; alkaloid, steroids, tannin and glycosides were moderately present while terpenoids and phenol were slightly present. This result is similar to the findings of Knez-Hrncic *et al.* (2019) who isolated various bio-active compounds from hops. Buckwold *et al.* (2014) also observed the presence of alkaloids, flavonoids, tannins, resins, glycosides from ethanol hop extract.

From therapeutic point of view, the most important role of flavonoids are their antioxidant properties, which is result of direct scavenging of reactive oxygen species. Flavonoids are also reported to be anti-inflammatory agents as the result of diminished formation of pro-inflammatory mediators (prostaglandins, leukotrienes, reactive oxygen species, and nitric oxide) (Kobus-Cisowska *et al.,* 2019). Phenols and tannins are reported to possess and antioxidant activity by Milligan *et al.* (2018). Yajima *et al.* (2014) demonstrated that the amount of galloyl groups, molecular weight, and the presence of an ortho-dihydroxy structure all increase the scavenging activity of tannins. Components of secondary metabolites in the extract can integrate with each other or work, respectively, to produce antibacterial activity against food pathogens.

The hop (*Humulus lupulus* L.) extract exhibited little antimicrobial activity against the test organisms (*Escherichia coli* and *Salmonella* spp). The results showed minimum inhibitory concentration (MIC) for *Escherichia coli* at 200mg/ml concentration with zone of inhibition 2.00mm while minimum inhibitory concentration (MIC) for *Salmonella* spp. at 100mg/ml concentration with zone of inhibition 1.00mm (Table 3). From these results, the hop (*Humulus lupulus* L.) extract was more active against *Salmonella* spp. than *Escherichia coli*. In contrast, Niknejad *et al.* (2014) showed that ethanolic extract of hops flower could inhibit the growth of microorganisms at 3 mg/ml and complete growth inhibition was showed at 2mg/ml. Bocquet *et al.* (2018) found that ethanolic extract of hops flower could inhibit the growth of different bacteria including *Escherichia coli* and *Salmonella* spp.

The antimicrobial activity of hop extracts and their inhibitory effects varies widely and depends on the type of extract, extraction method, age of extract, storage condition of extract and testing methods used to evaluate of the antimicrobial activity (Zanoli and Zavatti, 2018). The low performance of the hop extract as antimicrobial against

food pathogens is due to the fact that the hop extract has been available for a long time and are not properly stored. Hop plants are not grown in Nigeria and as such much efforts are put in place to make available this product from countries where they are grown.

The present study reveals the medical importance of hops extract as an alternative antimicrobial to control drug resistant bacteria which are becoming a threat to human health and economic burden worldwide. The activity of the little hops extract may be indicative of the presence of broad-spectrum bioactive compounds in the hops.

Conclusion

The extract obtained from hops flowers showed little antimicrobial activity against the tested food pathogens (*Escherichia coli* and *Salmonella* spp.). The findings showed that this plant, which is primarily used as a raw material in the brewing industry, has potential with regards to antibacterial and antioxidant activities. The phytochemical analysis revealed considerable levels of polyphenols with appreciable amounts of flavonoid and saponin, alkaloid, steroids, tannin and glycosides. Chemical compounds derived from hops are a good source of biologically active substances. Therefore, it may have future practical applications in the development of an antimicrobial product based on natural compounds, with multiple uses in industry. Use of hop extract that have not aged and that are properly stored prior testing. Nevertheless, further studies are needed to characterize the chemical composition and structures that contribute to the antimicrobial and antioxidant activity of hops extracts. In addition, it is recommended to evaluate the activity against other microorganisms with several other solvents, isolate the active ingredients, and investigate the mechanisms of action.

Acknowledgement

I sincerely express my appreciation for the efforts of the staff and students of Applied Microbiology and Brewing of the Enugu State University of Science and Technology in Enugu, Nigeria.

References

Adeleke, O. E., Adeniyi, B. A., & Akinrinmisi, A. A. (2013). Microbiological quality of local soymilk: A public health appraisal. *African Journal of Biomedical Research, 45*(1), 89-92.

Biendl, M. (2012). Pre-isomerised hop products - potential and practical use. *Brauwelt International, 4*(pp. 20-25).

Bocquet, L., Sahpaz, S., & Hilbert, J. L. (2019). *Humulus lupulus L., a very popular beer ingredient and medicinal plant: Overview of its phytochemistry, its bioactivity, and its biotechnology. Phytochemistry Reviews, 17*, 1047–1090.

Bocquet, L., Sahpaz, S., & Rivière, C. (2018). An overview of the antimicrobial properties of hop. In M. J. Mérillon & C. Riviere (Eds.), *Natural antimicrobial agents* (pp. 451-474). *Sustainable Development and Biodiversity, Volume 19*. Springer.

Buckwold, V. E., Wilson, R. J., & Nalca, A. (2014). Antiviral activity of hop constituents against a series of DNA and RNA viruses. *Antiviral Research, 61*(1), 57-62.

Butterweck, V., Brattström, A., Grundmann, O., & Koetter, U. (2017). Hypothermic effects of hops are antagonized with the competitive melatonin receptor antagonist luzindole in mice. *Journal of Pharmacy Pharmacology, 59*(4), 549-552.

Chadwick, L. R., Pauli, G. F., & Farnsworth, N. R. (2016). The pharmacognosy of *Humulus lupulus* L. (hops) with an emphasis on estrogenic properties. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 13*(1-2), 119–131.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries*. Part 2 (pp. 132-143). Cambridge University Press.

Di Lodovico, S., Menghini, L., Ferrante, C., Recchia, E., Castro-Amorim, J., Gameiro, P., Cellini, L., & Bessa, L. J. (2020). Hop extract: An efficacious antimicrobial and anti-biofilm agent against multidrug-resistant *Staphylococci* strains and *Cutibacterium acnes*. *Frontiers in Microbiology, 11*, 1852-1858.

Flythe, M. D. (2009). The antimicrobial effects of hops (*Humulus lupulus* L.) on ruminal hyper-ammonia-producing bacteria. *Letters in Applied Microbiology, 48*, 712-717.

Franco, L., Sanchez, C., Bravo, R., Rodriguez, A., Barriga, C., & Juánez, J. C. (2019). The sedative effects of hops (*Humulus lupulus*), a component of beer, on the activity/rest rhythm. *Acta Physiologica Hungarica, 99*(2), 133-139.

Frolich, S., Schubert, C., Bienzle, U., & Jenett-Siems, K. (2015). In vitro antiplasmodial activity of prenylated chalcone derivatives of hops (*Humulus lupulus*) and their interaction with haemin. *Journal of Antimicrobial Chemotherapy, 55*(6), 883-887.

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 Agriculture and Food Chemistry, 68*(1), 206-212.

Jaskula, B., Kafarski, P., Aerts, G., & Cooman, L. D. (2018). A kinetic study on the isomerization of hop alpha-acids. *Journal of Agricultural and Food Chemistry, 56*(15), 6408-6415.

Knez Hrnčič, M., Španinger, E., Košir, I. J., Knez, Ž., & Bren, U. (2019). Hop compounds: Extraction techniques, chemical analyses, antioxidative, antimicrobial, and anticarcinogenic effects. *Nutrients, 11*(2), 257-269.

Kobus-Cisowska, J., Szymanowska-Powałowska, D., Szczepaniak, O., Kmiecik, D., Przeor, M., Gramza-Michałowska, A., Cielecka-Piontek, J., Smuga-Kogut, M., & Szulc, P. (2019). Composition and in vitro effects of cultivars of *Humulus lupulus* L. hops on cholinesterase activity and microbial growth. *Nutrients, 11*(6), 1377-1384.

Kolenc, Z., Langerholc, T., Hostnik, G., Ocvirk, M., Štumpf, S., Pintarič, M., Košir, I. J., Čerenak, A., Garmut, A., & Bren, U. (2023). Antimicrobial properties of different hop (*Humulus lupulus*) genotypes. *Plants, 12*(1), 120-127.

Korpelainen, H., & Pietiläinen, M. (2021). Hop (*Humulus lupulus* L.): Traditional and present use, and future potential. *Economic Botany, 75*, 302–322.

Milligan, S. R., Kalita, J. C., Heyerick, A., Rong, H., De Cooman, L., & De Keukeleire, D. (2018). Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *The Journal of Clinical Endocrinology and Metabolism, 84*(6), 2249–2252.

Monte, J., Abreu, A. C., Borges, A., Simões, L. C., & Simões, M. (2014). Antimicrobial activity of selected phytochemicals against *Escherichia coli* and *Staphylococcus aureus* and their biofilms. *Pathogens, 3*(2), 473–498.

Niknejad, F., Mohammadi, M., Khomeiri, M., Razavi, S. H., & Alami, M. (2014). Antifungal and antioxidant effects of hops (*Humulus lupulus* L.) flower extracts. *Advances in Environmental Biology, 8*(24), 395-401.

Overk, C. R., Guo, J., & Chadwick, L. R. (2018). In vivo estrogenic comparisons of *Trifolium pratense* (red clover), *Humulus lupulus* (hops), and the pure compounds isoxanthohumol and 8-prenylnaringenin. *Chemical Biology Interaction, 176*(1), 30-39.

Rossini, F., Virga, G., Loreti, P., Iacuzzi, N., Ruggeri, R., & Provenzano, M. E. (2021). Hops (*Humulus lupulus* L.) as a novel multipurpose crop for the Mediterranean Region of Europe: Challenges and opportunities of their cultivation. *Agriculture, 11*(6), 484-494.

Yajima, H., Ikeshima, E., & Shiraki, M. (2014). Isohumulones, bitter acids derived from hops, activate both peroxisome proliferator-activated receptor alpha and gamma and reduce insulin resistance. *Journal of Biological Chemistry, 279*(32), 33456-33462.

Zanoli, P., & Zavatti, M. (2018). Pharmacognostic and pharmacological profile of *Humulus lupulus* L. *Journal of Ethnopharmacology, 116*(3), 383–396.