Pharmaceutical Sciences Asia

Pharm Sci Asia 2024; 51(4), 391-400

DOI:10.29090/psa.2024.04.24.AP0798

Research Article

Phytochemical screening, *in vitro* Antioxidant, and Antimicrobial Efficacy of *Humulus lupulus* L. flowers (Newport and Comet varieties) from Thailand

Phanida Suphiratwanich[1], Chonwipa Yarangsee[1], Pattamapan Lomarat[2], Nachtharinee Laosirisathian[1,*]

1 Department of Pharmaceutical technology, Faculty of pharmacy, Payap University, Chaing Mai, Thailand
2 Department of Food Chemistry, Faculty of pharmacy, Mahidol University, Bangkok, Thailand

ABSTRACT

Humulus lupulus L. (hop) has a rich history in beer brewing, prized for its contributions to flavor, aroma, and preservation qualities. Polyphenols in hops have been reported for various activities, especially antioxidant, anti-inflammatory antibacterial and anti-cancer properties. This study aims to explore the effects of flowers extracts of the Comet and Newport varieties of *H. lupulus* L. on antioxidant and antibacterial potential. Ethanolic hop extracts were prepared, and their antioxidant activity was assessed using the 2,2-diphenyl-1-picryhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation decolorization assays. Total phenolic and flavonoid contents were quantified via the Folin–Ciocalteu and colorimetric aluminum chloride assays, respectively. Antibacterial efficacy was evaluated using the microdilution method. Additionally, the chemical composition of hops from various varieties included in our investigation was analyzed using liquid chromatography-mass spectrometry (LC-MS). Ethanolic extracts from the Newport and Comet varieties of *H. lupulus* L. contained total phenolics content about 36.98±2.38 and 66.66±7.96 mg QE/g extract, respectively. Total flavonoid content of the Newport and Comet were 5.26±0.04 and 8.20±0.65 mg QE/g extract, respectively. Chemical analysis revealed phenolic and prenylflavonoid compounds in the extracts. Both varieties exhibited strong antioxidant activity, with DPPH assay IC50 values of 0.67 and 0.24 mg/mL, and ABTS assay IC50 values of 0.54 and 0.16 mg/mL, respectively. Additionally, the FRAP assay showed antioxidant capacity of 28.02 and 19.88 mgTE/g extract for Newport and Comet. Furthermore, the Newport and Comet extracts displayed significant antibacterial activity against *Stapphylococcus aureus* with MIC 0.061 and 0.146 mg/mL, respectively and *Cutibacterium acnes* with MIC 0.061 and 0.073 mg/mL, respectively. This study represents the first investigation into the phytochemical and biological activity of *H. lupulus* L. varieties in Thailand, revealing promising antioxidant and antibacterial properties in both Newport and Comet varieties against *S. aureus* and *C. acnes*.

Keywords:
Humulus lupulus; biological activities; phytochemical compounds; antioxidant; minimum inhibitory concentration (MIC)

1. INTRODUCTION

Humulus lupulus L. (hop) is an edible and medicinal plant belonging to the Cannabinaceae family. This climbing plant flourishes in temperate climates, exhibiting robust growth reaching heights of 6-9 meters, facilitated by sturdy, twining stems. Characterized by deeply lobed and serrated leaves, it yields cone-shaped flowers[1] (Figure 1A). Indigenous to Europe, western Asia, and North America[2,3], hop is best known for their critical role in brewing beer, where they impart bitterness, flavor, and aromatic qualities. With the availability of hundreds of hop varieties, each cultivar presents distinct characteristics that enhance the complexity of beer.

***Corresponding author:**
***** Nachtharinee Laosirisathian Email: nachtharinee_l@payap.ac.th

These varieties differ in their alpha and beta acid content, essential oil composition, and overall sensory profile. Newport and Comet varieties were developed in the United States by the United States Department of Agriculture (USDA) as high-alpha varieties[2,4]. Hop cones of Newport and Comet varieties are presented in Figures 1B and 1C, respectively.

All hops contain both alpha and beta-acids. The ratio of α/β-acids can be lower than 1.0 for some aroma hops, but for bitter hops, the ratio typically falls within a specific range. Hop bitter acids are broadly classed into two groups: α-acids and β-acids. Among the α-acids, three major compounds have been identified: humulone, cohumulone, and adhumulone. The significant β-acids include colupulone, lupulone, adlupulone, prelupulone, and postlupulone[5,6].

Several academic studies investigating pharmacological properties of hop have documented a range of effects, including antioxidant, antimicrobial, anti-inflammatory, anticancer activities, as well as sedative effect[3,4,7,8,9,10,11].

The pharmacological properties of hops are attributed to the presence of biologically active compounds, including the secondary metabolites such as phenolic, flavonoid, and terpenoid compounds which are found in lupulin glands of the female hop inflorescences[1,3,8] (see Figure1D). Hop bitter acids are broadly classified into two groups: α-acids and β-acids.

The α-acids (humulones) and their water-soluble isomers, as well as the iso-α-acids (iso-humulones) are reported as the major bitter flavor components in beer brewing, exhibiting strong antibacterial activity[5,12].

Isolated active compounds, xanthohumol and lupulones from hop, illustrated strong inhibitory activity against *P. acnes* with minimum inhibitory concentrations ranging from 0.1 to 3 μg/mL, and against *S. epidermidis*, *S. aureus*, *K. rhizophila*, *S. pyogenes*[12]. Additionally, in the 2008 study by Natarajan P, it was observed that the active compounds found in hops, namely lupulone and xanthohumol, demonstrated synergistic antibacterial effects when combined with antibiotics. This combination exhibited efficacy against both gram-positive and gram-negative bacteria[9].

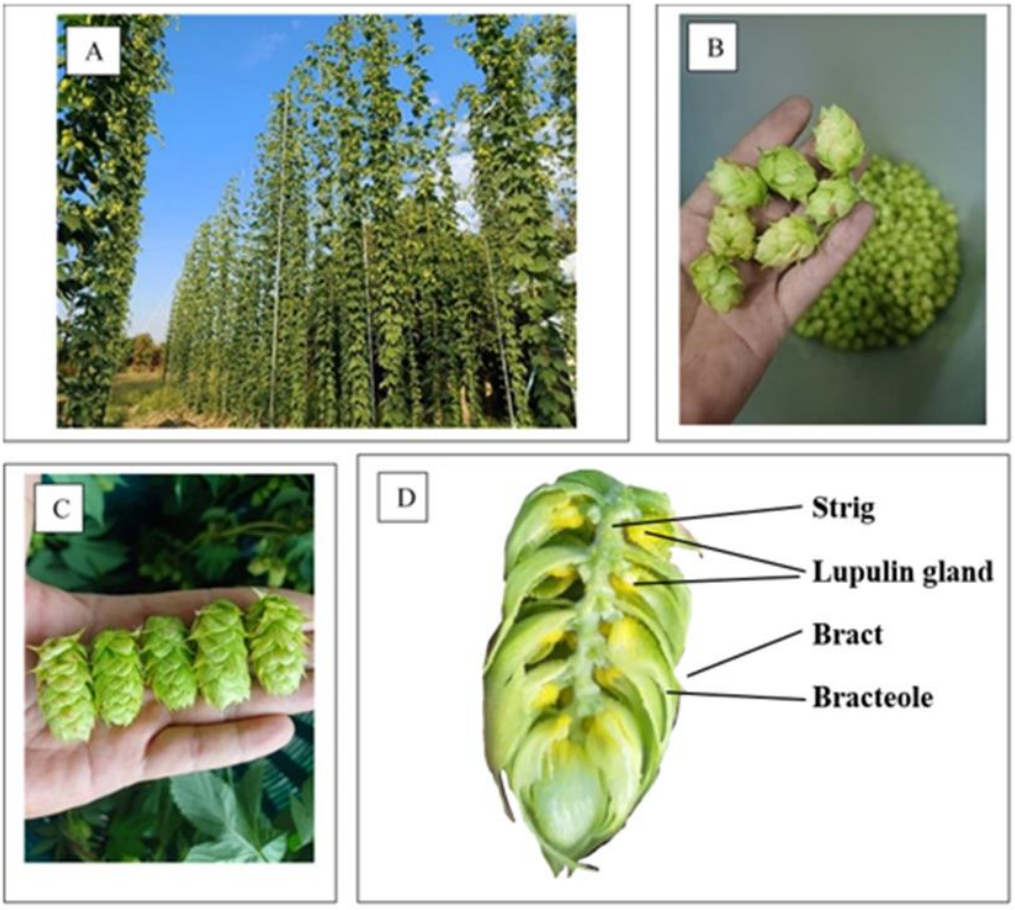

Figure 1. (A) *Humulus lupulus* L. (hop) plant growing on a trellis. (B) Newport variety. (C) Comet variety. (D) Hop anatomy.

The isolated compounds from Cascade, a variety of hops, including 4-hydroxycolupulone, humudifucol, and cascadone, were documented to possess anti-proinflammatory properties[13]. This finding parallels the investigation conducted by Young CC et al. in 2008, which examined the inhibitory mechanism of xanthohumol against inflammatory mediators such as IL-1β, TNF-α, and iNOS[14].

Xanthohumol and lupulones extracted from hops exhibited notable anticollagenase inhibitory properties, ranging from moderate to strong. Additionally, xanthohumol demonstrated the highest total oxygen radical absorbance capacity and singlet oxygen absorbance capacity, thus proving its significant antioxidative potential[12].

However, there have been no previous reports concerning the Comet and Newport varieties of *H. lupulus* L. flower in Thailand. This study would be the first to investigate for the bioactive active compounds extracted from Comet and Newport varieties of *H. lupulus* L. flower. Furthermore, it examines the antioxidant and antibacterial activities, as well as, the chemical composition of Comet and Newport varieties of *H. lupulus* L. flower extracts were investigated.

2. MATERIALS AND METHODS

2.1. Plant material

The Newport and Comet varities of *H. lupulus* L. flowers were collected from Ban Hong District, Lamphun province in Thailand. A specimen (voucher no. 0223383) was deposited in at the herbarium, Faculty of Pharmacy, Chiang Mai University, Thailand.

2.2. Chemicals reagents

Ammonium thiocyanate, dimethyl sulfoxide (DMSO), aluminum chloride, 2,2-diphenyl1-picryhydrazyl radical (DPPH), EDTA, ferrozine, ferric chloride ($FeCl_2$), methanol, ethanol, sodium carbonate, gallic acid, and Trolox were provided by Sigma Chemical Co. (St. Louis, MO, USA).

2.3. Plant extract preparation

Flowers of *Humulus lupulus* L. (Comet and Newport varieties) were dried at 45 °C in a hot air oven for 48 hours. Then, the dried plants were ground with a Moulinex® grinder. The resulting dried powder was macerated with 95% ethanol for 72 hours, followed by filtration of the extracts. The solvent was subsequently evaporated from the extract under vacuum using a rotary evaporator to obtain the dried extract. Thereafter, the extracts were stored at 4 °C for further use.

2.4. Total phenolic content evaluation

The total phenolic contents of the extracts was evaluated using the Folin–Ciocalteu assay, with minor modifications from the previous study from Saffoon N, et. al. (2014)[15]. The extract (20 µL) at a concentration of 100 mg/mL was mixed with 10% Folin–Ciocalteu's solution (100 µL) and left in the dark for 5 minutes. Subsequently, 80 µL of 7.5% w/v sodium carbonate solution was added and mixed at ambient temperature. The mixture was then incubated for an additional 30 minutes at room temperature. Next, the optical density was measured at 765 nm using a spectrophotometer (M200, Tecan, Switzerland). The total phenolic content was quantified as gallic acid equivalents per gram of extract (mg GAE/g extract) based on a gallic acid standard curve.

2.5. Total flavonoid content evaluation

A colorimetric aluminum chloride assay, with modifications from the previous study by Saffoon N, et al. (2014), was used to determine the flavonoid content of the extracts.[15]

An amount of 100 µL of 10% aluminum chloride was combined with the obtained extracts (100 µL). The resulting solution was then incubated at room temperature in the dark for 10 minutes. The optical density was measured at 415 nm. The total flavonoid content was quantified as quercetin equivalents per gram of extract (mg QE/g extract) based on a quercetin standard curve.

2.6. Anti-oxidant activity evaluation

DPPH radical scavenging method

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, a common method for evaluating the antioxidant activity of natural extracts by measuring their free radical scavenging ability, was adapted from a previous study by Zhou J, et. al. (2018)[16].

In brief, an aliquot of extract (10 µL) at concentrations ranging from 0.001 to 10 mg/mL was added to 195 µL of 120 µM DPPH in methanol. The reaction mixtures were placed in 96-well microtitre plates and incubated at room temperature in the dark for 30 minutes. Absorbance was measured at 517 nm. The assay was performed in triplicate, with Trolox used as the positive control.

The extract's capacity to scavenge the DPPH radical was calculated using the formula:

$$\% \text{ DPPH radical scavenging capacity} = [(AC - AT)/AC] \times 100$$

where AC stands for the control sample's

optical density (without the extract) and AT for the test sample's (extract) optical density. Calculating the sample concentrations using the graph plotting of the DPPH inhibition (%) and concentrations resulted in a 50% inhibition of DPPH radical scavenging (IC_{50}).

Ferrous iron chelating assay (FRAP)

The metal chelation activity of the extracts was determined using the ferrous iron chelating method. The FRAP assay was conducted using the study of Suphiratwanich P, et. al. (2023)[17]. In brief, the extracts were solubilized in DMSO to obtain the final concentrations of 0.001–10 mg/mL. Then, various concentrations of the extract (100 µL) were mixed with 2 µM of ferrous sulfate aqueous (50 µL), followed by adding 5 mM ferrozine (50 µL) and then adjusting the final volume to 300 µL with distilled water. After incubation for 15 minutes, the optical density was read at 570 nm. EDTA was used as a positive control, while the complex formation molecules of $FeCl_2$ and ferrozine were used as the negative control. The metal chelating activity (%) was determined via this equation:

% Metal chelating activity = [(AC − AT)/AC] × 100

where AC stands for the control sample's optical density (without the extract) and AT for the test sample's optical density. The graph plotting of the metal chelating activity (%) and the sample concentrations allowed for the calculation of the sample concentrations, which showed a result of 50% metal chelating activity (IC_{50})

ABTS radical scavenging capacity

Free radical scavenging capacity using a stable ABTS radical was performed according to a modification of the improved ABTS method of Zhou, et. al. (2018)[16]. The assay was carried out in a 96-well microtiter plates. Each extract (10 µL) was added to 195 µL of 120 µM ABTS radical solution and allowed to react at 25±2◦C for 30 minutes. The decrease in absorbance at 734 nm was measured against a blank (ethanol). The assay was conducted in triplicate, and Trolox was utilized as the positive control.

The sample concentrations were calculated using a graph plotting the ABTS inhibition (%) against concentrations, resulting in a 50% inhibition of ABTS radical scavenging (IC50).

2.8. Antibacterial activity

Microorganisms

The reference bacterial strains of *Staphylococcus aureus* and *Cutibacterium acnes* were purchased by American Type Culture Collections (ATCC; *S. aureus* ATCC29213 and *C. acnes* ATCC6919). Single colonies were cultured in Mueller-Hinton broth overnight at 37 °C. The bacteria were then diluted to an optical density of 0.01 at 600 nm (OD600) and cultivated until reaching a final concentration of 6 log CFU.

Determination of minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC)

The MIC and MLC were assessed using the broth microdilution method, as outlined by the Clinical and Laboratory Standards Institute (CLSI)[18]. Stock solutions of the extracts were initially dissolved in DMSO, followed by the preparation of a series of two-fold dilutions of each extract in Müller-Hinton broth medium. MIC was determined within the range of 5 µg/mL to 50 µg/mL using the microbroth dilution test. Subsequently, 100 µL of bacterial suspension was added to the wells of a 96-well plate containing 100 µL of serially diluted antimicrobials. Both negative (plain medium and DMSO) and positive control (plain medium and bacteria) experiments were conducted. The plates were covered with a lid and then incubated at 37°C for 18 hours. The MIC endpoint was identified as the lowest concentration of the antimicrobial at which turbidity was reduced. Turbidity readings were taken at 620 nm. The assay was carried out in triplicate with two biological replicates.

2.9. Liquid chromatography–mass spectrometry (LC-MS) analysis

The screening of phytochemical compounds in Newport and Comet varieties of *H. lupulus* flowers extract were performed by Liquid Chromatography equipped with Quadrupole Time of Flight Mass Spectrometry (LC/Q-TOF MSMS), using a Bruker Q-TOF impact II LC/MS system (Agilent Technologies Inc., Santa Clara, CA, USA). In brief, the liquid chromatography (LC) system (UltiMate™ 3000 Basic HPLC, Thermo Scientific™, USA) consisting of pump and autosampler. Mass spectrometry analysis was conducted on a QTOF-MS/MS instrument, connected-to the LC system through a dual ESI interface. A column of Acclaim RSLC 120 C18 (100 mm × 2.1 mm inner diameter, 2.2 µm particle size), maintained at 40°C, from Thermo Fisher Scientific, Waltham, MA, USA, was employed for the analysis.

Nitrogen served as both the drying and collision gas in the ESI source. The ion source parameters were configured as follow: the drying gas flowed at a rate of

8 liters per minute, the heated capillary maintained a 4500 V, the fragment voltage was established at 150 V, the skimmer voltage was regulated to 65 V, the Hexapole radio-frequency voltage was maintained at 50 V, and monitoring occurred across a wavelength spectrum ranging from 210 to 600 nm.

The detection was conducted in negative electrospray ionization mode, with spectra was recorded by mass spectrometer scanning within the range of m/z 50-1300.

The mobile phases consisted of solution A (0.1% v/v formic acid in water) and solution B (acetonitrile) at a flow rate of 0.4 mL/min. Experimental mass spectra were analyzed using POS_MS-DIAL and the Pos-ReSpect library library; Pos-BMDMS-NP was employed for phytochemical.

The identification process was conducted through comparison with mass spectral libraries, employing a weighted similarity score encompassing accurate mass and MS/MS spectra. A threshold of 80% was chosen as the cut-off value for matching.

2.10. Statistical analysis

The data are presented as the mean and standard deviation (SD) of measurements conducted in triplicate.

3. RESULTS AND DISCUSSION

3.1. Plant extraction, phenolics, and flavonoids content evaluation

The Newport and Comet varieties of *H. lupulus* demonstrated percentage yields of approximately 34.08% and 39.02% of dry weight, respectively. Regardless of the extraction technique employed for the flower of *H. lupulus*, both extracts exhibited high yields. The Newport and Comet varieties contained total phenolic compounds measured at 36.98±2.38 and 66.66±7.96 mg QE/g extract, respectively. Furthermore, the total flavonoid contents of the Newport and Comet varieties were determined to be 5.26±0.04 and 8.20±0.65 mg QE/g extract, respectively. These findings suggest that the Comet variety possesses higher total phenolic and total flavonoid contents compared to Newport (refer to Table 1).

The efficacy of conventional ethanol extraction in recovering polyphenols and prenylflavonoids from hops has been consistently demonstrated in prior research. The outcomes of this study align with these established findings, revealing substantial yields and noteworthy levels of phenolic and flavonoid compounds in both Newport and Comet varieties[19].

3.2. Antioxidant activity evaluation

temperature of 220°C, the capillary voltage was set to

Since both Newport and Comet extracts contained high phenolic and flavonoid compounds, it is probable that they contribute to the medicinal properties of the plant. Therefore, the antioxidant activity of Newport and Comet was investigated.

Newport and Comet varieties demonstrated significant anti-oxidant activity, with IC_{50} 0.67 and 0.24 mg/mL, respectively, compared to Trolox, as determined DPPH radical scavenging assay. Additionally, they exhibited IC50 values of 0.54 and 0.16 mg/mL, respectively, in the ABTS assay. Furthermore, the antioxidant effects of Newport and Comet extracts were determined to be 28.02 and 19.88 mgTE/g extract, respectively, using the ferrous iron chelating (FRAP) assay (see Table 1).

This research is informed by previous studies indicating that hop extract possesses significant antioxidant capabilities across various assays[20]. Notably, the principal constituents of hops, including phenolics, flavonoids, and terpenoids, have been identified as predominantly responsible for this activity. Noteworthy among these compounds is xanthohumol, a prenylflavonoid extracted from hop flowers, which has been recognized for its pronounced antioxidant potential[12,21].

3.3. Antibacterial activity

Microbial susceptibility to Newport and Comet extracts was assessed using the broth microdilution assay to determine Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC). The results, summarized in Table 2, demonstrate significant antibacterial activity of both extracts against *Staphylococcus aureu*s. Specifically, Newport extract exhibited an MIC value of 0.061 mg/mL (MLC value of 31.25 mg/mL), while Comet extract showed an MIC value of 0.146 mg/mL (MLC value of 4.688 mg/mL) against *S. aureus*. Similarly, both extracts displayed notable antibacterial efficacy against *C. acnes*, with Newport extract showing an MIC value of 0.061 mg/mL (MLC value of 0.122 mg/mL) and Comet extract exhibiting an MIC value of 0.073 mg/mL (MLC value of 0.146 mg/mL), respectively.

Recent research has revealed significant activity against acne-causing bacteria, including *S. aureus* and *C. acne*, by Newport and Comet extracts. The activity of Newport and Comet extracts against the test microorganisms revealed that *C. acne* exhibited greater sensitivity to both varieties compared to *S. aureus.* This finding is corroborated by a study demonstrating that CO_2 hop extract exhibited antibacterial activity against *P. acnes* and *S. aureus*, with MIC values ranging between 0.8 and 6.2 μg/mL and 6.25 μg/mL to 12.5 μg/mL, respectively[20].

Hops have a long history of use as a natural preservative in beer due to their high concentration of bitter acids, which inhibit bacterial growth. Similar to previous studies, which reported antibacterial activity against S. aureus by various hop genotypes with a MIC range of 9.8 to > 250 µg/mL, active compounds identified include α-acids, β-acids, and xanthohumol[11,22,23]. Bitter acids, including α-acids and β-acids, have been extensively studied for their antibacterial activity, with β-acids demonstrating significantly greater antimicrobial activity compared to alpha acids[24]. Correspondingly, lupulone (β-acids) and xanthohumol from hop flowers, have illustrated strong inhibitory activity against *P. acnes* (MIC 0.1–3μg/mL), *S. epidermidis*, *S. aureus*, *K. rhizophila*, and *S. pyogenes*[5,25].

The antibacterial activity of hop components is attributed to their ability to penetrate the bacterial cell wall, facilitated by the hydrophobic nature of compounds in hop extracts. Furthermore, interaction between these components and the inner membrane leads to structural damage, subsequently inhibiting the active transport of sugars and amino acids[25,26].

Table 1. Evaluation of total phenolic, total flavonoid, 2,2-diphenyl1-picryhydrazyl (DPPH), ABTS radical scavenging capacity, and ferrous iron chelating assay in the Newport and Comet varities of Humulus lupulus L. extracts

***Humulus lupulus* L**	**Total phenolic mg QE/g extract**	**Total flavonoid mg QE/g extract**	**DPPH IC50 (mg/mL)**	**ABTS IC50 (mg/mL)**	**FRAP mgTE/g extract**
Newport	36.98±2.38	5.26±0.04	0.67	0.54	28.02
Comet	66.66±7.96	8.20±0.65	0.24	0.16	19.88

IC_{50} stands for the concentration of the test sample that provided 50% inhibition.

Table 2. Antibacterial activity (Minimum Inhibitory Concentration, MIC and Minimum Lethal Concentration, MLC) of Newport and Comet varieties of *H. lupulus* extracts against *S. aureus* and *C. acnes*.

***Humulus lupulus* L**	***S. aureus* ATCC 29213**		***C. acne* ATCC 6919**	
	MIC (mg/mL)	**MLC (mg/mL)**	**MIC (mg/mL)**	**MLC (mg/mL)**
Newport	0.061	31.25	0.061	0.122
Comet	0.146	4.688	0.073	0.146

MIC; Minimum Inhibitory Concentration, MLC; Minimum Lethal Concentration

3.4. Liquid chromatography–mass spectrometry (LC-MS) analysis

Based on the results of the study, both Newport and Comet varieties of *H. lupulus* extracts exhibited high levels of total phenolic and total flavonoid contents, which correlate with their antioxidant activity. This finding is supported by previous studies demonstrating the strong antioxidant properties of *H. lupulus* and identifying active compounds such as xanthohumol, cohumulone, and lupulone[20,21,28].

Additionally, the amount of total phenolic and total flavonoid compounds, along with antioxidant activity, are also associated with antibacterial effects. Previous studies have demonstrated that *H. lupulus* extracts exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria, with active compounds identified as humulone, lupulone, and xanthohumol[5,22,23]. Therefore, this study proposes to analyze the active compounds in *H. lupulus* flowers to realize their chemical composition and to comprehend the correlation of these compounds with the observed biological activities. Furthermore, this chemical composition study represents the first investigation of two varieties of *H. lupulus* flowers were cultivated in Thailand providing a valuable reference chromatogram for hops grown in Thailand.

The phytochemical profiles of the ethanolic extracts from the Newport and Comet varieties of *H. lupulus* flowers were determined using Q-TOF LC/MSMS in the negative ion mode. The analysis revealed a diverse array of constituents present in both extracts, including xanthohumol, cohumulone**,** lupulone, desmethylxanthohumol, xanthohumol-C, quercrtin, epicatechin, luteolin, resveratrol, humulone, ferulic acid, and β-caryophyllene as shown in Table 3. Notably, xanthohumol, cohumulone, and lupulone emerged as the principal compounds in both extracts.

In the chromatographic analysis, the retention times for the Newport extract were observed at 15.82, 18.77, and 20.23 minutes, corresponding to xanthohumol, cohumulone, and lupulone, respectively, as evidenced in Figures 2 and 3. Similarly, the Comet extract exhibited retention times of 15.83, 18.41, and 20.25 minutes, aligning with xanthohumol, cohumulone, and lupulone, respectively, as depicted in Figures 2 through 5.

Xanthohumol, classified as a chalcone within the prenylflavonoid group, is characterized by the molecular formula $C_{21}H_{22}O_5$, as determined through mass spectrometry (m/z 353.1406). Xanthohumol, the primary prenylflavonoid in hops, is predominantly converted into its isomeric flavanone, isoxanthohumol, during the boiling of wort[29]. Xanthohumol has been reported to exhibit a range of biological activities,

including antioxidant, antimicrobial, anti-inflammatory, and anticancer effects. These biological activities suggest that xanthohumol and another prenylflavonoids from hops have significant potential for application in cancer prevention programs, as well as in the prevention or treatment of post-menopausal hot flashes and osteoporosis [8,9,10,12,30].

Cohumulone, a significant component of α-acids, shares an identical molecular formula of $C_{20}H_{28}O_5$ at 347.1864 m/z. Under high-temperature conditions, α-acids are isomerized into iso-α-acids. Iso-α-acids are considered the primary contributors to beer bitterness and also exhibit antibacterial activity, particularly against Gram-positive bacteria. The thermal isomerization of cohumulone to isocohumulone, occurs via an acyloin-type ring contraction[31].

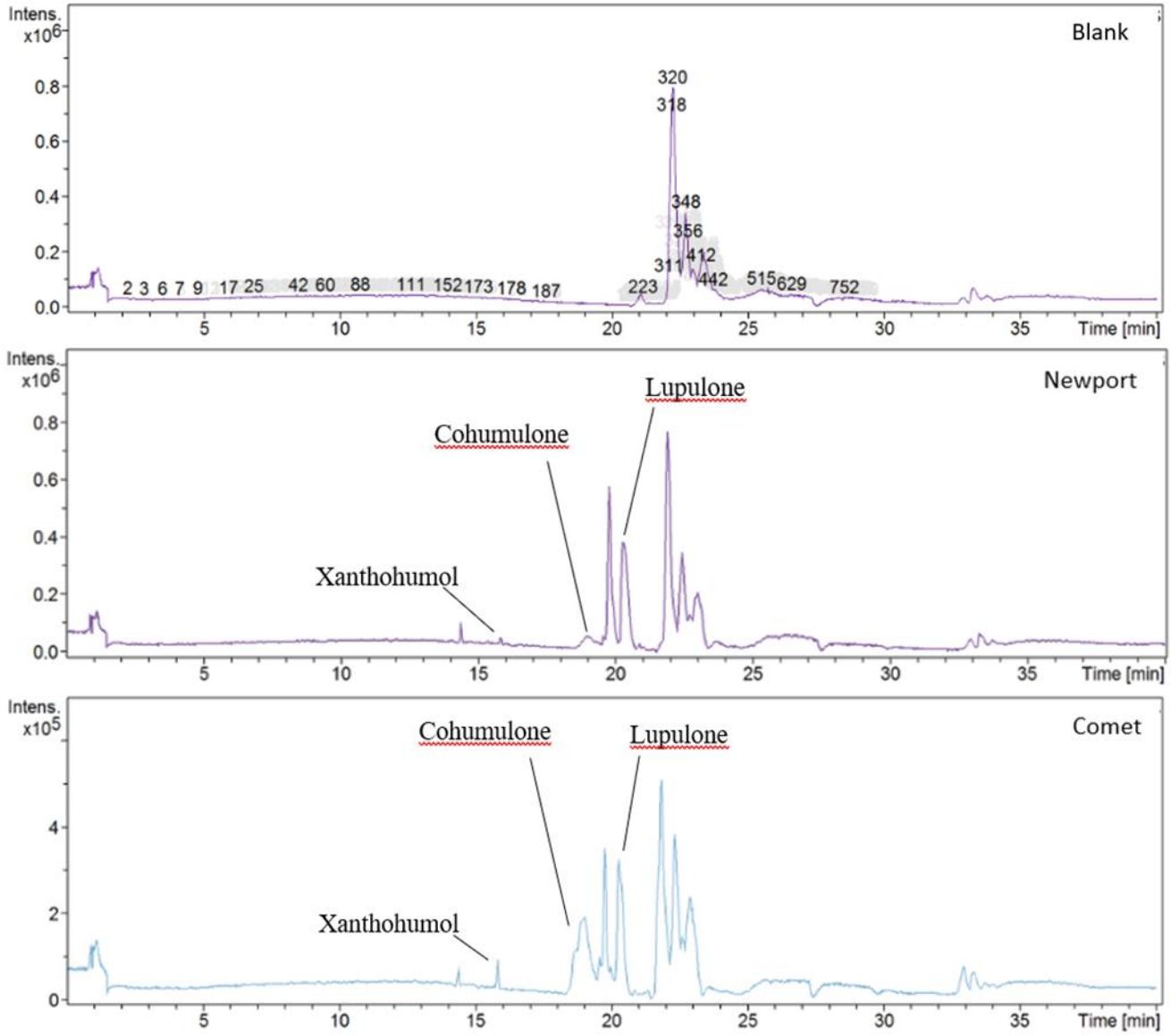

Figure 2. LC/MS-MS chromatograms of an ethanolic extract of Newport and Comet of *H. lupulus* extracts obtained using negative ion electrospray.

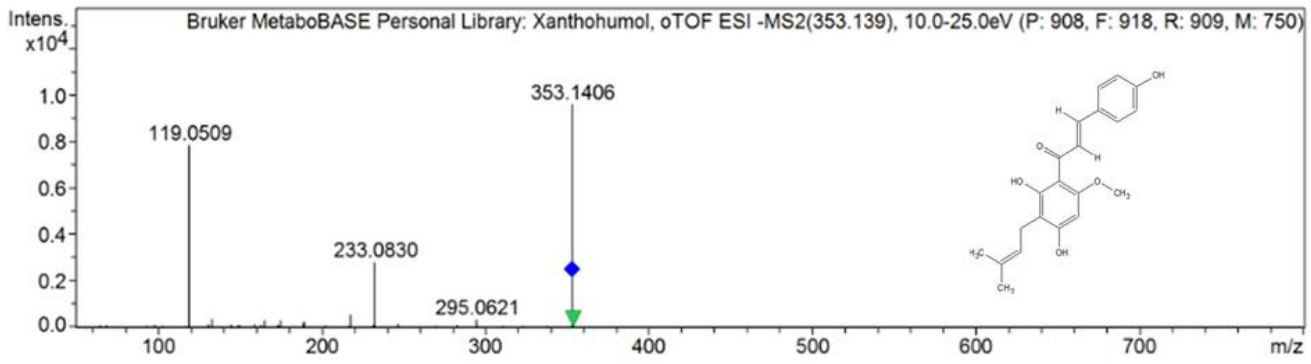

Figure 3. Mass spectrum of deprotonated molecular ion of xanthohumol, $C_{21}H_{22}O_5$, 353.1406 m/z

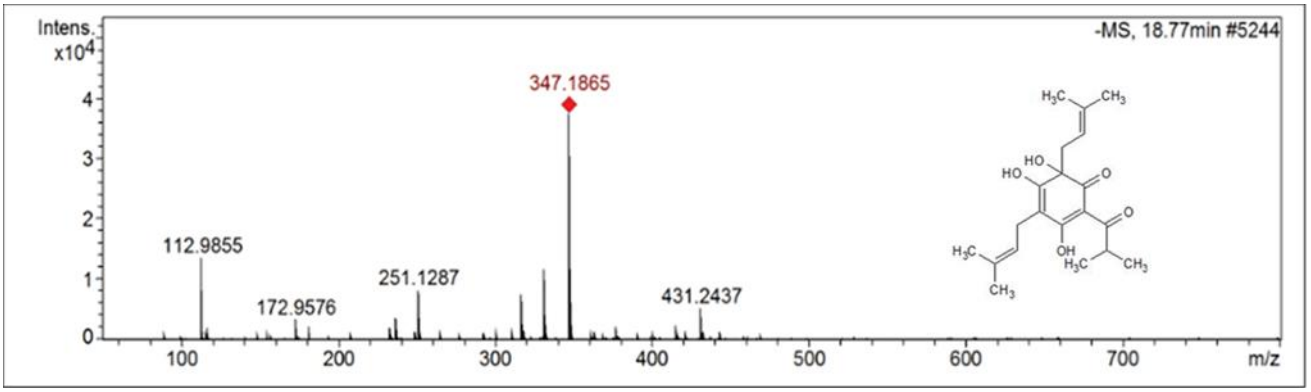

Figure 4. Mass spectrum of deprotonated molecular ion of cohumulone, $C_{20}H_{28}O_5$, 347.1864 m/z

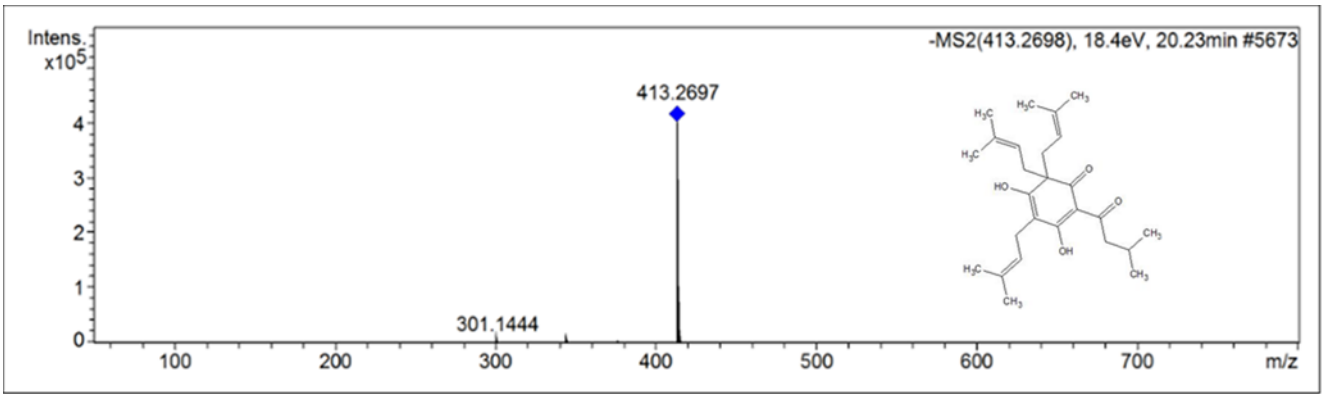

Figure 5. Mass spectrum of deprotonated molecular ion of lupulone, $C_{26}H_{38}O_4$, 413.2697 m/z

Lupulone, a key constituent of β-acids, was identified with a molecular formula of $C_{26}H_{38}O_4$ and a mass spectrum peak at m/z 413.2697. β-acids play a significant role in contributing to the plant's characteristic flavor and antimicrobial activity. Unlike α-acids, β-acids cannot isomerize in the same manner. The most crucial property of β-acids that influences their behavior during the brewing process is their susceptibility to oxidation, which is initiated by exposure to airborne oxygen[32].

The qualitative chemical composition analysis demonstrated that hop flowers from both varieties possess similar components. Compared to previous studies analyzing hop flowers from other countries, the chemical composition was similar[6,8]. However, some compounds were not detected in this experiment, possibly due to the analysis of isolated crude extracts.

As a result, compounds present in tiny amounts might have yet to be detected. These include 6-prenylnaringenin, 8-prenylnaringenin, catechin, and kaempferol.

The essential oil components, including myrcene, linalool, limonene, β-pinene, and β-caryophyllene oxide, are considered among the most identifiable and vital contributors to the aroma of hops, were undetectable due to the chosen extraction method and the utilization of ethanol as a solvent. Ethanol, known for its higher efficacy in extracting polar compounds than non-polar ones, likely contributed to this outcome.

Table 3. Liquid chromatography–mass spectrometry (LC-MS) analysis of Newport and Comet varieties of *H. lupulus* extracts

Proposed compounds	Retention time (min)		Chemical formula	Molecular formula (g/mol)	Observed (m/z)
	Newport	Comet			
xanthohumol	15.82	15.83	$C_{21}H_{22}O_5$	354.4	353.1406
cohumulone	18.77	18.41	$C_{20}H_{28}O_5$	348.4	347.1864
lupulone	20.23	20.25	$C_{26}H_{38}O_4$	414.6	413.2697
desmethylxanthohumol*	✓	✓	$C_{20}H_{20}O_5$	340.37	339.1283
xanthohumol-C*	✓	✓	$C_{21}H_{20}O_5$	352.38	351.1238
β-caryophyllene*	✓	✓	$C_{15}H_{24}$	204.35	203.1805
Ad-humulone / preumulone*	✓	✓	$C_{22}H_{32}O_5$	376.50	375.2177
quercrtin*	✓	✓	$C_{15}H_{10}O_7$	302.23	301.0354
luteolin*	✓	✓	$C_{15}H_{10}O_6$	286.24	285.0405
resveratrol*	✓	✓	$C_{14}H_{12}O_3$	228.24	227.0714
ferulic acid*	✓	✓	$C_{10}H_{10}O_4$	194.18	193.0506
epicatechin *	✓	✓	$C_{15}H_{14}O_6$	290.27	289.0718

* EIC; extracted ion chromatogram, ✓; minor

Table 4. Phytochemical constituents of Newport and Comet varieties of *H. lupulus* extracts comparing previous studies.

Compounds	Newport extract	Comet extract	Previous studies	Reference
α-acids				
cohumulone,	✓	✓	✓	6, 8
Ad-humulone / preumulone	✓	✓	✓	8
β-acids				
lupolone	✓	✓	✓	6, 8
Prenylated flavonoids				
xanthohumol	✓	✓	✓	6, 8, 21
xanthohumol-C	✓	✓	✓	6, 8, 21
desmethylxanthohumol	✓	✓	✓	6, 8, 21
Flavonoids				
epicatechin	✓	✓	✓	6, 8
quercetin	✓	✓	✓	6, 8
Phenolic				
ferulic acid	✓	✓	✓	6, 8
Stilbenes				
resveratrol	✓	✓	✓	6, 8
Essential oils				
β-caryophyllene	✓	✓	✓	6, 8

4. CONCLUSION

This study represents the first investigation into the phytochemical and biological activity of *H. lupulus* varieties in Thailand. The findings of this study demonstrate that hop flowers sourced from Thailand, specifically the Newport and Comet varieties, are rich in phenolic and flavonoid compounds. The primary phytochemical constituents identified through liquid chromatography-tandem mass spectrometry (LC-MS/MS) include xanthohumol, cohumulone, lupulone, as well as minor compounds such as desmethylxanthohumol, xanthohumol-C, quercetin, epicatechin, luteolin, resveratrol, humulone, ferulic acid, and β-caryophyllene. Additionally, extracts derived from Newport and Comet varieties exhibited notable antioxidant and potent antibacterial activities against *S. aureus* and *C. acnes*. These findings underscore the promising potential of Thai hop varieties, particularly Newport and Comet, for further investigation and potential applications in the development of natural pharmaceutical and cosmetic products.

5. ACKNOWLEDGMENT

The authors would like to thank the Faculty of Pharmacy, Payap University, and Faculty of Pharmacy, Mahidol University, for providing instruments. We are grateful to Dr. Nuttaluck Khumyong who provided hop samples.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This research received no external funding

Ethics approval

None to declare

Article info:
Received May 19, 2024
Received in revised form July 31, 2024
Accepted August 21, 2024

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