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RESEARCH ARTICLE

In Vitro and Bioinformatic Studies on The Phytochemical Constituent of Selected Zingiberaceae Plants Targeting Inhibitor Lipoxygenase (LOX)

Nur Amalia CHOIRONI[*°], Asep Gana SUGANDA[**], Kusnandar ANGGADIREDJA[***]

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SUMMARY

Lipoxygenase (LOX) is an enzyme essential for forming leukotriene, an endogenous substance involved in inflammatory pathological conditions, including asthma and rheumatoid arthritis. Plants that belong to the Zingiberaceae family have been empirically used as drugs in Indonesia. However, previous research has not spesifically identified the potential of this plant in inhibiting the lipoxygenase enzyme. This study aimed to screen the LOX-inhibiting activity of 18 plants from the Zingiberaceae family. The compounds of Curcuma zedoaria ((Chritsm.) Roscoe.) essential oil exhibiting the highest activity were identified for bioinformatic studies. Bioinformatics analysis to reveal and confirm the gene target of the active compound was acquired from the STITCH, STRING, and Swiss Target Prediction databases. The research result showed that the ethanol extract of C. zedoaria at 100 ppm had the highest inhibiting activity compared to other extracts, with a value of 54.8% and the IC50 value is 86.03 ppm. The further test also showed that the essential oil of C. zedoaria (EOCZ) in the selected plants was better than the extracts and their fractions. Based on GC-MS identification, the main components are longiverbenone, germacrene A, and α-pinene. The bioinformatics study showed that the potential target of EOCZ is MAPK3, MPO, HMOX1, ACE, PARP1, PPARG, ALOX 5 (5-LOX), PTGS1, PTGES, ESR, and NOS2. And one of the potential targets of EOCZ is 5-LOX. 5-LOX is essential in inflammatory reactions, and EOCZ, by inhibiting 5-LOX, may have the potential as an adjunct therapy for respiratory disorders.

Key Words: *Lipoxygenase, Zingiberaceae, Curcuma zedoaria.*

Seçilmiş Zingiberaceae Bitkilerinin İnhibitör Lipoksijenazı (LOX) Hedefleyen Fitokimyasal Bileşeni Üzerine In Vitro ve Biyoinformatik Çalışmalar

ÖZ

Lipoksijenaz (LOX), astım ve romatoid artrit dahil olmak üzere iltihaplı patolojik durumlarda rol oynayan endojen bir madde olan lökotrien oluşumu için gerekli bir enzimdir. Zingiberaceae ailesine ait bitkiler Endonezya'da deneysel olarak ilaç olarak kullanılmıştır. Ancak daha önce yapılan araştırmalarda bu bitkinin lipoksijenaz enzimini inhibe etme potansiyeli özel olarak belirlenmemiştir. Bu çalışma, Zingiberaceae ailesinden 18 bitkinin LOX inhibe edici aktivitesini taramayı amaçlamıştır. En yüksek aktivite sergileyen Curcuma Zedoaria ((Chritsm.) Roscoe.) uçucu yağının bileşikleri, biyoinformatik çalışmalara devam etmek için tanımlanmıştır. Aktif bileşiğin gen hedefini ortaya çıkarmak ve doğrulamak için biyoenformatik analizi STITCH, STRING ve Swiss Target Prediction veritabanlarından elde edilmiştir. Araştırma sonucu, C. zedoaria türünün 100 ppm'deki etanol özütünün %54,8'lik bir değerle diğer özütlere kıyasla en yüksek inhibe edici aktiviteye sahip olduğunu ve IC50 değerinin 86,03 ppm olduğunu göstermiştir. Ayrıca, daha ileri test için seçilen bitkilerden C. zedoaria'nın (EOCZ) uçucu yağının, özütlerden ve fraksiyonlarından daha iyi olduğunu göstermiştir. GC-MS kullanılarak yapılan tanımlamaya göre ana bileşenler longiverbenon, germakren A ve α-pinendir. Biyoenformatik çalışması, EOCZ'nin potansiyel hedefinin MAPK3, MPO, HMOX1, ACE, PARP1, PPARG, ALOX 5 (5-LOX), PTGS1, PTGES, ESR ve NOS2 olduğunu EOCZ'nin den önce ve EOCZ'nin potansiyel hedeflerinden birinin 5-LOX olduğunu göstermiştir. 5-LOX, inflamatuar reaksiyonlarda önemli bir enzimdir ve EOCZ, 5-LOX'u inhibe ederek solunum yolu hastalıklarına yönelik yardımcı tedavi potansiyeline sahip olabilir.

Anahtar Kelimeler: *Lipoksijenaz, Zingiberaceae, Curcuma zedoaria.*

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[*] ORCID: 0000-0003-3399-5019, Department of Pharmacy, Jenderal Soedirman University, Purwokerto, Indonesia.
[*] ORCID: 0000-0003-3399-5019, Research Centre of Rural Health, Jenderal Soedirman University, Purwokerto, Indonesia.
[**] ORCID: 0000-0003-0523-129X, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.
[***] ORCID: 0000-0001-9879-6112, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.

[°] Corresponding Author; Nur Amalia Choironi
E-mail: nur.choironi@unsoed.ac.id

INTRODUCTION

LOX is an enzyme that catalyzes the oxygenation of arachidonic acid and converts hydroperoxy-eicosatetraenoic acid (HPETE) into leukotriene-A_4 (LTA_4). Other leukotrienes formed include LTB4, LTC_4, LTD4, and LTE_4. Leukotrienes are involved in the mediation of various inflammatory disorders, including asthma (Hafner, Kahnt, & Steinhilber, 2019). The inflammatory response involves a biosynthetic pathway known as the arachidonic acid cascade. Cyclooxygenase (COX) and lipoxygenase (LOX) are two enzymes particularly important in the cascade. While there have been many studies on COX, only a few studies have been conducted to investigate LOX, let alone studies on enzyme-inhibiting substances (Leuti et al., 2020).

Lipoxygenases in the human body contribute significantly to the stimulation of inflammatory responses. Reactive oxygen species are key inflammatory disorders signaling molecules that trigger cytokine production and LOX activation. Illnesses, including asthma, cancer, stroke, cardiovascular and neurological diseases, are associated with inflammation. LOXs produce prostaglandins and leukotrienes. They are linked to the emergence of diseases, and preventing them is a step in preventing the disease. Linoleic, linolenic, and arachidonic acid are three polyunsaturated fatty acids (PUFAs) oxidized by lipoxygenases (LOXs), a class of monomeric proteins, to create hydroperoxides. LOXs are widely distributed in the kingdoms of cyanobacteria, plants, and animals. Additionally, 9-LOX and 13-LOX are plant LOXs that catalyze the oxygenation of linoleic and linolenic acid, whereas 5-LOX is pervasive in mammals and oxygenates carbon-5 on arachidonic acid (Leuti et al., 2020). The enzyme 5-lipoxygenase (5-LOX), also known as Arachidonate *5-lipoxygenase* (*ALOX5),* oxidizes arachidonic acid, which interacts with eicosapentaenoic acid to generate inflammation-causing leukotrienes. Drugs that inhibit 5-LOX are thought to prevent the production of inflammatory mediators from the arachidonic acid pathway, as 5-LOX plays a crucial role in the production of leukotrienes. Thus, it has been determined that inhibiting 5-LOX enzymes is a sensible strategy for antiinflammatory medications, especially for asthma. Zileuton, a synthetic inhibitor of 5-LOX, demonstrates hepatotoxic effects, similar to other synthetic medicines. This possible limitation, it is necessary to create inhibitory alternatives to 5-LOX (Loncaric et al., 2021). Due to the undesirable elements of the LOX pathway, research on lipoxygenase inhibition was conducted.

Plant phytochemicals have a significant protective function that may help prevent diseases brought on by oxidative stress. For thousands of years, several plants have been used as medicine. Plants which belong to the Zingiberaceae family, including Amomum compactum, Zingiber amaricans, Curcuma zedoaria, Curcuma xanthorriza, Kaempferia galanga, and Maranta galanga, have been empirically used in Indonesia to relieve pain, swelling, and asthma (Kloppenburg, 1983). Several *in vivo* pharmacological studies have demonstrated antiinflammatory activity of plants from the Zingiberaceae family (Leelarungrayub, Manorsoi, & Manorsoi, 2017; Karungkaran & Sadanandan, 2019). Early data from studies on *Zingiber purpureum*, *Zingiber officinale*, *Kaempferia galanga*, *Curcuma domestica*, *Zingiber zerumbet*, *Alpinia galanga*, *Curcuma xanthoriza*, *Curcuma aeruginosa*, and *Curcuma zedoaria* have so far been available (Wahuni, Sufiawati, Nittayanannta, & Levita, 2022; Iweala et al., 2023; Reanmongkol et al., 2011; Singh et al., 2012; Cahyono, Suzery, & Amalina, 2023; Rahaman et al., 2020). However, the study did not specifically determine the potential of Zingiberaceae plants in inhibiting the lipoxygenase enzyme. Therefore, further research is needed to determine its activity and the target protein that inhibits the enzyme. Based on this background, this study aims to verify the best LOX inhibitory activity

of several Zingiberaceae plant extracts and confirm the prediction of its molecular target.

MATERIAL AND METHODS

Preparation of Plant Material

Rhizomes and semen from 18 species of Zingiberaceae were selected based on ethnobotanical literature (Cumming et al., 2019; Kloppenburg, 1983). Plants used in this research were obtained from Manoko Garden, and their identification was carried out at Herbarium Bandungense, School of Life Sciences and Technology, ITB, Bandung, West Java, Indonesia. They were the rhizomes of *Curcuma manga*, *Kaempferia galangal*, *Curcuma xanthorrhiza*, *Zingiber officinale*, *Boesenbergia pandurata*, *Zingiber purpureum*, *Zingiber ottensii*, *Curcuma zedoaria*, *Curcuma aeruginosa*, *Zingiber aromaticum*, *Amomum zingiber*, *Alpinia galanga*, *Maranta galanga*, *Zingiber zerumbet*, *Zingiber amaricans*, *Curcuma domestica*, *Curcuma heyneana*, and the seed of *Amomum compactum*.

Extraction and Fractionation

The samples of 300 g were extracted by maceration using ethanol 95 % (in a 1:5 sample-to-solvent ratio) for 24 hours, followed by filtration. Residues were re-extracted twice with the same method and solvent. Ethanol extracts were concentrated using a rotavapor and dried using a freeze dryer. The extract with the highest inhibition activity, *C. zedoaria*, was fractionated using liquid-liquid extraction. The extract of *C. zedoaria* is dissolved in hot water at a ratio of 1:10. The aqueous solution is extracted using n-hexane solvent, then extracted with ethyl acetate solvent. The ratio between the aqueous solution and the solvent is 1:1. Three fractions were obtained: the n-hexane fraction, the ethyl acetate fraction, and the water fraction. The fractionation results were concentrated using a rotary vacuum evaporator and dried using a freeze dryer.

LOX-inhibiting Activity of Extracts

Assay of LOX inhibiting activity was adopted from Khan et al. (2012) with minor modifications. Test solution (100 ppm for extract and seven ppm for baicalein) 10 µL was pipetted into 1000 µL linoleic acid solution and 1690 µL of 0.2 M borate buffer solution. The solution was pre-incubated at 25 °C for 10 minutes. Three hundred microliters of 10000U/ml LOX were added, and the solution was then incubated for 5 minutes. After the second incubation, the formed HPETE can be measured for its absorbance at 234 nm using a UV spectrophotometer. Each sample was analysed in triplicate. The absorbance of the reference substance baicalein was used as a control. The percent of inhibition was calculated using the formula:

$$\%\in \text{hibition} = \frac{\text{Absorbance of control} - \text{absorbance of test subtance}}{\text{absorbance of control}} \times 100\%$$

The IC_{50} value represented the inhibiting activity for the highest activity; subsequently will be continued for fractionated and destillated. The lipoxygenase inhibition activity test on the fractions and essential oil of *C. zedoaria* was conducted using the same procedure as the test on the extract. Ethanol extract, n-hexane fraction, ethyl acetate fraction, water fraction, and essential oil of *C. zedoaria* were prepared into solutions with concentrations of 50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm to determine the IC_{50} value for lipoxygenase inhibition. For the positive control, baicalein, solutions were made with one ppm, three ppm, five ppm, and seven ppm.

Distillation and Identification of Essential Oils

The essential oil of *C. zedoaria* (EOCZ) was obtained through hydrodistillation using a modified apparatus. 2 kg of *C. zedoaria* produced 4,2 mL of 100% concentrated essential oil. The essential oil was stored in a brown glass bottle at 0–4°C after being dried using anhydrous sodium sulfate. Gas chromatography-mass spectrometry (GC-MS) examined the essential oil composition. The gas chromatograph is equipped with a split-splitless injector and 5% difenil-95% methylpolysiloxane, 30 m, 0.25 mm, 0.25 µm film thickness capillary column RTX5. Gas chromatography conditions include a temperature range of 60 to 290°C at 8°C/min, with a

solvent delay of 2 min. The injector was maintained at a temperature of 280°C. The inert gas was helium at a flow of 1.31 mL/min, and the injected volume in the splitless mode was 0.20 µL. The qualitative analysis was based on the percent area of each peak of the sample compounds. The mass spectrum of each compound was compared with the mass spectrum from the Wiley 7 and *National Institute of Standards and Technology* (NIST) databases.

Bioinformatics Analysis

This study involved a bioinformatic analysis to identify the gene targets of the active compound of EOCZ on asthma (Figure 1). The databases used included: NCBI to analyze gene expression in asthma (https://www.ncbi.nlm.nih.gov/), Swiss Target Prediction to identify the direct or indirect protein targets of EOCZ (http://www.swisstargetprediction.ch/) (Daina, Michielin, & Zoete, 2019), and Venny 2.1 for analyzing the overlap of EOCZ targets as an anti-asthma agent. STRING was used to analyze the protein-protein interaction network based on Venny 2.1 results (https://string-db.org/) (Szklarczyk et al., 2023), and WEBGESTALT was used to analyze GO and KEGG pathways (http://www.webgestalt.org/) (Elizarraras et al., 2024). Their results were visualized as a bar graph.

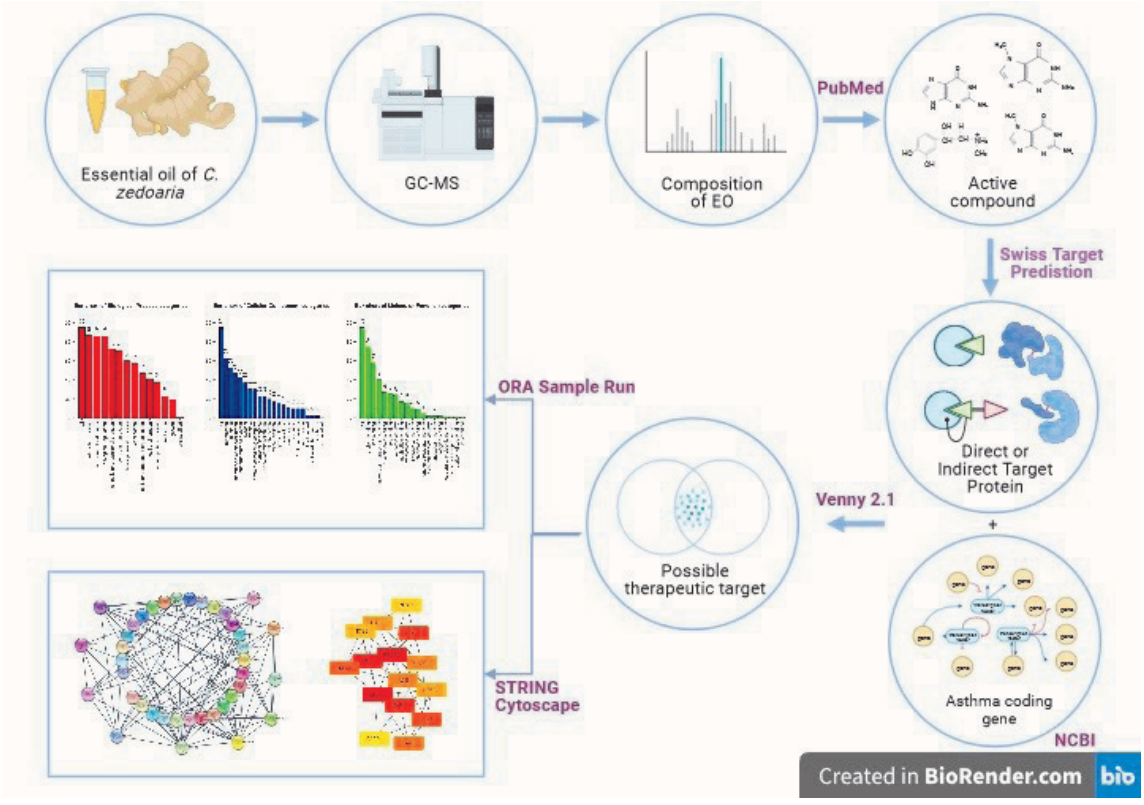

Figure 1. Scheme of bioinformatics analyses

RESULTS AND DISCUSSION

LOX-inhibiting Activity of Zingiberaceae Plant

The current study was carried out to screen plant extracts with lipoxygenase-inhibiting activity. Results showed that the ethanol extract of *Curcuma zedoaria* at 100 ppm had the highest inhibiting activity compared to the other extracts, with a value of 54.8% (Table 1).

Table 1. Inhibition activity of LOX of the Zingiberaceae species and baicalein

Species	Vern name	Part of the plant	Yield (%)	Inhibition (%)
Curcuma manga	Temu manga	Rhizomes	5.00	7.17 ± 1.55
Curcuma xanthorrhiza	Temulawak	Rhizomes	5.45	10.54 ± 2.63
Amomum zingiber	Jahe merah	Rhizomes	10.35	11.64 ± 2.52
Zingiber officinale	Jahe	Rhizomes	5.60	12.08 ± 1.26
Alpinia galanga	Lengkuas	Rhizomes	10.55	12.29 ± 1.71
Zingiber ottensii	Bangle hantu	Rhizomes	7.00	13.07 ± 3.41
Zingiber purpureum	Bangle	Rhizomes	6.45	13.52 ± 1.90
Boesenbergia pandurata	Temu kunci	Rhizomes	6.35	15.10 ± 4.27
Amomum compactum	Kapulaga	Seed	2.05	15.48 ± 6.86
Zingiber amaricans	Lempuyang emprit	Rhizomes	11.50	16.37 ± 1.55
Kaempferia galanga	Kencur	Rhizomes	5.10	16.94 ± 3.92
Curcuma domestica	Kunyit	Rhizomes	12.50	21.50 ± 1.37
Zingiber aromaticum	Lempuyang wangi	Rhizomes	8.75	40.98 ± 6.19
Maranta galanga	Laos merah	Rhizomes	10.80	42.46 ± 6.28
Curcuma heyneana	Temu giring	Rhizomes	12.55	47.92 ± 3.70
Zingiber zerumbet	Lempuyang gajah	Rhizomes	11.35	51.62 ± 1.17
Curcuma aeruginosa	Temu hitam	Rhizomes	7.35	52.71 ± 4.98
Curcuma zedoaria	Temu putih	Rhizomes	7.25	54.58 ± 1.02
Baicalein	-	-	-	54.90 ± 1.16

Subsequently, the highest activity is fractionated and distilled. IC_{50} was determined for the ethanol extract, *n*-hexane fraction, ethyl acetate fraction, water fraction, and essential oil of *C. zedoaria* (EOCZ). The inhibition value of *C. zedoaria* extract was 86.03 ppm, and the reference substance baicalein was 6.59 ppm (Table 2). LOX inhibiting activity was determined by comparing the absorbance of hydroperoxyeicosatetranoate (HPETE) formed in the presence or absence of the extracts. As the substrate of LOX, linoleic acid was used in the current study, as this is due to the structural similarity between linoleic acid and arachidonic acid. Both substrates are unsaturated fatty acids with methylene units between two double bonds. Both substrates are unsaturated fatty acids with methylene units between two double bonds. The mechanism of the LOX reaction consists of four steps. The LOX reaction's initial step is removing the hydrogen atom from the methylene unit between the double bonds in the substrate fatty acid, and the resulting electron is picked up by the ferric non-heme iron, which is reduced to the ferrous form. The second step is radical rearrangement, then oxygen insertion and peroxy radical reduction. The iron is reoxidized to its ferric form, and the peroxy anion is protonated (Cumming et al., 2019; Ivanov et al., 2010).

Table 2. LOX-inhibiting activity of *C. zedoaria* samples

Species	Yield (%)	IC_{50}
Ethanol extract	7.25	86.03 ± 2.15
Fraction of n-hexane	15.07	49.60 ± 2.78
Fraction of ethyl acetate	33.73	40.91 ± 3.14
Fraction of water	7.20	44.36 ± 2.97
Essential oil	0.21	17.03 ± 6.02
Baicalein (7 ppm)	-	6.59 ± 2.23

Phytochemicals naturally occur in plants with defense mechanisms and disease protection (Dosoky & Setzer, 2018). Phytochemical screening showed that the ethanol extract of *C. zedoaria* contained alkaloids, quinones, flavonoids, phenols, and steroid/triterpenoids is consistent with several studies that flavonoids and triterpenoids contribution to lipoxygenase-inhibiting activity (Eissa et al., 2020). An earlier study by Dosoky and Setzer showed that the ethanol extract of *C. zedoaria* had inflammatory activity *in vivo* (Dosoky and Setzer, 2018). *C. zedoaria* belongs to the family of Zingiberaceae, which has been reported to contain curcuminoids, flavonoids, and essential oils. Curcuminoid (constituted by curcumin, demethoxycurcumin, and bisdemethoxycurcumin) was shown to have a role in inhibiting the enzyme lipoxygenase (Yang et al., 2017). Work by Sroka et al. (Sroka, Sowa, & Drys, 2017) demonstrated that flavonoids also had lipoxygenase-inhibiting activity.

Identification of EOCZ

This study showed that the essential oil had better lipoxygenase inhibition than the extract and its three fractions. This shows that compounds that play a role in the lipoxygenase inhibition process are more abundant in essential oils than others. The identification of essential oil components (Table 3) using GC-MS shows that there are 26 peaks (Figure 2) and compares the mass spectrum with the Wiley 7 and *National Institute of Standards and Technology* (NIST) databases. In the study of Servi et al. (Servi, Sen, Servi, & Dogan, 2021) proposed that the presence of α-pinene, elemicin, and estragole, possibly synergistic action for the antiinflammatory effect. Corroborating these findings, Albano et al. (2012) showed the enzyme-inhibiting activity of essential oil, which terpenoid group containing **α**-thujene, **α**-pinene, camphene, sabine, **β**-pinene, 1,8-cineole, *p*-cymene, **β**-myrcene, **α**-phellandrene, limonene, **α**-terpinol, **γ**-terpinene, camphor, trans-linalool oxide, trans-anetol, carvacrol, and eucalyptol.

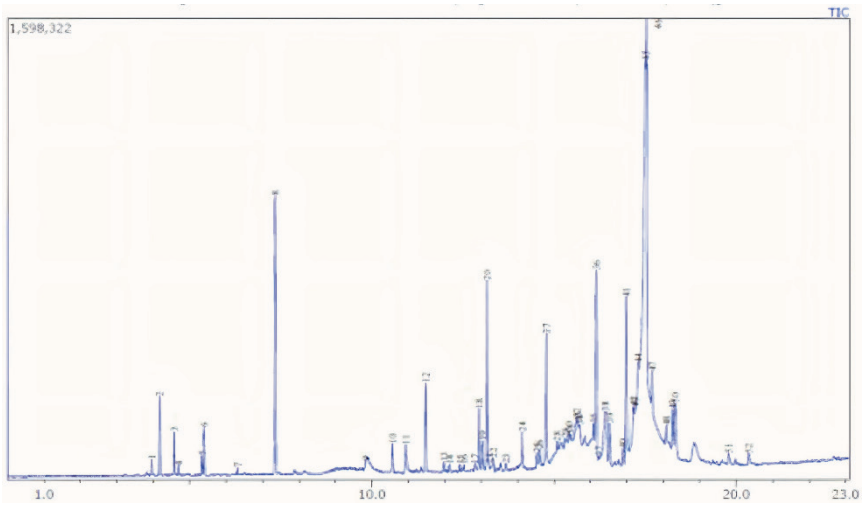

Figure 2. GC-MS Chromatogram of EOCZ

Juergens also hypothesized that 1,8-cineol inhibits 5-LOX and COXs to stop the production of inflammatory arachidonic acid metabolites such as LTB4 and PGE2 (Juergens, 2014). The camphoraceous oil of *Rosmarinus officinalis* showed the most remarkable ability to inhibit LOX in vitro (Cutillas, 2018). The inhibition of 5-LOX by α-pinene was also suggested by Kohoude et al. (Kohoude et al., 2017)..

Table 3. Chemical constituent of EOCZ

Retention time	Area	m/z	Constituent	RIcalc	RIdb
3.953	4.25	136	α-pinene	931	931
4.175	1.37	136	Camphene	944	948
4.572	0.77	136	β-pinene	976	978
4.692	0.15	136	β-myrcene	989	989
5.331	0.37	136	Limonen	1026	1028
5.390	0.91	154	Eucalyptol	1030	1030
6.309	0.13	142	2-nonanon	1088	1090
7.348	6.80	152	Camphor	1145	1145
11.476	1.92	204	β-elemene	1281	1281
11.977	0.17	204	Cariophyllene	1418	1418
12.132	0.16	204	Cyclohexane	1470	1471
12.518	0.18	204	α-humulen	1454	1454
12.942	1.47	204	Germacrene-D	1462	1463
13.330	0.59	204	β-elemene	1471	1471
14.126	0.75	204	Germacrene B	1558	1560
14.792	3.57	286	Boldenon	1561	1561
15.083	0.24	190	Triquinasen	1565	1565
15.455	0.74	220	Isobutyric acid	1566	1566
16.085	0.37	216	Furanodiena	1884	1886
16.160	5.21	204	Germacrene A	1885	1885
16.400	3.63	214	Azulene	1892	1892
16.523	1.00	204	Delta selinene	1897	1898
16.887	0.38	218	2,3,3-Trimethyl-2-(3-methylbuta-1,3-dienil)-6-methylencyclohexanone	1888	1899
17.181	2.35	176	Cyclolohеptatriene	1898	1900
17.311	4.14	214	1-(1-Methylamino-2-hydroxy-3-propyl)-dibenzo(b,e)-Bicyclo(2,2,2) octadiene Hydrochloride	1921	1921
17.539	12.29	218	Longiverbenon	1998	1998

RI_{calc}: Retention index determined concerning homologous series of n-alkanes, RI_{db}: Retention index from the database (Poudel et al., 2022; Quemel et al., 2021; Satyal, 2015)

Bioinformatics Analysis

To determine the mechanism of action of compounds in EOCZ (Figure 3), a bioinformatic study was carried out on the four dominant compounds in EOCZ, namely longiverbenon, camphor, α-pinene, and germacrene A (Table 3). The result showed that the potential target of these compounds was related to the expression of genes for asthma. Based on Venny's analysis, these four compounds affected 38 target genes encoding asthma. The protein-protein interaction network of EOCZ was analyzed using the STRING database. EOCZ interacted with proteins, namely MAPK3, MPO, HMOX1, ACE, PARP1, PPARG, ALOX 5 (5-LOX), PTGS1, PTGES, ESR, and NOS2 (Figure 3). In addition, ALOX5 has an impact on asthma activity. Swiss target analysis shows that longiverbenon, germacrene A, and α-pinene interaction with 5-LOX. The result of KEGG pathway analysis represents the inflammatory process. The KEGG pathway analysis indicates that there are pathways related to inflammation processes, such as prostaglandin receptor, GPCR, prostaglandin E receptor, eicosanoid receptor binding, and MAP kinase activity (Figure 4). KEGG pathway analysis described the interaction pathways that occur from proteins obtained by the STRING analysis. The enrichment ratio level represents the evidence-related pathways.

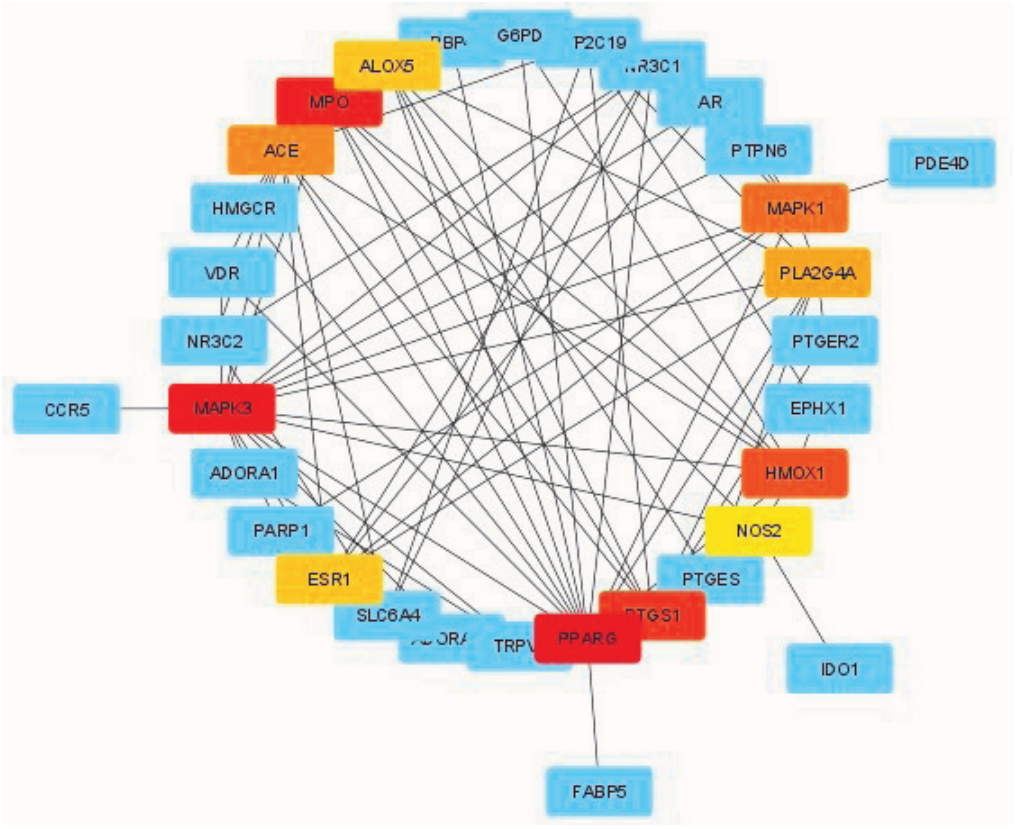

Figure 3. Top top-scoring displayed the target genes of EOCZ

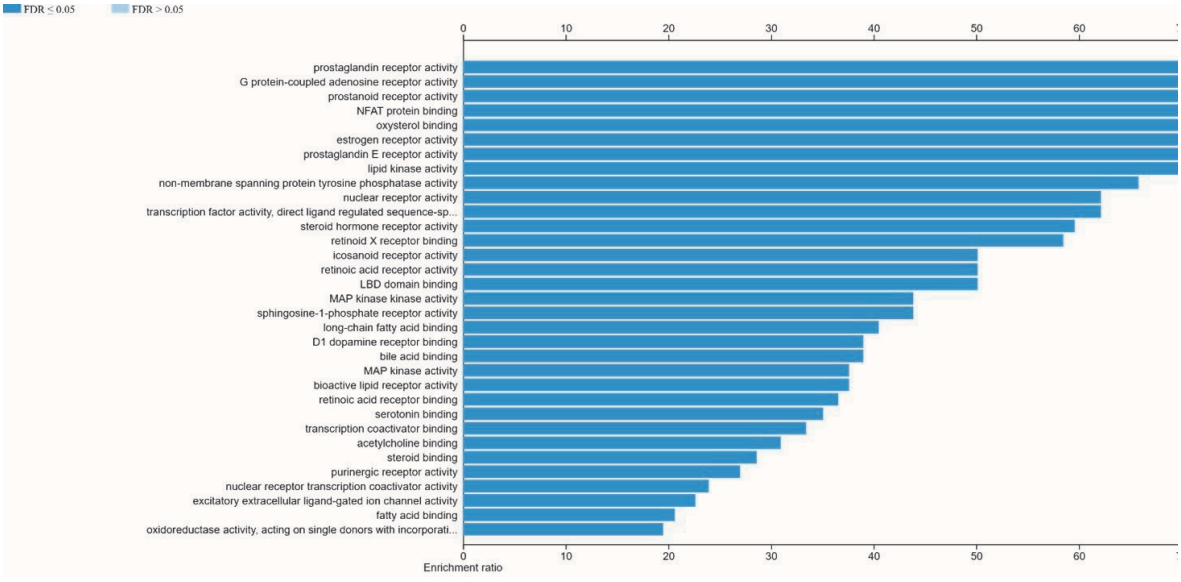

Figure 4. KEGG pathway of EOCZ

The GO analysis described the characteristics of a gene are biological process (BP), cellular component (CC), and molecular function (MF) (Figure 5). The analysis of KEGG pathway and gene ontology (GO) using the WEBGESTALT database was carried out to identify the involvement and role of these proteins, derived explicitly from wide-ranging molecular datasets generated by genome sequencing and other high-throughput experimental methods. The BP analysis showed the metabolic process, biological regulation, cell communication, and cell proliferation process. CC analysis describes the identified protein's localization sites at the membrane, nucleus, mitochondria, extracellular matrix, and Golgi apparatus.

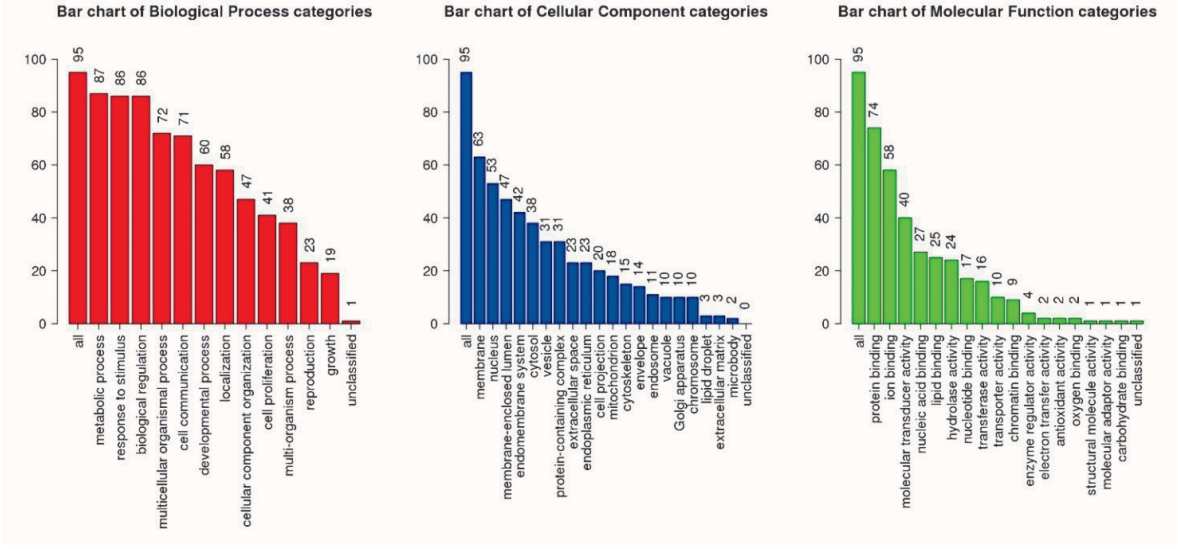

Figure 5. Gene ontology of EOCZ

The MF analysis reflects the molecular functions related to enzyme regulator activity and antioxidant activity (Figure 5). 5-LOX, which is found on chromosome 10q11.21, encodes 5-Lipoxygenase. The 5-LOX gene is located on chromosome 10q.11.21 and codes for an enzyme expressed in bone marrow-derived inflammatory cells. This enzyme catalyzes the conversion of arachidonic acid to LTA4, which is then converted by other enzymes in the LT pathway to LTB4 and the cysLTs (LTC4, LTD4, and LTE4). Asthma, atherosclerosis, and pulmonary hypertension are only a few of the inflammatory disorders that have been linked to 5-LOX function. Others have investigated the role of 5-LOX in predicting the phenotype of asthma and how well it responds to treatment. The association between 5-LOX expression and a patient's reaction to an anti-5-LOX drug was originally demonstrated. The impact of 5-LOX Sp1 promoter genotypes on leukotriene production and asthma outcomes may be influenced by other polymorphisms in 5-LOX, or leukotriene pathway genes like LTC4S and LTA4H. The powerful 5-LOX inhibitor prevents a significant percentage of cysteinyl leukotriene synthesis. Patients with bronchiolar asthma have been found to have the genetic E254K (760G4A) mutation in 5-LOX, despite the low incidence (Mashima & Torayuki, 2015; Mougey et al., 2013; Cai et al., 2019).

CONCLUSION

The current study's results show that the essential oil of *C. zedoaria* had the highest LOX-inhibiting activity among the tested plant extracts. The potential targets of EOCZ are 5-LOX, MAPK3, MPO, HMOX1, ACE, PARP1, PPARG, PTGS1, PTGES, ESR, and NOS2. 5-LOX is essential in inflammatory reactions and might be a potential adjunct therapy for this disease and respiratory disorder. Further research is needed to analyze its interaction with target receptors through molecular docking and dynamics.

AUTHOR CONTRIBUTION STATEMENT

Concept (NAC, AGS, KA), Design (NAC, AGS, KA), Supervision (AGS, KA), Resources (NAC, AGS), Materials (NAC, AGS), Data Collection and/or Processing (NAC), Analysis and/or Interpretation (NAC, AGS), Literature Search (NAC), Writing (NAC), Critical Reviews and proofreading (KA).

CONFLICT OF INTEREST

The authors declared no conflict of interest in this manuscript.

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