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ORIGINAL ARTICLE

Calamus caesius (Rattan) wood: Chemical constituents, biological activities' relative medicinal properties from Thai medicinal scriptures, and *in silico* antioxidant activity

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GC–MS

Abstract *Calamus caesius* is known as Rattan. It was found as a component of many formulas from evidence-based Thai medicinal scriptures but no research about their medicinal properties. We investigated the literature review analysis from Thai medicinal textbooks for proposed biological activity relatives including antioxidant, cytotoxic, anti-inflammatory, and antimicrobial activities, and chemical profiles using gas chromatography-mass spectrometry (GC–MS). In silico studies were inspected on tyrosinase and NAD(P)H oxidase actions. Thirty formulas from Thai medicinal textbooks found *C. caesius* as a component with a percent ratio in the range of 1.43 to 14.99, the formula's properties are antipyretic, followed by antidiarrhea, and cure abscesses related to inflammation and infection. Both water extracted and ethanol extracted showed high antioxidant activi-

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ties in all assays and showed no toxicity in macrophage-like cells and cancer cell lines. The ethanol extracted showed slightly bactericidal better than the water extracted, none of them inhibited against *C. albicans*. From GC–MS analysis, the highest components of water and ethanol extract are 3-*tert*-Butylamino-acrylonitrile and β-Sitosterol, respectively. Five chemical compounds revealed in both water and ethanol extracted of *C. caesius* are 1,3-di-*tert*-butylbenzene; 2,6-dimethoxyphenol; 2-propylphenol; 2,4-di-*tert*-butylphenol; methyl palmitate. Sterol compounds such as stigmasterol, beta-sitosterol, and campesterol from ethanol extracted showed outstanding interaction with both tyrosinase and NADPH oxidase *in silico* molecular docking study. All outcomes proven that *C. caesius* has potentially antioxidant effects to support health problems. Additionally, this is the first report on the scientific data of *Calamus caesius* wood related to its medicinal properties in the formula from Thai medicinal scriptures.

1. Introduction

Calamus caesius Blume (family Arecaceae) is widespread in South-East Asia, including Malaysia, Sumatra, Borneo, Palawan (the Philippines), and southern Thailand. *C. caesius* has a resilient and durable cane commonly used to make high-quality rattan carpets, mats, baskets, and in-house handicrafts or construction (Shim and Tan, 1993). In terms of medicinal use, the vine has a cold taste used to cure heat exhaustion, black fever, convulsions due to heat, relieve suffocation, stiff tongue and chin due to fever (Ayurvedic school, 2016).

To continue survival, the body maintains a consistent core temperature range of 37.2–37.7 °C or 99-100°F; fever or pyrexia or hyperthermia is defined as having a temperature above the average normal. Causes of fever are categorized by exogenous pyrogens (microbial substances, ex. LPS in the cell wall of bacteria) and endogenous pyrogens (cytokines of inflammatory mechanisms such as IL-1, IL-6, TNF-α). Some drugs such as antibiotics, antidepressants, and antihistamines also induce hyperthermia. Several signs and symptoms of fever are headaches, sweating, thirst, chills or shivering, nausea, lacking energy, poor appetite, etc (Ogoina, 2011). Antipyretic drugs, acetaminophen, aspirin, and ibuprofen are generally used for suppressing fever (Plaisance and Mackowiak, 2000).

Thai Traditional medicine (TTM) theory claims the human body is controlled by the balance of Tri-Dhatu (three elements) - consisting of Pit-ta (fire), Va-ta (wind), Sem-ha (water). Pit-ta represents all heat in the body involved in cell metabolisms, GI digestion, and thermal homeostasis. Va-ta describes fluids and airs throughout the body including blood circulation, respiratory, and nervous system. Sem-ha is all fluid in the body as saliva, tear, urine, blood, etc. They need to work together, so an imbalance of Tri-Dhatu can cause any illness that affects and manifest in the earth element. The reflection of higher fire (Pitta) in the body affected a high body temperature called "fever", which affects the skin showing red. In some cases, patients get a fever with papules, blisters, rashes, and diarrhea (Ayuraved-Wittayarai Foundation, 1988). Modern medicine considers fever with these symptoms to be involved in inflammation and infection mechanisms in the body, for example, dengue fever, chikungunya fever, chickenpox, hand and foot, and mouth disease, etc. (Ogoina, 2011). For clinical guideline practice of treatment in TTM, the most formulation is provided and recorded by Thai folk doctors and Thai traditional practitioners based on the Tri-Dhatu theory. The components might be slightly different according to available local herbals. The taste of herbs affected their pharmacological properties especially, for relieving fever usually use bitter, cold, and flavorless as the main constituent in the formula (Prommee et al., 2021).

Despite *C. caesius* has been reported as a component in the formulation of Thai traditional scriptures, the inscription of Thai historical pavilion walls and the stone. There is no research on their scientific reports in medicinal fields. Based on the literature review, we designed this research to investigate the preparation and purpose of using *C. caesius* in the formulation of Thai traditional medicine textbooks (Ayurvedic school, 2016). The results lead to a study on the pharmacological activities of their properties which include antioxidants, anti-inflammation, antimicrobials, and cytotoxicity. Additionally, the chemical constituents of the extract were also investigated by the Gas Chromatography-Mass Spectrometry (GC–MS) technique.

2. Materials and methods

2.1. Plant material and preparation of extract

Calamus caesius wood was purchased from Charoensuk Osod, a Thai herbal medicinal store in Nakorn Pathom province, Thailand. It was authenticated and deposited at the Thai traditional medicine herbarium, under the Thai Traditional medicine research institute, Ministry of public health of Thailand. The voucher specimen is TTM-c No. 1000721. Dried wood of *C. caesius* was ground and extracted using maceration and decoction following the previous study (Dechayont et al., 2021). All extracts have calculated the percentage of yield (% w/w) and were kept at −20 °C until further use.

2.2. Chemicals and reagents

DPPH and BHT were purchased from Fluka, Germany. ABTS, Trolox, potassium persulfate, DMSO, LPS, MTT, acetic acid, phosphoric acid, *N*-(1-naphthyl) ethylenediamine dihydrochloride, sulfanilamide, and resazurin sodium salt were purchased from Sigma-Aldrich, USA. Ferric chloride was purchased from Loba Chemie, India. RPMI 1640 Culture Medium, FBS, trypan blue stain 0.4%, and trypsin-EDTA were obtained from Gibco, USA. Hydrochloric acid and isopropanol were obtained from RCI Labscan, Thailand. PBS was provided by Biochrom, Germany. Nutrient agar and Mueller Hinton broth were purchased from Difco, USA. Amoxicillin and gentamicin were purchased from TCI, Japan.

2.3. Evaluation of antioxidant activities

2.3.1. DPPH radical scavenging assay

The DPPH solution at concentration 6×10^{-5} M was freshly prepared in absolute ethanol and protected from any light. Samples of stock at 1 mg/mL were prepared in a serial dilution (at least 4 concentrations). The ethanol extracts were prepared in absolute ethanol, the water extracts were prepared in dis-

tilled water. The 100 μL of sample solutions were added in 96 well-plates mixed with DPPH solution equally and put in the dark at room temperature for 30 min. BHT was prepared as same as the sample, as a positive control. The absorbance was measured at 520 nm using a microplate reader (Biotek, USA). The percentage of radical scavenging inhibition was calculated by the formula below (Phuaklee et al., 2021).

$$\text{The percentage of inhibition} = \frac{(\text{Abs.Control} - \text{Abs.Sample})}{\text{Abs.Control}} \times 100$$

A dose–response curve was created from the percentage of inhibition and calculated the EC_{50} using GraphPad software.

2.3.2. ABTS radical cation decolorization assay

The mixing of ABTS at concentration 7 mM with 2.45 mM potassium persulfate in deionized water was prepared and placed in the dark at room temperature for 12–16 h to generate the ABTS reagent (Re et al., 1999). It was diluted with deionized water to obtain an OD absorbance value of 0.700 ± 0.020 at wavelength 734 nm. The sample was dissolved in an appropriate solution as the ethanol extract in absolute ethanol and water extract in distilled water then prepared in five concentrations by serial dilutions technique. The 20 μL of sample solution was added in 96 well-plates mixed with the ABTS reagent 180 μL. The absorbance was measured 6 min later. The scavenging of the sample was calculated in percentage by this formula and generated the IC_{50} value by using GraphPad 4.0 software.

$$\text{The percentage of inhibition} = \frac{(\text{Abs.Control} - \text{Abs.Sample})}{\text{Abs.Control}} \times 100$$

2.3.3. Ferric reducing antioxidant power assay (FRAP)

The reducing powers of our extract can reflect their antioxidant activity by using a modified FRAP assay (Benzie and Strain, 1996). Concisely, freshly mixing of three solutions by 300 mM sodium acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl solution, and 20 mM ferric chloride ($FeCl_3$) solution in proportions of 10:1:1 (%v/v/v) was FRAP reagent. The 20 μL of sample solution (1 mg/ml) was added to 96 well plates. FRAP reagent was warmed in a water bath at 37 °C for 4 min before it (180 μL) was added to each well. After 8 min of mixing reaction, the absorbance was measured at 593 nm. Trolox and ferric sulfate were used as a standard to generate a calibration curve for ethanol extract and water extract, respectively. The results are expressed as ferrous ion equivalent or relative to a standard.

2.4. Evaluation of Anti-inflammatory activity

2.4.1. Nitric oxide inhibitory effect and MTT assay

The methodology of nitric oxide inhibition by lipopolysaccharide (LPS) induced from murine macrophage cell line (RAW 264.7) was evaluated as previously reported (Prommee et al., 2021). Briefly, the density of 1×10^5 cells/well was placed into 96 well plates and incubated at 37 °C with 5% CO_2 for 24 h to adhere. Freshly media with LPS (final conc. 5 ng/mL) was replaced with the test sample in various concentrations (max. 100 μg/mL) in equal volume and incubated. After 24 h, the supernatant nitrite was transferred into new 96 well-plates and evaluated by the Griess reagent. MTT assay was also evaluated to confirm cell viability which wasn't affected by our extracts. MTT solution was prepared at a concentration of 5 mg/mL in PBS added to the 96 well plate testing and placed in the incubator for 4 h. The excess supernatant was removed, then added 0.04 M HCl in isopropanol to dissolve the formazan product. The absorbance was measured at a wavelength of 570 nm. The percentage of inhibition and IC_{50} values were calculated using a formula and GraphPad software. In addition, cell survival was also calculated and presented by percentage following a formula.

$$\text{The percentage of inhibition} = \frac{(\text{Abs.Control} - \text{Abs.Sample})}{\text{Abs.Control}} \times 100$$

$$\text{The percentage of cell survival} = \frac{\text{Abs.Sample}}{\text{Abs.Control}} \times 100$$

2.5. Evaluation of cytotoxic activity

2.5.1. Sulforhodamine B (SRB) assay

Human breast cancer cell line (T-47D, ATCC® HTB-133™) and human cervical adenocarcinoma cell line (Hela, ATCC® CCL-2™) cell lines were used in this experiment. The estimated cells density detected by the total protein staining was evaluated based on SRB colorimetric assay following the procedure of Skehan et al. (1990); Juckmeta et al. (2019). The appropriate cell's density was seeded into 96 well plates and incubated at 37 °C with 5% CO_2 for 24 h, then the serial concentration of samples was added. After 72 h of incubation, the supernatant in plates was rinsed out and replaced with fresh media. The sample plates were placed in the incubation for 72 h (the recovery period) before fixing them with 10% trichloroacetic acid and stained with SRB. The excess dye was removed, and the protein-stained was dissolved in 10 mM Tris base solution. The absorbance was measured at 492 nm. The percentage of inhibition and IC_{50} values were analyzed using a formula and GraphPad software.

$$\text{The percentage of inhibition} = \frac{(\text{Abs.Control} - \text{Abs.Sample})}{\text{Abs.Control}} \times 100$$

2.6. Evaluation of antimicrobial activity

2.6.1. Microorganisms

Our study used standard microorganisms' cultures from the National Institute of Health of Thailand, including *Staphylococcus aureus* ATCC25923, Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC20651, *Streptococcus pyogenes* ATCC19615, *Pseudomonas aeruginosa* ATCC9097, and *Candida albicans* ATCC90028. The antimicrobial activities were tested using the disc diffusion method, minimum inhibitory concentration, and minimum bactericidal concentration.

2.6.2. Disc diffusion method

The disc diffusion method was performed as in the previous study (Dechayont et al., 2021). The ethanol extract (conc. 5 mg/disc) and water extract (1 mg/disc) were applied on a paper disc of 6 mm diameter and dried before testing. The 0.5 McFarland of inoculum was adjusted before being spread over an agar plate; MHA (*S. aureus, MRSA,* and *P. aeruginosa*), MHA with 5% sheep blood (*S. pyogenes*), and SDA (*C. albicans*). The air-dried sample discs were placed on the inoculum. The zone of inhibition was measured after incubating at 37 °C for 24 h (all bacteria) and 48 h (only *C. albicans*). Amoxicillin and gentamicin (conc. 10 µg/disc) were positive controls. The mean value of the three replicates measuring is shown in Table 3**.**

2.6.3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The lowest concentration that can inhibit visible microbial growth (bacteriostatic activity) called MIC was determined using the colorimetric resazurin microtiter plate-based antibacterial assay (Sarker et al., 2007). All microorganisms have been grown in their appropriate conditions before being transferred to media broth. For testing, they were adjusted to 0.5 McFarland by the McFarland densitometer. Bacteria suspension and sample in a ratio 1:1 was transferred into each well of the 96-well plate and incubated at 37 °C for 24 h (all bacteria) and 48 h (only *C. albicans*). A resazurin solution, blue dye (1 mg/mL) was added into each well and incubated for 3 h. The lowest concentration with no change of resazurin color was determined as MIC value. After that, all concentrations with blue color were transferred onto nutrient agar and incubated at 37 °C for 24 h. The lowest concentration with no growth of bacteria was recorded as MBC. All bacteria and fungi were performed in triplicate. The MIC and MBC values were reported as mg/mL.

2.7. Statistical analysis

All the experiments were conducted in triplicate. Results are expressed as mean ± standard error of the mean. The IC_{50} value and statistical significance with $p < 0.05$ were calculated using one-way ANOVA, followed by Dunnett's multiple comparison test using the GraphPad software.

2.8. Gas Chromatography-Mass Spectrometry (GC–MS) analysis

The chemical composition of both ethanol extract (CCE) and water extract (CCW) of *C. caesius* were investigated by Gas Chromatography-Mass Spectrometry (GC–MS) technique. Scion 436-GC model coupled with a single quadruple mass spectrophotometer comprising of a CP-8410 autosampler with a fused silica capillary column SCION-5MS (5% phenyl/95% dimethyl polysiloxane) 30 m × 0.25 mm × 0.25 µM run on helium gas with a flow rate of 1 mL/min (Konappa et al., 2020). The sample (10 µL) was injected into the capillary column and was run for 60 min throughout the experiment. The initial temperature was maintained at 80 °C and gradually increased up to 250 °C at a rate of 5 °C/min. (until 38 min.) and finally raised to 280 °C at a rate of 20 °C/min. The components' identification was confirmed by comparing the mass spectra with reference data from the National Institute Standard and Technology (NIST) library.

2.9. In silico – Molecular docking analysis

Molecular docking studies were using PyRx 0.8 and BIOVIA Discovery Studio 2021 software programs. The available 3D configuration compounds found from both ethanol and water extracts of *C. Caesius* were retrieved from PubChem as ligands in these studies (https://pubchem.ncbi.nlm.nih.gov). The force field and optimization with the lowest energy of ligands were prepared for docking. The crystal structures of receptor proteins, tyrosinase from *Bacillus megaterium* (3NM8) and NADPH oxidase from *Lactobacillus sanfranciscensis* (2CDU) were retrieved from the Protein Databank, RCSB PDB (https://www.rcsb.org). The proteins were processed by removing ligands, and water molecules (H_20) and then adding polarity. PyRx was used to dock 26 compounds derived from CC into the target sites. Nordihydroguaiaretic acid (NDGA) and dextro-methorphan (DEX) were used as the positive control of 3NM8 and 2CDU proteins, respectively. The results are expressed in the binding affinities (kcal/mol), the number of interactions, and the types of interaction. Docking effects were considered when the binding energy values were less than those of NDGA and DEX to the target proteins. Ligand interaction and visualization were carried out via BIOVIA Discovery Studio 2021.

3. Results and discussion

3.1. Analytical a literature review of Thai traditional scriptures

Thai medicinal textbooks, gathered from many local and royal scriptures, record knowledge of traditional medical management and treatment throughout the history. The principle of medicinal materials in Thai medicinal formulations includes plants, animals, and elements. The medicinal properties of *C. caesius* have also been reported in several scriptures used for a similar purpose to reduce heat thereby balancing temperature from fever in the body. The Thai medicinal textbook named "Paet-Saat- Song-Kroh (Thai word)" combined various Thai scriptures, including That Wi Phang, Pra Thom Jinda, Maha Chotarat, Artisan, and Ya-Taret scriptures, etc (Ayurvedic school, 2016; Ayuraved-Wittayarai Foundation, 1988; Mulholland, 1979). Moreover, other local medicinal textbooks from Wat-Pho have also been reported (Phisal and Phisal, 1917). *C. caesius* was used as part of the thirty formulas from Thai scriptures, pavilion walls, and the inscription stones. All formulas were conveyed as ingredients, methodology for preparation which were related chemical constituents, and their properties shown in Table 1. Remarkably, many formulations can cure similar ailments depending on the difference in locations and herbal diversity. Various liquids are added to prepare a specific purpose based on properties like Traditional Chinese medicine and Ayurveda beliefs. For example, rice water helps to cure fever, relieve faintness, and diuretics (Ayurvedic school, 2016). In Ayurveda, varieties of rice were also used for cooling effect, reducing fever and blood pressure, relieving pimples and small boils in infants (Chaudhari et al.,

Table 1 Thai traditional scriptures found Calamus caesius (CC) is a component and their method of preparation before use. (Ayurvedic school, 2016; Ayuraved-Wittayarai Foundation, 1988; Mulholland, 1979; Phisal and Phisal, 1917).

No.	Scripture / other resources	Formula name, page	Components	%Ratio (plants/ animals/ elements)	%CC in formula	Preparation before use	Properties
1	That Wi Phang	Unnamed1, 164	10	68.75/ 31.25/ -	6.25	ground to be a powder, dissolved in a hot water, some borneol was added before eat.	heats the body throughout the limbs
2		Jak kra wan fah krob, 175	65	78.46/ 16.92/ 4.62	1.54	the herbal powder was prepared to a stick, animal bile was added properly before eat.	antipyretic, cure abscess
3	Pra Thom Jinda	Nan ta Krai wad, 4	26	76.92/ 19.23/ 3.85	3.85	some flower water was added to the herbal powder for prepared to a stick, for edible and topical use.	relieving seizures in children and adults
4		Thep mong khol, 19	23	52.18/ 34.78/ 13.04	4.35	the herbal powder was prepared to a stick.	relieve blisters in the mouth (Infants under 3 months old)
5		Unnamed2, 59	7	57.14/ 42.86/ -	14.29	ground to be a powder, used as a powder or the herbal powder was prepared to a stick.	relieve symptoms of granules on the body, diarrhea (infants from 3 days of age to 1 year and 6 months)
6		Unnamed3, 452	3	100.00/ -/ -	33.33	all herbals were rubbed with python oil for topical use.	antipyretic, antiemetics, antidiarrhea in children
7		Kae sang lohn long pai tam thong, 473	8	75.00/ 25.00/ -	12.50	the herbal powder was prepared to a stick, dissolved in water before eat.	antipyretic, antiemetics, antidiarrhea
8		In ta john, 528	15	66.67/ 26.67/ 6.67	6.67	*Areca catechu* L. water was added in the herbal powder for prepared to a stick.	antipyretic, antiemetics, antidiarrhea in children
9		Loam Tab Dab Pit, 537	12	83.33/ 16.67/ -	8.33	the herbal powder was prepared to a stick, dissolved in alcohol before eat.	antidiarrhea in children (5–12 years old)
10		Kae sang daeng sang fai, 539	17	58.82/ 41.18/ -	5.88	python bile was added in the herbal powder for prepared to a stick.	antipyretic, antiemetics, antidiarrhea in children
11		Ma ha wong, 547	48	62.50/ 36.07/ 1.43	1.43	alcohol was added in the herbal powder for prepared to a stick.	relieve hiccups in children and adults
12		Chan Pid Ruea, 548	22	86.36/ 4.55/ 9.09	4.55	some of animal fangs water was added in the herbal powder for prepared to a stick.	antipyretic, antidiarrhea
13		Kwad sang kho, 575	6	16.67/ 66.66/ 16.67	16.67	the herbal powder was prepared to a stick.	antipyretic, relieve body rash in children
14		Unnamed4, 580	11	100.00/ -/ -	9.09	the herbal powder was prepared to a stick, for edible and topical use.	relieve white pathes in the mouth, throat, cheekbones, or on the tongue (newborns to children up to 5 years and 6 months)
15		Thep mong khol, 600	22	50.00/ 31.82/ 18.18	4.55	the herbal powder was prepared to a stick.	relieve symptoms of granules on the body, diarrhea, vomit, thirsty, cough, inedible
16		Jud lah sang ka dook, 609	7	28.58/ 57.13/ 14.29	14.29	the herbal powder was prepared to a stick, dissolved in lime before eat.	relieve blisters in the mouth (Infants under 3 months old)
17		Unnamed5, 609	10	90.00/ 10.00/ -	10.00	the herbal powder was prepared to a stick, dissolved in flower water before eat or apply on skin.	relieve seizures in babies
18	Maha Chotarat	Unnamed6, 148	17	88.24/ 5.88/ 5.88	5.88	ground to be a powder, dissolved in rice water before eat.	prevent liver damage, relieve hiccups and vomiting

(*continued on next page*)

Table 1 (*continued*)

No.	Scripture / other resources	Formula name, page	Components	%Ratio (plants/ animals/ elements)	%CC in formula	Preparation before use	Properties
19		Thep ni mit, 159	33	87.74/ 3.77/ 8.49	3.77	the herbal powder was prepared to a stick, dissolved in wood water with some borneol before eat.	relieve joint and bone aches before menstruation
20	Atisan	Pra sa chan yai, 900	27	81.49/ 14.81/ 3.70	3.70	Sandalwood equal all components ground to be powder, five-part of pomegranate water was used for prepared a stick, rubbed with sandalwood water before eating.	antipyretic, antiemetics, antidiarrhea in children
21		Pra sa chantara monthol, 904	22	81.81/ 13.64/ 4.55	4.55	Sandalwood equal all components ground to be powder, prepare as stick for rub with flower water.	
22		Pra sa chan, 912	20	85.00/ 10.00/ 5.00	5.00		
23	Ya-Taret	Unnamed7, 962	27	100.00/ -/ -	3.70	the herbal powder was prepared to a stick, dissolved in rice water before eat.	antipyretic
24		Unnamed8, 964	4	100.00/ -/ -	25.00	the herbal powder was prepared to a stick.	
25		Unnamed9, 990	5	80.00/ 20.00/ -	20.00	the herbal powder was dissolved in alcohol before eat.	relieve headache
26	PW name guardian spirit of a newborn baby No.46	Unnamed10, 158	10	60.00/ 40.00/ -	10.00	all equal components are ground to powder, dissolved in alcohol before eating.	relieve symptoms of granules on the body (infants 3 months)
27	stone inscription	Unnamed11, 173	7	100.00/ -/ -	14.29	all equal components are ground to powder, dissolved in water for eating and bath.	antipyretic
28		Unnamed12, 176	23	69.57/ 30.43/ -	4.35	all equal components are ground and prepared to a stick, dissolved in rice water with some borneol before eating.	
29	PW name smallpox or massage plan no.70	Ta-rat-thann-phee-yod-deaw, 225	9	77.78/ 11.11/ 11.11	11.11	all equal components are ground to powder and dissolved in alcohol before applying to the abscess.	cure abscess
30	PW name smallpox or massage plan no.79	Unnamed13, 227	14	50.00/ 50.00/ -	7.14		

2018). Flower water has a cool scent that helps to relieve fever and fatigue and nourish the heart (Ayurvedic school, 2016). Sandalwood is known as an anti-inflammatory, anti-microbial, and anti-proliferative agent (Moy and Levenson, 2017). It has also been used in Ayurveda by ground into a paste and applied on local inflammations, skin diseases, and on the forehead during fever. Animal bile has a bitter and mao bua taste that helps nourish the blood and bile, treat fever, and increase appetite (Mulholland, 1979). The animal biles of goat, pig, dog, crow, raven, python, and black snake were important drugs in traditional Chinese medicine. Python bile was used for biliary colic disease, infantile malnutrition, infectious skin and eye diseases, gingivitis, and high fever in children (Wang and Carey, 2014).

The percentage of *C. caesius* in each formula was calculated and the grouping of ratio was shown in Fig. 1. There are 19 formulas (64%) that used *C. caesius* as a small part of recipes with less than 10% ratio. However, *C. caesius* was used as the principal component of 33.33% in Unnamed3 from Pra Thom Jinda scripture for antipyretic antiemetics, antidiarrheal in children, 25% in Unnamed8 from Ya-Taret scripture for antipyretic, and 20% in Unnamed9 from Ya-Taret scripture for relieving headaches.

In the preparation of some formula ingredients, which were animal products, elements, or plants were burned before grinding them. Carbonized wood, shell, horn, fang, and the jaw of animals are components in various formulations. All preparations were ground into a powder form before dilution in liq-

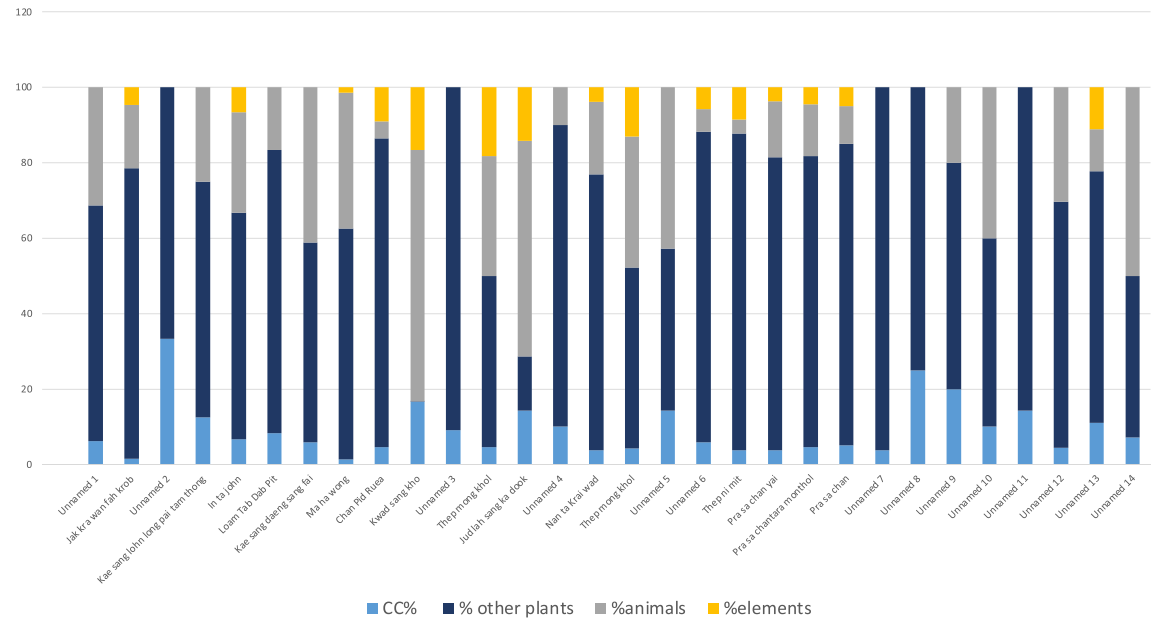

Fig. 1 The ratio of *C. caesius* in thirty formulas with the classification of ingredients in a recipe, categorized into plants, animals, and elements.

uids such as water or alcohol solution. Depending on appropriate purposes, many types of liquid were added to the formulation, such as hot water, rice water, flower water, wood water, and animal bile. The statistical frequency of formula properties (Fig. 2) found that 14 formulas were used for antipyretic and seizure in babies, followed by 11 formulas used for antidiarrheal. Nine formulas used for skin infections (anti-bacterial and anti-inflammatory relevance) which showed symptoms are cure abscess, rashes, blisters, etc. Other purposes are to relieve hiccups in children and adults (2 formulas), relieve joint and bone aches (1 formula), and warm the body throughout the limbs (1 formula).

C. caesius is no research on its medicinal properties although it normally is a component in the formulas according to Thai traditional medicinal textbooks. Not only dried wood but carbonized *C. caesius* used as a component found in Kwad sang kho from Pra Thom Jinda scripture and unnamed with 4 components from Ya-Taret scripture (Phisal and Phisal, 1917). Moreover, carbonized *C. caesius* is an ingredient of the Mahanintangtong remedy in Thailand's National List of Essential Medicines (NLEM). It was reported antioxidant activity by ABTS and DPPH assay, anti-inflammatory, and antibacterial activities of both carbonized *C. caesius* ethanolic and aqueous extract (Dechayont et al., 2021). As Thai traditional medicines use polyherbal for treating diseases, each herb has its own status in a formula. The main drug is herbs with a major ratio for curing the symptoms. The secondary drug is the herbs for curing any symptoms related to or aiding the main drug through additive or synergistic activities. The ratio in the formula varies depends on the number of herbs in the formulation. The complementary drug is the herbs for enhancing immunity or nourishing the power of the body. Also, the flavoring drug is a small amount in the formulation. It is used to improve the taste and is easy to consume (Ayurvedic school, 2016). *C. caesius* acts as the main drug with a percentage ratio of more than 15 in four formulas (13.33%). Mostly, *C. caesius* has been used as a secondary drug for treating antipyretic and antidiarrheal formulations. It implied that *C. caesius* might show additive or synergistic activities with the main drug in the formulation. Thai traditional medicines use this herb to

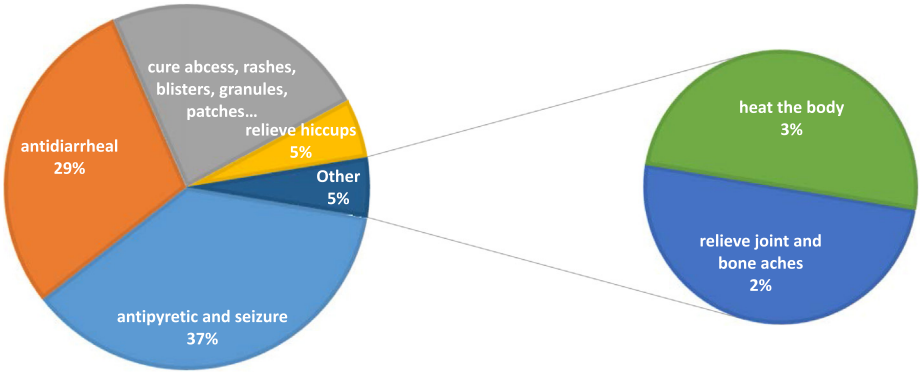

Fig. 2 The statistical frequency of formula properties found *C. caesius* as a component.

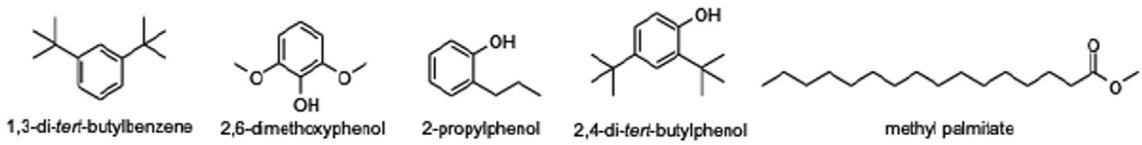

Fig. 3 The chemical compounds found in both ethanol and water extract of *C. caesius*.

Table 2 Percentage yield, and antioxidant capacity assay (DPPH, ABTS, and FRAP) of the *C. caesius* extracts.

Code name	Extraction	%Yield	Antioxidant activity		
			DPPH EC_{50} ± SEM (μg/mL)	ABTS EC_{50} ± SEM (μg/mL)	FRAP (mg Trolox equivalent/g)
CCE	EtOH, maceration	5.54	28.77 ± 3.87*	56.75 ± 0.91*	73.23 ± 2.49
CCW	Water, decoction	9.80	23.33 ± 2.48*	32.15 ± 3.65*	144.47 ± 6.77
BHT[a]	–	–	13.72 ± 2.08	5.66 ± 0.26	–

[a] = positive control.
* = $p < 0.05$.

increase the potential of antipyretic, it displays a mechanism of action to cure diarrhea which is a general symptom from septic patients. Our scientific evidence showed that *C. caesius* might have the potential to inhibit *P. aeuginosa* which is related to diarrhea in children (Chuang et al., 2017). Conversely, the small amount of *C. caesius* in the formulation aimed to use for tonic properties related to our results found that this herb showed leading inhibiting free-radical protecting oxidative stress in the body instead of killing any infectious organisms. So, our preliminary research was conducted based on literature reviews to screen the possibility of *C. caesius* for medicinal use.

3.2. Effect of antioxidant activities

The result of estimated antioxidant activity using radical scavenging methods of the extracts showed similar activity, CCW showed a higher potential than CCE in all assays (Table 2). CCW exhibited a similar result to CCE in the DPPH radical scavenging activity with IC_{50} values of 23.33 and 28.77 μg/mL, respectively. In previous study, the carbonized wood of CCE and CCW reported lower antioxidant activity in DPPH assay with IC_{50} values of 58.93 and >100 μg/mL (Dechayont et al., 2021). Salusu et al., (2018) researched on several part of *C. caesius* fruits which showed greater DPPH activity ordered as following pericarp (IC_{50} = 15.34 μg/mL), seed (IC_{50} = 10.66 μg/mL), and flesh (IC_{50} = 8.80 μg/mL). In ABTS assay, CCW exhibited better results with IC_{50} value of 32.15 μg/mL than CCE (IC_{50} = 56.75 μg/mL). However, the positive control, BHT showed the best one with an IC_{50} value of 5.66 μg/mL. Amount of FRAP values calculated as standard equivalent from the calibration curve as linear regression formula; $y = mx + c$, $R^2 = 0.999$. Interestingly, the results of CCW represented two-fold higher than CCE with FRAP values of 144.47 and 73.23 mg standard equivalent/g, respectively. Regarding results of the dried stem of *Calamus quiquesetinervius* was isolated compounds and investigated antioxidants by Chang et al., (2010a). EtOAc fraction of *C. quiquesetinervius* ethanol extract revealed highest antioxidant possibility in total polyphenols (273.5 mg/g gallic acid eq.) and DPPH assay (IC_{50} = 21.9 μg/mL) led to a purified active compound. Only Quiquelignan E from isolated 8 compounds showed stronger OH and O_2 free radical scavenging powers with IC_{50} value of 6.2 and 53.8 μg/mL while Trolox, a positive control remained the best (4.4 and 32.8 μg/mL, respectively). In summary, both CCE and CCW showed strong antioxidant activities in three different assays.

3.3. Effect of anti-inflammatory activity in vitro

Neither CCE nor CCW had an inhibitory effect on nitric oxide production in RAW264.7 cell line with an IC_{50} values of greater than 100 μg/mL. The cell viability at a concentration of 100 μg/

Table 3 *In vitro* anti-inflammatory activity against macrophage cell line (RAW264.7) and cytotoxic activity against breast (T47D) and cervical (CCL-2) cancer cells of the *C. caesius* extracts.

Sample	**Anti-inflammatory activity**		**Cytotoxic activity** IC_{50} ± SEM (μg/mL)	
	IC_{50} ± SEM (μg/mL)	%Survival (conc. 100 μg/mL)	T47D	CCL-2
CCE	>100	97.46 ± 6.15	>100	>100
CCW	>100	106.02 ± 3.16	>100	>100

Table 4 Inhibition zone, MIC and MBC of the *C. caesius* extracts against five organisms.

Sample	Antimicrobial activity showed inhibition zone/ MIC/ MBC (mm, mg/ml, mg/ml)				
	S.aureus	***S.aureus* MRSA**	***P.aeruginosa***	***S.pyogenes***	***C.albicans***
CCE	6/ 0.625/ 0.625	6/0.625/ 0.625	0/ 0.625/ > 5	7/ 0.313/ 0.625	0/ > 5/ > 5
CCW	0/ 0.625/ 0.625	0/ 0.625/ 1.25	0/ 5/ > 5	0/ > 5/ > 5	0/ > 5/ > 5
Amoxicillin[a]	NT	NT	NT	35/ 0.016/ 0.025	NT
Gentamicin[a]	15/ 0.195/ 0.195	10/ > 200/ > 200	12/ 0.39/ 0.39	NT	NT

[a] = positive control.

mL was more than 97%. All extracts did not show cytotoxicity in RAW264.7 cell line using MTT assay. Similarly, the extract of *C. caesius* carbonized showed inhibitory effects on NO, TNF-∝, and IL-6 production with IC_{50} values > 100 μg/mL. It also showed no toxicity on macrophage cell lines (Dechayont et al., 2021). Other researchers studied suppressing LPS-stimulated production of nitric oxide (NO) of isolated compounds from *C. quiquesetinervius*. Chang et al. (2010b) revealed phenylpropanoid glycosides named Quiquesetinerviuside D and E exhibited potent activity with IC_{50} values of 9.5 and 9.2 μM while a positive control, quercetin showed an IC_{50} value of 34.5 μM. Quiquelignan D and F had anti-inflammatory potency 2.7 to 4.5-fold higher compared to quercetin in RAW264.7 cell lines (Chang et al., 2010a). Carapanolide J, limonoid compound from *C. guianensis* showed similar inhibitory activities compared to positive control $_L$-NMMA with non-toxicity (Dias et al., 2023). Total saponin compound at the concentration in the range of 6.25 to 25 μg/ml from *Dioscorea nipponica* showed a dose-dependent significant reduce the level of NO on the RAW 264.7 cell lines (Chang et al., 2023). Nonetheless, the numerous phytochemical constituents found from *C. caesius* ethanol extract such as heptadecane (Kim et al., 2013), *n*-hexadecanoic acid (Aparna et al., 2012), 9(E),11(E)-conjugated linoleic acid (Lee et al., 2009), β-Sitosterol (Loizou et al., 2010) and glutinol (Adebayo et al., 2017) had reported anti-inflammatory in various studies.

Table 5 Phytochemical constituents identified in the water extract of *C. caesius* using Gas Chromatography-Mass Spectrometry (GC–MS). CAS chemical abstract service, SI No. serial number.

SI no.	RT (min.)	CAS	Name of the compound	R. Match	Molecular formular	Molecular weight	%Peak area	Biological and pharmacological activities
1	5.786	3658-77-3	Furaneol	818	$C_6H_8O_3$	128.13	3.37	Flavoring agent (Buechi et al., 1973)
2	6.719	77376-84-2	3-*tert*-Butylamino-acrylonitrile	–	$C_7H_{12}N_2$	124.18	25.81	Antifungal (Ayed et al., 2021))
3	9.064	3232-39-1	Diacetyl sulphide	967	$C_4H_6O_2S$	118.15	8.47	–
4	10.16	1014-60-4	Benzene, 1,3-bis(1,1-dimethylethyl)-	930	$C_{14}H_{22}$	190.32	9.03	–
5	11.796	31853-85-7	2-Methoxy-4-vinylphenol	921	$C_9H_{10}O_2$	150.17	8.26	Aromatic substance, flavoring agent (EU Food Improvement Agents, 2020)
6	12.663	91-10-1	Phenol, 2,6-dimethoxy-	955	$C_8H_{10}O_3$	154.16	8.90	Flavoring agent (Yannai, 2012)
7	13.189	644-35-9	Phenol, 2-propyl-	903	$C_9H_{12}O$	136.19	12.65	–
8	15.896	629-78-7	Heptadecane	887	$C_{17}H_{36}$	240.46	0.68	Anti-inflammatory (Kim et al., 2013)
9	16.067	498-07-7	β-D-Glucopyranose, 1,6-anhydro-	919	$C_6H_{10}O_5$	162.14	6.67	–
10	16.446	96-76-4	2,4-Di-*tert*-butylphenol	955	$C_{14}H_{22}O$	206.32	5.33	Antioxidant (Yoon et al., 2006)
11	17.792	-	Phenol, 4-ethyl-2,6-dimethoxy	924	$C_{10}H_{14}O_3$	182.21	2.49	Phenolic compound (Tymchyshyn and Xu, 2010)
12	18.708	642-71-7	Phenol, 3,4,5-trimethoxy-	900	$C_9H_{12}O_4$	184.18	2.80	–
13	20.895	20675-95-0	(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	912	$C_{11}H_{14}O_3$	194.22	4.31	–
14	25.477	112-39-0	Methyl palmitate	948	$C_{17}H_{34}O_2$	270.45	1.23	Antibacterial, Fatty acid (Shaaban et al., 2021), Antifungal (Abubacker and Deepalakshmi, 2013)

Note: SI no; serial number, MW; Molecular weight, (-); not detected, (–); not reported.

Table 6 Phytochemical constituents identified in the ethanolic extract of *C. caesius* using Gas Chromatography-Mass Spectrometry (GC–MS). CAS chemical abstract service, SI No. serial number.

SI no.	RT (min.)	CAS	Name of the compound	R. Match	Molecular formular	MW	% Peak area	Biochemistry and pharmacology
1	10.158	1014-60-4	Benzene, 1,3-bis(1,1-dimethylethyl)-	930	$C_{14}H_{22}$	190.32	0.62	–
2	11.801	31853-85-7	2-Methoxy-4-vinylphenol	921	$C_9H_{10}O_2$	150.17	0.76	Aromatic substance, flavoring agent (EU Food Improvement Agents, 2020)
3	12.662	91-10-1	Phenol, 2,6-dimethoxy-	955	$C_8H_{10}O_3$	154.16	0.71	Flavoring agent (Yannai, 2012)
4	13.189	644-35-9	Phenol, 2-propyl-	903	$C_9H_{12}O$	136.19	0.96	–
5	16.444	96-76-4	2,4-Di-*tert*-butylphenol	955	$C_{14}H_{22}O$	206.32	1.12	Antioxidant (Yoon et al., 2006)
6	17.791	-	Phenol, 4-ethyl-2,6-dimethoxy	924	$C_{10}H_{14}O_3$	182.21	0.18	Phenolic compound (Tymchyshyn and Xu, 2010)
7	18.568	629-78-7	Heptadecane	887	$C_{17}H_{36}$	240.46	0.39	Anti-inflammatory (Kim et al., 2013)
8	21.342	124-10-7	Methyl tetradecanoate	910	$C_{15}H_{30}O_2$	242.39	0.25	–
9	22.924	629-94-7	Heneicosane	964	$C_{21}H_{44}$	296.57	0.43	Antimicrobial (Vanitha et al., 2020)
10	23.797	502-69-2	2-Pentadecanone, 6,10,14-trimethyl-	910	$C_{18}H_{36}O$	268.47	0.33	–
11	25.477	112-39-0	Methyl palmitate	948	$C_{17}H_{34}O_2$	270.45	5.82	Antibacterial, Fatty acid (Shaaban et al., 2021), Antifungal (Abubacker and Deepalakshmi, 2013)
12	26.184	57-10-3	n-Hexadecanoic acid	955	$C_{16}H_{32}O_2$	256.42	1.52	Anti-inflammatory (Aparna et al., 2012)
13	26.788	628-97-7	Hexadecanoic acid, ethyl ester	907	$C_{18}H_{36}O_2$	284.47	16.91	Palmitic acid ester (Hungund et al., 1988), Antimicrobial (Krishnaveni et al., 2014)
14	26.899	630-01-3	Hexacosane	950	$C_{26}H_{54}$	366.70	0.53	Antimicrobial (Rukaiyat et al., 2015)
15	28.782	112-62-9	9-Octadecenoic acid (Z)-, methyl ester	923	$C_{19}H_{36}O_2$	296.48	1.50	–
16	29.256	112-61-8	Methyl stearate	943	$C_{19}H_{38}O_2$	298.50	0.46	Fatty acid (Bancquart et al., 2001)
17	29.863	544-71-8	9(E),11(E)-Conjugated Linoleic Acid	923	$C_{18}H_{32}O_2$	280.50	7.46	Anti-inflammatory (Lee et al., 2009), Anticancer (Kelley et al., 2007)
18	30.438	111-61-5	Octadecanoic acid, ethyl ester	901	$C_{20}H_{40}O_2$	312.53	2.06	–
19	44.409	474-62-4	Campesterol	850	$C_{28}H_{48}O$	400.68	2.12	Antiangiogenic (Choi et al., 2007)
20	44.685	83-48-7	Stigmasterol	865	$C_{29}H_{48}O$	412.69	11.98	Anti-osteoarthritic (Gabay et al., 2010), Antiperoxidative and Hypoglycemic (Panda et al., 2009)
21	45.435	83-46-5	β-Sitosterol	895	$C_{29}H_{50}O$	414.70	28.38	Anti-inflammatory (Loizou et al., 2010)
22	46.769	545-47-1	Lupeol	916	$C_{30}H_{50}O$	426.71	9.41	Triterpene compound
23	46.982	545-24-4	Glutinol	921	$C_{30}H_{50}O$	426.70	6.08	Anti-inflammatory (Adebayo et al., 2017)

Note: SI no; serial number, MW; Molecular weight, (-); not detected, (–); not report.

3.4. Effect of SRB cytotoxic activity

Our results revealed that the ethanol and water extracts of *C. caesius* wood at concentrations of 50 and 100 µg/mL did not show cytotoxic activity against breast and cervical cancer cell lines (IC_{50} > 100 µg/mL). The percentage of cell viability was more than 90 in both cell lines. From Thai traditional medicine wisdom, Thai doctors have been using this herb in the formula for treating pyretic and diarrheal. The main action of this herb is to support inhibiting free radicals and antimicrobial which is not used to inhibit cancer cells directly. Although there is no research on the cytotoxic activity of *C. caesius*, some research in the same genus of *Calamus was* reported. Yu et al., (2008) informed that the methanol extract of *Calamus ornatus* tender shoots and isolated steroidal saponin compound 2, 3 inhibited cell proliferation of breast (MCF7), CNS

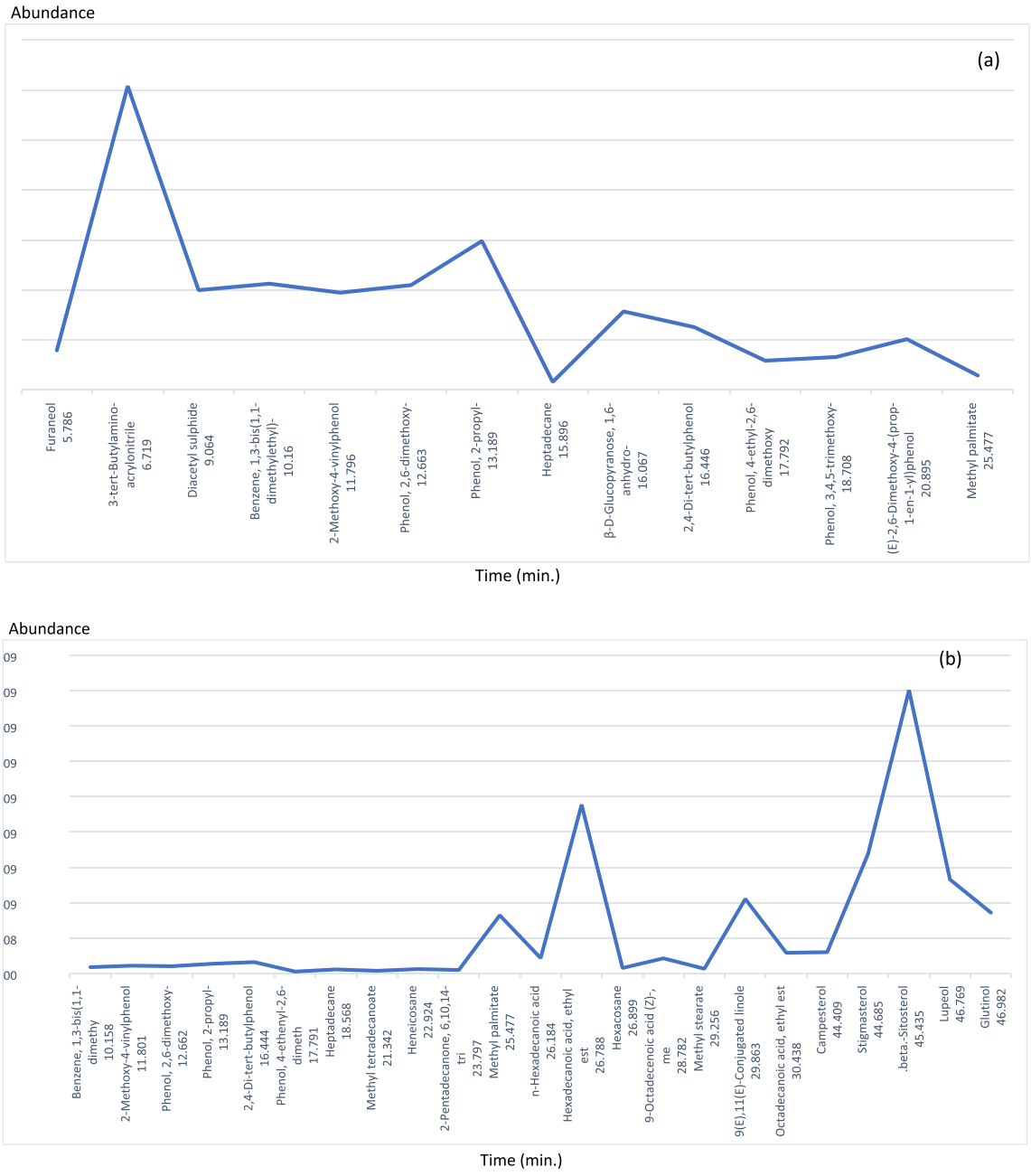

Fig. 4 GC–MS chromatogram showing the peaks and retention time the water extract (a) and the ethanolic extract (b) of *C. caesius*.

(SF-268), lung (NCI-46), colon (HCT-116) and gastric (AGS) cancer cell lines. Thakur et al., (2017) presented that the methanolic supernatant and methanolic precipitate of *Calamus tenuis* shoots, MSCT and MPCT, had potent against lung carcinoma (A549) and breast carcinoma (MCF7) cell lines. Besides, some chemical constituents in these extracts were reported to have cytotoxicity. β-sitosterol, a phytosterol that is present in the plant cell membrane. Recently, Alvarez-Sala et al., 2019 reported that β-sitosterol displayed cytotoxicity against the HeLa cancer cell line. The mechanism of this compound is an elevated level of p53 mRNA and a reduced level of oncogenic HPV E6. Also, Vundru et al., (2013) showed that β-sitosterol treatment led to G1 arrest in human breast cancer MDA-MB-231 cells corresponding to reduced levels of cyclin D1 and cyclin-dependent kinase (CDK) and increased levels of p21/ Cip1 and p27/Kip1 proteins involved in inhibiting the kinase activity of CDK. Furthermore, lupeol and campesterol were reported to inhibit cancer cell lines. Lupeol also showed cytotoxic against Hela, KB, MCF-7, and A549 cell lines (Bednarczyk-Cwynar et al., 2016). Moreover, Kang et al., (2013) supported that lupeol reduced the viability of HeLa cells, and campesterol displayed to inhibit MDA-MB-231 human breast cancer (Awad et al., 2000). Although, the phytochemicals of CC showed the possibility of anti-

Table 7 Binding energy of phytochemical compounds from *C. caesius,* NDGA, and DEX.

Compounds	Binding Affinity (kcal/mol)	
	Tyrosinase 3NM8	**NAD(P)H Oxidase** 2CDU
(E)-2,6-Dimethoxy-4-(prop-1-en-1-yl)phenol	−7.1	−5.8
2,4-Di-*tert*-butylphenol	−5.9	−6.3
2-Methoxy-4-vinylphenol	−6.4	−5.5
2-Pentadecanone, 6,10,14-trimethyl-	−7.3	−5.8
2-Propylphenol	−7.0	−5.6
3-*tert*-Butylamino-acrylonitrile	−5.2	−4.6
9(E),11(E)-Conjugated Linoleic Acid	−7.4	−6.5
Benzene, 1,3-bis(1,1-dimethylethyl)-	−5.8	−6.5
Beta-Sitosterol	−8.3	−8.6
Campesterol	−8.0	−8.7
Diacetyl sulphide	−4.2	−4.0
Furaneol	−5.6	−5.3
Glutinol	−5.0	−8.6
Heneicosane	−7.0	−4.8
Heptadecane	−6.4	−5.3
Hexacosane	−7.3	−4.9
Hexadecanoic acid, ethyl ester	−6.8	−5.5
Levoglucosan	−5.7	−5.3
Lupeol	−3.1	−8.6
Methyl palmitate	−6.4	−5.5
Methyl tetradecanoate	−6.7	−5.4
n-Hexadecanoic acid	−6.8	−5.4
Phenol, 2,6-dimethoxy-	−5.7	−4.9
Phenol, 3,4,5-trimethoxy-	−6.0	−5.2
Phenol, 4-ethyl-2,6-dimethoxy	−6.4	−5.3
Stigmasterol	−8.0	−9.1
Nordihydroguaiaretic acid, NDGA*	−8.7	−7.7
Dextromethorphan, DEX*	−5.7	−7.6

* = positive control.

Table 8 Ligand-protein bonding interaction classify by numbers and types.

		Interaction type			
Enzymes	**Ligands**	**Total number of interaction**	**Hydrogen bonding**	**Hydrophobic bonding**	**Unfavorable bonding**
Tyrosinase	Beta-Sitosterol	10	0	10	0
	Campesterol	6	0	6	0
	Stigmasterol	9	0	7	2
	Nordihydroguaiaretic acid	9	3	6	0
NAD(P)H Oxidase	Beta-Sitosterol	4	0	4	0
	Campesterol	5	1	4	0
	Glutinol	2	1	1	0
	Lupeol	2	0	2	0
	Stigmasterol	8	0	6	2
	Nordihydroguaiaretic acid	6	2	3	1
	Dextromethorphan	6	0	6	0

inflammatory and anticancer in several compounds. Both extracts showed no potential therapeutic effect on the chronic inflammatory mechanism and cytotoxicity against woman's cancer cell lines. It suggests that the quantity of compounds may affect their biological activity.

3.5. Effect of antimicrobial activity

The antimicrobial activity against gram-positive, gram-negative bacteria and gram-positive fungus was reported in the inhibition zone, MIC, and MBC values of the extract

which are shown in Table 4. None of extracts demonstrated anti-microbial effects against *C. albicans*. The extract from *C. caesius*, especially ethanol extract, represented a productive inhibition activity against *S. aureus*, *S.aureus* MRSA, *P.aeruginosa,* and *S.pyogenes*. Our results supported Thai traditional doctors' use of *C. caesius* to treat fever from bacteria. This data is the first report on *C. caesius*. Furthermore, some chemical constituents influence inhibiting bacteria. Triazole rings in several compounds including Triadimefon, Triazolam, Ribavirin, Fluconazole, and Tazobactum displayed antiviral and antimicrobial activities (Al-Ghulikah et al., 2023). Benzothiazole is a heterocyclic compound that has also been reported in the field of antimicrobial activity (Alheety et al., 2021). Besides, the silver nanoparticle ions with benzisothiazolinone can cause uncontrolled regulation of cell permeability that results in bacterial cell death (Alheety et al., 2019). Heterocyclic sulfur-containing ligands (1-Phenyl-1H-tetrazole-5-thiol) exhibited *C. albicans* and *A. niger* (Al-Janabi et al., 2020). Furaneol has been well-known as the main aroma compound that showed antimicrobial activities against gram-positive and gram-negative bacteria (Sung et al., 2006). Previous studies have reported the antibacterial activity of heptade-

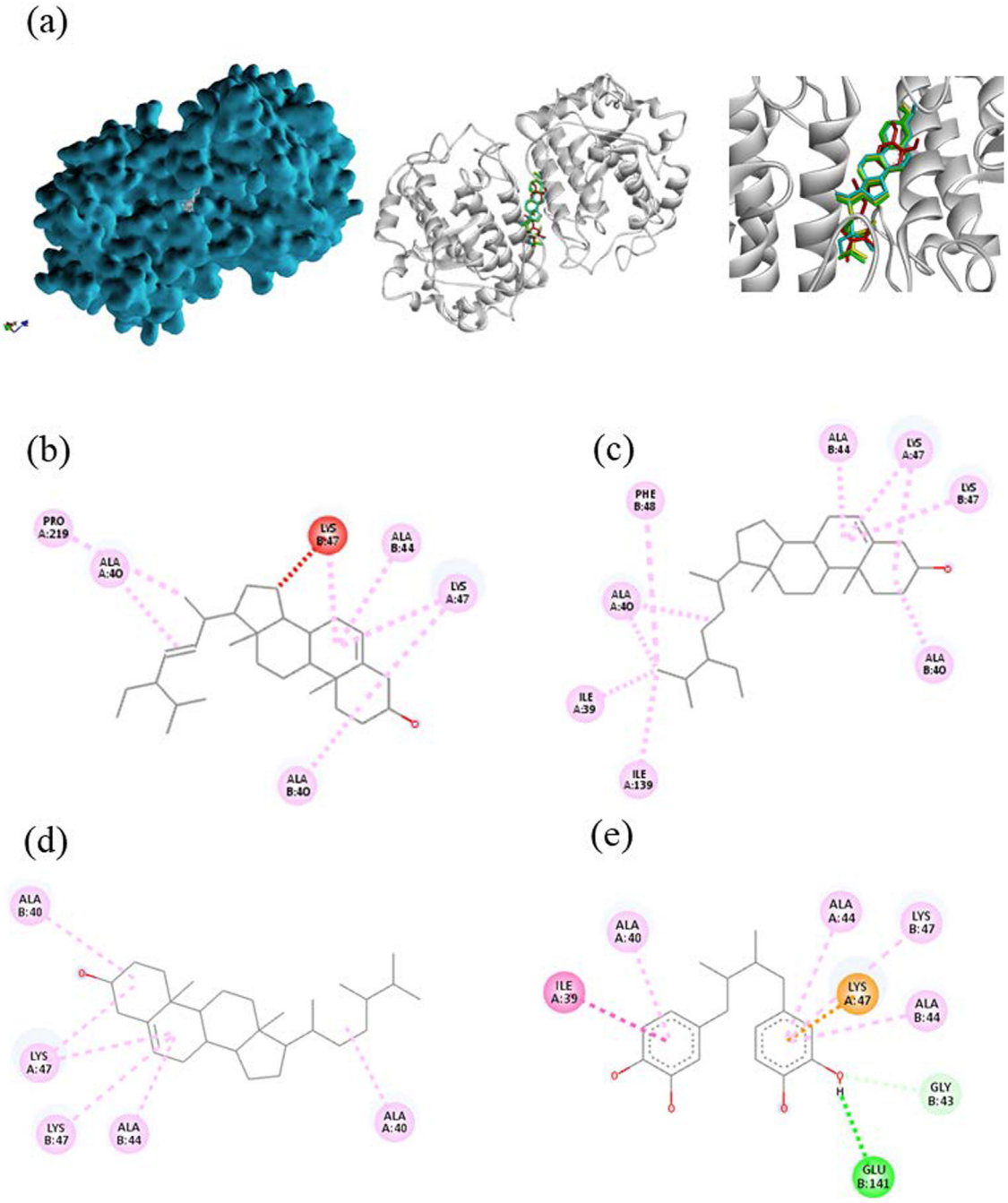

Fig. 5 Interaction visualization of four ligands binding 3NM8 with BIOVIA discovery studio - stigmasterol in blue, beta-sitosterol in yellow, campesterol in green, NDGA in red (a), 2D interaction of stigmasterol (b), beta-sitosterol (c), campesterol (d), NDGA (e).

cane to possess potent activity against gram-positive and gram-negative bacteria (Naeim et al., 2020). Also, Vanitha et al., (2020) investigated the antimicrobial activity of heneicosane against *S. pneumoniae*, *M. tuberculosis*, *B. cereus*, *S. enteritidis*, *A. baumannii,* and *A. fumigatus*. The results showed that heneicosane at 10 µg/mL exhibited *S. pneumoniae* with a maximum zone of inhibition (31 mm), followed by *M. tuberculosis*(28 mm), *B. cereus*(27 mm), *S. enteritidis*(26 mm) and *A. baumannii*(24 mm), respectively. For the antifungal activity, heneicosane at the same concentration also exhibited *A. fumigatus* excellent activity (29 mm). Stigmasterol and β-Sitosterol, are known to possess antimicrobial properties. Several research claimed that Stigmasterol had the potential to inhibit *S. aureus*, *B. subtilis*, *E. coli* and *C. albicans (*Odiba et al., 2014; Yusuf et al., 2018*)*. β-Sitosterol has exhibited antimicrobial activity against *S. aureus*, *B. subtilis,* and *K. phemoniae* with the zone of inhibition of 27 mm, 34 mm, and 26 mm, respectively (Alawode et al., 2021). Simultaneously, diacetyl and campesterol displayed antimicrobial activity (Lanciotti et al., 2003; Achika et al., 2020). These major compounds may be responsible for antibacterial activity against *S.aureus*, *S.aureus* MRSA, *P.aeruginosa,* and *S.pyogenes*.

3.6. The profile of GC–MS

The GC–MS chromatogram of CCW and CCE demonstrated a total of 14 and 24 peaks corresponding to the phytochemical constituents shown in Table 5 and Table 6. The data were recognized by molecular weight relating the name of the compound and percentage of peak area to that of the known compounds provided by the National Institute of Standards and Technology (NIST) library as shown in Fig. 4. The 14 chemical constituents of CCW with peak area were 3-*tert*-Butylamino-acrylonitrile (25.81%), phenol, 2-propyl- (12.65%), benzene, 1,3-bis(1,1-dimethylethyl)- (9.03%), phenol, 2,6-dimethoxy- (8.90%), diacetyl sulphide (8.47%), 2-Methoxy-4-vinylphenol (8.26%), β-D-glucopyranose, 1,6-anhydro- (6.67%), 2,4-di-*tert*-butylphenol (5.33%), (E)-2,6-di methoxy-4-(prop-1-en-1-yl)phenol (4.31%), furaneol (3.37%), phenol, 3,4,5-trimethoxy- (2.80%), phenol, 4-ethyl-2,6-dimethoxy (2.49%), hexadecanoic acid, methyl ester (1.23%), heptadecane (0.68%), respectively. The major chemical constituents of CCW revealed 3-*tert*-butylamino-acrylonitrile which was shown antifungal activity in the previous study (Ayed et al., 2021). The chemical constituents of CCW were shown several bioactivities for example 2,4-di-*tert*-butylphenol exhibited antioxidant in TBARS assay (Yoon et al., 2006), heptadecane exhibited a potent anti-oxidative effect and suppressed NF-kB signal pathway in aged rats (Kim et al., 2013), hexadecenoic acid methyl ester or methyl palmitate showed high antimicrobial effect against clinical pathogenic bacteria (Shaaban et al., 2021) and inhibited fungal pathogen cause of leaf spot and rot diseases (Abubacker and Deepalakshmi, 2013).

In contrast, the major chemical constituents of CCE were phytosterol compounds such as β-sitosterol and stigmasterol and triterpenoid compound as lupeol. According to the peak areas, seven major phytochemical constituents of *C. caesius* ethanol extract (CCE) included β-Sitosterol (28.38%), hexadecanoic acid, ethyl ester (16.91%), stigmasterol (11.98%),

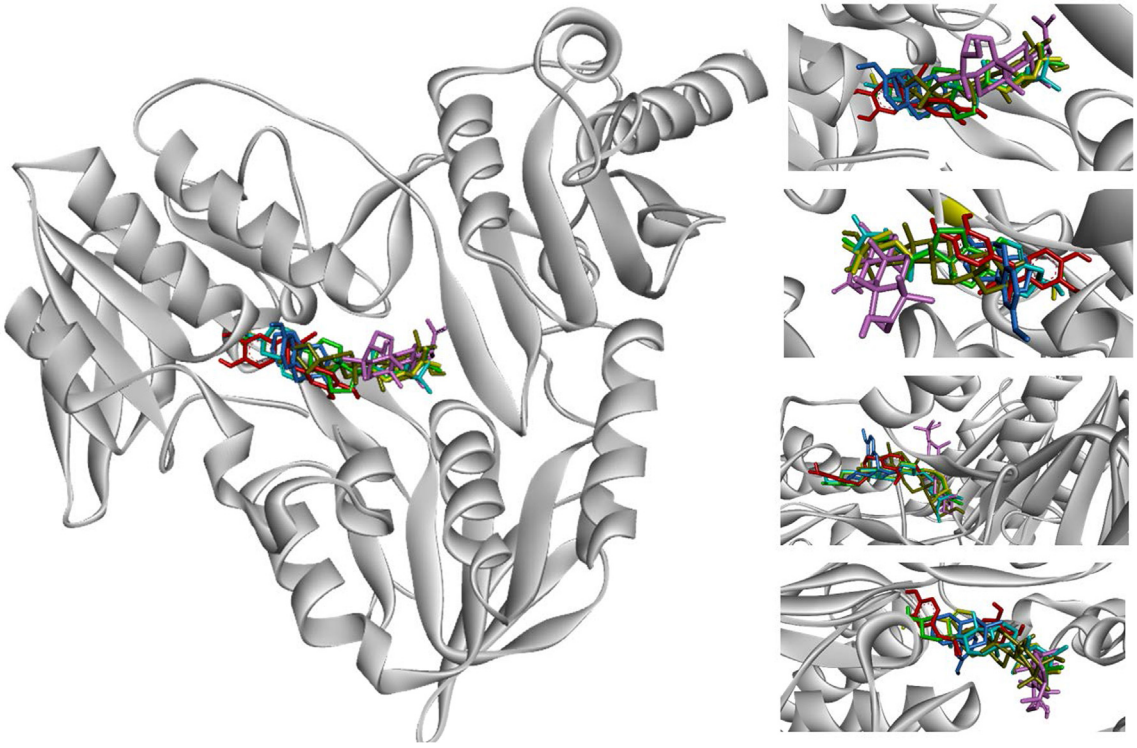

Fig. 6 Interaction visualization of seven ligands binding active site of 2CDU with BIOVIA discovery studio- stigmasterol (blue, #55ffff), beta-sitosterol (yellow, #ffff00), campesterol (green, #00ff00), lupeol (light pink, #ffaaff), glutinol (light yellow, #aaaa00), NDGA (red, #ff0000), DEX (light blue, #55aaff).

lupeol (9.41%), 9(E),11(E)-conjugated linoleic acid (7.46%), lutinol (6.08%), hexadecanoic acid, methyl ester (5.82%), respectively. An isomer of linoleic acid as 9(E),11(E)-conjugated linoleic acid found in CCE has reported potential anticancer and anti-inflammatory activities (Lee et al., 2009). Interestingly, β-Sitosterol and hexadecanoic acid, ethyl ester demonstrated anti-inflammatory and antibacterial activities (Loizou et al., 2010; Krishnaveni et al., 2014) which is related to our study that used a CCE for treating fever and anti-inflammatory disorder. The investigation of CCE revealed the presence of various phytoconstituents, including phenolic compounds and fatty acids as well as CCW. Five compounds found in both ethanol and water extract of *C. caesius*showed in Fig. 3. These bioactive phytoconstituents of CCW and CCE could be responsible for the therapeutic capability of *C. caesius* prescribed in Thai traditional medicine by following Thai scriptures and textbooks.

3.7. Molecular docking analysis

Both ethanol and water extracts of *C. Caesius* have dominant antioxidant in three free-radical scavenging activities which is interesting to develop as ingredient into the pharmaceutical drugs, food additives and cosmetic industries. The primary function of NADPH oxidase is to produce reactive oxygen species that is regard as important factor to pathogenesis of various diseases such as vascular diseases, cancer, inflammation, CNS diseases, and other degenerative diseases (Maraldi, 2013; Sui et al., 2019). Besides, ROS inducing from UV irradiation lead to induce hyperpigmentation on skin by activating tyrosinase enzyme (Muddathir et al., 2017). For antioxidant *in silico,* phytochemical constituents from GC–MS analysis were focused on inhibiting properties of these two enzymes. So, the molecular docking approaches of NADPH oxidase and tyrosinase were conceived under budget constraints of research. The active sites of proteins in this study defined from PDB site records via Discovery studio Visualizer 2021. The results of the docking score were reported as binding affinity with kcal/mol (Table 7), the interaction of ligand–protein bonding classified by numbers and types was also shown in Table 8. In addition, an interactive visualization of molecular docking studies represented the hydrophobic and hydrogen bond interactions between ligands and proteins. The different colors performed diverse interactions as following green-

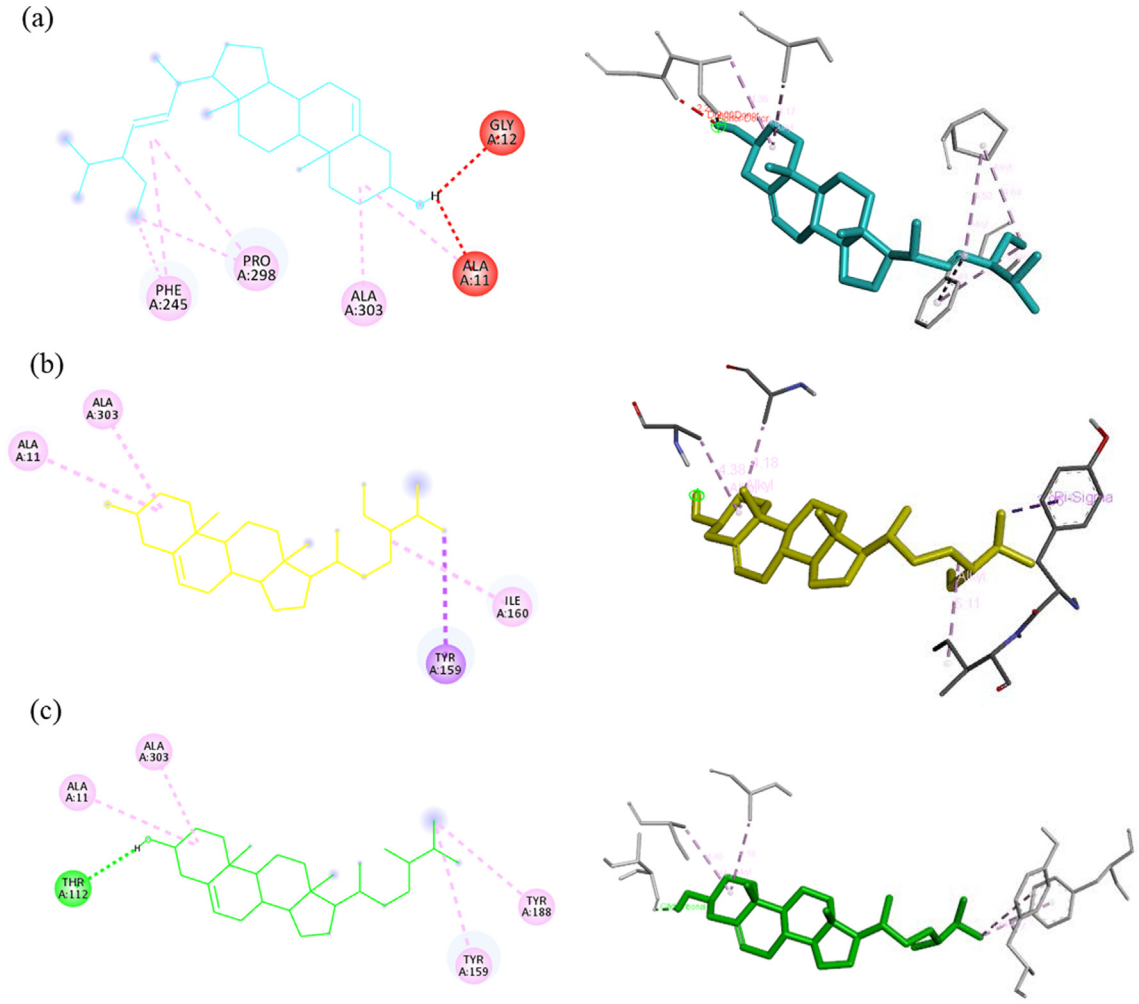

Fig. 7 2D and 3D interaction of 2CDU binding stigmasterol (a), beta-sitosterol (b), campesterol (c), glutinol (d), lupeol (e), NDGA (f), DEX (g) 2D and 3D interaction of 2CDU binding stigmasterol (a), beta-sitosterol (b), campesterol (c), glutinol (d), lupeol (e), NDGA (f), DEX (g).

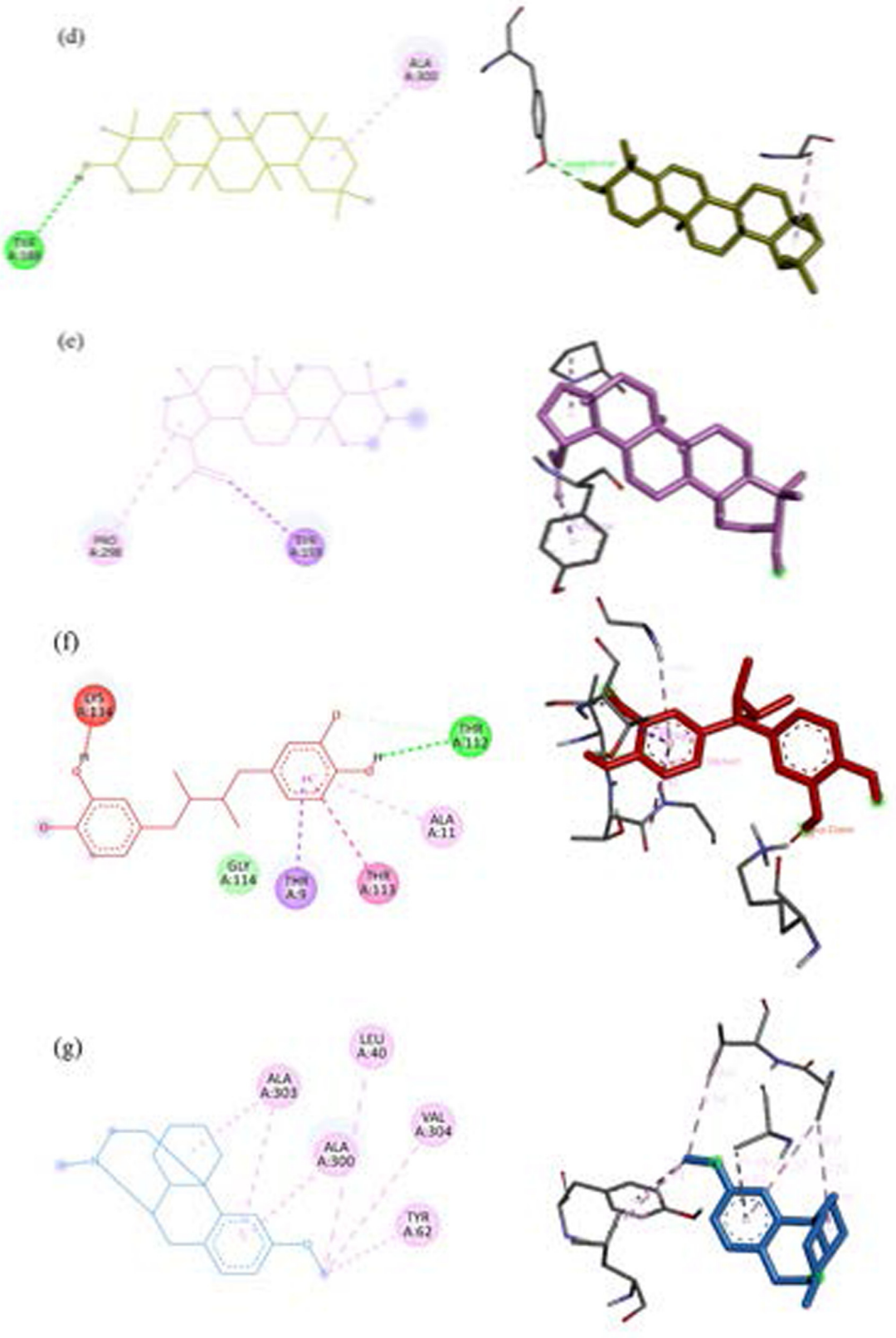

Fig. 7 (*continued*)

hydrogen bonding, pink-alkyl/hydrophobic bonding, and red-unfavorable bonding. In tyrosinase (PBD CID: 3NM8) binding simulation, Nordihydroguaiaretic acid (NDGA) was used as a standard (Al-Salahi et al., 2019) has a docking score of −8.7 kcal/mol in this experiment. Three ligands showed the best efficient docking energy values β-sitosterol with a binding affinity score of −8.3 kcal/mol followed by campesterol and stigmasterol with the same score of −8.3 kcal/mol. Analogous to Ghalloo et al., (2022) research, β-sitosterol revealed a binding affinity score of around −9.0 kcal/mol while Kojic acid (standard) had −5.0 kcal/mol. None of them had hydrogen bond interaction. Stigmasterol and campesterol similarly activate hydrophobic interaction with LYS47, ALA44, and ALA40. In the case of docked NDGA and β-sitosterol, hydrophobic bonds with LYS47, ALA44, ALA40, and ILE39 were also formed but H-bonds interaction with GLU141 was not found in beta-sitosterol (Fig. 5). Similarly, Yousuf et al., (2022) reported campesterol had alkyl bond interaction with ALA155. Based on our results NDGA appeared to bind more strongly to the active site of tyrosinase than beta-sitosterol, campesterol, and stigmasterol, respectively. Docked conformation of seven ligand structures in the binding site of NADPH oxidase (PBD CID: 2CDU) is visualized in Fig. 6**.** The standard compounds of the 2CDU model were dextromethorphan (DEX) (Farouk et al., 2021) and NDGA with binding affinity score of −7.6 and −7.7 kcal/mol. The candidate compounds had a better affinity of the binding score more than NDGA and DEX. Stigmasterol revealed the greatest docking score (−9.1 kcal/mol) followed by campesterol (−8.7 kcal/mol), β-sitosterol and two triterpenoids, glutinol and lupeol with balanced scores (−8.6 kcal/mol). An interaction of ligands and 2CDU presented by 2D and 3D visualization via BIOVIA Discovery studio was shown in Fig. 7**.** DEX appears to interact with ALA303 ALA300 LEU40 VAL304 TYR62 with hydrophobic bonding. Phytosterol compounds are stigmasterol, β-sitosterol, and campesterol also formed with ALA303 in the same interaction. In addition, stigmasterol interact the nature ligand (GSH) into the active pocket of NADPH-dependent human carbonyl reductase-1 (hCBR-1) with hydrophobic bonding to VAL96, MET141, TYR193, TRP229 (Andriani et al., 2022). From the list of residues, five interact with NDGA are ALA11, THR113, and THR9 with hydrophobic bonding, THR112 with hydrogen bonding, and LYS unfavorable bonding. Campesterol also interacts with THR112 similar to NDGA interface. Our results discovered the possibility of antioxidant activities of ethanol compounds were phytosterols and triterpenoids from *C. caesius*.

4. Conclusion

This is the first scientific report on the ethnopharmacological analysis of *C. caesius*. From Thai traditional formulations in Thai traditional scriptures and Thai medicinal textbooks (TMT), *C. caesius* was mostly used as a secondary drug in the formula with a percentage ratio in the range of 5 to 14.99 act as a component for antipyretic, antidiarrhea, and cure abscess treatment. Based on GC–MS analysis, the chemical profiles reported the scientific data associated with antioxidant, anti-inflammatory, and antimicrobial activities. *In vitro* results of biological activities in this study support that *C. caesius* displayed antioxidants and antimicrobial activities in the formulas. Furthermore, *in silico* molecular docking study of phytochemical constituents revealed the phytosterols from ethanol extract are promising antioxidant agents (tyrosinase and NADPH oxidase). According to Thai traditional formulations analysis, *C. caesius* is used for oral and topical drugs that is supposed to against oxidative stress for immune support in the human body. Hence, our results in vitro and *in silico* supported *C. caesius* acts as an antioxidant agent in drug formulas.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

TJ conceived and designed most of the experiments. BD, PP, and JC performed most of the experiments. OP and KY carried out cytotoxic activity. NP performed GC-MS analysis and wrote this part. SN and TJ analyzed Thai traditional scriptures, analyzed the remaining data, and wrote the manuscript. TJ performed Molecular docking analysis. TJ, BD, PD reviewed and modified the paper. All authors read and approved the manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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