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Aquilaria species as potential anti-inflammatory agents–A review on *in vitro* and *in vivo* studies

Manar A. Eissa[1*], Yumi Z. H-Y. Hashim[1,*], Hamzah Mohd. Salleh[1], Saripah S.S. Abd-Azziz[2], Muhammad Lokman Md. Isa[3], Nor Malia Abd Warif[4], Yusilawati Ahmad Nor[5], Dina M. El-Kersh[6] and Muhamad Shirwan Abdullah Sani[1]

[1]International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, (IIUM), Jalan Gombak, 53100 Kuala Lumpur, Malaysia

[2]Faculty of Science and Mathematics, Sultan Idris Education University, 35900 Tanjung Malim, Perak, Malaysia

[3]Kulliyah of Nursing, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia

[4]Biomedical Sciences Program, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

[5]Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), Jalan Gombak, 53100 Kuala Lumpur, Malaysia

[6]Pharmacognosy Department, Faculty of Pharmacy, The British University in Egypt, 11837 Cairo, Egypt

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In the current review article, the studies conducted to investigate the anti-inflammatory activity of *Aquilaria* species are compiled and summarized. Since inflammation is the underlying cause of many diseases, the encounter of effective and safe biomedical anti-inflammatory compounds has become the focus of recent researches. *Aquilaria* species were known to possess a wide spectrum of pharmacological activities, among which anti-inflammatory activity has been reported in many *in vitro* and *in vivo* studies. Chromones, sesquiterpenoids, flavonoids, benzophenones and phorbol esters were the major anti-inflammatory compounds isolated from *Aquilaria* species. The objective of this review paper is to extend researches on the anti-inflammatory activity of different parts of *Aquilaria* species and support their future use in natural pharmaceutical preparations for the treatment of inflammation-associated conditions.

Keywords: *Aquilaria*, Agarwood, Inflammation, Anti-inflammatory, Secondary metabolites.

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Introduction

Inflammation is a vital biological phenomenon that occurs in response to internal and external injurious stimuli to mitigate foreign triggers, initiate damaged tissue repair and restore the normal body homeostasis[1].

Although the role of inflammation is to safeguard the body, inflammation has been proven to be among the underlying etiologies of chronic and degenerative diseases such as diabetes, atherosclerosis, rheumatoid arthritis, cancer and cardiovascular diseases[2,3]. The most common drugs for the treatment of inflammatory disorders are non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids. However, high doses and prolonged use of synthetic anti-inflammatory medications may lead to intolerable side effects, most commonly, gastrointestinal bleeding. Across the world, traditional medicines in the form of herbal drinks were known to relieve inflammation. Accordingly, the discovery of effective and safe bio-based alternative compounds for the prevention and treatment of inflammation has attracted the attention of researchers, aiming to obviate the adverse effects of synthetic drugs.

Aquilaria is a tropical evergreen tree that belongs to family Thymelaeaceae and is native to Southeast Asian rainforests and some parts of China and India[4]. The genus embraces 47 species, among which 22 are stated as accepted in The Plant List[4]. The tree is famous for its resinous and fragrant heartwood known as agarwood that is commonly used in religious ceremonies, perfumes and traditional Ayurvedic remedies. The dark fragrant agarwood occurs naturally in response to natural injuries such as lightning, insects and mould attacks[5]. However, the economic interest in agarwood has encouraged the establishment of plantations where sustainable resin

*Correspondent author
Email: manareissa1210@gmail.com, yumi@iium.edu.my
Tel: (+6)0126125692

production can be induced by physical means such as nailing and cutting, chemical means or fungi[5]. Besides, other parts of the tree became targets for the discovery of secondary metabolites and bioactive compounds. In the comprehensive ethnobotanical reviews, of Wang *et al.*[6] and Hashim *et al.*[7], the phytochemicals found in *Aquilaria* species and their pharmacological actions were documented. The most common phytochemicals found in *Aquilaria* species were alkaloids, tannins, saponins, terpenoids, flavonoid glycosides, sesquiterpenes and 2-(2-phenylethyl) chromone derivatives[6,7]. Extracts of different parts of the tree and the isolated compounds were reported to exert several pharmacological potentials such as anti-cancer[8–10], anti-diabetic[11,12], antimicrobial[13,14], cardioprotective[15], antioxidant[16,17] and anti-inflammatory[18,19] effects. Of particular interest, the present report aims to provide a comprehensive review of the anti-inflammatory activity of *Aquilaria* extracts and the species-isolated compounds, tested *in vitro* and *in vivo*. The information is important to researchers interested in further exploration of the anti-inflammatory activity of the plant.

In vitro and *in vivo* models for studying anti-inflammatory effects

Based on the pathophysiology and the apparent signs of inflammation, many *in vitro* tests and *in vivo* animal models have been approved to affirm the potential anti-inflammatory activity of newly discovered agents. Human macrophages and polymorph nuclear neutrophils (PMNs) are the major cells involved in the pathogenesis of inflammatory diseases. The aberrant stimulation of the macrophages induces the release of pro-inflammatory mediators such as NF-kβ, TNFα, IL6, reactive oxygen species (ROS), superoxide anions and proteases[20–22]. All above mentioned inflammatory molecules are considered possible targets for anti-inflammatory drugs. Inflammation is also associated with elevation of nitric oxide (NO) levels and protein denaturation. Therefore, the subsidence of NO levels and the inhibition of egg albumin or Bovine Serum Albumin (BSA) denaturation have been widely applied as *in vitro* screening assays for anti-inflammatory action.

Accordingly, several herbal extracts and compounds are thought to be able to reduce pro-inflammatory mediators, diminish NO levels or inhibit protein denaturation, and thus can be accepted as new potential anti-inflammatory agents. At *in vitro* level, lipopolysaccharide (LPS)-activated macrophages RAW 264.7 has been broadly used as a cell line model to study inflammation and identify anti-inflammatory compounds.

Animal models were extensively used in biomedical research to study the pharmacological and toxicological effects of anti-inflammatory drugs. The complex cascade in the pathogenesis of inflammation involves interaction between leucocytes, blood vessels and tissue cells. Increased permeability of blood vessels occurs accompanied by exudation of fluid from the blood into interstitial spaces and infiltration of leukocytes into the tissues[2,23] resulting in the five hallmark signs of inflammation: redness, heat, swelling, pain and loss of function[1]. The reduction in vascular permeability and neutrophil infiltration which purges in the form of signs suppression was assessed *in vivo* among different animal models, most commonly, Xylene-induced ear swelling and carrageenan-induced paw oedema in mice[19]. Recently, the zebrafish animal model was used due to its morphological and physiological similarities to mammals and its high genetic similarities with humans. Yang *et al.*[24] suggested that the zebrafish LPS (Lipopolysaccharide) *in vivo* inflammation model is a promising screening model that can be applied to study suppressors of inflammation.

Studies on the anti-inflammatory activity of *Aquilaria* species

Evidence in literature confirmed that extracts of different parts of *Aquilaria* species and some of the purified compounds demonstrate noticeable anti-inflammatory potential. Dried *Aquilaria* leaves have gained wide popularity as traditional herbal tea in Asian countries. It was reported that tea processed from *Aquilaria* species is applied in traditional medicine to promote health and relief morbid conditions including inflammatory-related disorders[25]. Based on previous studies, the chemical constituents of different parts of *Aquilaria* species include 2-(2-phenylethyl) chromones, fatty acids, benzophenones, flavonoids, terpenoids, and esters[6,7]. The presence of these compounds in herbal extracts is thought to be responsible for several pharmacological actions including anti-inflammatory. Conversely, the review on the anti-inflammatory activity of *Aquilaria* species is still incomprehensive. Therefore, this paper aims to compile the findings of previous studies conducted to investigate the anti-inflammatory activity *in vivo* and *in vitro* of different *Aquilaria* species. Four species

dominate this review for being the most popular species documented in various reports; and with proven anti-inflammatory activity, namely, *Aquilaria sinensis, Aquilaria malaccensis, Aquilaria agallocha* and *Aquilaria crassna.* The findings of the *in vitro* and *in vivo* studies are summarized in Tables 1-2, respectively. The chemical structures of the identified compounds in *Aquilaria* species that demonstrated anti-inflammatory activity are shown in Fig. 1.

Table 1 — *In vitro* anti-inflammatory studies on *Aquilaria* species

Part	Extract/ compounds	Experimental protocol	Quantitative response	Ref.
Aquilaria sinensis				
Leaves	Ethanolic leaf extract: 50, 100, and 200 µg/mL	Inhibition of LPS-induced NO release from mouse peritoneal macrophages	IC_{50}= 80.4 µg/mL Positive control: hydrocortisone (IC_{50}= 0.1 µM)	19
	(1) Aquilarinoside A (2) Iriflophenone (3) Mangiferin (4) 7-b –D-glucoside of 5-O-methylapigenin (5) 5-O-xylosylglycoside of 7-O-methylapigenin (6) Luteolin (7) Genkwanin (8) Hydroxygenkwanin	Inhibition of PMA-stimulated polymorphonuclear neutrophils (PMNs) respiratory burst	(1) IC_{50}= 89.92 µmol/L (2) IC_{50}= 52.59 µmol/L (3) IC_{50}= 50.34 µmol/L (4) IC_{50}= 61.25 µmol/L (5) IC_{50}= 293.06 µmol/L (6) IC_{50}= 2.03 µmol/L (7) IC_{50}= 265.41 µmol/L (8) IC_{50}= 0.80 µmol/L Positive control: NA	26
	(9) Aquisiflavoside	Inhibition of LPS-induced NO production in RAW 264.7 cells	(9) IC_{50}= 34.95 µM Positive Control: LN 6-(1-iminoethyl)lysine (IC_{50}= 27.12 µM)	27
Agarwood	(10) Aquilarone A (11) Aquilarone B (12) Aquilarone C (13) Aquilarone D (14) Aquilarone E (15) Aquilarone F (16) Aquilarone G (17) Aquilarone H (18) Aquilarone I (19) (Known analogue) (20) (Known analogue)	Inhibition of LPS-induced NO production in RAW 264.7 cells	(10) IC_{50}= 9.03 µM (11) IC_{50}= 5.12 µM (12) IC_{50}= 7.71 µM (13) IC_{50}= 7.49 µM (14) IC_{50}= 22.26 µM (15) IC_{50}= 13.09 µM (16) IC_{50}= 7.94 µM (17) IC_{50}= 5.95 µM (18) IC_{50}= 7.59 µM (19) IC_{50}= 7.94 µM (20) IC_{50}= 6.59 µM Positive control: ibuprofen (IC_{50}= 94.12 µM)	28
	(21) 5-hydroxyl-7-methoxy-2-[2-(40-methoxyphenyl) ethyl] chromone (22) 5_,6_-epoxy-7_,8_,30-trihydroxy-40-methoxy-2-(2-phenylethyl)chromone	Inhibition of LPS-induced NO production in RAW 264.7 cells	(21) IC_{50}= 4.6 µM (22) IC_{50}= 84 µM Positive control: aminoguanidine (IC_{50}= 1.8-0.2 µM)	29
	(23) 1,10-dioxo-4_H-5_H-7_H-11_H-1,10-secoguaia-2(3)-en-12,8_-olide	Inhibition of LPS-induced NO production in RAW 264.7 cells	(23) IC_{50}= 8.1 µM Positive control: aminoguanidine HCl (IC_{50}= 11.6µM)	30
	(24) (5R,6R,7R,8S)-8-Chloro-5,6,7-trihydroxy-2-(4-methoxyphenethyl)-5,6,7,8- tetrahydrochromone (25) (5R,6R,7R,8R)-8-Chloro-5,6,7-trihydroxy-2-(4-methoxyphenethyl)-5,6,7,8- tetrahydrochromone (26) (5S,6S,7S,8S)-8-Chloro-5,6,7-trihydroxy-2-(2-phenylethyl)-5,6,7,8- tetrahydrochromone (27) [29], 8-chloro-2-(2-phenylethyl)-5,6,7-trihydroxy-5,6,7,8- tetrahydrochromone (28) [4], 8-dihydroxy-2-(2-phenylethyl) chromone (29) [10], rel-(1aR,2R,3R,7bS)- 1a,2,3,7b-tetrahydro-2,3-dihydroxy-5-[2-(4-methoxyphenyl)ethyl]-7H-oxireno[f][1] benzopyran-7-one	Inhibition of LPS-induced NO production in RAW 264.7 cells	(24) IC_{50}= 4.5 µM (25) IC_{50}= 7.3 µM (26) IC_{50}= 3.8 µM (27) IC_{50}= 4.5 µM (28) IC_{50}= 6.4 µM (29) IC_{50}= 1.6 µM Positive control: indomethacin (IC_{50} = 23.6 µM)	31

(*Contd.*)

Table 1 — *In vitro* anti-inflammatory studies on *Aquilaria* species (*Contd*.)

Part	Extract/ compounds	Experimental protocol	Quantitative response	Ref.
Aquilaria sinensis				
	(30) (+)-Aquisinenone A (31) (-)-Aquisinenone A (32) 4′-methoxyaquisinenone A (33) (+)-Aquisinenone B (34) (-)-Aquisinenone B (35) (+)-6″-hydroxy-4′,4‴-dimethoxyaquisinenone B (36) (-)-Aquisinenone D (37) (+)-4′-demethoxyaquisinenone D (38) (-)-4′-demethoxyaquisinenone D (39) (-)-Aquisinenone F (40) (−)-Aquisinenone G (41) (+)-4′-methoxyaquisinenone G	LPS-induced NO production in RAW 264.7 cells	(30) IC_{50}= 11.5 µM (31) IC_{50}= 7.6 µM (32) IC_{50}= 9.3 µM (33) IC_{50}= 8.8 µM (34) IC_{50}= 8.6 µM (35) IC_{50}= 10.5 µM (36) IC_{50}= 7 µM (37) IC_{50}= 8.5 µM (38) IC_{50}= 8.5 µM (39) IC_{50}= 12 µM (40) IC_{50}= 11.4 µM (41) IC_{50}= 8 µM Positive control: GYF-17 (IC_{50}= 4.4 µM)	32
	(42) 5,6-dihydroxy-2-[2-(3´-hydroxy-4´-ethoxyphenyl) ethyl] chromone (43) 7-methoxy-2-(2-phenylethyl)chromone (44) 6,7-dimethoxy-2-(2-phenylethyl)chromone (45)6,7-dimethoxy-2-[2-(4´-methoxyphenyl)ethyl] chromone (46) Neopetasan (47) 7α-H-9(10)-ene-11,12-epoxy-8-oxoeremphilane (48) Dehydrokaranone	Luciferase activity in lipopolysaccharide (LPS)-induced NF-κB activation in RAW 264.7/Luc-P1 cells	(42) 0.74 (43) 0.54 (44) 0.31 (45) 0.38 (46) 0.55 (47) 0.75 (48) 0.72 Positive control: andrographolide (0.35)	33
	(49) Aquisinenone H (50) Aquisinenone I (51) 7″-Methoxyaquisinenone I (52) 4′,7″-Dimethoxyaquisinenone I (53) Aquisinenone K (54) 4′,4‴-Dimethoxyaquisinenone K (55) Aquisinenone L (56) Aquisinenone M (57) Aquisinenone O (58) 7,4′-Dimethoxyaquisinenone O (59) 2′-di-(2-phenylethyl)-8,6′-dihydroxy-5,5′-bichromone	LPS-induced NO production in RAW 264.7 cells	(49) IC_{50}= 4.3 µM (50) IC_{50}= 1.9 µM (51) IC_{50}= 1.6 µM (52) IC_{50}= 5.8 µM (53) IC_{50}= 0.7 µM (54) IC_{50}= 0.6 µM (55) IC_{50}= 8.0 µM (56) IC_{50}= 37.1 µM (57) IC_{50}= 7.6 µM (58) IC_{50}= 2.3 µM (59) IC_{50}= 7.4 µM Positive control: indomethacin (IC_{50}= 45.6 µM)	34
Stem bark	(60) 3’-O-Geranylpolloin (61) 7-Hydroxy-6-methoxy-2- (2-phenylethyl) chromone (62) 5-Hydroxy-7,31,41-trimethoxyflavone (63) Velutin (64) 3’-Hydroxygenkwanin (65) Sakuranetin (66) 6,7-Dimethoxy-2-(2-phenylethyl) chromone (67) Ergosta-4,6,8(14),22-tetraen-3-one	Superoxide anion (SOA) generation, Elastase release in fMLP/CB-activated human neutrophils	(60) IC_{50}= 12.51, 3.91 µM (61) IC_{50}= 4.62 µM (62) IC_{50}= 4.69 µM (63) IC_{50}= 1.78, 4.26 µM (64) IC_{50}= 7.96, 4.56 µM (65) IC_{50}= 1.74 µM (66) IC_{50}= 11.54 µM (67) IC_{50}= NA, 15.25 µM Positive control (SOA inhibition): diphenyleneiodonium (IC_{50}= 1.73 µM) Positive control (Elastase release inhibition): phenylmethylsulfonyl fluoride (IC_{50}= 199.6 µM)	21

(*Contd*.)

Table 1 — *In vitro* anti-inflammatory studies on *Aquilaria* species (*Contd*.)

Part	Extract/ compounds	Experimental protocol	Quantitative response	Ref.
Aquilaria sinensis				
Flower buds	Methanolic flower bud extract: 100 ug/mL (68) Aqulasides B (69) Aqulasides C	Inhibition of iNOS activity Inhibition of NF-KB-mediated transcription in SW1353 cells	Extract: 22% inhibition of iNOS Positive control: parthenolide (97% at 100 ug/mL) (68) 31% at 100 µM (69) 60% at 100 µM Positive control: parthenolide (83% at 2.5 ug/mL)	35
Pericarp	(63) Velutin (70) Pilloin (71) β-sitostenone	Inhibition of lipopolysaccharide (LPS)-induced NF-κB activation by macrophages	(63) IC_{50}= 23.36 µM (70) IC_{50}= 25.58 µM (71) IC_{50}= 11.51 µM Positive control: NA	36
Aquilaria malaccensis				
Leaves	Ethanolic leaf extract: 16000 µg/mL Hexanoic leaf extract: 16000 µg/mL Supercritical fluid leaf extraction: 16000 µg/ML	Inhibition of albumin denaturation	70.045% 55.9% 52.47%	38
Seeds	(79) (2′E,4′E)-6-oxohexa-2′,4′-dienoylphorbol-13-acetate. (80) 12-O-deoxyphorbol 13-decanoate (81) mellerin A.	Inhibition of elastase release by fMLP/CB stimulated human neutrophils	(79) IC_{50}= 2.7 µM (80) IC_{50}= 0.8 µM (81) IC_{50}= 2.1 µM Positive Control: PI3K inhibitor LY294002 (IC_{50}= 3.3 µM)	39
Aquilaria agallocha				
Agarwood	Oil: 100, 250, and 500 mcg/mL	Human red blood cell membrane stabilization method	Protection of human RBC in hypotonic solution ranging from 39.66 to 78.50% Positive control: diclofenac (43.74 to 86.73%)	41
	Oil: 100, 250, and 500 µg/mL	BSA denaturation assay	23.68, 48.21, and 56.71% inhibition of protein denaturation Positive control: diclofenac (39.58, 75.83, and 77.51%)	43
Leaves	Ethanolic extract: 100, 250, and 500 µg/mL	BSA denaturation assay	34.09, 36.95, and 43.13% inhibition of protein denaturation	43
Aquilaria crassna				
Agarwood	Ethyl acetate extract: 1.5 mg/mL	LPS-induced TNF-α production by human polymorphonuclear cells (hPBMC)	1.5 mg/mL significantly reduced TNF-α level. Positive control: NA	44

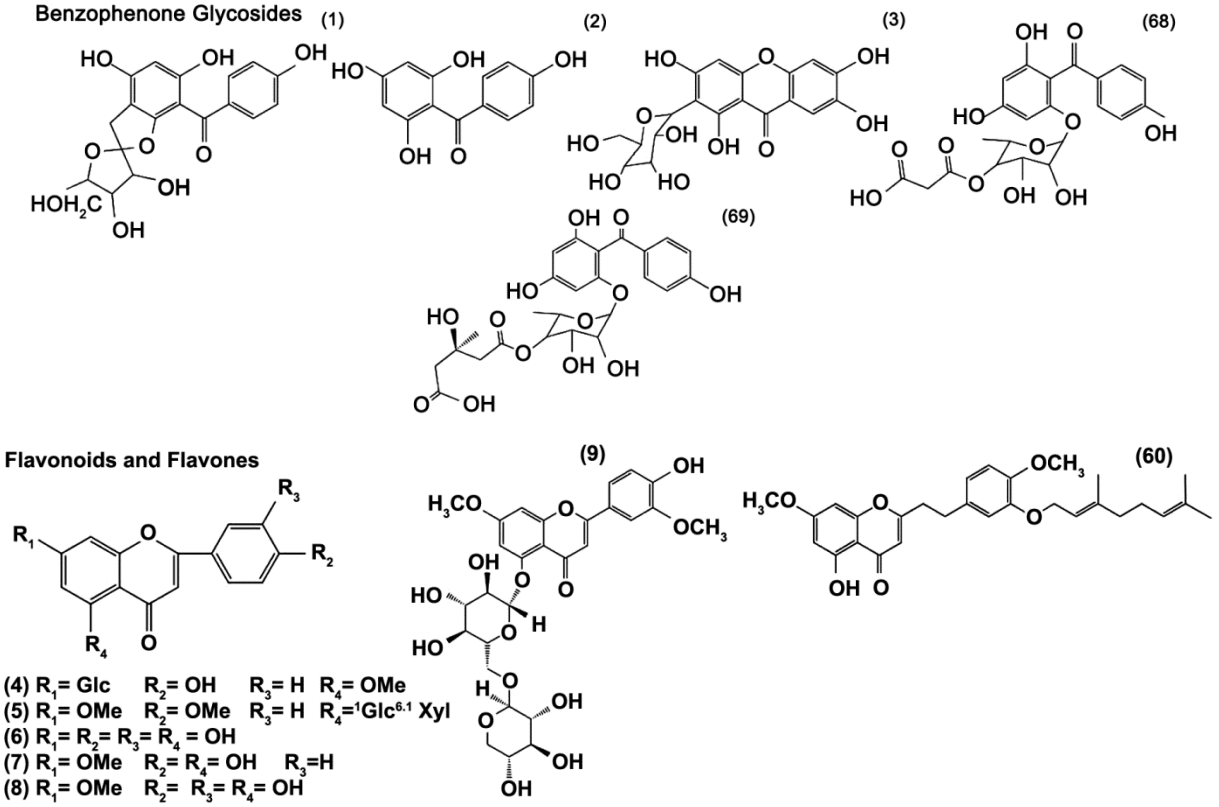
Table 2 — *In vivo* anti-inflammatory studies on *Aquilaria* species

Part	Extracts/ compounds (Dose)	Experimental protocol	Quantitative/ qualitative Response	Ref.
Aquilaria sinensis				
Leaves	Ethanolic extract (424 and 848 mg/ kg)	Xylene-induced ear swelling (ICR mice) Carrageenan-induced paw oedema (ICR mice) CMC-Na induced leukocyte migration (ICR mice)	31.4 and 49.50% inhibition of ear swelling Positive control: indomethacin 20 mg/kg (63.9%) 24.34 and 16.40 inhibition of paw oedema Positive control: indomethacin 20 mg/kg (14.76%) 68.8 and 90.6% inhibition of leukocytes migration Positive control: dexamethasone 20 mg/kg (96.84%)	19
Aquilaria agallocha				
Leaves	Ethanolic extract (200 and 400 mg/kg)	Freund's adjuvant-induced paw oedema (Rats)	21.20 and 25.34% inhibition in paw oedema on day 21 Positive control: ibuprofen 50 mg/kg, p.o. (42.12%)	43

(*Contd*.)

Table 2 — *In vivo* anti-inflammatory studies on *Aquilaria* species (*Contd*.)

Part	Extracts/compounds (Dose)	Experimental protocol	Quantitative/ qualitative Response	Ref.
Aquilaria sinensis				
Agarwood	Ethyl acetate extract orally (50, 100, and 200 mg/kg)	Carrageenan induced paw oedema (Wister Rats) Cotton pellets induced granuloma (Wister Rats)	51.38, 55.09, and 56.25% inhibition in paw oedema Positive control: diclofenac Na 25 mg/kg, p.o (% of inhibition not specified) 43.46, 68.24, and 77.18% reduction in granuloma weight Positive control: diclofenac Na 10 mg/kg, p.o. (80.87%)	40
	Oil (125 and 250 mg/kg)	Freund's adjuvant-induced paw oedema (Rats)	18.12 and 27.88% inhibition in paw oedema on day 21 Positive control: ibuprofen 50 mg/kg, p.o. (42.12%)	43
	(Synonym: *A. malaccensis*) Oil (Topical) 20 µL/ear/time 30 min after TPA administration three times till 24 h after the TPA administration	TPA-induced ear inflammation (Swiss mice)	Significant reduction of ear oedema and pro-inflammatory cytokines production. (1.0% agarwood oil had an equivalent effect to indomethacin). Positive control: indomethacin (200 µg/ear).	42
Wood	Oil hexanoic extract (50 and 100 mg/kg) sub plantar injection	Carrageenan-induced paw oedema (Rats)	58.6 and 62.11% inhibition in paw oedema after 3 h Positive control: diclofenac 10 mg/kg (68.94%)	41
Aquilaria crassna				
Agarwood	(65) β-caryophyllene	Carrageenan-induced rat hind paw edema model (Sprague Dawley Rats)	(65) 56.2, 71.4, and 87.6% inhibition in paw oedema Positive control: indomethacin 10 mg/kg (75.5%)	45



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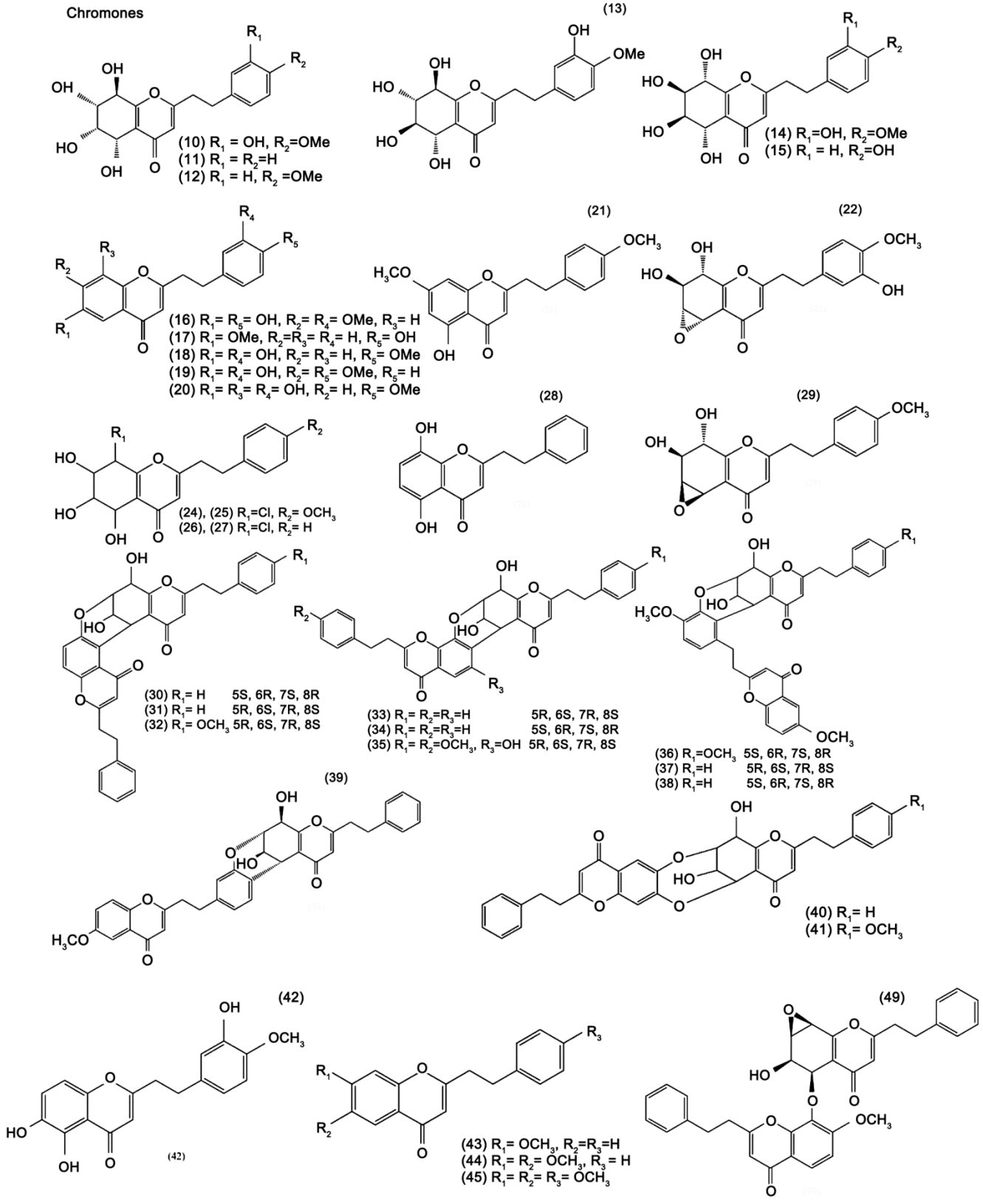
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(13)
(14) R1=OH, R2=OMe
(15) R1 = H, R2=OH
(16) R1= R5= OH, R2= R4= OMe, R3= H
(17) R1= OMe, R2=R3= R4= H, R5= OH
(18) R1= R4= OH, R2= R3= H, R5= OMe
(19) R1= R4= OH, R2= R5= OMe, R5= H
(20) R1= R3= R4= OH, R2= H, R5= OMe
(21)
(22)
(24), (25) R1=Cl, R2= OCH3
(26), (27) R1=Cl, R2= H
(28)
(29)
(30) R1= H 5S, 6R, 7S, 8R
(31) R1= H 5R, 6S, 7R, 8S
(32) R1= OCH3 5R, 6S, 7R, 8S
(33) R1= R2=R3=H 5R, 6S, 7R, 8S
(34) R1= R2=R3=H 5S, 6R, 7S, 8R
(35) R1= R2=OCH3, R3=OH 5R, 6S, 7R, 8S
(36) R1=OCH3 5S, 6R, 7S, 8R
(37) R1=H 5R, 6S, 7R, 8S
(38) R1=H 5S, 6R, 7S, 8R
(39)
(40) R1= H
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(42)
(43) R1= OCH3, R2=R3=H
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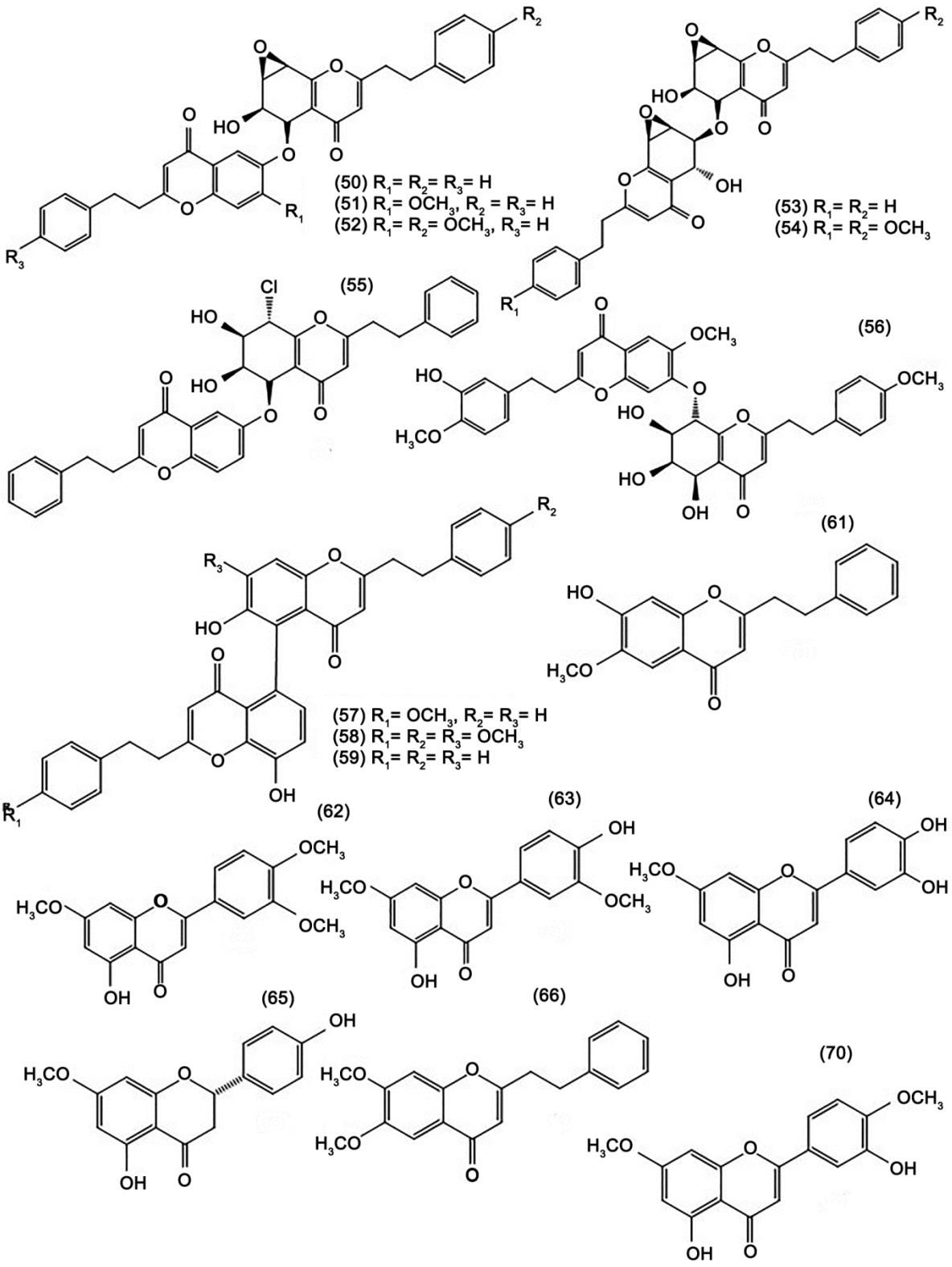
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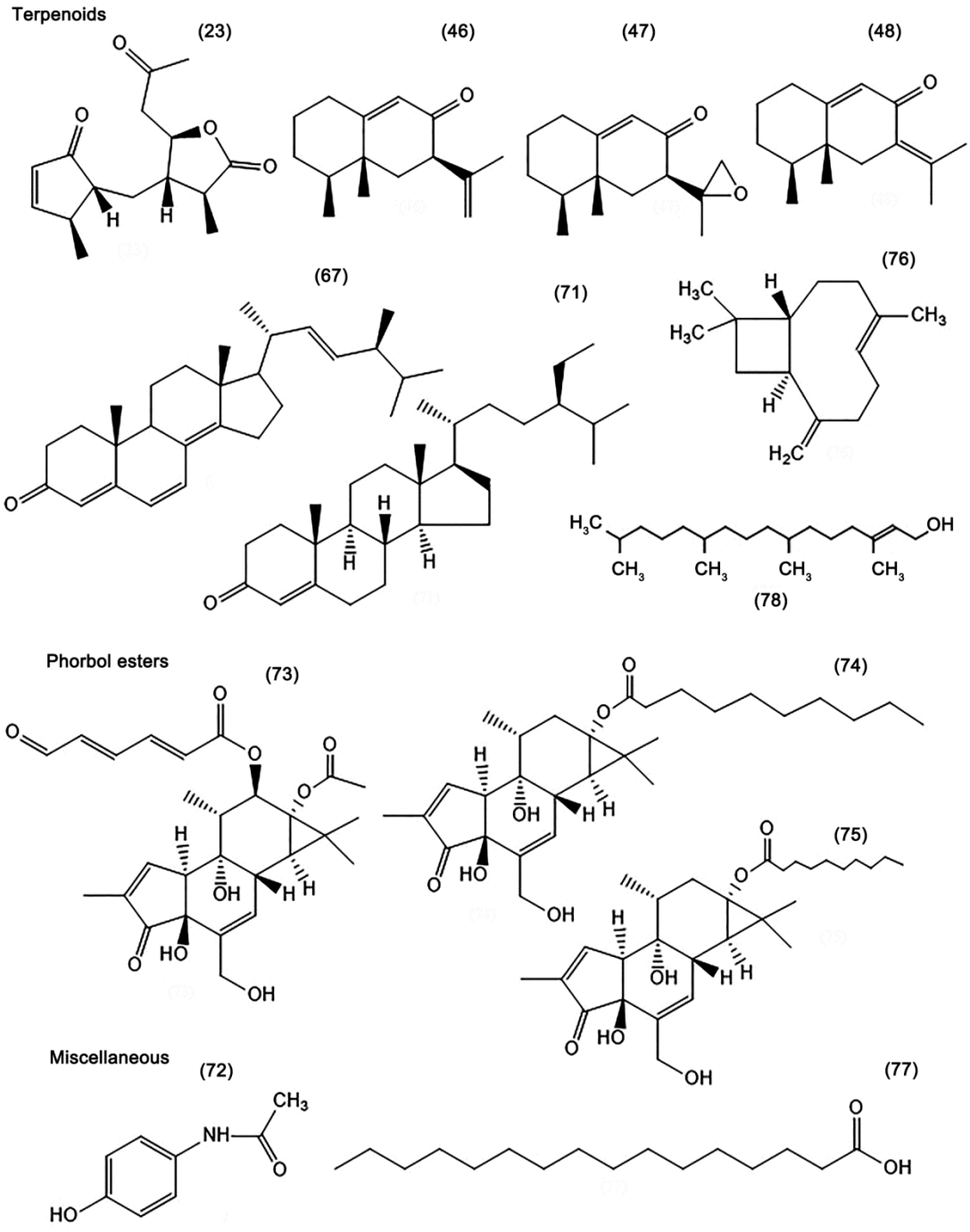

Fig. 1 — Chemical structures of anti-inflammatory compounds isolated from *Aquilaria* species. The number indicated for each compound correspond to the number in the text.

Aquilaria sinensis

The anti-inflammatory potency of *A. sinensis* ethanolic leaf extract was evaluated using *in vivo* and *in vitro* models[19]. Using mice as an animal model, the extract demonstrated inhibition the xylene-induced ear swelling (848 mg/kg, 49.5%), carrageenan-induced paw oedema (424 mg/kg, 25.02%), and carboxymethyl cellulose sodium (CMC-Na)-induced leukocyte emigration (848 mg/kg, 90.60%)[19]. Meanwhile, in *in vitro* study, the NO production induced by LPS was reduced by the extract in a concentration-dependent manner with an IC_{50} of

80.4 µg/mL[19]. Flavonoids and benzophenone glycosides are majorly found in the aerial parts of the plants such as leaves and flowers and are considered to possess anti-inflammatory activity. For instance, the benzophenone glycosides **(1-3)** and the flavonoids **(4-8)** isolated from the ethanolic extract of *A. sinensis* leaves demonstrated inhibition against PMA-induced polymorphonuclear neutrophils respiratory burst with an IC_{50} values ranging from 0.80 to 89.92 µmol/l[26]. Aquisiflavoside flavonoid **(9)** was also isolated from *A. sinensis* leaves and showed potent inhibition of nitric oxide production *in vitro* with an IC_{50} value of 34.95 µM[27].

The bioactive constituents of the resinous heartwood of *A. sinensis* were determined and multiple chromones and sesquiterpenoids with anti-inflammatory activities were identified. Chen *et al*. discovered novel chromone derivatives in *A. sinensis* resinous heartwood identified as aquilarones (A – I) and another two analogues **(10-20)** which were reported to exhibit potent inhibitory activity against LPS-induced NO production in RAW 264.7 cells, with an IC_{50} between 5.12–22.26 µM[28]. Similarly, two other chromones **(21, 22)** were isolated from agarwood produced through agarwood-inducing techniques demonstrated moderate to significant anti-inflammatory activity with an IC_{50} (4.6 and 84 µM), respectively[29]. The sesquiterpenoid **(23)** was also identified in *A. sinensis* heartwood and reported to have observable anti-inflammatory activity against LPS-induced NO production with an IC_{50} value of 8.1 µM[30]. The ethyl acetate fraction obtained from 95% ethanolic extract of *A. sinensis* agarwood demonstrated potent inhibition of NO production at a concentration of 20 µg/mL[31]. The chromones isolated by the same researchers in two different studies were tested for their anti-inflammatory activity against LPS-induced NO production by RAW 264.7 cells, and the compounds **(24-41)** showed inhibition of NO production with an IC_{50} (1.6 µM – 12 µM)[31,32]. The anti-inflammatory activity of the aforementioned compounds can be attributed to the presence of chlorine atom **(24-27)** and the epoxy group **(29)** on the A-ring[31] or the dioxepine moiety as in compounds **(40)** and **(41)**[32]. In another recent study, many chromones **(42-45)** and sesquiterpenoids **(46-48)** that were isolated from ethyl acetate fraction obtained from the methanolic extract of the resinous agarwood of *A. sinensis* were tested for their ability to suppress LPS-induced NF-KB activation correlated with luciferase gene reporter expression[33]. Some of the isolated compounds were reported to reduce Luciferase activity. Besides, the chromones showed an extra ability to inhibit NO production in RAW 264.7 macrophages over sesquiterpenoids[33]. The strongest compound was 6,7-dimethoxy-2-(2-phenylethyl) chromone **(44)** which showed the lowest relative luciferase activity (0.31±0.05)[33]. It is possible that the presence of two Methoxy ($-OCH_3$) groups supports the surpassing inhibition of NF-KB activation of compound **(44)** over compounds **(43)** and **(45)** with single and triple Methoxy groups, respectively. To search for more 2-(2-phenylethyl) chromones with potent anti-inflammatory activity, Huo *et al*. conducted a recent study that resulted in the discovery of eleven compounds **(49-59)** that showed inhibition of NO production in LPS-stimulated RAW 264.7 cells with an IC_{50} ranging from 0.6–37.1 µM[34].

The stem barks for *A. sinensis* were also investigated for their anti-inflammatory activity. Some of the chromatographically isolated flavones **(60),** chromones **(61-66)** and sesquiterpenoids **(67)** were reported to have strong inhibitory effects against superoxide anion generation and elastase release in fMLP/CB-activated human neutrophils, with compounds **(61-63)** shown to be most effective[21]. The methanolic extract of the flower buds of *A. sinensis* demonstrated moderate inhibition of NO production and comprises benzophenone glycosides **(57, 58)**, that showed weak inhibition of NF-KB[35]. The pericarp of *A. sinensis* was also tested for its anti-inflammatory activity and some of the isolated compounds **(63, 70, 71)** exhibited anti-inflammatory activity with an IC_{50} between 11.51–25.58 µM[36].

Aquilaria malaccensis

Relatively few studies have been conducted to assess the anti-inflammatory activity of *A. malaccensis* extracts or isolated compounds. 4'-hydroxyacetanilide **(72)** or acetaminophen, which is a well-known anti-inflammatory agent, was determined in *A. malaccensis* leaves extract obtained by hydrodistillation[37]. A preliminary study demonstrated the ability of different *A. malaccensis* leaves extracts to inhibit albumin denaturation as a potential sign of anti-inflammatory effect[38]. Meanwhile, phorbol esters discovered in *A. malccensis* seed extract demonstrated anti-inflammatory activity by inhibiting elastase release in human neutrophils **(73-75)**[39]. On the contrary, some phorbol esters induced inflammation by enhancing the generation of superoxide anions. The study added a new notion to the literature on the dual role of phorbol esters in the inflammation process.

Aquilaria agallocha

The anti-inflammatory activity of *A. agallocha* was observed *in vivo* against Carrageenan induced paw oedema in rats and cotton pellets induced granuloma, revealing statistically significant results up to 56.25% inhibition in paw oedema at a dose of 200 mg/kg[40]. It was also stated that *A. agallocha* oil demonstrated *in vivo* and *in vitro* anti-inflammatory activity which is comparable to standard diclofenac[41]. Another *in vivo* study concluded that topical treatment with agarwood oil obtained from *A. agallocha* significantly reduced oedema and pro-inflammatory cytokines production (IL-1β, IL-6, and TNF-α) in TPA-induced mouse ear inflammation model which validates its use as an anti-inflammatory topical medication[42]. The anti-arthritic activity of *A. agallocha* was studied *in vitro* using BSA denaturation assay and *in vivo* using Freund's adjuvant-induced arthritic rat model[43]. The findings of both *in vitro* and *in vivo* assays revealed that the ethanolic extract of *A. agallocha* leaves and heartwood oil exhibited strong anti-inflammatory activity. Further details can be described in Table 1.

Aquilaria crassna

Studies on *A. crassna* showed its remarkable anti-inflammatory activity. For instance, the ethyl acetate extract of *A. crassna* demonstrated anti-inflammatory response through inhibition of TNF-α production[44]. β-caryophyllene **(76)** isolated from *A. crassna* essential oils was proven to have anti-inflammatory activity *in vivo*[45]. However, the leaves extract of *A. crassna* failed to demonstrate any anti-inflammatory effect at a dose of 800 mg/kg *in vivo*[18].

Miscellaneous common compounds with anti-inflammatory activity

In addition to the aforementioned compounds discovered in *Aquilaria* species, Hexadecanoic acid **(77)**, which is a well-known anti-inflammatory compound, was predominant in the characterization of almost all extracts of *Aquilaria* species. Initial studies confirmed that Hexadecanoic acid controls inflammation through inhibition of phospholipase A2 enzyme by binding to its active site[46]. Hexadecanoic acid was found as a major compound in the extracts of *A. malaccensis* leaves[38,47], essential oil of *A. sinenis*, *A. crassna* and *A. agallocha*[48-50]. Yet, there is no specific study in literature targeting the anti-inflammatory activity of Hexadecanoic acid isolated from *Aquilaria* species *in vivo* or *in vitro*. Likewise, phytol **(78)**, diterpene alcohol with reported anti-inflammatory activity[51], appeared in the GCMS of different *A. malaccensis* leaves extracts[38,47].

Toxicological studies

Few studies have been conducted to examine the safe consumption of herbal extracts and the possible toxic effects of *Aquilaria* extracts. Zhou *et al*. reported the relative safety of *A. sinensis* leaf extract when administered in mice intra-gastrically with a maximum tolerated dose of 20.4 g/kg[19]. The essential oil, hexane and methanol extracts of *A. sinensis*, *A. malaccensis*, and *A. crassna* leaves have been tested against human's peripheral blood mononuclear cells (PBMCs) using MTT assay for cytotoxicity and comet assay for genotoxicity[52]. It has been shown that no cytotoxic or genotoxic effects were reported upon testing the essential oils and the extracts of the three species, except for the methanolic leaf extract of *A. malaccensis* which reported slight toxicity[52]. On the contrary, Liyana *et al*. reported that the methanolic extract of *A. malaccensis* leaves demonstrated no signs of acute or sub-chronic toxicity when administered orally in rats[53]. The ethanolic leaf extract and oil obtained from *A. agallocha* wood was safe up to a dose of 2000 mg/kg in rats when tested following the OECD 423 protocol for testing chemicals[41,43]. In an acute oral toxicity study in mice following the same protocol, the ethanolic extract of *A. crassna* leaves showed no signs of toxicity[54]. In another study, the methanolic leaf extract of *A. crassna* was found to be safe with repeated oral doses up to 8000 mg/kg[46]. The ethyl acetate extract of *A. crassna* was found not to exert any cytotoxicity of human peripheral blood mononuclear cells at a dose of ≥1.5 mg/mL[44]. Interestingly, Alam *et al*. testified that the ethanolic extract of *A. agallocha* leaves exerted a hepatoprotective effect against paracetamol-induced liver toxicity[55].

Current aspects and future prospects

In the present review, our attempt was not only to summarize the research studies on the anti-inflammatory effects of *Aquilaria*, but also to pinpoint areas that require further investigations aiming to highlight possible developments that will eventually lead to advancements in prevention or treatment of inflammatory disorders.

Based on prior research, the four species included in this article were proven to exhibit anti-inflammatory activities. Some issues are associated with the nomenclature of *A. agallocha*. It is worth to highlight that the studies included in this article about this

species were supported with voucher specimen from recognized herbariums to confirm the identification of the species unless otherwise mentioned.

An inadequate number of studies has been published on the anti-inflammatory activity of different parts of *Aquilaria*. From Tables 1 and 2, it is obvious that a greater number of studies were performed on *A. sinensis* than the three other species in the focus area of this review. Besides, the highly valuable agarwood resin received more attention by researchers in former studies than other parts of the tree which have not yet been evaluated for their anti-inflammatory activity. Fig. 2 shows the number of anti-inflammatory studies conducted on each species reported between the years 2007-2019. Assumptions about the action of drugs in humans can be anticipated once-promising *in vitro* and *in vivo* findings have been achieved. Based on the current review, 19 *in vitro* studies and 7 *in vivo* studies have been carried out to identify the anti-inflammatory activity of *Aquilaria*. The *in vivo* studies are narrow and limited compared to the *in vitro* studies. Since inflammation is a vascular phenomenon, research directed towards *in vivo* studies would be more informative. More planned *in vitro* and *in vivo* studies to provide insights into the anti-inflammatory effect of *Aquilaria* species are hypothesized to be beneficial. The researchers have used several analytical methods to assess the anti-inflammatory property of the plant. The LPS-induced NO production in RAW 264.7 is the most commonly used method *in vitro*. Animal models used *in vivo* were mainly rats and mice. Other animal models such as zebrafish are recommended for further studies.

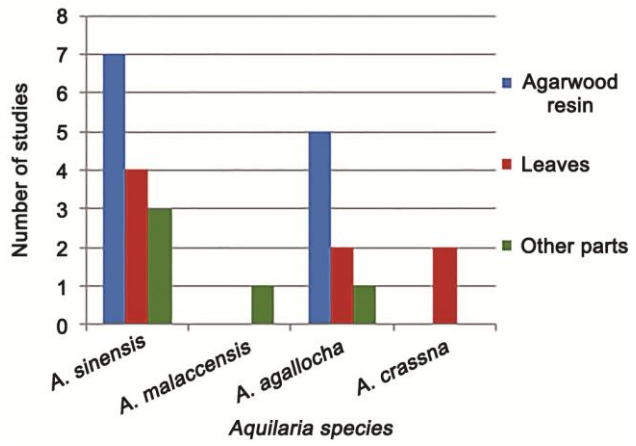

Fig. 2 — Relative anti-inflammatory studies performed on different parts of selected *Aquilaria* species reported between the years 2007-2019

According to the findings, the anti-inflammatory properties of *Aquilaria* extracts and recognized compounds qualify the genus to become an attractive anti-inflammatory agent. It is concluded that the species exert their anti-inflammatory effect by interfering with inflammatory pathways and/or suppression of inflammatory mediators. The species, the parts investigated and the methods of extraction are among the factors that influence the anti-inflammatory response and the isolated compounds. The species-derived compounds that are majorly responsible for the anti-inflammatory activity are Chromones, sesquiterpenoids, flavonoids, benzophenones and phorbol esters. To date, almost 78 anti-inflammatory compounds were identified. However, more isolated compounds can be further discovered by targeting other parts of the plant and can be assessed for suppressing inflammatory-related disorders.

Toxicological studies carried out on *Aquilaria* species are still deficient. Most studies confirmed the safe use of different extracts of the plant. To a lesser extent, other evidence revealed that extracts from certain *Aquilaria* species could be unsafe to humans. Therefore, further examination of the toxicological effects is still needed to provide guidelines for their safe intake.

Deeper research into the phytochemical composition of the parts that are not yet explored is needed and may introduce new compounds able to ameliorate inflammatory responses. It is likewise essential to identify the mechanism by which the anti-inflammatory actions are attained for future studies that can involve synergism between different mechanisms. The relationship between the chemical structures of the active compounds and their pharmacokinetics need to be identified to develop their clinical use. The information assembled from previous studies suggests that research is still open in this field.

Conclusion

Extensive researches are directed towards finding natural lead compounds for the treatment of inflammatory disorders. The review of the aforementioned studies not only provided evidence on the anti-inflammatory potential of *Aquilaria* but also would be beneficial to the future studies and exploitation of different parts of the plant as a potential source for treatment and prevention of inflammatory-associated disorders. *Aquilaria* species

were investigated for their anti-inflammatory activity *in vitro* and *in vivo*, and the results qualified *Aquilaria* tree to become a natural source for anti-inflammatory drugs. The present report is considered the first comprehensive review of the anti-inflammatory activity of *Aquilaria*. Based on the investigated parts of different species and the isolated compounds, the report highlighted certain gaps that can be areas for further studies.

Conflict of interest

The author declares no conflict of interest in this work.

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