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REVIEW ARTICLE

A Review on the Pharmacological Activities and Phytochemicals of *Alpinia officinarum* (Galangal) Extracts Derived from Bioassay-Guided Fractionation and Isolation

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ABSTRACT

The rhizomes of *Alpinia officinarum* Hance have been used conventionally for the treatment of various ailments, triggering a wide interest from the scientific research community on this ethnomedicinal plant. This review summarizes the phytochemical and pharmacological properties of the extracts and fractions from *A. officinarum*, a plant species of the Zingiberaceae family. Different parts of the plant – leaves, roots, rhizomes, and aerial parts – have been extracted in various solvents – methanol, ethanol, ethyl acetate, hexane, dichloromethane, aqueous, chloroform, and petroleum ether, using various techniques – Soxhlet extraction, maceration, ultrasonication, and soaking, whereas fractionation of the plant extracts involves the solvent–solvent partition method. The extracts, fractions, and isolated compounds have been studied for their biological activities – antioxidant, antibacterial, anti-inflammatory, anticancer, antiproliferative, inhibition of enzymes, as well as the inhibition of nitric oxide production. More findings on *A. officinarum* are certainly important to further develop potential bioactive drug compounds.

Key words: *Alpinia officinarum*, ethnomedicinal plant, lesser galangal, pharmacological, phytochemicals, Zingiberaceae

INTRODUCTION

Alpinia officinarum Hance, also known as lesser galangal, is indigenous to Southeast China (Guangdong, Guangxi, Hainan) and Indochina, and the plant is cultivated in the plains of West Bengal, Assam, and Eastern Himalayas in India.[1] *A. officinarum* belongs to the Zingiberaceae family. It is a perennial herb with thick, creeping reddish-brown rhizomes, lineolate acuminate ornamental leaves, and showy white flowers in racemes.[2] It has been used conventionally both in Ayurvedic and Chinese medicine since the very early times and in Europe since the Middle Ages.[1,3] The rhizome has been used in China for relieving stomach ache, treating colds, invigorating the circulatory system, and reducing swelling.[4] The dry root and rhizome have been used for their antioxidant, antidiabetic, antiulcer, antidiarrhea, anti-emetic, analgesic, anti-inflammatory, and anticoagulation effects.[5-7]

Different solvents are available to extract the bioactive compounds from natural products.[8] Various methods such as sonication, heating under reflux, Soxhlet extraction, maceration, and modern extraction techniques including supercritical fluid extraction are commonly used for plant sample extraction.[9-11] Alcoholic (methanol or ethanol) solutions frequently provide satisfactory results for the extraction process.[12] It is a common practice when isolating bioactive compounds that a number of different separation techniques such as thin layer chromatography, column chromatography, flash chromatography, Sephadex chromatography, and high-performance liquid chromatography (LC) are used to obtain pure compounds for the determination of structure and biological activity. Besides that, non-chromatographic techniques such as phytochemical screening assay can also be used to obtain and facilitate the identification of the bioactive compounds.[8] These compounds have been reported to possess biological activities due to the presence of various potentially active groups in their molecular structure.[12]

Diarylheptanoid (DAH) is a group of compounds found to have the potential in the development of natural products, with a special characteristic of bearing the 1,7-diphenylheptane skeleton.[13] There have been numerous DAH compounds isolated and reported for their structural characterization and biological activities.[13-21] Another group of compounds, polyphenols and flavonoids, are of interest because of their ability to scavenge reactive oxygen species (ROS).[22] The reduction capability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals is determined by the decrease in their absorbance at 517 nm induced by antioxidants.[23] Many antioxidants that react quickly with peroxyl radicals may react slowly or may even be inert to DPPH.[24]

Carrageenan paw edema test is used to screen anti-inflammatory drugs as it involves the inhibition of the release and/or action of several mediators – histamine, serotonin, kinin, and prostaglandin.[25,26] The bioactive compounds within *A. officinarum* may also be responsible for the antiproliferative activity, which have shown to exert anticancer

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effects on numerous cancer cell lines.[27-30] It was reported that the galangal extracts could penetrate into the bacterial cell, causing the bacterial membrane to rupture, and resulted in bacterial death.[31] Herein we report the phytochemical and biological activities exerted by the different solvent extracts and fractions and the identified compounds of *A. officinarum*.

SOLVENT EXTRACTS/FRACTIONS AND ISOLATED COMPOUNDS OF *ALPINIA OFFICINARUM*

Methanol

Tan *et al.* identified 16 chemicals consisting of 12 flavonoids and 4 DAHs from a methanol extract of *A. officinarum* leaves using LC-mass spectrometry (MS)/MS with a selected reaction monitoring mode.[32] The 12 flavonoids included chrysin (1), pinocembrin (2), tectochrysin (3), apigenin (4), galangin (5), 3-*O*-methylgalangin (6), acacetin (7), kaempferol (8), kaempferide (9), quercetin (10), isorhamnetin (11), and rutin (12). The four DAHs were yakuchinone A (13), oxyphyllacinol (14), hexahydrocurcumin (15), and hannokinol (16). In another study, they identified 17 components in the aerial parts and rhizome of a 3-year-old *A. officinarum*.[33] They extracted the plant material by maceration and ultrasonic extraction in methanol, and an aliquot was injected into the LC-MS/MS system. The 17 plant metabolites were compounds 1–5, 7–13, 15, nootkatone (17), DAH (18), luteolin (19), and izalpinin (20). Their study concluded that the contents of these compounds, except for compound 10, were higher in the rhizomes than in the aerial parts, and the six major constituents for both the aerial parts and rhizomes were compounds 1, 2, 5, 9, 11, 15.

Dried rhizomes of *A. officinarum* were extracted by maceration in methanol and were subsequently screened for *in vivo* anti-inflammatory and *in vitro* antioxidant activity.[34] The extract showed inhibition of right hind paw edema on carrageenan-induced inflammation in rats and promising free radical scavenging effect of DPPH in a concentration-dependent manner up to a concentration of 100 μg/ml. Ghil reported the ability of *A. officinarum* rhizome methanolic extract to inhibit cell proliferation in a dose- and time-dependent manner against human breast cancer cell line MCF-7, by promoting cell cycle arrest, hence triggering cell apoptosis.[35] In another study on the antiproliferative activity on *A. officinarum* leaf and rhizome, the 100% methanol extracts at a concentration of 2 mg/ml were tested against the AMoL cell line THP-1 and were reported to have significantly higher anti-proliferative activity for the leaf extract compared to the rhizome extract, with the solvent 100% methanol considered to be the least toxic extraction solvent on the cell culture, among other extraction solvents (hexane, chloroform, dichloromethane, acetone and aqueous), when tested *in vitro* against the cell culture.[36] Chang *et al.* prepared the methanol extract of *A. officinarum* dried rhizomes by ultrasonic extraction, which has demonstrated good antioxidant activity based on the scavenging effect on DPPH assay.[37] The roots of *A. officinarum* were extracted at 80°C in 70% methanol for 3 h, also displayed high DPPH radical scavenging activity in a dose-dependent manner, and effectively inhibited the lipid peroxidation in H_2O_2-treated V79-4 cells.[38] The summary of activities from methanol extracts/fractions and the isolated compounds, as well as their chemical structures, is shown in Table 1 and Figure 1, respectively.

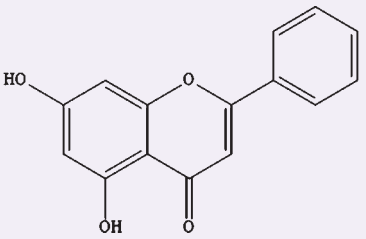
Ethyl acetate

The screening of crude methanol extract of *A. officinarum* rhizomes for *in vivo* anti-inflammatory and *in vitro* antioxidant activity showed promising results, and the extract was further fractionated to isolate its marker compounds.[34] The ethyl acetate fraction from this extract had isolated compound 5 and 5-hydroxy-7-(4'-hydroxy-3'-methoxyphenyl)-1-phenylheptan-3-one (21) that has shown its effectiveness in acute inflammatory animal model, comparable to a clinical non-steroidal anti-inflammatory drug, diclofenac, that acts as the positive control. The compounds have displayed a significant inhibition of the increase in the carrageenan-induced paw edema in a time-dependent manner, as well as *in vitro* scavenging activity in a concentration-dependent manner.[34] In another study, the ethyl acetate fraction of acetone crude extract showed a more potent activity compared to other solvent extracts (acetone and aqueous) and was responsible for the isolation of compounds 5, 9, 21, pinobaksin (22), 5-hydroxy-1,7-diphenyl-3-heptanone (23), 7-(4"-hydroxy-3"-methoxyphenyl)-1-phenylhept-4-en-3-one (24), 3,5-dihydroxy-1,7-diphenylheptane (25), 3-phenylpropanoic acid (26), and zingerone (27). Only compounds 5, 24, and 25 showed the inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-induced murine RAW 264.7 macrophage cell line while, as melanogenesis inhibitors, compounds 5, 9, 21, 23, 24, and 25 substantially inhibited melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells, and in addition, compounds 5, 9, and 24 inhibited the enzyme activity of mushroom tyrosinase.[39]

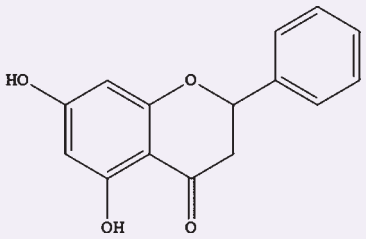
Zhao and Liu *et al.* reported several new DAH compounds isolated from the ethyl acetate fraction of *A. officinarum* dried and powdered rhizomes ethanolic extract.[40-46] All of the compounds were evaluated for their *in vitro* cytotoxic activity against several cancer cell lines. Compound 1,7-diphenylhept-4-en-3-one (28) showed cytotoxicity against the human glioblastoma T98G cell line with IC_{50} of 27 μmol/L, while compound alpinin B (29) was inactive against the cell lines tested (human glioblastoma T98G and B16-F10 murine melanoma cell lines).[44] Compound alpinin C (30) showed selective cytotoxicity against human breast cancer MCF-7 and human glioblastoma T98G cell lines, and compound 5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-phenylhepta-4,6-dien-3-

Table 1: Summary of activities from methanol extracts/fractions of *Alpinia officinarum*

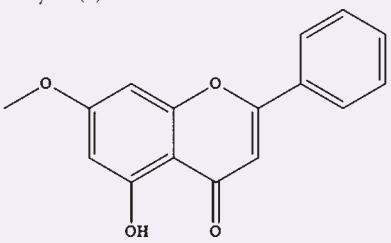
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Leaves	Maceration and ultrasonic extraction	1-16	-	[32]
Aerial parts and rhizome	Maceration and ultrasonic extraction	1-5, 7-13, 15, 17-20	High contents of compounds found in rhizomes compared to aerial parts	[33]
Rhizomes	Maceration	-	Anti-inflammatory and *in vitro* antioxidant activity	[34]
Rhizomes	Soaking in 99.8% methanol at r.t. for 72 h	-	Anticancer activity	[35]
Leaf and rhizomes	Sonication in 100% methanol for 2 h	-	High antiproliferative activity in leaf extract compared to rhizome extract	[36]
Rhizomes	Ultrasonic extraction	-	Antioxidant activity based on DPPH assay	[37]
Roots	Extracted in 70% methanol at 80°C for 3 h	-	Antioxidant activity	[38]



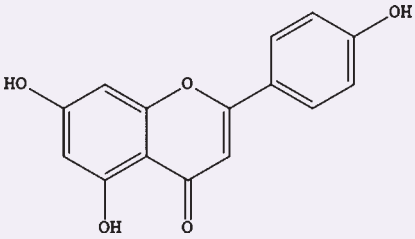
Chrysin (1)[32,33]



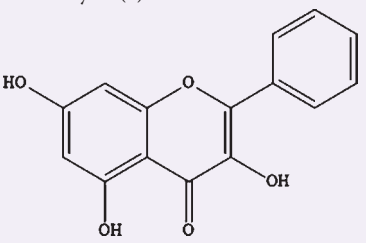
Pinocembrin (2)[32,33]



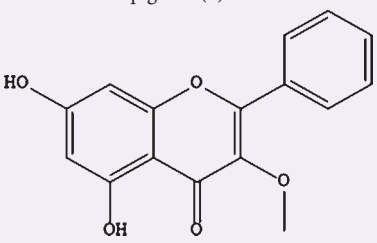
Tectochrysin (3)[32,33]



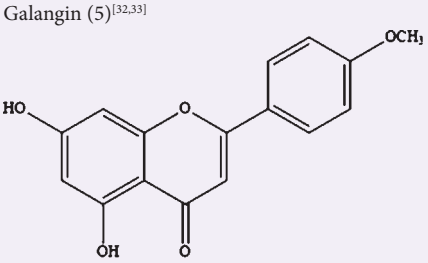
Apigenin (4)[32,33]



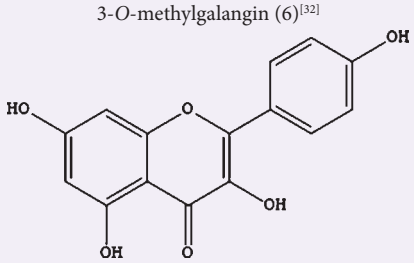
Galangin (5)[32,33]



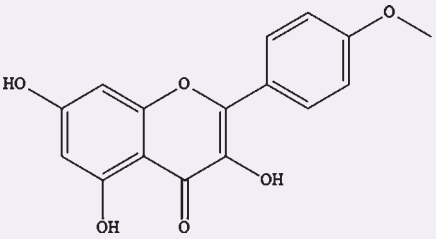
3-*O*-methylgalangin (6)[32]



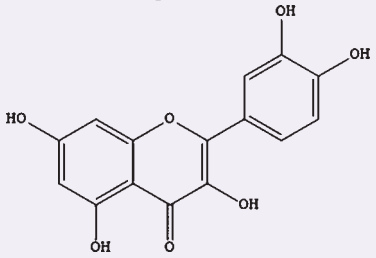
Acacetin (7)[32,33]



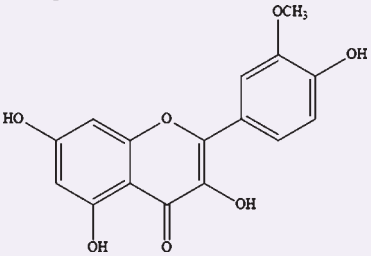
Kaempferol (8)[32,33]



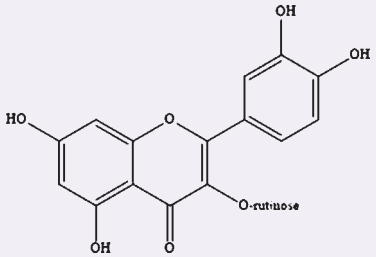
Kaempferide (9)[32,33]



Quercetin (10)[32,33]



Isorhamnetin (11)[32,33]



Rutin (12)[32,33]

Figure 1: Isolated compounds from methanol extracts/fractions of *Alpinia officinarum*

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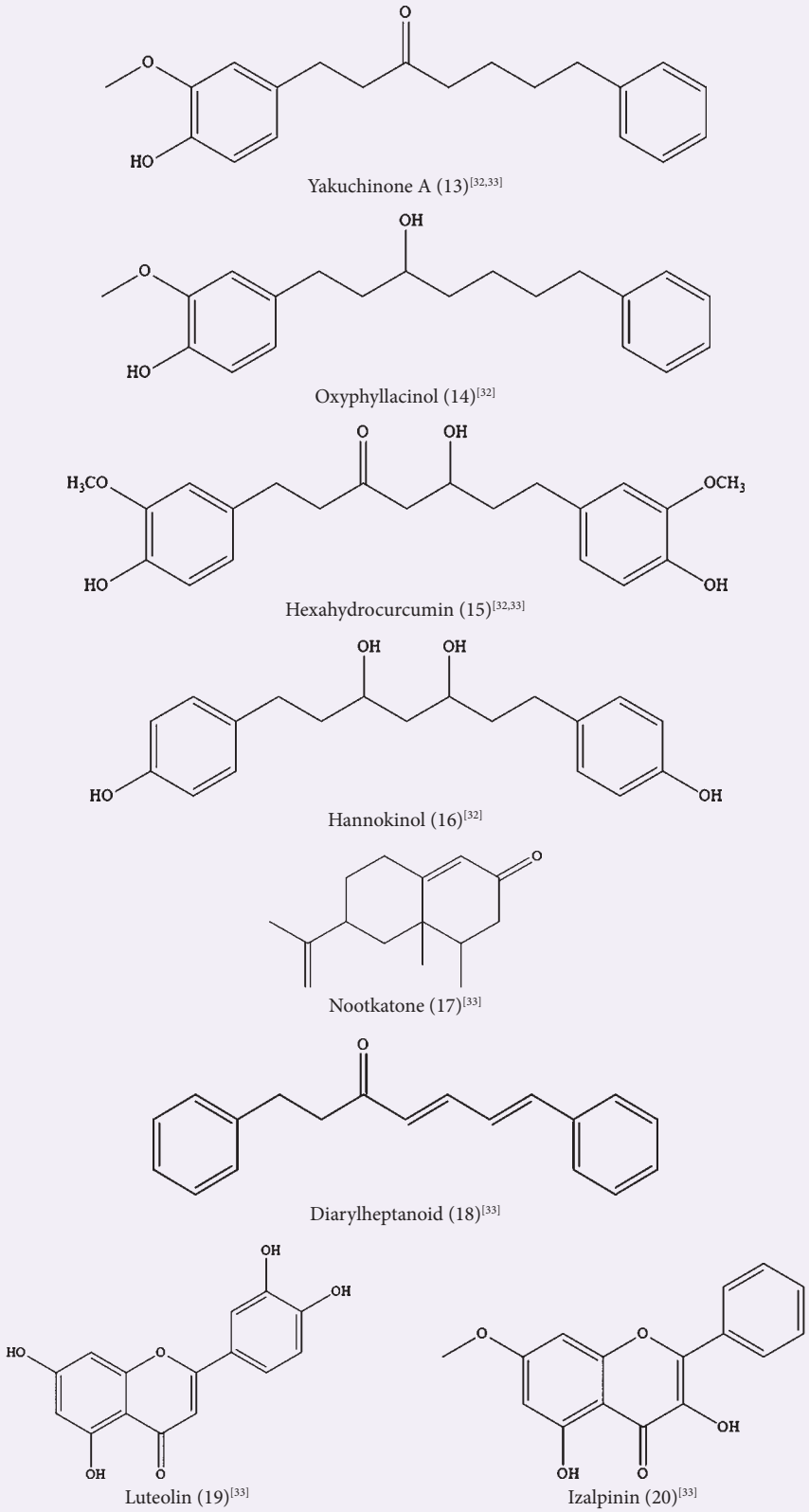


Figure 1: Contd...

one (31) showed significant cytotoxicity to human hepatoma HepG2, human breast cancer MCF-7, human glioblastoma T98G, and human murine melanoma B16-F10 cell lines with IC_{50} values of 8.46, 12.37, 22.68, and 4.44 μmol/L, respectively.[45] Table 2 and Figure 2 summarize the activities of ethyl acetate extracts/fractions and the isolated compounds and chemical structures.

Ethanol

Zhang *et al*. identified five flavonoids which were compounds 2, 5, 6, 9, and 22 from an ethanol extract of the aerial parts of *A. officinarum*.[47] A Chinese patent (CN104138368A) provided a protocol for producing a purified extract from the aerial parts by ethanol extraction and subsequent purification *via* macroporous adsorptive resins.[48] The extract displayed antiproliferative activity *via* a mitochondrial pathway-induced cell apoptosis. It has been reported that *A. officinarum* rhizome ethanolic extract possessed potent anti-inflammatory, anticarcinogenic, antinociceptive, and antipsychiatric activities in animal model of carrageenan-induced paw edema due to the presence of DAHs.[6,49,50] Dried rhizomes of *A. officinarum* were powdered and extracted with 50% ethanol by either hot or cold maceration, with the former found to contain more phenol and flavonol compared to the latter.[51] The hot macerated ethanolic extract showed better antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*, compared to the cold macerated ethanolic extract. The former also showed better antioxidant activity by inhibiting DPPH-free radical with moderate reducing power when compared to ascorbic acid, as the antioxidant reference standard. However, both extracts did not show any antifungal activity against *Aspergillus niger* and *Candida albicans*.[51]

An *A. officinarum* ethanol extract prepared in 95% ethanol at 70°C for 6 h was shown to have more total phenolic and flavonoid contents compared to the aqueous extract although with lower free radical scavenging activity when studied for antioxidant activity using the DPPH assay.[52] In another study, *A. officinarum* rhizomes were soaked in 40% ethanol for 2 h, and the extract was found to inhibit the reaction of bacterial fatty acid synthase, β-ketoacyl-ACP reductase enzyme (FabG). It also showed effective inhibition against the proliferation of Gram-positive bacterial strains – *S. aureus*, *α-Hemolytic streptococcus*, *β-Hemolytic streptococcus*, and *Streptococcus pneumoniae*.[53]

Suja and Chinnaswamy prepared ethanolic extracts of *A. officinarum* dried roots using a Soxhlet extractor.[54] In their study, the ethanolic extract revealed the highest activity on an MTT analysis against a prostate cancer cell line PC-3, compared to the other solvent extracts (petroleum ether, chloroform, and aqueous), with the ethanolic extract displaying an effective reduction in the growth of the cancer cells. The list of activities of ethanol extracts/fractions and the isolated compounds and chemical structures are summarized in Table 3 and Figure 3, respectively.

Hexane

An MTS-assay-based antiproliferative study on *A. officinarum* leaf and rhizome extracts, which were tested against the AMoL cell line THP-1, showed that the hexane leaf extract had distinctly higher antiproliferative activity at a concentration of 2 mg/ml compared to the hexane rhizome extract. However, when the *A. officinarum* leaf extract was diluted to a concentration of 0.1 mg/ml, its antiproliferative activity was reduced dramatically.[36]

A study on the anti-inflammatory activity of *A. officinarum* rhizome hexane extract and its isolated compound 18 revealed the inhibition of NO production in LPS-induced murine RAW 264.7 macrophage cell line, which was found to be mediated by the inhibition of the transcriptional activity of nuclear factor-κβ, a gene regulator involved in cell proliferation, cell adhesion, and inflammatory responses.[55] The activities of hexane extracts/fractions and the chemical structures of the isolated compounds are shown in Table 4 and Figure 4, respectively.

Dichloromethane

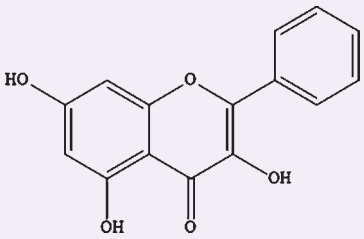
An *A. officinarum* dichloromethane extract showed a highly significant antiproliferative activity against AMoL THP-1 cell line using the MTS assay, with 100% cell death from both leaf and rhizome extracts, at both tested concentrations of 0.1 and 2 mg/mL and within 24 h.[36] Lee and Houghton studied the anticancer activity of *A. officinarum* dichloromethane rhizome extract using sulforhodamine

Table 2: Summary of activities from ethyl acetate extracts/fractions of *Alpinia officinarum*

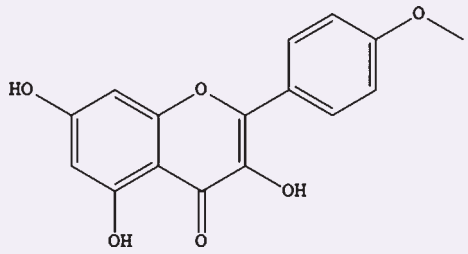
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Rhizomes	Solvent partition from methanol extract	5, 21	Anti-inflammatory activity and antioxidant activity	[34]
Rhizomes	Solvent partition from acetone extract	5, 9, 21-27	5, 24, 25: Inhibition of nitric oxide production 5, 9, 21, 23–25: Anticancer activity 5, 9, 24: Enzyme inhibition	[39]
Rhizomes	Solvent partition from ethanolic extract	28-31	28: Anticancer activity 29: Inactive anticancer activity 30: Selective anticancer activity 31: Significant anticancer activity	[44,45]

Table 3: Summary of activities from ethanol extracts/fractions of *Alpinia officinarum*

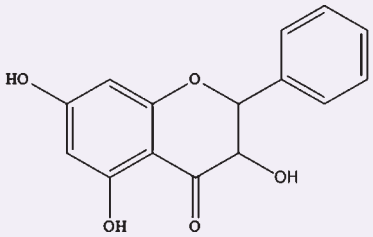
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Aerial parts	-	2, 5, 6, 9, 22	-	[47]
Rhizomes	Hot and cold maceration in 50% ethanol	-	Hot macerated ethanolic extract: High content of phenol and flavonol, better antibacterial and antioxidant activity. No antifungal activity	[51]
-	Extracted in 95% ethanol at 70°C for 6 hrs	-	High content of total phenolics and flavonoids Low antioxidant activity	[52]
Rhizomes	Soaking in 40% ethanol for 2 h	-	Enzyme inhibitory activity Antibacterial activity	[53]
Roots	Soxhlet extraction	-	Anticancer activity	[54]



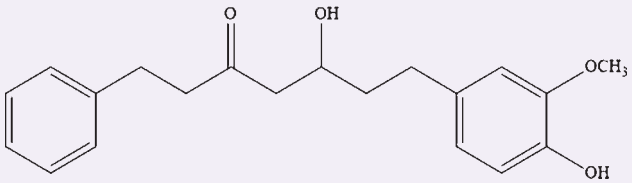
Galangin (5)[34,39,58]



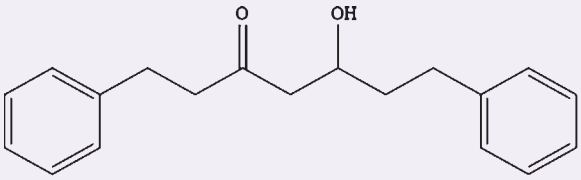
Kaempferide (9)[39,58]



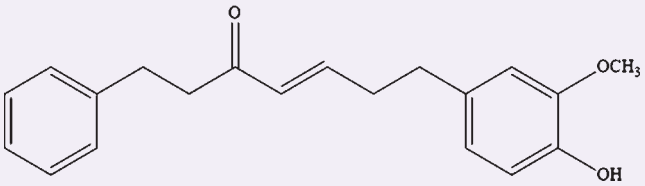
Pinobaksin (22)[39,58]



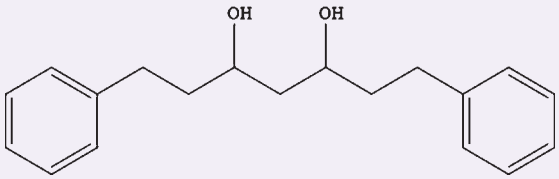
5-hydroxy-7-(4'-hydroxy-3'-methoxyphenyl)-1-phenylheptan-3-one (21)[34,39,58]



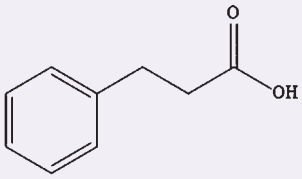
5-hydroxy-1,7-diphenyl-3-heptanone (23)[39,58]



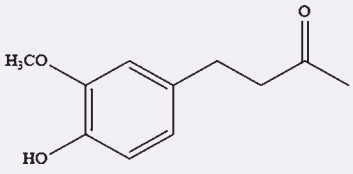
7-(4"-hydroxy-3"-methoxyphenyl)-1-phenylhept-4-en-3-one (24)[39,58]



3,5-dihydroxy-1,7-diphenylheptane (25)[39,58]



3-phenylpropanoic acid (26)[39,58]



Zingerone (27)[39,58]

Figure 2: Isolated compounds from ethyl acetate extracts/fractions of *Alpinia officinarum*

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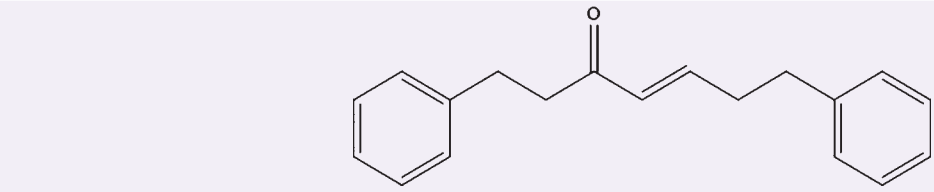
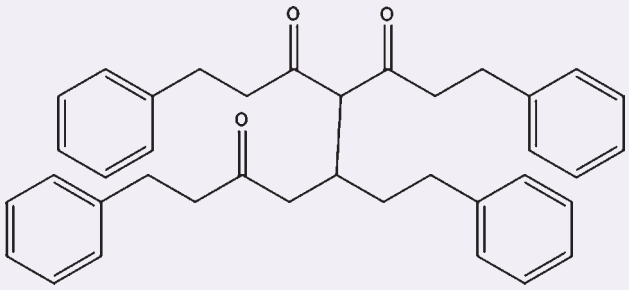
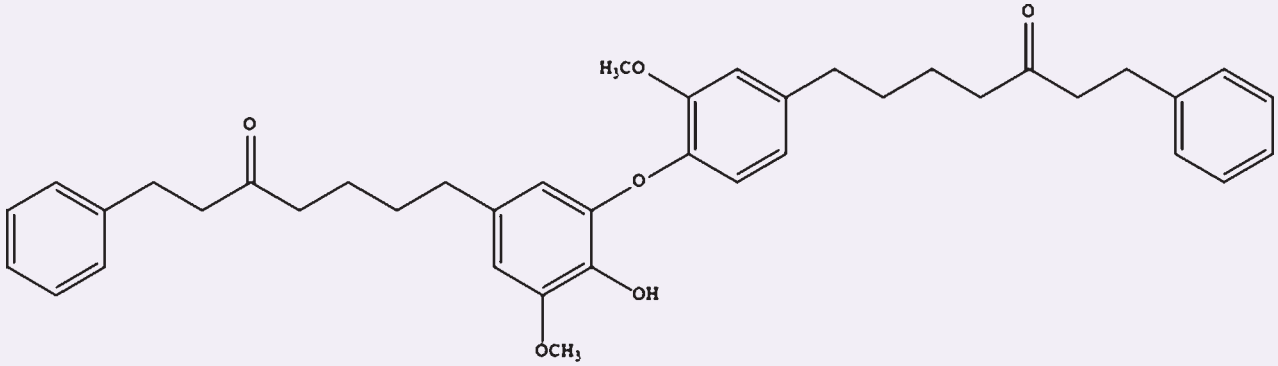


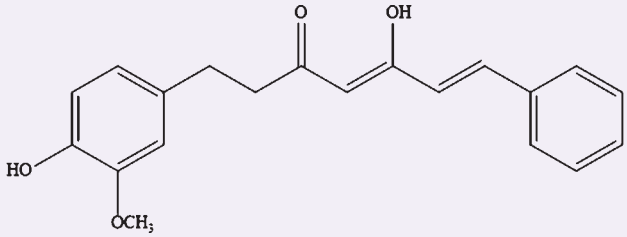
1,7-diphenylhept-4-en-3-one (28)[44,45]



Alpinin B (29)[44,45]



Alpinin C (30)[44,45]



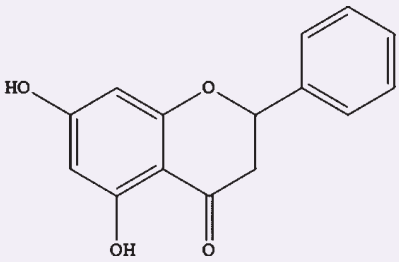
5-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-phenylhepta-4,6-dien-3-one (31)[44,45]

Figure 2: Contd...

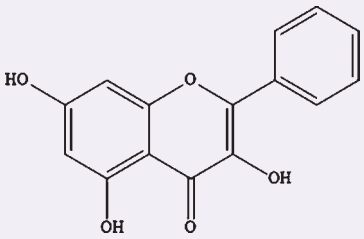
B assay, and the extract exhibited the highest cytotoxicity against human nonsmall lung cancer COR L23 cell line following 48 h treatment, with an IC_{50} value of 5.4 ± 0.51 μM.[27] A number of pure compounds were isolated from the extract, which were 1'-acetoxychavicol acetate (32), trans-*p*-coumaroyl diacetate (33), 4-hydroxycinnamaldehyde (34), and β-sitosterol (35), and were also tested for their cytotoxic activities. Compound 32 demonstrated the highest activities, with IC_{50} values of 5.8 ± 0.2 μM and 8.6 ± 0.0 μM, against the COR L23 and human breast adenocarcinoma MCF7 cancer cell lines, respectively. When tested against a noncancer MCF5 cell line, compound 32 showed higher cancer cell selectivity toward the COR L23 cell line compared to the MCF7 cell line, with the selectivity factor of 2.83 and 1.91, respectively, whereas compound 35 displayed no cytotoxic activity toward all the cell lines tested.[27,56] The summary of activities displayed by dichloromethane extracts/fractions are shown in Table 5, and the chemical structures of the isolated compounds are shown in Figure 5.

Aqueous

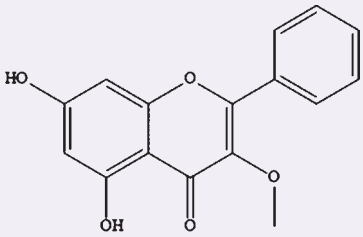
An aqueous fraction of methanolic *A. officinarum* rhizome extract prepared by Ly *et al.* had isolated *p*-coumaryl alcohol (36) and 1,5-*bis* -(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol (37), with the structures of the compounds as shown in Figure 6.[57] The compounds were studied to determine the antioxidant activity by autoxidation of methyl linoleate, and it was found that compound 36 has higher antioxidant activity than compound 37. Omoregie *et al.* reported that the ultrasonication-derived aqueous extracts of *A. officinarum* leaves and rhizomes, which was administered at both concentrations of 0.1 and 2 mg/mL, had no distinct antiproliferative activity against the AMoL THP-1 cell line within 24 and 48 h, in comparison with the other solvent extracts – methanol, hexane, chloroform, dichloromethane, and acetone.[36] In addition, the boiled aqueous extract of *A. officinarum* rhizomes was also prepared considering the traditional practice of brewed *A. officinarum* rhizome tea. However, the extract did not show



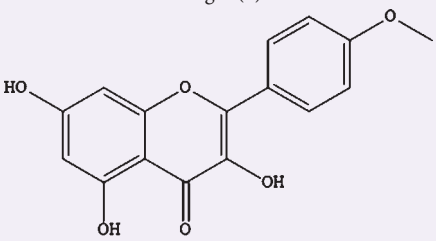
Pinocembrin (2)[47]



Galangin (5)[47]



3-*O*-methylgalangin (6)[47]



Kaempferide (9)[47]

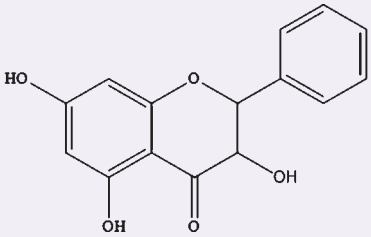
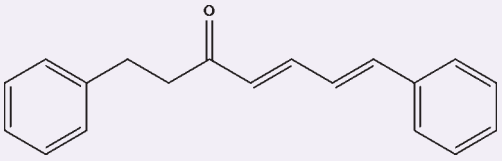


Figure 3: Isolated compounds from ethanol extract/fraction of *Alpinia officinarum*



Diarylheptanoid (18)[55]

Figure 4: Isolated compounds from hexane extract/fraction of *Alpinia officinarum*

any significant antiproliferative activity against the AMoL THP-1 cell line within 24 h. Similarly, a refluxed aqueous extract of *A. officinarum* rhizomes collected in Songkla, Thailand, only displayed <50% inhibition of growth against human nonsmall cell lung cancer COR L23 cell line and human breast adenocarcinoma MCF7 cell line, when tested at the highest concentration of 25 μg/mL and at 48 h exposure.[27]

Matsuda *et al.* studied an aqueous fraction of *A. officinarum* acetone extract and reported to have no anti-inflammatory activity as there was no inhibition of NO production on LPS-induced murine RAW 264.7 macrophage cell line,[58] as well as no inhibition of melanogenesis and proliferation in B16 melanoma 4A5 cell line.[39] However, in another antioxidant and anti-inflammatory study of *A. officinarum* aqueous extract prepared by autoclaving with deionized water, the aqueous extract was found to have significant activities compared to the *A. officinarum* ethanol extract.[52] Table 6 shows the summary of activities by aqueous extracts/fractions and the isolated compounds.

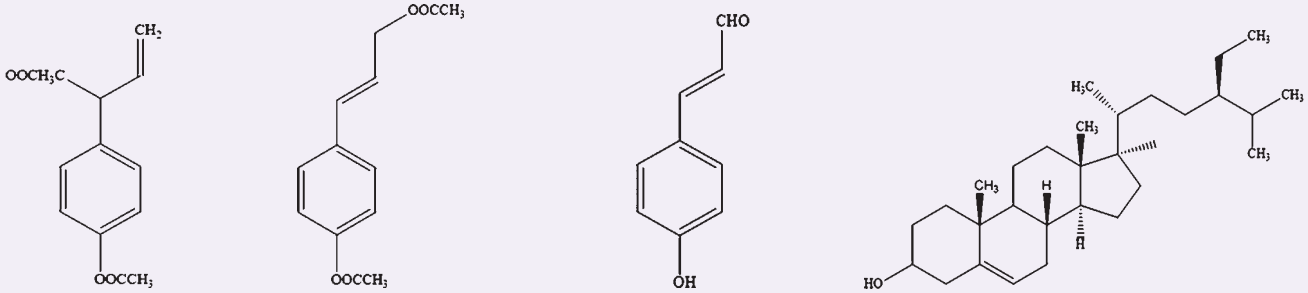
Chloroform

Ly *et al.* reported the isolation of compounds from the chloroform fraction of methanolic *A. officinarum* rhizome extract, which were used for antioxidant studies by autoxidation of methyl linoleate.[57] These compounds were *p*-coumaryl alcohol γ-*O*-methyl ether (38), 1,5-*bis*-(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl)-4-pentene (39),1,5-*bis*-(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene(40),1,5-*bis*-(4-hydroxyphenyl)-1-[3-(4-acetoxyphenyl)-2-propenoxy]- 2-(methoxymethyl)-4-pentene (41), and 1,5-*bis*- (4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol (42). Compound 38 was shown to have the highest antioxidant, whereas compounds 39–42 exhibited lower antioxidant activities than that of α-tocopherol, an antioxidant reference standard. Figure 7 shows the structure of the compounds isolated from the chloroform fraction.

Wei *et al.* isolated a new DAH compound, alpinisin A (43), from the chloroform fraction of *A. officinarum* rhizome ethyl acetate extract.[59] The cytotoxicity of the compound was tested by MTT assay against human gastric cancer SGC-7901, human breast cancer MCF-7, and cervical carcinoma Caski cell lines, and it was shown that the compound possessed anticancer activities and had a significant inhibitory effect with IC_{50} values of 11.42, 15.14, and 14.78 μM, respectively. In a separate study, chloroform extracts of *A. officinarum* leaves and rhizomes showed a very high antiproliferative activity against AMoL THP-1 cell line with 100% cell death, at both concentrations of 0.1 and 2 mg/mL within 24 h.[36] Table 7 summarizes the activities observed from chloroform extracts/fractions and the isolated compounds.

Petroleum ether

Wen *et al.* isolated two novel diterpene compounds, as shown in Figure 8, from the rhizomes of *A. officinarum* through a petroleum ether fraction of 95% ethanol rhizome extract.[60] Compounds (12*S*)-15–16-epoxy-8 (17),13 (16),14-labdatrien-12-ol (44), and (12*E*)-labda-8 (17),12 (13)-dien-15,16-olide (45) were shown to exhibit strong anti-inflammatory effect and antioxidant activity *in vitro*.



1'-acetoxychavicol acetate (32)[27] *trans-p*-coumaroyl diacetate (33)[27] 4-hydroxycinnamaldehyde (34)[27] β-sitosterol (35)[27]

Figure 5: Isolated compounds from dichloromethane extracts/fractions of *Alpinia officinarum*

p-Coumaryl alcohol (36)[57] 1,5-*bis*-(4-hydroxyphenyl)-2-(hydroxymethyl)-4-penten-1-ol (37)[57]

Figure 6: Isolated compounds from aqueous extracts/fractions of *Alpinia officinarum*

Table 4: Summary of activities from hexane extracts/fractions of *Alpinia officinarum*

Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Leaf and rhizome	Sonication for 2 h	-	High antiproliferative activity in rhizome	[36]
Rhizomes	Soxhlet extraction	18	Inhibition of nitric oxide production	[55]

Table 5: Summary of activities from dichloromethane extracts/fractions of *Alpinia officinarum*

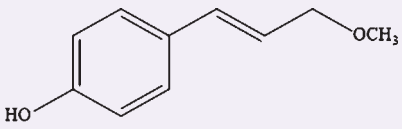
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Rhizomes	Soxhlet extraction	32-35	32: Highest anticancer activity 35: No anticancer activity	[27,56]
Leaf and rhizome	Sonication for 2 h	-	High antiproliferative activity in both leaf and rhizome extracts	[36]

Table 6: Summary of activities from aqueous extracts/fractions of *Alpinia officinarum*

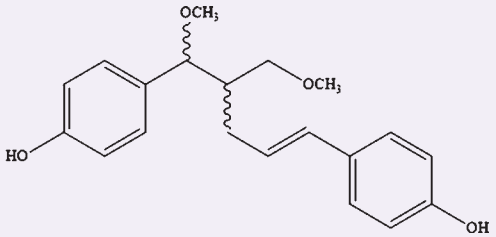
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Rhizome	Reflux at 100°C for 3 h	-	No anticancer activity	[27]
Leaf and rhizome	Sonication for 2 h	-	No antiproliferative activity	[36]
Rhizome	Solvent partition from 80% acetone extract	-	No anti-inflammatory activity No antiproliferative activity	[39,58]
-	Autoclave at 121°C for 1 h	-	Significant antioxidant and anti-inflammatory activities	[52]
Rhizome	Solvent partition from methanolic extract	36-37	36: High antioxidant activity	[57]

Table 7: Summary of activities from chloroform extracts/fractions of *Alpinia officinarum*

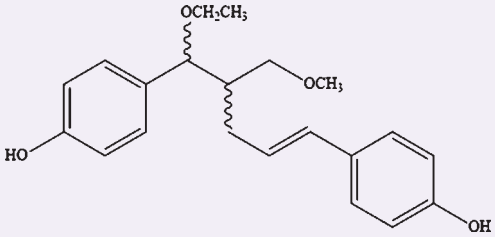
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Leaf and rhizome	Sonication for 2 h	-	High antiproliferative activity	[36]
Rhizome	Solvent partition from methanolic extract	38-42	38: High antioxidant activity 39-42: Low antioxidant activity	[57]
Rhizome	Solvent partition from ethyl acetate extract	43	Anticancer activity	[59]



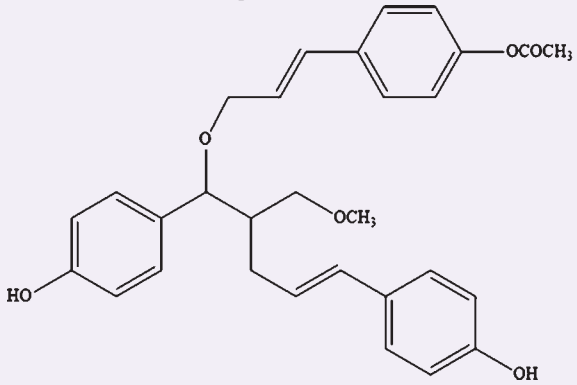
p-Coumaryl alcohol γ-*O*-methyl ether (38)[57]



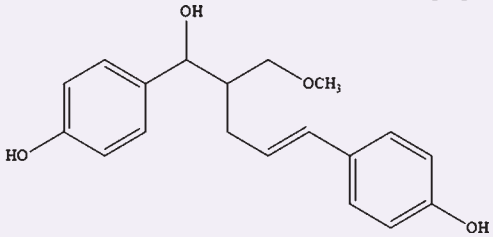
1,5-*bis*-(4-hydroxyphenyl)-1-methoxy-2-(methoxymethyl) -4-pentene (39)[57]



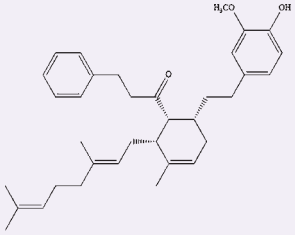
1,5-*bis*-(4-hydroxyphenyl)-1-ethoxy-2-(methoxymethyl)-4-pentene (40)[57]



1,5-*bis*-(4-hydroxyphenyl)-1-[3-(4-acetoxyphenyl)-2-propenoxy]-2-(methoxymethyl)-4-pentene (41)[57]



1,5-*bis*-(4-hydroxyphenyl)-2-(methoxymethyl)-4-penten-1-ol (42)[57]



Alpinisin A (43)[59]

Figure 7: Isolated compounds from chloroform extracts/fractions of *Alpinia officinarum*

Table 8: Summary of activities from petroleum ether extracts/fractions of *Alpinia officinarum*

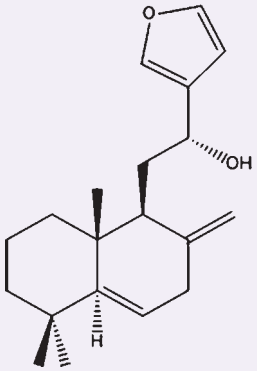
Parts of *Alpinia officinarum*	Extraction method	Identified compounds	Observation	References
Rhizome	Solvent partition from ethanolic extract	44-45	Strong anti-inflammatory and antioxidant activity	[60]

The activities and chemical structures are summarized in Table 8 and Figure 8, respectively.

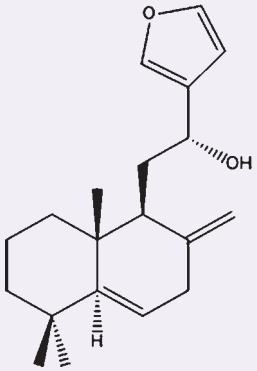
DISCUSSION AND FUTURE DIRECTIONS

A. officinarum has been traditionally used for the treatment of various ailments. This review has presented a wide range of supporting scientific results to validate the traditional usage of *A. officinarum* as herbal medicine. The screening of *A. officinarum* solvent extracts revealed a high proportion of various biological activities that include antioxidant, antibacterial, anti-inflammatory, anti-cancer, anti-proliferative, enzyme inhibition, as well as, the inhibition of NO production. This proves that *A. officinarum* is enriched with bioactive chemical constituents, laying a solid foundation for its pharmacological research.

Many studies have focused on the rhizomes of *A. officinarum*, due to its known traditional uses for medicinal purposes such as to relieve fever and stomach ache. Consequently, other parts of the plant, which



(12*S*)-15-16-epoxy-8 (17),13 (16),14-labdatrien-12-ol (44)[60]



(12*E*)-labda-8 (17),12 (13)-dien-15,16-olide (45)[60]

Figure 8: Isolated compounds from petroleum ether extracts/fractions of *Alpinia officinarum*

may have valuable potential, are being disposed of as waste while only its rhizomes are being collected. Therefore, to maximize the use of this medicinal plant species, it would be useful to carry out studies on the other parts of the plant such as the aerial parts, roots, and leaves, which could also contain potential bioactive metabolites. Several previous studies have successfully identified the bioactive compounds in *A. officinarum* as were summarized in this review. Higher percentage of compounds were found in the rhizomes than in the aerial parts of *A. officinarum*.[33] The identified compounds in this review are mainly found to be DAH, flavonoids, phytosterols, and terpenes. A few recent studies on novel dimeric DAH compounds 29 and 30 were reported showing only selective or inactive cytotoxicity that could be due to the lack of its α, β-unsaturation in its molecular structure.[45] An α, β-unsaturated unit is defined as the pi bond between the α and β carbons adjacent to the carbonyl (=CO) group[61] and is often employed as an active moiety in the design of enzyme inhibitors.[62] Further biological studies on the novel compounds are essential to reveal other potential bioactivities of the new dimeric DAH compounds.

Molecules that consist of phenolic hydroxyl groups are believed to act as antioxidants due to their hydrogen donating ability[23,63-68] and as prooxidants that contributes towards their anticancer activities.[69,70] A study in this review revealed the differences in the antioxidant behavior of phenylpropanoid compounds 36–42, and the results suggested that their activities might be influenced by the number of hydroxyl groups that were present in the molecule.[57] Galangin, compound 5, a flavonol of flavonoids, appears to be the predominant constituent in all parts of *A. officinarum* – leaves, aerial parts, and rhizomes, showing anti-inflammatory, antioxidant, and anticancer properties, as well as the inhibition of NO production and enzymes.[32-34,39,47,58,71-73] There have been much studies on the compound galangin;[74-81] however, its molecular mechanism is still unknown. It has been reported that flavonoids and terpenes act by inhibiting the cytoplasmic membrane functions, such as altering the influx of calcium, hence promoting the disruption of the cellular membrane.[21,82-85] It has also been reported that phenolics and flavonoids are able to enter the hydrophobic layer of the cell membrane, causing the disruption of the membrane's lipid packing.[85-87] It would be useful to carry out further studies to investigate the efficacy of galangin and elucidate its mechanism (s) of action that underlie the observed pharmacological effects, as well as to reflect the traditional uses of *A. officinarum*.

Some of the *A. officinarum* isolated compounds even showed discriminatory tolerance against normal cells, especially compound 32, isolated from the rhizome dichloromethane extract of *A. officinarum*,[27] in which the 1'-acetoxyl group in the chavicol analog was found to majorly contribute toward its cytotoxic activity.[88,89] The ideal drug candidate would be those that are found to be selective and only target a specific region within the human body, as well as to not cause genetic and chromosomal aberrations that could lead to toxicity and unwanted side effects. This finding is, therefore, a promising step in the search for a safe treatment and management of patients in cancer therapy. By understanding the mechanisms of the biologically active compounds toward their respective therapeutic potentials, it will be able to support in preventing the possible adverse effects of the compounds, hence maximizing their medicinal benefit.

Bioassay-guided fractionation and isolation have been the most widely used approach for evidence-based pharmacological *in vitro* and *in vivo* studies, where each solvent extract/fraction is investigated for their potential biological activities. The studies on the different solvent extracts/fractions may lead to the identification of novel compounds in the field of pharmaceutical medicine. Various solvents were used for the extraction of *A. officinarum* as reported in this review. It can be seen that methanol is found to be the most preferred solvent used as the initial crude solvent extraction before they are further fractionated using other solvents. Methanol is also considered to be the least toxic extraction solvent toward an *in vitro* cell culture line,[36] indicating that the observed anticancer activities were not due to the interference from the methanol solvent itself.

Many studies have also been done on the aqueous *A. officinarum* extracts as to mimic the traditional practice of brewing the rhizomes of the plant for tea consumption; however, in contrast to the methanol extracts, the aqueous extracts were shown to exert the least biological activity, showing no presence of anticancer, antiproliferative, and anti-inflammatory activities. Previous studies have shown that some plant species extracted using organic solvents were found to give more consistent scientific results when compared to their aqueous extracts.[90] Furthermore, some water-soluble compounds, such as flavonoids and phenolics, only showed either selective or no significant biological activities at all.[91,92] However, depending on the extraction method,[93] significant antioxidant and anti-inflammatory activity could also be observed.[52] It was revealed that the plant materials extracted by either shaking or refluxing in a hydroalcoholic solvent system gave higher yields, with higher phenolic contents and better antioxidant activities compared to when using a 100% aqueous or 100% alcoholic solvent.[6,38,51,53,94,95] The results could also suggest that the aqueous extracts might contain different components of bioactive compounds compared to the contents of the other solvent extracts, or the various extracts may contain similar compounds, however, with

different concentrations, hence leading to different values of their biological activities.

There has not been much work specifically on *A. officinarum* petroleum ether extract, and also the studies on the isolation of diterpene compounds are very rare. Further studies could be done on the solvent extract to further clarify the chemical compositions of *A. officinarum*, especially diterpenes, as well as to discover new biologically active compounds. Two novel labdane diterpenes have been shown to have strong anti-inflammatory activities that could be linked with the inhibition of ROS.[60] The production of ROS can cause damage to cells and tissues, activating oxidative stress, and triggering inflammation.[96-98] This leads to several disorders that include inflammatory, cardiovascular, and neurodegenerative diseases that have shown a significant increase in their occurrence worldwide.[99-101] This finding warrants further study on the two labdane diterpenes, as well as other bioactive plant constituents in *A. officinarum* especially in relation to both antioxidant and anti-inflammatory activities due to its potential application in disease treatment. Moreover, toxicity and immunological studies of *A. officinarum* are also beneficial to further authenticate the traditional claims of the uses of the plant species, as well as for the potential of clinical drug development.

CONCLUSION

We have reviewed the phytochemical and biological activities exerted by the different solvent extracts and fractions, as well as the isolated and identified compounds of *A. officinarum*. Most solvent extracts had shown significant biological activities, and a few novel compounds had been successfully isolated. There have been much studies on the methanol extract of this plant species, which were used for crude extraction before further fractionation using other solvents. The scientific results have provided evidence to support the traditional uses of *A. officinarum* in the treatments of various diseases, as well as to offer new therapeutic possibilities, such as antioxidant, anti-inflammatory, anticancer, and antimicrobial activities. These pharmacological activities are mainly exerted by the bioactive metabolites of the plant species. Flavonoids, DAHs, and terpenes were among the compounds isolated, and some were found to have significant biological activities, as well as being selective that shows good potential as natural drug candidates.

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Conflicts of interest

There are no conflicts of interest.

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