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Antioxidant and Anti Inflammation Activity of Methanolic Extracts of Alpinia Rhizomes

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Abstract

Cancer, RA, asthma, and diabetes are largely caused by oxidative stress-induced inflammation. Natural products, notably phytochemicals such diarylheptanoids and flavonoids, have garnered interest for their antioxidant and anti-inflammatory activities. This study employed methanolic Alpinia officinarum rhizome extracts to measure total phenolic content and antioxidant and anti-inflammatory activities. We also used molecular docking to analyze bioactive compounds' COX-2 active site binding affinity. Maceration with methanol extracted A. officinarum rhizomes. Using ^1H NMR, ^13C NMR, and MS, five compounds were recovered from the pure methanolic extract for structural analysis. The carrageenan-induced paw edema model in rats studied anti-inflammatory effects, whereas in vitro tests examined antioxidant activity. The Glide module of the Schrödinger program was used to conduct molecular docking in order to find COX-2 binding relationships. The methanolic extract was used to isolate the following compounds: galangin, kaempferide, 1,7-diphenylhept-4-en-3-one, 5-hydroxy-1,7-diphenyl-3-heptanone, and 5-hydroxy-7-(4′-hydroxy-3″-methoxyphenyl)-1-phenyl-3-heptanone. When administered at a dosage of 10 mg/kg, Galangin and the fifth chemical showed the most antioxidant and anti-inflammatory effects, with p-values less than 0.001. Phenolic compounds were abundant (72.96 mg gallic acid equivalent and 51.18 mg total). Docking scores averaged -9.03, showing strong binding affinity to the COX-2 active site. Alpinia officinarum rhizome methanolic extracts, rich in phenolics including galangin, are antioxidant and anti-inflammatory. These findings show that A. officinarum may produce selective COX-2 inhibitors and support its use in treating inflammatory disorders and oxidative stress.

Keywords: Alpinia officinarum; Alpinia galangal; Methanolic extract; Antioxidant activity; Anti-inflammatory activity; Phenolic compounds; Flavonoids (Galangin, Kaempferide)

Abbreviations: ROS: Reactive oxygen species, MEAO: methanolic extract of A. officinarum, ANOVA: analysis of variance, SEM: standard error of the mean, RMSD: root-mean-square deviation

Introduction

Reactive oxygen species (ROS) and endogenous antioxidant defenses are often out of whack, which may lead to serious health problems including diabetes, cancer, RA, asthma, and other chronic disorders. An important factor in the development and advancement of many illnesses is this imbalance [1]. The activation of inflammatory pathways by chronic oxidative stress has the potential to harm tissues and lead to various medical disorders.

Plant-Derived Phytochemicals: Natural Protectants

A considerable amount of study is being conducted on natural phytochemicals to investigate their anti-inflammatory and antioxidant capabilities in an attempt to reduce the negative impacts of these substances. Flavonoids and diarylheptanoids stand out among these substances because of their ability to regulate oxidative stress and reduce the amount of pro-inflammatory mediators it produces [2,3].

Alpinia Species: A Source of Bioactivity

The species of the genus Alpinia, such as Alpinia galanga (greater galangal) and Alpinia officinarum (lesser galangal), have been used in traditional Asian medicine for the purpose of treating inflammatory conditions, digestive disorders, and respiratory conditions [4]. It has been shown that compounds such as galangin and kaempferide, which were extracted from methanolic extracts of A. officinarum rhizomes, has characteristics that are both anti-inflammatory and antioxidant [5]. It has been shown that extracts of A. galanga rhizome possess an antioxidant capacity, as evidenced by their high phenolic content and their ability to scavenge free radicals [6,7]. Further evidence of the anti-

inflammatory properties of methanolic extracts of A. galanga has been shown by in vivo studies that used carrageenan-induced rat paw edema models [8].

Phytochemical Profiles and Mechanisms

Research into the phytochemistry of A. officinarum rhizomes has uncovered a wealth of flavonoids (galangin, kaempferide, and quercetin) and diarylheptanoids (yakuchinone A, 1,7-diphenylheptane derivatives), which have been shown to have strong antioxidant and anti-inflammatory effects [9,10]. Extraction of bioactive substances from A. officinarum's methanolic extract and subsequent testing for dual action was the subject of much research. Rats' paw edema caused by carrageenan was prevented by compounds like galangin and diarylheptanoid derivatives. Not only that, but these chemicals also successfully decreased oxidative stress. A second mechanism for their anti-inflammatory activity was revealed by molecular docking, which further demonstrated their significant binding affinity to the COX-2 enzyme. They were able to decrease inflammation by this technique.

Objectives

a. To evaluate the anti-inflammatory and antioxidant qualities of methanolic extracts of Alpinia rhizomes utilizing both in vitro and in vivo methods.

b. To discover and investigate the bioactive phytochemicals responsible for these processes, with a focus on their phenolic content and potential COX-2 inhibitory action.

Research Methodology

Chemicals

We only used solvents of the AR grade. This chromatographic analysis made use of pre-coated RP-18 TLC plates and silica gel GF254 gel. All column chromatography procedures made use of Silica gel 100-200 mesh (Merck). Lab-Grade Diclofenac was given out free of charge by Indian Symed Pharmaceutical Pvt. Ltd. The gallic acid and DPPH were supplied by Sigma-Aldrich of St. Louis, MO, USA, and are both 97% pure.

Test animals

The National Institute of Biosciences in Pune provided female Swiss albino mice (20-25 g) and Wistar rats (150-200 g). These rats were 10-12 weeks old. The animal habitat was kept at 24 ± 1 °C with 65 ± 10% relative humidity, and the animals were exposed to a 12-hour light and 12-hour dark cycle. Water and pellet rodent food were freely available to the rats. The Institutional Animal Ethical Committee (CPCSEA/43/2014) approved all research experiments.

Isolation and extraction

Dry A. officinarum rhizomes from Kerala, India, were imported. The laboratory has a voucher specimen (R-138) of plant material verified by the Department of Botany, Agharkar Research Institute, Pune. One kilogram of ground rhizomes was macerated in methanol at room temperature for 48 hours to extract their important components. The viscous extract was filtered and concentrated in a rotary evaporator under reduced pressure at 40 °C to give a crude methanol extract (71.42 g). Its anti-inflammatory properties were tested, and it worked. So, it was purified and fractionated using numerous solvents. The 65 g methanol extract was redissolved in 80:20 methanol: water (1.0 lit) and partitioned with n-hexane and ethyl acetate. These components and the aqueous methanol layer were vacuum-concentrated. Column chromatography on silica gel was used to separate the 25.42 g ethyl acetate fraction using a hexane-ethyl acetate gradient (0-100%) with increasing polarity [11]. Four fractions were obtained: AO-1 (7.62 g), AO-2 (0.54 g), AO-3 (5.21 g), and AO-4 (8.45 g). Chromatographic purification yielded compounds (1, 21 mg) and (2, 32 mg) from fraction AO-1, while preparative TLC with 25% ethyl acetate in hexane yielded compound (3144 mg) from fraction AO-2. From portions AO-3 and AO-4, compounds (4, 43 mg) and (5, 86 mg) were purified by column chromatography on silica gel with 10% ethyl acetate and hexane. Also utilized was preparative TLC.

Study of acute oral toxicity

According to OECD guidelines-425, healthy male and female Swiss albino mice were tested for acute oral toxicity [12,13]. After fasting the night before, the animals were divided into five groups. The oral methanolic extract of A. officinarum (MEAO) was dosed at 55, 175, 550, 1750, and 2000 mg/kg of body weight. For 2 hours, mice's behavioral and autonomic features were observed. Toxicology and death were reported up to 48 hours later. The vehicle control group administered 10 mg/kg oral 0.1% CMC for maintenance. The animals were monitored for 48 hours for behavioral abnormalities, autonomic or respiratory responses, agitation, seizures, tremors, salivation, diarrhea, or death.

Rats with carrageenan-induced paw edema

Ten groups of female Wistar rats were created, with six rats in each:

- **Group I:** Vehicle control (0.1% CMC, 10 mg/kg, p.o.)
- **Group II:** Diclofenac (10 mg/kg, p.o.)
- **Group III-V:** MEAO (methanolic extract of Alpinia officinarum) at 100, 200, and 400 mg/kg, p.o.
- **Group VI-X:** Isolated compounds AO-1 to AO-5 (10 mg/kg, p.o.).

One hour before injecting subplantarly with 0.1 ml of 1% carrageenan to cause acute paw edema, all medicines were administered orally. Using a plethysmometer, paw volume was measured at 0, 1, 2, 3, 4, 5, and 6 hours to calculate edema percentage [14].

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In vitro antioxidant activity

DPPH, hydrogen peroxide (H2O2), and hydroxyl (•OH) radical scavenging assays were used in order to assess the antioxidant capacity of MEAO, compound-3, compound-5, and ascorbic acid [15-17]. Standard protocols were performed in order to accomplish this evaluation.

Phenolic content

The Folin-Ciocalteu reagent was used in order to determine the total phenolic content of MEAO, as well as those of gallic acid, compound-3, and compound-5 [18]. (GAE) stands for milligrams of gallic acid equivalent, which was used to show the data.

Statistical analysis

The data is shown using the mean plus or minus the standard error of the mean (SEM). For the purpose of statistical comparisons, a two-way analysis of variance (ANOVA) was used, along with Bonferroni's post hoc test (GraphPad Prism, San Diego, California, United States of America). In order for the p-value to be considered significant, it had to be lower than 0.05.

Computational studies

Schrödinger's Glide module was utilized for molecular docking [19,20]. The Protein Preparation platform was used to create the COX-2 crystal structure complexed with diclofenac (PDB ID: 1PXX) from Protein Data Bank [21]. The ligands were created in Maestro, refined using LigPrep, and optimized and minimized with OPLS2005 to achieve an RMSD of 0.001 Å and 0.30 Å, respectively. Glide was used to build receptor grids with a 10 $Å^3$ binding box, centered on the co-crystallized ligand diclofenac. Docking exploited input partial charges and van der Waals.

Result and Discussion

Characterization and isolation

Compounds: 1,7-diphenylhept-4-en-3-one (compound-1), 5-hydroxy-1,7-diphenyl-3-heptanone (compound-2), 3,5,7-trihydroxyflavone (Galangin), 3,5,7-trihydroxy4′-methoxyflavone (Kaempferide), and 5-hydroxy-7- (4″-hydroxy-3A-methoxyphenyl). Substance 5, also known as 1-phenyl-3-heptanone, was detected by FT-IR, 1 H NMR, 13C NMR, and MS. The details of spectroscopic compound identification: Compound-1 is a yellow liquid having FT-IR (CHCl3) cm−1 values of 3028 (aliphatic), 1669 (CO), and 1496 (aromatic). In CD3OD: δ (ppm), 1H NMR (400 MHz). The chemical shifts are: 2.54 (2H, q, J = 7.3 Hz, H-6), 2.78 (2H, t, H-7), 2.87 (4H, s, H1,2), 6.10 (1H, d, J = 16 Hz, H-4), 6.80 (1H, double t, J = 16.6 Hz, H5), 7.10-7.32 (10H, m, H2′-6′, 2) Chemical shifts recorded in the 13C NMR spectrum at 100 MHz in CD3OD mode: 30.2 (C-1), 34.2 (C-7), 34.4 (C-6), 41.4 (C-2), 125.2 (C-4″), 125.4 (C-4′), 128.4 (C-2′,2″,6′,6″), 128.6 (3′, 3″, 5′, 5″), 130.7 (C-4), 140.3 (C-1′), 141.2 (C-1″), 146.4 (C-5), 199. ESI-Ms. [M + Na]+ = 287. After evaluating spectrum data and literature [22] (Figure 1A), compound-1 was identified as 1,7-diphenylhept-4-en-3-one. Compound-2: Pale yellow crystals in EtOAc, melting point 48-50 °C, chemical shift 9.9 degrees (C = 0.010, CHCl3), and FT-IR peaks 3434 (OH), 1700 (CO), and 1629.

1 H NMR (400 MHz) in CD3OD: δ (ppm) 7.15-7.29 (10H, m, H2′-6′, H2 -6), 4.04 (1H, m, H-5) The chemical shifts are: 2.91 (1H, br s, 5-OH), 2.89 (2H, t, J = 7.5 Hz, H-1), 2.75 (2H, m, H-2), 2.62-2.82 (2H, m, H-4), 2.54 (2H, m, H-7), 1.61-1.84. 13C NMR (100 MHz) in CD3OD: δ (ppm): 29.51 (C-1), 31.75 (C-7), 38.04 (C-6), 45.03 (C-2), 49.29 (C-4), 66.88 (C-5), 125.91 (C-4′), 126.26 (C4′), 128.3 (C-2′, 2″, 6′, 6″), 128.45 (C-3′, 3″, 5′, 5″), 140.68 (C-1′), 141.82 (C-1 Our ESI-Ms ratio is 305 (M + Na]+). Comparing spectrum data with literature [23], compound-2 was 1,7-diphenyl-5-hydroxy-hept-3-one (Figure 1B). Compound 3 is a yellow solid with a melting point of 213-215 °C (or 213-214 °C as described in literature). 1 H NMR (400 MHz) in CD3OD shows δ (ppm) values of 8.20 (2H, dd, H-2′, 6′), 7.62 (2H, dd, H-3′, 5′), 7.53 (1H, m, H-4′), 6.43 (JH8/H6 = 1.5 Hz, d, 1H, H-8), and 6.21 Results from FT-IR (KBr) cm−1 analysis: OH 3410, CO 1656, Aromatic 1450, 1526. The chemical shifts of CD3OD in 13C NMR (100 MHz) are 148.5 (C2), 141.3 (C-1′), 131.3 (C-2′, 6′), 133.4 (C3′, 5′), and 132. Figure 2 shows MS's highest value, 271 [M + 1+]. Comparing spectral data to literature [24] (Figure 1C) revealed galangin as a 3,5,7-trihydroxy flavone. Compound 4 is a yellow solid with a melting point of 223-225 °C (or lit. 224-226).

The FT-IR (KBr) cm−1 patterns are 3457 (OH), 1645 (CO), 1495, and 1026. The 1 H NMR spectrum at 400 MHz in CD3OD showed the following chemical shifts: 8.07 (2H, H-2′, 6′), 6.94 (2H, H-3′, 5′), 6.35 (1H, H-8), 6.19 (1H, 2.H-6), 3.81 (3H, S). The 13C NMR (100 MHz) chemical shifts of CD3OD are: 123.3 (C-1′), 129.3 (C-2′, 6′), 114 (C-3′, 5′), 160.5 (C-4′). MS [M + 1+] peak = 301. Comparing spectrum data with literature [25] (Figure 1D), compound-4 was identified as 3,5,7-trihydroxy-4′-methoxy flavone (kaempferide). A yellow liquid having FT-IR (CHCl3) cm−1 values of 3537 (OH), 1702 (CO), 1515, 1216, and 1035. The chemical shifts in CDCl3's 1H NMR spectra are: The peaks are: 1.75-1.88 (2H, m, H-6), 2.64-2.96 (8H, m, H-1, 2, 4, 7), 3.90 (3H, s, 3″-OCH3), 4.19 (1H, m, H-5), 6.78 (6H, bd, J = 8 and 2 Hz), 6.81 (6H, 8 Hz, H-5″), 6.92 (6H, 2 Hz, H-2″), and 7.25-7.37 (5 The following chemical shifts were found in 100 MHz CDCl3 13C NMR experiments: Chemical elements: 29.5 (C-1), 31.4 (C-7), 38.5 (C-6), 45.0 (C-2), 49.4 (C-4), 211.0 (C-3), 55.9 (3-OCH3), 67.0 (C-5), 114.6 (C-2), 111.4 (C-5), 120.9 (C-6), 126.2 (C-4′), 128.56 (C-2′,6′), 128.58 (C-3′,5′), 133.7 (C-1), 140.7 (C-1′), 146.7 (C-4), 143.8 (C-3). ESI-Ms have [M + Na]+ 351 (Figure 3). Comparing spectra data with literature, compound-5 was identified as 1-phenyl-5-hydroxy-7-(4-hydroxy-3-methoxy phenyl)-hept-3-one (Figure 1E).

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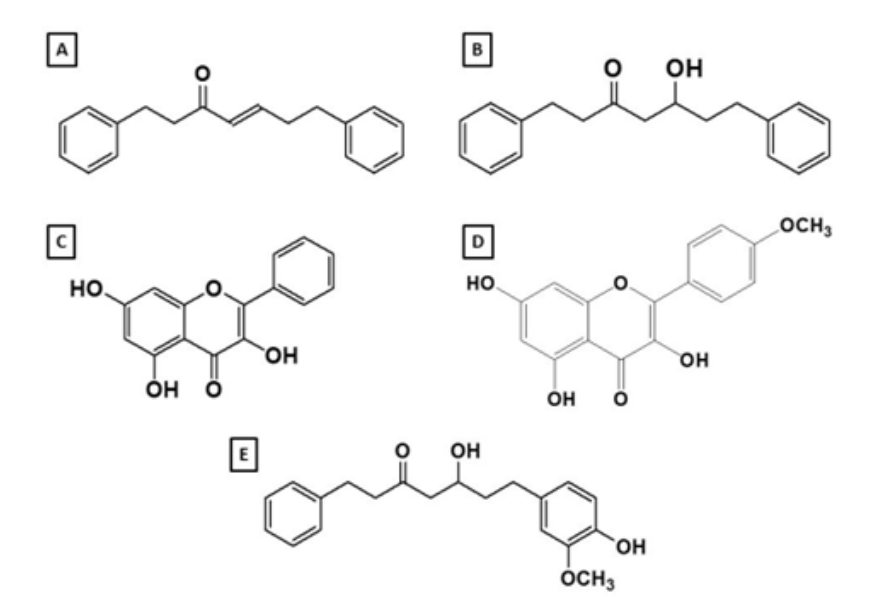

Figure 1: Isolated substances' molecular structures: 3-galangin, 4-kaempferide, 5 hydroxyphenylhydrohydantoin, and 5-HPH.

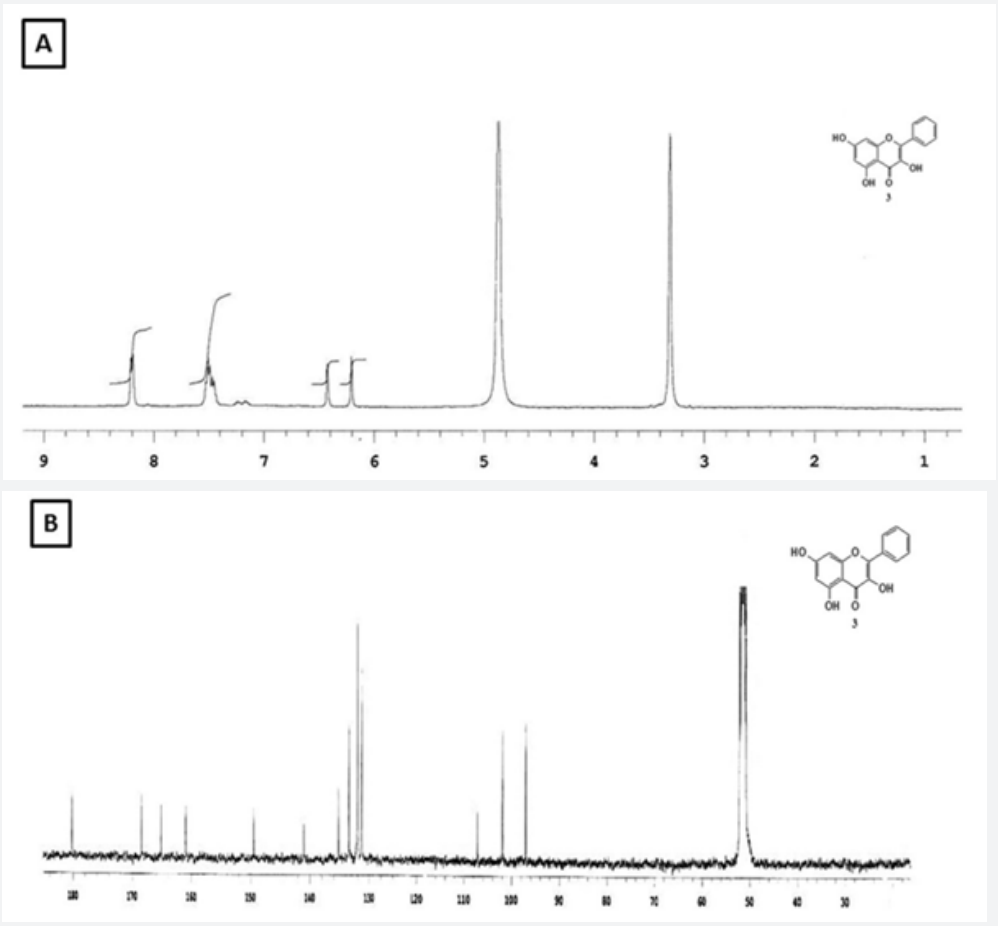

Figure 2: Combined 13C and 1H NMR spectra of compound-3 (A and B, respectively).

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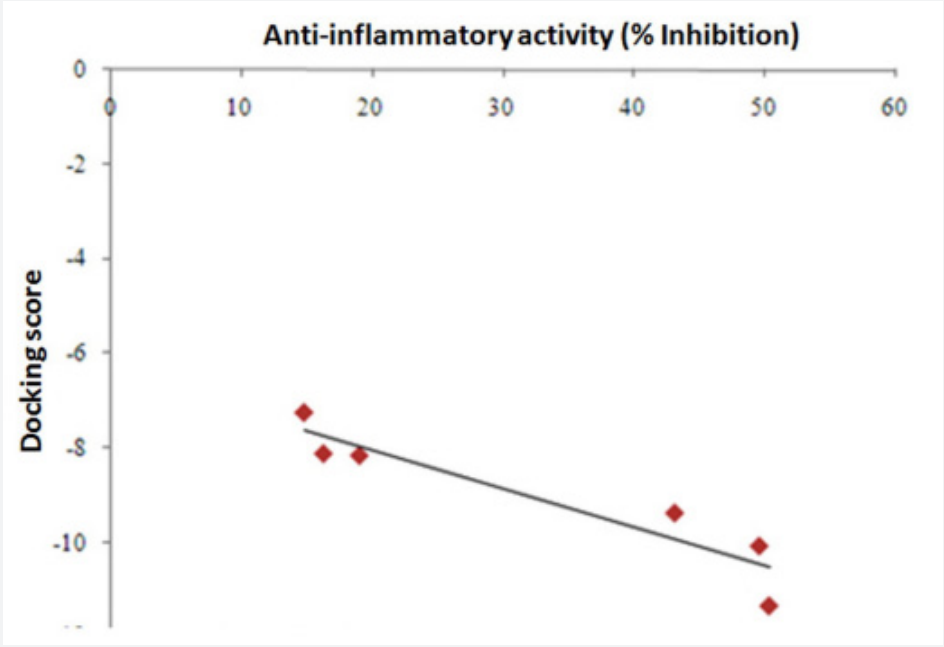

Figure 3: Glide docking scores (1–5) of the extracted chemicals and diclofenac, coupled with their anti-inflammatory efficacy (% inhibition after 5 h.), are correlated in a plot.

Acute toxicity in the mouth

No behavioral issues or fatalities were reported with MEAO at 2000 mg/kg p.o. The following study used 100, 200, and 400 mg/kg MEAO with 10 mg/kg compounds-1, 2, 3, 4, and 5.

In-vitro anti-inflammatory activity

The carrageenan-induced edema test examined the anti-inflammatory effects of the methanolic extract of A. officinarum (MEAO) and its components (Table 1). The multi-mediator carrageenan paw edema test screens anti-inflammatory drugs [26]. Carrageenan-induced edema develops in three stages: histamine and serotonin release in the first 90 minutes, kinin in the second 90-150 minutes, and prostaglandin after 180 minutes [27]. After MEAO, compound-3, and compound-5 for 1 hour, carrageenan injection reduced paw edema dose-dependently from 1 to 5 hours. MEAO, compound-3, and compound-5 at 200 and 400 mg/kg inhibited after 3 and 5 hours. Compounds-1, 2, and 4 at 10 mg/kg did not reduce paw edema. Diclofenac (10 mg/kg) substantially reduced paw edema 3 and 5 hours after carrageenan injection (p b 0.001). Diclofenac reduced rat paw edema by 32.67% and 39.47% after 3 and 5 hours. Compound-3 and compound-5 blocked diclofenac-like processes. In an acute inflammatory model, MEAO, compound-3, and compound-5 effectively decrease paw edema development at 3 and 5 hours.

In-vitro antioxidant activity

Several inflammatory diseases are brought on by reactive oxygen species [28,29]. Assays for hydroxyl radical scavenging, DPPH, and hydrogen peroxide were used in the current investigation in order to establish the level of antioxidant activity (Table 2).

Impact of ascorbic acid, MEAO, compounds three and five on the DPPH free radical scavenging test

DPPH exhibited concentration-dependent free radical scavenging at 100 µg/ml, as seen with compound-5, compound-3, and MEAO. MEAO scavenged more than compounds 3 and 5. Ascorbic acid, the gold standard, scavenged radicals better. According to Table 2, the IC50 values for MEAO, compound-3, compound-5, and ascorbic acid were 84, 90, 93.5, and 58 µg/ml, respectively. Antioxidants' hydrogen donation may affect DPPH radical scavenging. The dip in absorbance at 517 nm generated by antioxidants was utilized to test DPPH radical reduction. Fast peroxyl radical neutralizers don't always work against DPPH [30]. This study found concentration-dependent scavenging activity for MEAO, compound-3, and compound-5 (Table 2). Most importantly, hydrogen peroxide triggers nuclear translocation of transcription factors NFkB, which allows gene transcription, inflammation, and Syndrome X [31-33]. Hydrogen peroxide may cross plasma membranes. In this study, MEAO, compound-3, and compound-5 demonstrated concentration-dependent free radical scavenging from hydrogen peroxide up to 100 µg/ml. MEAO scavenged more than compounds 3 and 5. Ascorbic acid, the gold standard, scavenged radicals better. According to Table 2, the IC50 values for MEAO, compound-3, compound-5, and ascorbic acid were 90.5, 4.5, over 100 µg/ml, and 63.5 µg/ml, respectively. Ascorbic acid was less efficient than compounds 3, 5, and MEAO at scavenging EDTA/H2O2 hydroxyl radicals. MEAO, compound-3,

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and compound-5 scavenged more OH radicals' dose-dependently. Similar scavenging effects were seen with standard ascorbic acid (10-100 µg/ml). Table 2 shows that MEAO, compound-3, compound-5, and ascorbic acid showed IC50 values of 65, 74.5, 77, and 47 µg/ml, respectively.

Table 1: Effect of MEAO and compounds 1-5 on rat carrageenan-induced hind paw edema.

Treatment groups	Change in paw edema volume (ml)			% inhibition		
	1 h	3 h	5 h	1 h	3 h	5 h
Vehicle control	0.655 ± 0.025	1.125 ± 0.028	1.235 ± 0.034	-	-	-
Diclofenac (10 mg/kg)	0.653 ± 0.018	0.758 ± 0.017***	0.748 ± 0.023***	0.38	32.67	39.47
MEAO 100 mg/kg	0.658 ± 0.026	0.985 ± 0.028*	1.020 ± 0.032***	0.38	12.44	17.41
MEAO 200 mg/kg	0.658 ± 0.034	0.915 ± 0.038***	0.933 ± 0.039***	0.38	18.67	24.49
MEAO 400 mg/kg	0.655 ± 0.042	0.808 ± 0.031***	0.830 ± 0.033***	0	28.22	32.79
Compound-1 (10 mg/kg)	0.650 ± 0.056	1.070 ± 0.047	1.150 ± 0.057	0.76	4.89	6.88
Compound-2 (10 mg/kg)	0.650 ± 0.033	1.095 ± 0.018	1.178 ± 0.019	0.76	2.67	4.66
Compound-3 (10 mg/kg)	0.648 ± 0.035	0.768 ± 0.029***	0.805 ± 0.033***	1.15	31.78	34.82
Compound-4 (10 mg/kg)	0.658 ± 0.020	1.070 ± 0.007	1.165 ± 0.005	0.38	4.89	5.67
Compound-5 (10 mg/kg)	0.645 ± 0.024	0.750 ± 0.032***	0.715 ± 0.031***	1.53	33.33	42.11

Notes: The results (n = 6) are shown as mean ± SEM. A two-way ANOVA and Bonferroni's post hoc test were used to evaluate the data. Comparing vehicle control, *p < 0.05 and ***p < 0.001 were found.

Table 2: In vitro DPPH, H2O2, and OH radical scavenging experiments including compound-3, compound-5, and ascorbic acid.

Conc. (µg/ml)	DPPH scavenging assay (% inhibition)				H_2O_2 scavenging assay (% inhibition)				OH scavenging assay (% inhibition)			
	MEAO	Comp-3	Comp-5	AA	MEAO	Comp-3	Comp-5	AA	MEAO	Comp-3	Comp-5	AA
10	18.15	16.97	19.70	22.68	2.44	9.57	5.09	15.48	25.66	21.29	19.10	29.37
20	20.01	26.09	22.21	27.70	13.44	18.13	10.18	28.51	30.46	26.09	23.91	38.65
40	24.10	34.48	27.14	45.38	22.81	23.22	15.07	39.92	38.54	34.17	31.99	45.74
60	27.46	41.82	34.79	50.62	35.23	31.77	22.20	46.64	47.49	42.69	42.03	56.88
80	40.59	49.89	46.33	61.84	44.81	41.34	27.09	51.32	57.64	52.95	51.53	66.92
100	52.83	58.91	57.65	64.88	54.99	53.97	38.29	62.32	64.08	61.68	58.84	70.85
IC_{50}	84.0	90.0	93.5	58.0	90.5	94.5	-	63.5				

Ascorbic Acid, MEAO, Compound-3, and Compound-5 on Hydrogen Peroxide and Hydroxyl Radical Scavenging Analysis.

Effect of MEAO, compound-3, compound-5, and gallic acid on total phenolics

Due to their hydroxyl groups, phenolic substances are excellent antioxidants that break chains [34,35]. This study used Folin-Ciocalteu to measure total phenolics. Table 3 shows gallic acid equivalents of phenols in MEAO at 77.63 mg, compound-3 at 72.96 mg, and compound-5 at 51.18 mg.

Docking of molecules

A docking approach's accuracy may be checked by comparing the object scoring function's predicted lowest energy pose (binding conformation) to an experimental binding mode from X-ray crystallography. This study validated docking by re-docking the chemical to the COX-2 binding site after removing it. Docking studies showed a substantial link between the inhibitor's docked position and crystal structure. Based on the 0.28 Å root-mean-square deviation (RMSD) of the diclofenac conformation, Glide docking parameters are suitable for reproducing the X-ray structure and receptor-bound conformation of other molecules in the dataset. The medicines' COX-2 active site binding orientations were shown by molecular docking. Docking simulations preserved 10 configurations for each molecule. Table 4 shows each isolation's ideal orientation. Ligands are ranked on Glide score and anti-inflammatory efficacy. The docking results are analyzed using glide score, glide energy, Hbonds, and non-bonded interactions (electrostatic and van der Waals). We can determine the ligand's receptor binding affinity from this. This shows that docking simulation bound all isolates to the same place. Docking

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experiments showed that isolated compounds 1-4 matched COX-2 well, putting them near diclofenac in the crystal structure complex. The shortest RMSD (1.80 Å) for all ligands in COX-2's active site indicates that they bind in the same orientation and position within the enzyme. The five isolates were efficient COX-2 binders with an average docking score of -9.03. At -9.36, diclofenac had the lowest docking score of the isolates. This shows these isolates might be viable next-generation drugs. Figure 3 plots these isolates' anti-inflammatory efficacy against diclofenac by Glide docking score. Docking scores and inhibitory activities of isolates were substantially associated. Isolates with weak inhibition had lower docking scores, whereas active molecules with high scores had high inhibitory activities.

Table 3: Impact of gallic acid, MEAO, compound-3, and compound-5 on total phenolic content.

Conc. (µg/ml)	Total phenolic content (mg)			
	MEAO	Comp-3	Comp-5	Gallic acid
10	4.50	4.25	3.49	11.19
20	15.38	15.03	9.46	19.47
40	38.00	34.66	26.45	41.48
60	44.66	42.19	32.15	52.71
80	62.44	56.21	39.72	92.75
100	77.63	72.96	51.18	96.35

Table 4: Isolated Compounds' Anti-Inflammatory Activity and Their Combination with Molecular Docking (1-5).

Compound / Group	Docking Score	Glide Score (Rank*)	Glide Energy	vdW Energy	Coulombic Energy	Key Per-residue Interactions	% Inhibition (1h)	% Inhibition (3h)	% Inhibition (5h)
Diclofenac (10 mg/kg)	-9.36	-	-34.79	-31.09	-3.70	Standard NSAID reference	-12.67	37.97	43.17
Compound-1 (10 mg/kg)	-8.10	(4)	-34.29	-31.32	-2.97	Ala527, Gly526, Val523, Trp387, Tyr385, Leu359, Tyr355, Ser353, Leu352, Val349, Arg120, Val116 (H-bonds: Ser530, Glu524, Tyr355)	-49.29	4.92	16.37
Compound-2 (10 mg/kg)	-7.23	(5)	-33.49	-31.99	-1.49	Leu531, Ser530, Ala527, Gly526, Val523, Met522, Tyr385, Leu384, Tyr355, Ser353, Leu352, Val349, Ile345, Val116 (Coulombic: Ser353)	-25.35	-12.75	14.88
Compound-3 (10 mg/kg)	-11.33	(1)	-39.27	-32.38	-6.88	Ser530, Ala527, Gly526, Val523, Trp387, Phe381, Leu359, Tyr355, Ser353, Leu352, Val349, Tyr348, Ile345 (Coulombic: Ser530, Arg513, Tyr385, Ser353)	17.6	16.52	50.37

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Compound-4 (10 mg/kg)	-8.14	(3)	-33.85	-28.34	-5.51	Ser530, Gly526, Val523, Met522, Tyr385, Leu352, Leu531, Ala527, Leu359, Tyr355, Val349 (Coulombic: Ser530, Phe518, Tyr385)	-70.42	-8.12	19.11
Compound-5 (10 mg/kg)	-10.06	(2)	-35.91	-29.41	-6.50	Ser530, Leu531, Ala527, Gly526, Val523, Met522, Trp387, Tyr385, Ser353, Leu352, Val349, Val116 (Coulombic: Glu524, Tyr385)	-7.04	36.23	49.6

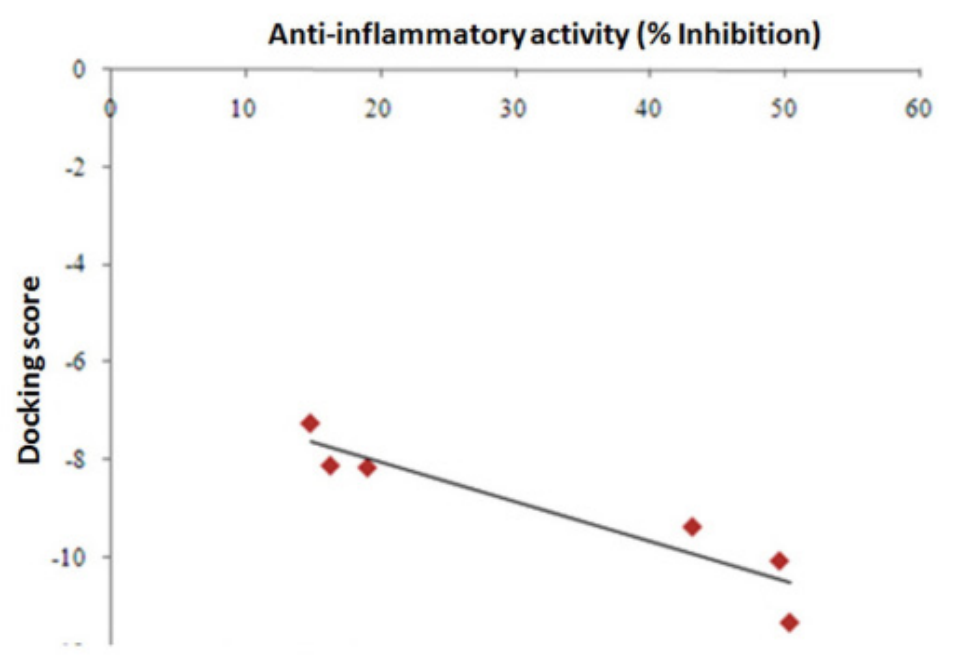

Figure 3: Glide docking scores (1–5) of the extracted chemicals and diclofenac, coupled with their anti-inflammatory efficacy (% inhibition after 5 h.), are correlated in a plot.

Conclusion

It was found that the methanolic extracts of Alpinia rhizome exhibited significant anti-inflammatory and antioxidant properties. Compounds such as 5-hydroxy-7-(4″-hydroxy-3 -methoxyphenyl)-1-phenyl-3-heptanone (compound-5) and Galangin (compound-3) had considerable anti-inflammatory effects. These effects were most likely brought about by the compounds' capacity to inhibit the generation and/or action of histamine, serotonin, and kinin. It is possible that the extracts' strong antioxidant effect in vitro might also be attributed to the high phenolic content of the extracts. In addition, molecular docking studies shown that these compounds had a strong affinity for the COX-2 active site, which led researchers to conclude that they have the potential to be effective as selective inhibitors of COX-2. It has been a long-held opinion that Alpinia rhizomes might be effective in the treatment of inflammatory illnesses and oxidative stress. The findings of this investigation provide support to this view.

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