Ciência Rural, Santa Maria, v.52:10, e20210372, 2022 http://doi.org/10.1590/0103-8478cr20210372
ISSNe 1678-4596
BIOLOGY

Chemical profile, anti 5-lipoxygenase and cyclooxegenase inhibitory effects of ginger (*Zingiber officinale*) rhizome, callus and callus treated with elicitors

Ammar Mohammed Ahmed Ali[1] Mawahib ElAmin Mohamed El-Nour[2] Abdulrahman Abdullah Al-Atar[3] Owais Mohammad[4] Mohamed Abdel-Raouf El-Sheikh[3] Ahmed Ali Qahtan[3] Eslam Mohamed Abdel-Salam[3*] Sakina Mohamed Yagi[5]

[1]Department of Biology, Faculty of Education, Hajjah University, Hajjah, Iêmem.
[2]Department of Biology and Biotechnology, Faculty of Science and Technology, AL Neelain University, Khartoum, Sudão.
[3]Department of Botany and Microbiology, College of Science, King Saud University, 11451, Riyadh, Arábia Saudita. E-mail: eabdelsalam@ksu.edu.sa.
*Corresponding author.
[4]Molecular Immunology Lab1, Biotechnology Unit, Aligarh Muslim University, Aligarh, India.
[5]Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudão.

ABSTRACT: *The present study investigated the chemical profiles and evaluated the inhibitory effect against 5-Lipoxygenase (5-Lox) activity for extracts of ginger rhizome, callus, and callus treated with the elicitors; yeast extract (100, 300 and 500 mg/L), glycine (100, 200 and 300 mg/L) and salicylic acid (100 and 200 mg/L). Oils and chloroform: methanol (CM) extracts were prepared by maceration in petroleum ether and CM (1:1, v/v), respectively. Chemical profiles were determined by gas chromatography/mass spectrometry (GC/MS) analysis. Oil of the callus recorded higher 5-Lox inhibitory effect (IC_{50} 58.33±4.66 µg/mL) than the oil of rhizome (IC_{50}168.34±15.64 µg/mL) and comparable to that of the positive control; Nordihydroguaiaretic acid (IC_{50} 61.25±1.02 µg/mL). The chemical profile of the callus oil contained large amounts of fatty acids, mainly the unsaturated fatty acid oleic acid (31.11%) and saturated fatty acid palmitic acid (28.56%). Elicitors modified the chemical profile of the callus and ameliorated the anti-5-Lox activity of CM extract of the callus. CM extracts of callus treated with 100 and 300 mg/L yeast extract and 50 mg/L salicylic acid significantly suppressed ($P \leq 0.05$) the 5-Lox activity by 33.16%, 25.46% and 16%, respectively as compared to the CM extract of untreated callus. In conclusion, ginger callus could be considered as a valuable dietary supplement in the treatment of various inflammatory disorders.*
Key words: *ginger, anti-5-lipoxygenase activity, yeast extract, salicylic acid, glycine.*

Perfil químico, anti-5-lipoxigenase e efeitos inibitórios da ciclooxegenase do rizoma de gengibre (*Zingiber officinale*), do calo e calos tratados com elicitores

RESUMO: *O presente estudo teve como objetivo investigar os perfis químicos e avaliar o efeito inibitório da atividade da 5-Lipoxigenase (5-Lox) em extratos de rizoma, calo e calo de gengibre tratados com os eliciadores; extrato de levedura (100, 300 e 500 mg / L), glicina (100, 200 e 300 mg / L) e ácido salicílico (100 e 200 mg / L). Extratos de óleos e clorofórmio: metanol (CM) foram preparados por maceração em éter e CM (1: 1, v / v), respectivamente. Os perfis químicos foram determinados por análise de cromatografia gasosa / espectrometria de massa (GC / MS). O óleo do calo registrou maior efeito inibitório de 5-Lox (IC50 58,33 ± 4,66 µg / mL) do que o óleo de rizoma (IC50168,34 ± 15,64 µg / mL) e comparável ao do controle positivo; Ácido nordi-hidroguaiarético (IC50 61,25 ± 1,02 µg / mL). O perfil químico do óleo de calo continha grandes quantidades de ácidos graxos, principalmente o ácido graxo insaturado ácido oleico (31,11%) e ácido graxo saturado palmítico (28,56%). Os elicitores modificaram o perfil químico do calo e melhoraram a atividade anti-5-Lox do extrato de CM do calo. Extratos de CM de calos tratados com 100 e 300 mg / L de extrato de levedura e 50 mg / L de ácido salicílico suprimiram significativamente ($P \leq 0,05$) a atividade de 5-Lox em 33,16%, 25,46% e 16%, respectivamente, em comparação com o extrato de CM de calo não tratado. Em conclusão, o calo de gengibre pode ser considerado um suplemento dietético valioso no tratamento de vários distúrbios inflamatórios.*
Palavras-chave: *gengibre, atividade anti-5-lipoxigenase, extrato de levedura, ácido salicílico, glicina.*

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is widely consumed as spice and to cure a wide range of diseases (KARAMAN et al., 2019). Pharmacological studies revealed that ginger has broad healthy benefits such as anti-emetic (KARAMAN et al., 2019; KALAVA et al., 2013; JEENA et al., 2013), antioxidant, anti-inflammatory and antinociceptive activities (KULKARNI & DESHPANDE, 2016), preventing diabetes complications (ARABLOU et al., 2014; SHIDFAR et al., 2015), reliving dysmenorrheal pain (SHIRVANI et al., 2015; RAD et al., 2018), and treating ulcerative colitis (NIKKHAH-BODAGHI et al., 2019).

Received 05.11.21 Approved 11.17.21 Returned by the author 02.13.22
CR-2021-0372.R1
Editors: Leandro Souza da Silva Juliane Welke

The healthy properties of ginger are frequently imputed to some pungent or non-volatile components in ginger as gingerols and shogaols or to volatile components as zingiberene, which is mainly responsible of the distinct aroma of ginger (PRASAD & TYAGI, 2015). These phytocompounds exist with varying concentrations depending on ginger form used, either fresh or dry, and method of extraction. Fresh rhizome contains high amount of gingerol which is converted to shogaol form via heating or drying process (PRASAD & TYAGI, 2015). Lipoxygenases are a family of nonheme iron-containing enzymes that catalyze the deoxygenation of polyunsaturated fatty acids (PUFAs). Various lipoxygenases are involved in the metabolism of leukotrienes, a family of eicosanoid inflammatory mediators. For example, leukotrienes are synthesized in the cell from arachidonic acid by arachidonate 5-lipoxygenase (5-Lox). 5-LOX plays a pivot role in asthma and inflammation, as it causes the constriction of bronchioles in response to cysteinyl leukotrienes such as LTC4, thus leading to asthma. It also induces neutrophilic inflammation by its recruitment in response to LTB4. Ginger extracts as well as gingerol, shogaol, and other structurally-related substances in ginger exhibited a broad spectrum of anti-inflammatory activities through multiple mechanisms as suppressing prostaglandin synthesis through inhibition of cyclooxygenase-1 and cyclooxygenase-2 and suppresses leukotriene biosynthesis by inhibiting 5-lipoxygenase. These dual inhibitors of cyclooxygenase and 5-Lox consequently distinguished ginger to exert fewer side effects than non-steroidal anti-inflammatory drugs (GRZANNA et al., 2005; MASHHADI et al., 2013).

The high request on ginger worldwide as spice and reliable medicinal herb, especially with anti-inflammatory properties, is associated with increasing loss of ginger productivity when it is propagated under natural conditions in the field, because it is easily infected by many pathogenic factors including fungi, bacterial wilt, and nematodes. To overcome these problems, plant tissue culture was employed as an efficient technique to initiate microbial free plants or to induce masses of undifferentiated cells (callus tissue) *in vitro* as sustainable and steady sources of phytochemicals for industrial and commercial purposes. To increase the *in vitro* yield of bioactive components, elicitation represents the most effective strategy applied to rise the yield of bioactive secondary metabolites in different *in vitro* cultures.

It is well established that flavonoids and phenolic acids inhibit the activity of various cyclooxygenases and lipoxygenases (LAUGHTON et al., 1991). Conversely, PUFAs have the ability to control inflammation and leukotriene synthesis via affecting cyclooxygenases and lipoxygenases (ARAUJO et al., 2019). Moreover, PUFAs are known to inhibit arachidonic acid metabolism. A recent study showed that callus derived from ginger rhizome as well as callus treated by elicitors significantly suppress the LPS-induced production of TNF-α, IL-1 and IL-6 and production of the IL-10 and TGF-β anti-inflammatory cytokines (ALI et al., 2019). The present study, in continuation to evaluate the anti-inflammatory capacity of ginger callus, was undertaken to assess the 5-Lox inhibitory effect of ginger rhizome, callus and callus elicited by yeast extract, glycine and salicylic acid and to investigate their chemical profile by GC/MS analysis.

MATERIALS AND METHODS

Plant materials

Ginger rhizomes were obtained from the botanical garden at Biology and Biotechnology Department, Faculty of Science and Technology, Al-Neelain University, Khartoum, Sudan. Rhizomes were well washed, cut into thin slices, and dried at room temperature.

Callus initiation and proliferation

Ginger callus initiation and proliferation were previously described by ALI et al. (2016). The best callus fresh weight was developed and proliferated on MS medium augmented by 0.5 mg/L 2,4- dichlorophenoxyacetic acid (2,4-D).

Elicitor's treatments

Treatment of callus with different concentrations of elicitors: yeast extract (YE) 100, 300 and 500 mg/L; glycine (GL) 100, 200 and 300 mg/L, and salicylic acid (SA) 100 and 200 mg/L were prepared using the protocol published by ALI et al. (2018).

Preparation of extracts

The extracts of ginger rhizome and callus were prepared by maceration in petroleum ether and chloroform: methanol (CM) (1:1, v/v) for 72 h at room temperature (ALI et al., 2018).

Gas chromatography/mass spectrometry (GC/MS) analysis

Analysis of the chemical composition of ginger rhizome and callus extracts were performed by gas chromatography coupled to mass spectrometry (Model GC-MS-QP2010 Plus, Shimadzu, Japan). Separation was performed using Rtx-5MS capillary column (5% diphenyl-95% dimethylsilicone, 30 m × 0.25 mm × 0.25 µm) and a temperature program of 50 °C (1 min) ramped to 300°C (3 min) at 5 °C/min. Identification of compounds was based on comparison of mass spectra with the GC/MS system data bank (NIST 08 library), comparison with published data, and retention indices. The relative amount of each compound was expressed as percent peak area relative to the total peak area of the GC chromatogram.

Lipoxygenase assay

5-lipoxygenase assay was performed following the procedure described by FRUM & VILJOEN (2019). Briefly, 12.5 µL of extract was mixed with 50 µL of linoleic acid (0.003 g/10 mL) and made up to 1 mL with 0.1 M phosphate buffer with Tween (0.005%). To initiate the reaction, 1.5 µL of 5-lipoxygenase from soybean (0.054 g/mL) was added to mixture. The increase in absorbance at 234 nm was recorded for 5 min in a Shimadzu 160-UV spectrophotometer. Nordihydroguaiaretic acid was used as positive control. The % enzyme inhibition was calculated by the following equation: % = [(A0 - A1) / A0] x 100 where A0 was the absorbance of the control without extract and A1 was the absorbance of the sample.

Statistical analysis

Data were statistically analyzed using SPSS version 19. The 5-lox experiment were performed in triplicate and the results were expressed as mean ± standard deviation (SD) values. Significant differences between samples were analyzed using analysis of variance (ANOVA) and Duncan's multiple-range test ($P < 0.05$).

RESULTS

Extraction yield

Different extraction yields (percentage of mass of extract/mass of dry matter) of ginger rhizome and untreated and treated callus are depicted in table 1. Oils of rhizome and untreated callus yielded low amount than their respective CM extracts and with higher quantity of rhizome oil than that of the callus. Treatment of callus with different elicitors highly increased the yield of CM extracts but did not provide measurable oil content. The ranking order of CM extracts of the untreated and treated callus was in the following decreasing order: callus treated by SA50 mg/L (27.2%) > callus treated by YE500 mg/L (23.49%) > callus treated by YE100 mg/L (23.25%) > callus treated by GL100 mg/L (19.86%) > callus treated by GL200 mg/L (18.58%) > callus treated by GL300 mg/L (17%) > callus treated by YE300 mg/L (12.18%) > callus treated by SA100 mg/L (10.75%) > untreated callus (5.74%).

Table 1 - Extractive yields of ginger rhizome and treated and untreated callus extracts.

Extract	Yield (%)
Oil of rhizome	3.82
Oil of callus	1.60
CM extract of rhizome	5.05
CM extract of callus	5.74
CM extract of callus treated by 100 mg/L yeast extract	23.25
CM extract of callus treated by 300 mg/L yeast extract	12.18
CM extract of callus treated by 500 mg/L yeast extract	23.49
CM extract of callus treated by 100 mg/L glycine	19.86
CM extract of callus treated by 200 mg/L glycine	18.58
CM extract of callus treated by 300 mg/L glycine	17.00
CM extract of callus treated by 50 mg/L salicylic acid	27.20
CM extract of callus treated by 100 mg/L salicylic acid	10.75

CM, Chloroform: Methanol (1:1, v/v).

Anti-5-lipoxygenase activity

The anti-5-Lox activity of the oil and CM extracts of ginger rhizome and untreated callus was evaluated, and results are presented in figure 1-a. The highest anti-5-Lox activity was exerted by callus oil (58.33 ± 4.66 µg/mL) and its CM extract (87.68±7.32 µg/mL) respectively. In fact, the callus oil was significantly ($P < 0.05$) more active than the positive control; Nordihydroguaiaretic acid (IC_{50} 61.25±1.02 µg/mL) and was 2.9-fold more active than the rhizome oil. Moreover, the CM callus extract exhibited significant ($P < 0.05$) 1.7–fold higher anti-5-Lox activity than that obtained from of the rhizome CM extract and was comparable to that exerted by 6-shogaol (87.65 ± 4.35 µg/mL).

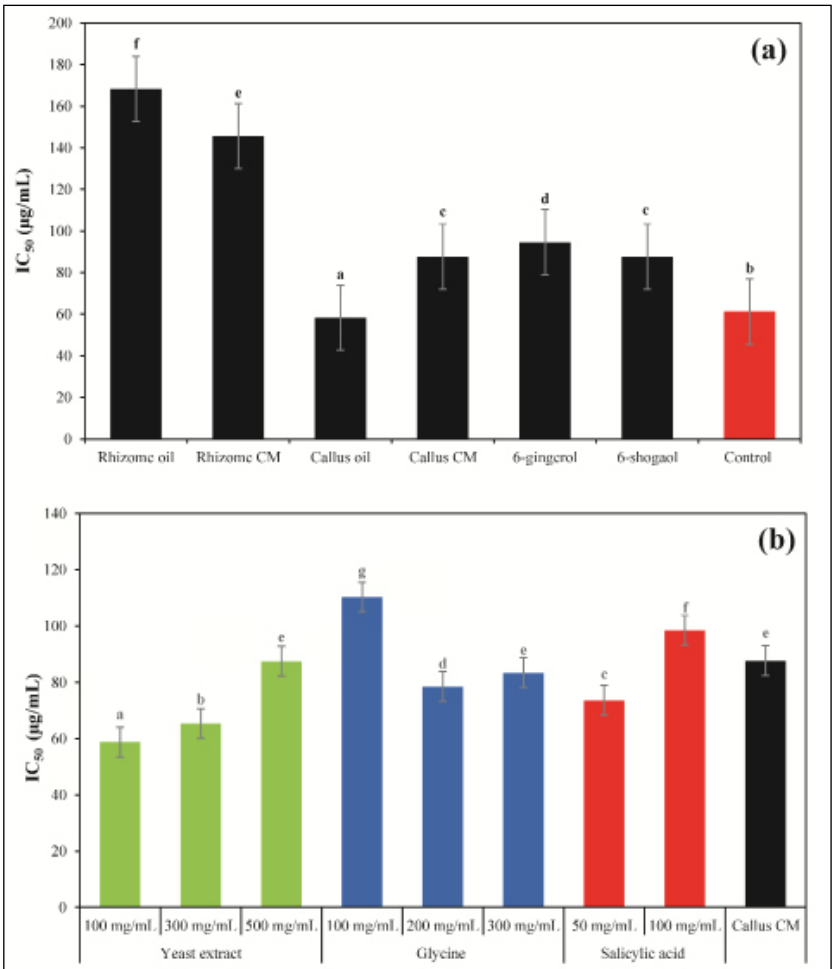

Figure 1 - Anti5-Lipoxygenase activity of ginger rhizome, callus (a) and treated callus extracts (b). CM, Chloroform: Methanol (1:1, v/v).

Further, the callus was treated with elicitors (YE, GL and SA) in an attempt to increase its anti-5-Lox activity. Results of anti-5-Lox activity of CM extracts from treated calli are presented in figure 1-b. Generally, elicitors enhanced significantly ($P < 0.05$) the callus inhibitory effect on 5-Lox activity. Treatment of callus with YE recorded highest inhibition levels against 5-Lox activity compared to other treatments of elicitors. One hundred and 300 mg/L YE decreased the 5-Lox activity by 33.16% and 25.46% respectively. SA 50 mg/L inhibited the 5-Lox activity by 16% while treatment of callus with GL reduced it by 10.46% and 4.82% at concentrations 200 and 300 mg/L respectively.

GC/MS profile of ginger rhizome and callus

Based on the results of anti-5-Lox activity, the GC/MS profile of the callus oil was determined and compared with that of the rhizome. Also, GC/MS profile of CM extracts of treated callus was compared with that of untreated callus. Results are presented in tables 2, 3, 4, and 5. Rhizome oil, with a yield of 3.82% (w/w) on dry weight basis, was brownish in colour with a pleasant aroma. The callus gave a yellow-coloured oil with an agreeable perfumery odour and a yield of 1.60% (w/w) on dry weight basis. GC/MS chromatogram of the rhizome oil revealed the presence of 46 identified components comprised 100% of the total oil. The oil was dominated by the presence of oxygenated sesquiterpenes (58.39%)

Table 2 - Gas chromatography/mass spectrometry (GC/MS) profile of ginger rhizome and callus oil.

No.	RI	RT	Compound name	----------Area (%)----------	
				Rhizome	Callus
1	907	3.34	Methyle Hexanoate	0.35	_
2	965	3.72	Camphene	0.03	_
3	940	4.09	5-Hepten-2-one,6-Methyle-	0.09	_
4	987	4.14	β-Myrcene	0.02	_
5	1011	4.24	1,2,3-Trimethylbenzene	0.02	_
6	1007	4.30	Octanal	0.09	_
7	1024	4.37	α-Phellandrene	0.02	_
8	995	4.63	2-Ethylhexanol	0.15	1.83
9	1038	4.71	β-Phellandrene	0.40	_
10	1055	4.75	Eucalyptol	0.39	_
11	1100	5.49	Acetamide,N-[1-methyl-1-(4-methylcyclohex-3-enyl) ethyl]	0.06	_
12	1106	5.61	Linalol	0.07	_
13	1083	5.92	Methyl octanoate	0.17	0.04
14	1166	6.65	Endo-Borneol	0.38	_
15	1188	6.97	α-Terpineol	0.17	_
16	1200	7.08	Decanal	0.41	_
17	1242	7.62	β-Citral	0.08	_
18	1260	7.77	Geraniol	0.07	_
19	1272	8.02	α-Citral	0.09	_
20	1290	8.29	2-Undecanone	0.26	_
21	1306	8.68	Methyl Caprate	0.84	_
22	1330	8.97	Cyclohexene,4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methyl ethyl)-,(3R-trans)	0.06	_
23	1345	9.43	1,2,4-Metheno-1Hindene,octahydro-1,7a-dimethyl-5-(1-methylethyl)-,[1S-(1.α-.,2.α .,3a.beta.,4.α-.,5.α-.,7α-a.β.8S	0.18	_
24	1352	9.52	α-Cubebene	0.32	_
25	1370	9.71	7-Isopropenyl-1-methyl 4-methyl enedecahydroazulene	0.63	_
26	1450	9.82	β-Curcumene	0.20	_
27	1494	10.24	γ.-Elemene	0.64	_
28	1400	10.43	(E)-β-Famesene	0.35	_
29	1524	10.49	β-Sesquiphellandrene	0.22	_
30	1471	10.67	Alloaromadendrene	0.21	_
31	1480	10.83	Ar-Curcumene	6.93	_
32	1508	10.91	Germacrene D	1.37	_
33	1496	10.98	Zingiberene	29.6	_
34	1505	11.08	α-Farnesene	4.68	_
35	1520	11.14	β-Bisabolene	7.31	_
36	1481	11.25	Methyl dodecanoate	_	0.05
37	1446	11.35	Cedr-8(15)-ene	12.62	_
38	1779	14.64	Methyl pentadecanoate	_	0.12
39	1541	13.28	7-epi-trans-sesquisabinene hydrate	0.81	_

Table 3 - Gas chromatography/mass spectrometry (GC/MS) profile of ginger rhizome and callus oil (continued).

No.	RI*	RT	Compound name	----------Area (%)----------	
				Rhizome	Callus
40	1680	13.56	Methyl myristate	0.29	0.5
41	1886	15.36	7,10-Hexadecadienoic acid	0.14	0.24
42	1886	15.47	Palmitoleic acid	_	0.15
43	1878	15.66	Palmitic acid	2.68	28.56
44	2093	17.31	Linoleic acid	7.19	18.63
45	2085	17.36	Oleic acid	0.61	31.11
46	2154	17.38	Linolenic acid	1.32	_
47	2077	17.57	Stearic acid	0.72	4.12
48	2085	18.97	17-Octadecynoic acid	_	0.34
49	4007	19.12	Gingerol	16.47	_
50	2284	19.13	cis-11-Eicosenoic acid	_	0.61
51	2212	19.33	Arachidic acid	_	0.94
52	2228	19.73	Oleamide	_	0.55
53	2788	20.25	Phenol,2,2-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	0.29	1.28
54	2411	20.95	Methyl behenate	_	0.66
55	2510	21.71	Methyl tricosanoate	_	0.28
56	2739	22.12	Stigmasterol	_	0.89
57	2674	22.45	Methyl lignocerate	_	1.08
58	2731	23.22	Δ^7-Stigmastenol	_	7.51
59	2398	23.59	Cholestane	_	0.51
			Total hydrogenated monoterpenes	0.47	_
			Total oxygenated monoterpenes	2.06	_
			Total sesquiterpenes hydrocarbons	6.93	_
			Total oxygenated sesquiterpenes	58.39	_
			Total fatty acids	14.31	87.43
			Total phenols	16.76	1.28
			Total steroids	_	8.91
			Total alcohol	_	1.83
			Total	98.92	98.82

*RI: retention indices, RT: retention time.

followed by phenols (16.76%), fatty acid (14.31%), sesquiterpenes hydrocarbons (6.93%), oxygenated monoterpenes (2.06%) and monoterpenes (0.47%). Zingiberene (sesquiterpene) was the most prominent compound found in highest concentration (29.6%) followed by gingerol (16.47%), cedr-8(15)-ene (12.62%), β- bisabolene (7.31%), linoleic acid (7.19%), Ar-Curcumene (6.93%) and α-farnesene (4.68%) respectively.

Twenty-two components were characterized from the callus oil and was dominated by high percent of fatty acid (87.43%) followed by steroids (8.91%), alcohol (1.83%) and phenols (1.28%) respectively. The major constituent of the callus oil was oleic acid (31.11%) followed by palmitic acid (28.56%), linoleic acid (18.63%), Δ7-Stigmastenol (7.51%) and stearic acid (4.12%) sequentially.

The CM extract of the untreated callus contained only four compounds dominated by Ethyl.α.Dglucopyranoside (92.96%) and followed by trimethyl citrate (3.29%), ethyl.α. palmitic acid (2.5%) and 1-dodecene (1.25%) respectively. GC/MS profile of treated callus CM extracts revealed the

Table 4 - Gas chromatography/mass spectrometry (GC/MS) profile of treated and untreated callus chloroform: methanol (1:1, v/v) extracts.

No.	RT	Compound name	Untreated callus	Yeast extract ----------(mg/L)----------			Glycine ----------(mg/L)----------			Salicylic acid ----(mg/L)----	
				100	300	500	100	200	300	50	100
1	3.28	Glycerol	_	_	_	_	_	_	_	5.19	8.87
2	4.55	4-Hydroxybutanoic acid	_	1.84	0.76	0.37	_	_	_	_	_
3	9.21	3,5-dihydroxy-6-methyl-2,3-dihydro -4H-Pyran-4-one	_	2.88	_	_	_	_	_	_	_
4	10.03	1-Dodecene	1.25	_	_	_	_	_	_	_	_
5	10.30	Nitroisobutylglycerol	_	_	_	_	31.34	38.73	33.47	_	_
6	15.33	Nitroisobuty glycerol	_	19.71	_	_	_	_	_	_	_
7	15.66	Trimethyl citrate	3.29	_	_	_	_	_	_	_	_
8	18.57	Ethyl.α.D- glucopyranoside	92.96	2.69	70.85	22.95	14.99	_	_	_	_
9	18.63	Methyl.β.-D-gluco pyranoside	_	68	3.87	_	_	31.74	60	_	_
10	18.66	β-Sitosterol	_	_	_	_	24	_	_	_	_
11	23.12	Palmitic acid	2.5	3.65	_	_	_	_	_	_	
12	23.77	Stigmasterol	_	_	_	_	11.29		_	_	25.3
13	24.91	Lupeol	_	_	11.58	_	_	_	_	9.78	_
14	25.33	Betulic acid	_	_	7.27	_	_	_	_	_	_
15	25.80	Betulin	_	_	_	_	_	_	_	4.15	_
16	25.84	Δ^7-Stigmastenol	_	_	_	_	_	15.23	_	44.54	_
17	26.35	β-Amyrin	_	_	_	_	_	_	_	6.81	_
18	26.49	2-Hydroxy-4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecan-9-one	_	_	_	_	_	_	_	14.14	_

presence of 3 to 7 compounds (Table 4, 5). Callus extracts treated with YE were mainly characterized by the presence of glucosides (22.95% - 74.72%), and those treated with GL by nitroisobutylglycerol (31.34% - 38.73%) and glucosides (14.99% – 60%) while those treated by SA were dominated by steroids (44.54% - 47.68%).

DISCUSSION

Plant tissue culture is considered as an effective technique to produce microbial free plants and a steady source of bioactive molecules for industrial and commercial purposes. Moreover, elicitation was proven as an excellent strategy to improve the *in vitro* yield of bioactive components in cultures (ALI et al., 2018). In the present study callus was obtained from ginger rhizome to evaluate their 5-Lox inhibitory property and then callus was treated with elicitors to determine their effect on callus yield, anti-5-Lox activity, and chemical profile. Treatment of callus with the three elicitors; YE, GL and SA highly increased the yield of callus CM extracts but not its oil content. Many previous studies reported the effects of elicitors on enhancing the yield and production of high-added value plant compounds (ALI et al., 2018; CAI et al., 2014; RAMIREZ-ESTRADA et al., 2016).

Results of the anti-5-Lox activity of ginger rhizome and callus revealed that the callus oil displayed remarkable anti-5-Lox activity with IC_{50} value (58.33±4.66 µg/mL) significantly ($P < 0.05$) lower than that of the standard control Nordihydroguaiaretic acid (61.25±1.02 µg/mL). Even the CM extract of the callus showed remarkable anti-5-Lox activity with IC_{50} value comparable to that exerted by 6-shogaol (87.65±4.35 µg/mL). Previous studies reported that, the anti-5-Lox activity of ginger

Table 5 - Gas chromatography/mass spectrometry (GC/MS) profile of treated and untreated callus chloroform: methanol (1:1, v/v) extracts (continued).

No.	RT*	Compound name	Untreated callus	Yeast extract ---------(mg/L)---------			Glycine --------(mg/L)--------			Salicylic acid ----(mg/L)---	
				100	300	500	100	200	300	50	100
19	26.53	1a.2,5,5Tetramethyl-trans1a,4a,5,6,7,8-hexahydro-gamma-chromene	_	_	_	29.38	_	_	_	_	_
20	26.62	Desmosterol	_	_	_	_	_	_	_	_	22.4
21	26.79	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7, 11,15-tetraenyl)-cyclohexanol	_	_	_	24.74	_	_	_	_	_
22	26.92	Humulane-1,6-dien-3-ol	_	_	_	11.68	_	_	_	_	_
23	27.06	Urs-12-en-28-al,3-(acetyloxy)-,(3.β,)-	_	_	_	10.43	_	_	_	_	_
24	27.83	Phenol,2,2-methylenebis{6-(1,1-dimethylethyl)-4-methyl-	_	0.69	_	_	3.7	6.79	2.01	_	_
25	27.78	9.β.-Acetoxy-4-hydroxy-3,4, 8-trimethyl-5.α.Htricyclo [6.3.1.0(1,5)] dodecane	_	_	5.66	_	_	_	_	_	_
26	27.92	Nonacosane	_	_	_	_	_	_	_	_	4.3

*RT: retention time.

rhizome is mainly attributed to its major pungent compounds 6-gingerol and 6-shogaol with the latter exhibited potent anti-inflammatory. They exert their anti-inflammatory activity through the suppression effect on leukotriene biosynthesis by inhibiting 5-Lox (EZZAT et al., 2018; FLYNN et al., 1986; GRZANNA et al., 2005; KIUCHI et al., 1992). Chemical profile of the oil and CM extract of the callus indicated the absence of 6-gingerol, 6-shogaol, zingiberene and gingerol which were known as the major compounds of rhizome (KAMALIROOSTA et al., 2013; KIZHAKKAYIL & SASIKUMAR, 2012; NAMPOOTHIRI et al., 2012). Instead, the callus oil was rich in fatty acids (87.43 %). The unsaturated fatty acids, oleic acid (31.11%) and linoleic acid (18.63%) representing 49.9 % of the total fatty acid content and the saturated fatty acid, palmitic acid represented 28.56%. Many studies have demonstrated that callus extracts produced large amounts of fatty acids (BERNABÉ-ANTONIO et al., 2015; JACOMINI et al., 2015). The differences in fatty acids compositions in the oil of callus tissues and their mother plant may be related to variations in the gene expression of different cells, which showed alterations in their metabolism (DA LUZ COSTA et al., 2015). The anti-inflammatory activity of fatty acids was reported by HENRY et al. (2002) where they found that oleic acids possessed COX-I inhibitory activities while linoleic and linolenic acids showed appreciable COX-I and COX-II inhibitory activities. Linoleic acid inhibited the COX and LOX pathways of arachidonate metabolism (SINGH & MAJUMDAR, 1997). However, palmitic acid was reported to have marginal COX-I and COX-II inhibitory activities (HENRY et al., 2002). Thus, it could be suggested that the high fatty acid contents of the callus might also play considerable role in its anti-5-Lox activity.

Treatment of callus with different elicitors increased the yield of callus CM extracts and significantly ($P < 0.05$) improved its capacity to inhibit the 5-Lox activity by 4.82% to 33.16% according to elicitor used and its concentration. The highest effect was obtained from the treatment of the callus by YE at 100 and 300 mg/L respectively followed by 50 mg/L of SA. Callus treated by GL showed the least effect. The chemical profiling of CM extracts of callus elicited with elicitors was generally different and revealed an enhancement in the production of some bioactive compounds that were not detected in untreated callus. Plant cells *in vitro*, displayed physiological and morphological responses

to the elicitors that could induce or enhance synthesis of secondary metabolites in plant cells or tissue to ensure their survival, persistence, and competitiveness (NAMDEO et al., 2002). Furthermore, some of the identified compounds in the treated callus extracts were proven to possess anti-inflammatory properties like lupeol which was identified in the CM extracts of callus treated with YE300 mg/L (11.58 %) and SA50 mg/L (9.78 %). A study carried out by THIRUMALAISAMY et al. (2020) showed that lupeol exhibited anti-inflammatory activity against the five targets of inflammation: COX-2, MPO, TNFα, IL1β and IL6, respectively. Also, β-amyrin (6.81% from callus treated with SA50 mg/L) significantly inhibited PGE2, IL-6 secretion, and NF-κB activation (KRISHNAN et al., 2014). Moreover, a previous study showed a significant increase in the total polyphenolic content of CM extracts of ginger callus especially those treated with YE100 mg/L and SA50 mg/L suggesting that phenolic compounds could also contributed to the observed activity (ALI et al., 2018).

CONCLUSION

In conclusion, this is the first study on the anti-5-Lox activity and chemical profile of ginger callus and callus treated with YE, SA and GL as elicitors. Results showed that the oil extracted from the callus was rich in fatty acids and exerted anti-5-Lox activity higher than the oil extracted from the intact rhizome and standard control as well. Elicitors modified the chemical profile of the callus and ameliorated the anti-5-Lox activity of CM extract of the callus. Further study is warranted to determine the phytochemical(s) responsible for this current observed bioactivity; and consequently, could lead to the development of potential natural-based anti5-Lox agent from ginger callus.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this research through research group no. (RGP-1438-053).

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

ALI, A. M. A. et al. Callus induction, direct and indirect organogenesis of ginger (*Zingiber officinale* Rosc). **African Journal of Biotechnology**, v.15, n.38, p. 2106-2114, 2016. Available from: <https://www.ajol.info/index.php/ajb/article/view/144451>. Accessed: Mar. 22, 2021. doi: 10.5897/AJB2016.15540.

ALI, A. M. A. et al. *In vitro* anti-inflammatory activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors. **Journal of Medicinal Plants Research**, v.13, n.10, p.227-235, 2019. Available from: <https://academicjournals.org/journal/JMPR/article-abstract/B19AD1C61046>. Accessed: Mar. 22, 2021. doi: 10.5897/JMPR2019.6758.

ALI, A. M. A. et al. Total phenolic and flavonoid contents and antioxidant activity of ginger (*Zingiber officinale* Rosc.) rhizome, callus and callus treated with some elicitors. **Journal of Genetic Engineering and Biotechnology**, v.16, n.2, p.677-682, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S1687157X18300210>. Accessed: Mar. 22, 2021. doi: 10.1016/j.jgeb.2018.03.003.

ARABLOU, T. et al. The effect of ginger consumption on glycemic status, lipid profile and some inflammatory markers in patients with type 2 diabetes mellitus. **International Journal of Food Sciences and Nutrition**, v.65, n.4, p.515-520, 2014. Available from: <https://www.tandfonline.com/doi/abs/10.3109/09637486.2014.880671?journalCode=iijf20>. Accessed: Apr. 10, 2021. doi: 10.3109/09637486.2014.880671.

ARAUJO, P. et al. The Effect of Omega-3 and Omega-6 Polyunsaturated Fatty Acids on the Production of Cyclooxygenase and Lipoxygenase Metabolites by Human Umbilical Vein Endothelial Cells. **Nutrients**, v.11, n.5, p.966, 2019. Available from: <https://www.mdpi.com/2072-6643/11/5/966>. Accessed: Oct. 12, 2021. doi:10.3390/nu11050966.

BERNABÉ-ANTONIO, A. et al. Fatty acid profile of intact plants of two different sites and callus cultures derived from seed and leaf explants of *Calophyllum brasiliense* Cambess: A new resource of non-edible oil. **Industrial Crops and Products**, v.77, p.1014-1019, 2015. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0926669015304490>. Accessed: Mar. 10, 2021. doi: 10.1016/j.indcrop.2015.10.004.

CAI, Z. et al. Chitosan or yeast extract enhance the accumulation of eight phenolic acids in cell suspension cultures of *Malus* × *domestica* Borkh. **The Journal of Horticultural Science and Biotechnology**, v.89, n.1, p.93-99, 2014. Available from: <https://www.tandfonline.com/doi/abs/10.1080/14620316.2014.1151305>. Accessed: Feb. 10, 2021. doi: 10.1080/14620316.2014.11513054.

DA LUZ COSTA, J. et al. Callus growth kinetics of physic nut (*Jatropha curcas* L.) and content of fatty acids from crude oil obtained *in vitro*. **Applied Biochemistry and Biotechnology**,

v.176, n.3, p.892-902, 2015. Available from: <https://link.springer.com/article/10.1007%2Fs12010-015-1618-y>. Accessed: Mar. 29, 2021. doi: 10.1007/s12010-015-1618-y.

EZZAT, S. M. et al. The hidden mechanism beyond ginger (*Zingiber officinale* Rosc.) potent *in vivo* and *in vitro* anti-inflammatory activity. **Journal of Ethnopharmacology**, v.214, p.113-123, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0378874117328763>. Accessed: Mar. 23, 2021. doi: 10.1016/j.jep.2017.12.019.

FLYNN, D. L. et al. Inhibition of human neutrophil 5-lipoxygenase activity by gingerdione, shogaol, capsaicin and related pungent compounds. **Prostaglandins, Leukotrienes and Medicine**, v.24, n.2-3, p.195-198, 1986. Available from: <https://www.sciencedirect.com/science/article/abs/pii/0262174686901265>. Accessed: Apr. 19, 2021. doi: 10.1016/0262-1746(86)90126-5.

FRUM, Y. & VILJOEN, A. M. *In vitro* 5-lipoxygenase activity of three indigenous South African aromatic plants used in traditional healing and the stereospecific activity of limonene in the 5-lipoxygenase assay. **Journal of Essential Oil Research**, v.18, n.1, p.85-88, 2019. Available from: <https://www.tandfonline.com/doi/abs/10.1080/10412905.2006.12067127>. Accessed: Mar. 01, 2021. doi: 10.1080/10412905.2006.12067127.

GRZANNA, R. et al. Ginger—An Herbal Medicinal Product with Broad Anti-Inflammatory Actions. **Journal of Medicinal Food**, v.8, n.2, p.125-132, 2005. Available from: <https://www.liebertpub.com/doi/10.1089/jmf.2005.8.125>. Accessed: Apr. 13, 2021. doi: 10.1089/jmf.2005.8.125.

HENRY, G. E. et al. Antioxidant and Cyclooxygenase Activities of Fatty Acids Found in Food. **Journal of Agricultural and Food Chemistry**, v. 50, n. 8, p. 2231-2234, 2002. Available from: <https://pubs.acs.org/doi/10.1021/jf0114381>. Accessed: Mar. 21, 2021. doi: 10.1021/jf0114381.

JACOMINI, D. et al. Lipid profile and antiproliferative activity of callus cultures of *Cereus peruvianus* Mill. **Industrial Crops and Products**, v.69, p.408-414, 2015. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0926669015001296>. Accessed: Mar. 10, 2021. doi: 10.1016/j.indcrop.2015.02.034.

JEENA, K. et al. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. **Indian Journal of Physiology and Pharmacology**, v.57, n.1, p.51-62, 2013. Available from: <http://naturalingredient.org/wp/wp-content/uploads/51-62.pdf>. Accessed: Apr. 1, 2021.

KALAVA, A. et al. Efficacy of ginger on intraoperative and postoperative nausea and vomiting in elective cesarean section patients. **European Journal of Obstetrics & Gynecology and Reproductive Biology**, v.169, n.2, p.184-188, 2013. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0301211513001024>. Accessed: Mar. 12, 2021. doi: 10.1016/j.ejogrb.2013.02.014.

KAMALIROOSTA, Z. et al. Isolation and identification of ginger essential oil. **Journal of Food Biosciences and Technology**, v.3, p.73-80, 2013. Available from: <https://naturalingredient.org/wp/wp-content/uploads/JFBT-Ginger.pdf>. Accessed: Mar. 29, 2021.

KARAMAN, S. et al. A randomized placebo-controlled study of aromatherapy for the treatment of postoperative nausea and vomiting. **Complementary Therapies in Medicine**, v.42, p.417-421, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0965229918312330>. Accessed: Mar. 10, 2021. doi: 10.1016/j.ctim.2018.12.019.

KIUCHI, F. et al. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. **Chemical and Pharmaceutical Bulletin**, v.41, n.2, p.387-391, 1992. Available from: <https://www.jstage.jst.go.jp/article/cpb1958/40/2/40_2_387/_article>. Accessed: Apr. 19, 2021. doi: 10.1248/cpb.40.387.

KIZHAKKAYIL, J. & SASIKUMAR, B. Characterization of ginger (*Zingiber officinale* Rosc.) germplasm based on volatile and non-volatile components. **African Journal of Biotechnology**, v.11, n.4, p.777-786, 2012. Available from: <https://www.ajol.info/index.php/ajb/article/view/100208>. Accessed: Mar. 01, 2021. doi: 10.5897/AJB11.292.

KRISHNAN, K. L. et al. Anti-inflammatory potential of β-amyrin, a triterpenoid isolated from *Costus igneus*. **Inflammopharmacology**, v.22, n.6, p.373-385, 2014. Available from: <https://link.springer.com/article/10.1007%2Fs10787-014-0218-8>. Accessed: Mar. 28, 2021. doi: 10.1007/s10787-014-0218-8.

KULKARNI, R. A. & DESHPANDE, A. R. Anti-inflammatory and antioxidant effect of ginger in tuberculosis. **Journal of Complementary and Integrative Medicine**, v.13, n.2, p.201-206, 2016. Available from: <https://www.degruyter.com/document/doi/10.1515/jcim-2015-0032/html>. Accessed: Mar. 15, 2021. doi: 10.1515/jcim-2015-0032.

LAUGHTON, M. J. et al. Inhibition of mammalian 5-lipoxygenase and cyclo-oxygenase by flavonoids and phenolic dietary additives: relationship to antioxidant activity and to iron ion-reducing ability. **Biochemical pharmacology**, v.42, n.9, p.1673-1681, 1991. Available from: <https://www.sciencedirect.com/science/article/abs/pii/000629529190501U>. Accessed: Oct. 12, 2021. doi: 10.1016/0006-2952(91)90501-u.

MASHHADI, N. S. L. et al. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. **International Journal of Preventive Medicine**, v.4, n.1, p.S36-S42, 2013. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3665023>. Accessed: Apr. 13, 2021.

NAMDEO, A. et al. Influence of fungal elicitors on production of ajmalicine by cell cultures of *Catharanthus roseus*. **Biotechnology Progress**, v.18, n.1, p.159-162, 2002. Available from: <https://aiche.onlinelibrary.wiley.com/doi/abs/10.1021/bp0101280>. Accessed: Mar. 28, 2021. doi: 10.1021/bp0101280.

NAMPOOTHIRI, S. V. et al. Comparison of essential oil composition of three ginger cultivars from sub-Himalayan region. **Asian Pacific Journal of Tropical Biomedicine**, v.2, n.3, p.S1347-S1350, 2012. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S2221169112604146>. Accessed: Mar. 10, 2021. doi: 10.1016/S2221-1691(12)60414-6.

NIKKHAH-BODAGHI, M. et al. *Zingiber officinale* and oxidative stress in patients with ulcerative colitis: A randomized, placebo-controlled, clinical trial. **Complementary Therapies in Medicine**, v.43, p.1-6, 2019. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0965229918310008>. Accessed: Mar. 13, 2021. doi: 10.1016/j.ctim.2018.12.021.

PRASAD, S. & TYAGI, A. K. Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer.

Gastroenterology Research and Practice, Article ID. 142979, p.11, 2015. Available from: <https://www.hindawi.com/journals/grp/2015/142979/>. Accessed: Mar. 15, 2021. doi: 10.1155/2015/142979.

RAD, H. A. et al. Effect of Ginger and Novafen on menstrual pain: A cross-over trial. **Taiwanese Journal of Obstetrics and Gynecology**, v.57, n.6, p.806-809, 2018. Available from: <https://www.sciencedirect.com/science/article/pii/S1028455918302304>. Accessed: Apr. 13, 2021. doi: 10.1016/j.tjog.2018.10.006.

RAMIREZ-ESTRADA, K. et al. Elicitation, an Effective Strategy for the Biotechnological Production of Bioactive High-Added Value Compounds in Plant Cell Factories. **Molecules**, v.21, n.2, p.182, 2016. Available from: <https://www.mdpi.com/1420-3049/21/2/182>. Accessed: Mar. 1, 2021. doi: 10.3390/molecules21020182.

SHIDFAR, F. et al. The effect of ginger (*Zingiber officinale*) on glycemic markers in patients with type 2 diabetes. **Journal of Complementary and Integrative Medicine**, v.12, n.2, p.165-170, 2015. Available from: <https://www.degruyter.com/document/doi/10.1515/jcim-2014-0021/html>. Accessed: Mar. 13, 2021. doi: 10.1515/jcim-2014-0021.

SHIRVANI, M. A. et al. The effect of mefenamic acid and ginger on pain relief in primary dysmenorrhea: a randomized clinical trial. **Archives of Gynecology and Obstetrics**, v.291, n.6, p.1277-1281, 2015. Available from: <https://link.springer.com/article/10.1007/s00404-014-3548-2>. Accessed: Apr. 13, 2021. doi: 10.1007/s00404-014-3548-2.

SINGH, S. & MAJUMDAR, D. K. Evaluation of anti-inflammatory activity of fatty acid of *Ocimum sanctum* fixed oil. **Indian Journal of Experimental Biology**, v.35, n.4, p.380-383, 1997. Available from: <https://europepmc.org/article/med/9315239>. Accessed: Apr. 18, 2021.

THIRUMALAISAMY, R. et al. *In-Vitro* and *In-Silico* Anti-inflammatory Activity of Lupeol Isolated from *Crateva adansonii* and Its Hidden Molecular Mechanism. **International Journal of Peptide Research and Therapeutics**, v.26, p.2179-2189, 2020. Available from: <https://link.springer.com/article/10.1007/s10989-019-10006-5>. Accessed: Mar. 13, 2021. doi: 10.1007/s10989-019-10006-5.