Journal of Complementary and Integrative Medicine

Volume 9, *Issue* 1 2012 *Article* 11

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Recommended Citation:
Wijaya, Sumi; Jin, Khoo Teng; Nee, Ting Kang; and Wiart, Christophe (2012) "In Vitro 5-LOX Inhibitory and Antioxidant Activities of Extracts and Compounds from the Aerial Parts of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm.," *Journal of Complementary and Integrative Medicine*: Vol. 9: Iss. 1, Article 11.
DOI: 10.1515/1553-3840.1615

In Vitro 5-LOX Inhibitory and Antioxidant Activities of Extracts and Compounds from the Aerial Parts of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm.

Sumi Wijaya, Khoo Teng Jin, Ting Kang Nee, and Christophe Wiart

Abstract

The aim of this study was to investigate the antioxidant and anti-inflammatory potentials of crude extracts of Lopholaena coriifolia (Sond.) E. Phillips & C.A. Sm. and its isolated compounds. Separation and structure elucidation of Lopholaena coriifolia (Sond.) E. Phillips & C.A. Sm. were conducted using chromatographic and spectroscopic method. The antioxidant activities of the extracts in this study were determined by the ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching assays meanwhile the anti-inflammatory activity was evaluated using the 5-lipoxygenase assay. Seven known compounds quercetin 3-O-glucoside (1), naringenin 7-O-glucoside (2), seneciphylline-O-glucoside (3), chrysoeriol (4), retrorsine (5), adonifiline (6) and 5,4′-di-O-methyl alpinumisoflavone (7) were isolated from ethanol extract of Lopholaena coriifolia (Sond.) E. Phillips & C.A. Sm. The ethanol and water extracts of Lopholaena coriifolia (Sond.) E. Phillips & C.A. Sm. elicited potent antioxidant and anti-inflammatory properties. Amongst the isolated compounds quercetin 3-O-glucoside gave strong antioxidant activity and adonifiline strongly inhibited 5-lipoxygenase activity.

KEYWORDS: Lopholaena coriifolia, flavonoids, alkaloids, antioxidant, anti-inflammatory

1. Introduction

Free radicals and reactive oxygen species (ROS) cause tissue damages that account for stroke, heart attack, artery disease, neurodegenerative diseases, diabetes, cancer and inflammation (Eposito et al., 2002). Antioxidants can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, and therefore, they appear to be very important in the prevention of many age-related diseases (Halliwell et al., 1992; Ames et al., 1990).

Inflammatory is a complex biological response of vascular tissue to harmful stimuli, such as pathogens, damaged cells or irritants (Khan et al., 2010). The process of inflammation plays a detrimental role in the pathogenesis of most chronic illnesses, including neurodegenerative diseases. The therapeutic potential of anti-inflammatory agents in the prevention and treatment of chronic disorders has been highlighted (Aggarwal and Harikumar, 2008). Several methods are available to measure anti-inflammatory activities. In this study, the 5-lipoxygenase (5-LOX) inhibitory assay was used to measure the anti-inflammatory properties. 5-LOX is a lipid-peroxidising enzyme that plays an essential role in the biosynthesis of leukotrienes, which mediate inflammatory and allergic reactions (Baylac and Racine, 2003; Choudhary et al., 2009) and is theoretically very sensitive to antioxidants because of its non-heme iron atom in the active site of the enzyme which undergoes redox recycling for activation (Young, 1999; Schneider and Bucar, 2005).

Inflammatory processes also believe involved reactive oxygen species, started by leukocytes activation. Grabmann et al. (2000) found that eucalyptus and myrtle essential oils attenuated leucocytes activation by scavenging hydroxyl radicals indirectly produced by leukocytes degranulation, thereby interfering with inflammatory processes by acting as antioxidants. Therefore, screening of antioxidant properties may provide important information about the potential activity of a drug on inflammatory processes (Njenga and Viljoen, 2006).

In recent years, much attention has been devoted to natural antioxidants and their association with health benefits (Arnous et al., 2001). Plants and natural products possess antioxidant activities. These antioxidants are used to protect plants against the damage caused by active oxygen formed due to exposure to ultraviolet radiation (Craig, 1999). *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm., a member of the Asteraceae family, is known as small-leaved fluff bush. This plant is used to treat convulsions and wound healing in Zimbabwe and is also used as a sedative and for epilepsy treatment in Southern African (Gelfand et al., 1985). Previous phytochemical analysis of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. resulted in the isolation of caryophyllene, furanoeremophilanes, germacrene, bicyclogermacene, α-humulene, polyisoprene,

α-zingiberene and α-curcumene (Bohlmann & Wallmeyer, 1982). The aim of this study was to investigate the antioxidant and anti-inflammatory potential of the crude extracts of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. and its chemical constituents. The correlation between the activities was also examined. The antioxidant activities of the extracts in this study were determined by the ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene bleaching assays meanwhile the anti-inflammatory activity was examine using 5-lipoxygenase assay.

2. Materials and Methods

2.1. Plant material and extraction

The aerial part of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. was collected from Broga, Selangor, Malaysia in March 2009, and identified by Dr. Christophe Wiart, University of Nottingham Malaysia campus. Voucher specimens (UNMC49W) was deposited in the herbarium of School of Pharmacy, Faculty of Science, University of Nottingham Malaysia Campus. Air-dried and finely milled samples (500 g) were extracted by hexane, ethyl acetate, ethanol and water sequentially. The extracts were concentrated using rotary evaporator (Buchi, USA) under reduced pressure at 40°C. Dried extracts were kept at -20°C until further tests were carried out. For stock solutions, 100 mg/ml of each extract was dissolved in DMSO (dimethyl sulfoxide).

2.2. Fractionation and isolation

The ethanol extract (6 gram) was fractionated by column chromatography on silica gel using a linear gradient from $CHCl_3$-MeOH to yield 100 fractions. The fractions obtained were grouped and coded A (1-40) and B (41-100). Separation on fraction B (2.2 gram) on Sephadex LH-20 using ethanol 100% and ethanol-water (9:1 to 7:3, v/v) successively, yielded 66 fractions. Fraction 37-66 were combined and fractionated by HPLC (Varian, Australia) series LC-940 liquid chromatography system with PDA. The separation was achieved on a Pursuit XRs C_{18} column (150 x 4.6 mm; i.d.: 10 μm) eluted with a linear gradient of methanol-water containing 1% formic acid from 30:70 to 70:30 in 15 min. The flow rate was 0.5 ml/min and UV detection (PDA) was recorded between 190-400 nm. Structure elucidation of the isolated compounds was employed spectroscopic techniques of mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy. Mass spectrometry was performed on triple quadrupole mass spectrometer, Varian 325-MS with ESI interface, 212-LC pumps (Varian Inc.,

USA) meanwhile NMR spectrometry was performed on Bruker DRX 500 spectrometer for ^1H proton using CD_3OD solutions.

2.3. Ferric reducing antioxidant power (FRAP) assay

The antioxidant activity of the extracts was estimated by the FRAP method of Benzie and Strain (1996) with slight modifications. The working FRAP reagent was prepared freshly by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) and 20 mM $FeCl_3.6H_2O$ in a 10:1:1 ratio. Briefly, 180 µl of the FRAP reagent was mixed with 20 µl of the test sample, to obtain a final concentration of 1/10. Readings were taken after 90 minutes (λ: 593 nm) using a spectrophotometer (Dynex MRX-Revelation, USA). Ferrous sulphate concentrations in the range 1 µM to 125 µM ($FeSO_4.7H_2O$) were used for calibration. Trolox and quercetin were used as positive controls. FRAP values were calculated as Ferrous Equivalents: the concentration of trolox/quercetin or extracts which produced an absorbance value equal to 1 mM of $FeSO_4$.

2.4. β-Carotene bleaching assay

The *β*-carotene bleaching assay was conducted according to Miller (1971) with some modifications. A solution of *β*-carotene was prepared by dissolving 2 mg of β-carotene in 10 ml of chloroform. Two ml of this solution was pipetted into a 100 ml round-bottom flask. After removal of chloroform in vacuo, 40 mg of linoleic acid, 400 mg of tween 80, and 100 ml of distilled water were added to the flask with vigorous shaking. The zero time absorbance was measured at λ: 490 nm using a spectrophotometer (Dynex MRX-Revelation, USA). Absorbance readings were recorded at 20 min intervals for 240 minutes. A blank, devoid of *β*-carotene, was prepared for background subtraction. Percentage of antioxidant activity (AA) was calculated using the following equation: % AA = ((DR control-DR sample)/DR control) x 100, where DR is degradation rate of sample (DR = ln (initial absorbance at time zero)/(absorbance at 240 minutes)/t (time in minutes)). The effective concentration values exhibiting 50% of the antioxidant activity of samples (EC_{50}) were calculated from the graph of antioxidant activity percentage against concentration of the extracts.

2.5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

The DPPH assay was conducted according to the method of Juan-Badaturuge et al. (2011). Twenty µl of sample (1 mg/ml to 0.003 mg/ml) were pipetted into each well. One hundred and eighty µl of DPPH (0.1 mM) were added. The plates were kept in the dark at room temperature for 30 minutes. The percentage of

decolourisation was measured spectrophotometrically at λ: 550 nm. The DPPH scavenging effect (%) was calculated using the following equation: % Scavenging effect = ((A control- A sample)/A control) x 100.

2.6. Anti-inflammatory – 5-Lipoxygenase assay

The anti-inflammatory activity was determined using the method of Baylac and Racine (2003) with linoleic acid as the substrate. Crude plant extracts (50 mg/ml) were prepared. Five μl of extract was mixed with 970 μl of phosphate buffer (pH 9) and 17 μl of linoleic acid in a 1 ml cuvette maintained at 25° C. The mixture was shaken and 4 μl of the aliquot enzyme and 4 μl of the phosphate buffer (4° C) were pippeted to initiate enzyme reaction. Absorbance was measured at λ: 234 nm over a period of 10 minutes using spectrophotometer (Libra, USA). Absorbance was plotted graphically against the different concentrations used. Nordihydroguaiaretic acid (NDGA) was used as the positive control. The slopes of the straight-line portions of the sample and the control curves were used to determine the percentage activity of the enzyme (Lourens et al., 2004).

2.7. Statistical analysis

All data were expressed as mean ± standard deviation. Data were analyzed using one way ANOVA followed by Tukey test using GraphPad Prism5 software. A significant difference was considered at the level of $P < 0.01$.

3. Results and discussion

3.1. Antioxidant and anti-inflammatory properties of the crude extracts

The antioxidant properties of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. extracts were determined using the FRAP, DPPH and β-carotene bleaching assays. The FRAP assay is commonly used to determined the ferric reducing ability of biological fluids and aqueous solutions of active compounds from plants (Alothman et al., 2009; Pulido et al., 2000). The mechanism of action of this method is based on the reduction of Fe^{3+} - TPTZ complex to ferrous form at low pH (Benzie and Strain, 1996). The results were defined as FRAP value, the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L $FeSO_4.7H_2O$. Results obtained in the present study revealed that the reducing ability of the extracts were in the range of 0.74 – 71.57 μg/ml (Table 1). Lower FRAP value indicate greater antioxidant activities. The antioxidant activities of all extracts (except for hexane) were comparable to those of quercetin

and trolox and the rank order of antioxidant activity was E =W > trolox > quercetin > EA > H.

On the other hand, the antioxidant capacity of lipophilic compounds was determined using the *β*-carotene bleaching method. The mechanism of action of the *β*-carotene bleaching assay is based on *in vitro* bleaching of *β*-carotene. Radicals released upon the oxidation of linoleic acid in the emulsion will attack *β*-carotene molecules and leads to discoloration of the emulsion. The extent of discoloration is measured at 490 nm (Koleva et al., 2002; Cao et al., 2009). The rank order of potency observed in the *β*-carotene bleaching assay was trolox > quercetin > E > EA > H > W (Table 1). The lower EC_{50} indicate the higher antioxidant activity. The ethanolic extract of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. was able to interact with the stable free DPPH radicals efficiently and quickly by hydrogen atom transfer (Abbasi et al., 2011), with IC_{50} values of 106.14 µg/ml. Overall, the ethanol extract exhibited the promising value for antioxidant activities.

Table 1. Antioxidant and anti-inflammatory properties of crude extracts and isolated compounds of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm.

Sample		FRAP assay	DPPH assay	*β*-carotene bleaching assay	Anti-inflammatory assay
		FRAP value (*µ*g/ml)	IC_{50} (*µ*g/ml)	Ec_{50} (*µ*g/ml)	IC_{50} 5-LOX (*µ*g/ml)
Lopholaena coriifolia	H	71.57 ± 0.01A	1434.85 ± 0.00A	68.66 ± 0.49A	27.93 ± 0.01A
(Sond.) E. Phillips & C.A.	EA	1.57 ± 0.04B	158.14 ± 0.001B	17.07 ± 0.37B	15.24 ± 0.00B
Sm.	E	0.74 ± 0.05B	106.14 ± 0.01C	0.11 ± 0.02C	176.75 ± 0.11C
	W	0.74 ± 0.11B	177.45 ± 0.01D	148.9 ± 0.57D	12.02 ± 0.00B
Quercetin 3-O-Glucoside		n.d	0.03 ± 0.01E	1.04 ± 0.04E	29.26 ± 0.00A
Naringenin-7-O-Glucoside		n.d	0.65 ± 0.01F	6.31 ± 0.01F	34.57 ± 0.01D
Seneciphylline-O-Glucoside		73.11 ± 0.09A	n.d	8.71 ± 0.00F	46.38 ± 0.01E
Chrysoeriol		42.47 ± 0.02C	n.d	3.50 ± 0.01G	71.11 ± 0.02F
Adonifiline		85.65 ± 0.08D	n.d	24.76 ± 0.01H	26.84 ± 0.00A
Retrorsine		64.07 ± 0.01E	n.d	22.44 ± 0.01H	31.26 ± 0.00A
5,4′-di-O-methyl alpinumisoflavone		47.39 ± 0.08C	n.d	8.42 ± 0.02F	47.13 ± 0.01E
Quercetin (standard)		1.31 ± 0.02B	0.11 ± 0.17E	0.13 ± 0.17C	-
Trolox (standard)		1.14 ± 0.02B	0.09 ± 0.17E	0.05 ± 0.17C	-
NDGA (standard)		-	-	-	5.33 ± 0.05G
Aspirin (standard)		-	-	-	13.90 ± 0.07B

Data were obtained from three independent experiments, each performed in triplicates (n=9) and represented as mean ± SD. Values with the same superscript letter are not significantly different (P<0.01) according to Tukey multiple comparison test.

H: Hexane extract; EA: Ethyl acetate extract; E: Ethanol extract; W: Water extract

Inhibition of the biosynthesis of inflammatory mediators by blocking the activities of 5-lipoxygenase and cyclooxygenase-1 is considered as a promising approach to treat inflammatory diseases. 5-LOX inhibitors are potential new drugs to treat inflammation, since they act by blocking the formation of both prostaglandins and leukotrienes (Flamand et al., 2006). The *in vitro* anti-

inflammatory activity of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. extracts, are shown in Table 1. Lower IC_{50} 5-LOX values indicate greater anti-inflammatory activities. The water extract displayed the highest inhibitory activity, with an IC_{50} value of 12.02 μg/ml.

3.2. Isolation of bioactive compounds

The study of bioactive compounds from the ethanol extract of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. led to identification of seven known compounds (Figure 1) which were first time isolated and reported for this plant. The description of the compounds was as follow:

Compound-1, Quercetin 3-O-Glucoside: The chromatographic behavior of this compound proved that it is an aglycone. This compound had characteristic MS fragment at *m/z* 303, 179 and 151 in positive mode confirms the identity of quercetin. It exhibited peak with *m/z* 465, losing 162 u (hexose unit) at *m/z* 303 $[M+H-162]^+$ confirmed the identity of the compound was quercetin-3-O-Glucoside or known as isoquercetrin with the molecular formula $C_{21}H_{20}O_{12}$. The ^{1}H-NMR data showed signals at δ in ppm 6.97 (1H, *d*, J = 8 Hz, H-2'), 7.56 (1H, *dd*, J = 9 Hz, H-4'), 7.77 (1H, *d*, J = 8.5 Hz, H-5'), 5.30 (1H, *d*, H-3′, H-5, H-7, H-4′), 6.53 (1H, *d*, J = 3 Hz, H-8) and 6.32 (1H, *d*, J = 3 Hz, H-6) which are in agreement with those reported for quercetin 3-O-glucoside (Kashif et al., 2009), so compound 1 could be identified as quercetin 3-O-glucoside.

Compound-2, Naringenin-7-O-Glucoside: A pale yellow needles [MeOH]; UV λmax (MeOH) nm: 286, 332; ^{1}H NMR spectral data : aglycon δ 12.06 (1H, *brs*, 5-OH), 9.61 (1H, *brs*, 4`-OH), 7.33 (2H, *d*, *J*= 8.5 Hz, H-2`,6`), 6.81 (2H, *d*, *J*= 8.5 Hz, H-3`,5`), 6.16 (1H, *d*, *J*= 2.1 Hz, H-8), 6.14 (1H, *d*, *J*= 2.1 Hz, H-6), 5.51 (1H, *dd*, *J*= 12.7/3.0 Hz, H-2), 3.35 (1H, *dd*, *J*=17.1/12.7 Hz, H-3_{ax}), 2.76 (1H, *dd*, *J*= 17.1/3.0 Hz, H-3_{eq}); sugar moiety d 4.97 (1H, *d*, *J*= 7.7 Hz, H-1``), 3.68 (1H, *dd*, *J*= 11.8/3.3 Hz, H-6b``), 3.46 (1H, *dd*, *J*= 11.8/5.8 Hz, H-6a``), 3.39 (1H, *m*, H-5``), 3.28 (1H, *t*, *J*= 9.2 Hz, H-3``), 3.23 (1H, *dd*, *J*= 7.7/9.2 Hz, H-2``), 3.16 (1H, *dd*, *J*= 9.2/9.1 Hz, H-4``). The mass spectrum of this compound displayed a molecular ion peak at *m/z* = 434.4 $[M+H]^+$ corresponding to the molecular formula of $C_{20}H_{20}O_{10}$ also the peaks at *m/z* = 272 $[M+H - \text{hexose}]^+$ confirm the identity of naringenin-7-O-glucoside. These data were in agreement with that reported for naringenin-7-O-glucoside (Ragab et al., 2010), so we can identify compound-2 as naringenin-7-O-glucoside.

Compound-3, Seneciphylline-O-Glucoside: Positive ion electrospray mass spectrometry revealed compound-3 with a prominent $[M + H]^+$ ion at *m/z* 496 and fragmentation ion showed up at *m/z* 334 as base peak. The loss of 162 *u* formally corresponds to the elimination of a hexose unit at formation of the protonated alkaloid. From these data we conclude, that the compound with a $[M + H]^+$ at *m/z*

496 represents an *O*-glycoside composed of a hexose and seneciphylline. The ^{1}H NMR data (Table 2) similar with the NMR data from Segal and Dallas (1983).

Compound-4, Chrysoeriol: The UV absorption spectrum of the compound-4 in spectroscopic methanol displayed band-I at 337 nm, which indicates the flavone nature of this compound. The mass spectrum of this compound displayed a molecular ion peak at *m/z* = 300 $[M+H]^+$corresponding to the molecular formula of $C_{16}H_{12}O_6$ also the peaks at *m/z* = 286 $[M+H - CH3]^+$, *m/z* = 272 $[M+H - CO]^+$ and *m/z* = 152 confirm the identity the Chrysoeriol. The ^{1}H-NMR data showed signals at δ in ppm 7.63 (1H, *d*, J = 8.9 Hz, H-2'), 4.00 (1H, *s*, J = 3 Hz, H-4'OCH_3), 7.0 (1H, *d*, J = 8.5 Hz, H-5'), 7.60 (1H, *dd*, H-6′), 6.55 (1H, *d*, J = 4.5 Hz, H-8) and 6.25 (1H, *d*, J = 3.5 Hz, H-6) which are in agreement with those reported for chrysoeriol (Khaled et al., 2009).

Compound-5, Adonifiline: yellow powder with melting point 263°C. The full scan MS spectra of compound-5 exhibited the corresponding protonated molecular ion $[M+H]^+$ of 366.3 with two specific fragment ions for pyrrolizidine alkaloid of the retronecine-type (at *m/z* 120 and *m/z* 138). The ^{1}H NMR results (Table 2) compared with the literature review identified that compound-5 was adonifoline (Witte et al., 1992).

Compound-6, Retrorsine: white solid material with meliting point 208-211°C. The full scan MS spectra exhibited the corresponding protonated molecular ion $[M+H]^+$ of 352 with two prominent fragment ions for pyrrolizidine alkaloid of the retronecine-type (at *m/z* 120 and *m/z* 138). The ^{1}H NMR results was presented in Table 2, adding the information for compound-6 and as a result, compound-6 was identified as retrorsine (Segall and Dallas, 1983).

Compound-7, 5,4′-di-O-methyl alpinumisoflavone: yellow powder with UV max (MeOH) at 224, 271 and 326 (sh) nm, suggested an isoflavone skeleton. The ESI-MS analysis showed a molecular ion at *m/z* 365.1 $[M+H]^+$ in agreement with a molecular weight of 364 amu, whilst the ESI-MS analysis showed a fragmental ion at *m/z* 350.1 $[M+H-CH3]^+$ corresponding to the loss of a methyl unit. ^{1}H NMR: δ 7.74 (^{1}H, *s,* H-2), 7.42 (2H, *d, J* = 8.1 Hz, H-2′, H-6′), 6.92 (2H, *d*, *J* = 8.1 Hz, H-3′, H-5′), 6.71 (1H, *d, J* = 10.0 Hz, H-4″), 6.58 (1H, *s,* H-8), 5.69 (1H, *d*, *J* = 10.0 Hz, H-3″), 3.87 (3H, *s*, 5-OMe), 3.80 (3H, *s*, 4′-OMe), 1.45 (6H,*s*, 2″Me_2) (Stewart et al., 2000).

3.3. Antioxidant and anti-inflammatory properties of the isolated compounds

It is well established that the efficacy of flavonoids as antioxidants based on the number of and position of the hydroxyl substitutions on the basic structure, an increase in number of hydroxyl groups (directly correlated with increasing activity) and the 3',4'-dihydroxy substitution (Rice-Evans et al., 1996). These explains the order of antioxidant potencies of isolated compounds: quercetin 3-O-

glucoside, naringenin 7-O-glucoside, chrysoeriol followed by 5,4′-di-O-methyl alpinumisoflavone. Pyrrolizidine alkaloids, which were isolated from ethanol extract inhibited weak antioxidant properties (Table 1).

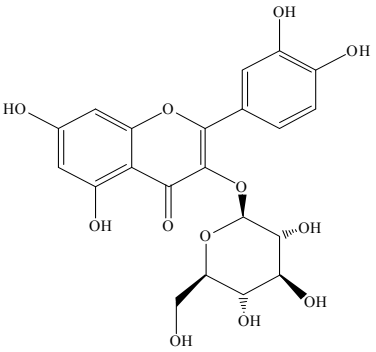
Table 2. ^{1}H proton of compound-3,5 and 6

Proton assignment	Compound-3		Compound-5		Compound-6	
	δ (ppm)	proton	δ (ppm)	proton	δ (ppm)	proton
2	6.18	1H, *d*	6.13	1H, *m*	6.20	1H, *d*
3a	3.93	1H, *d*	4.07	1H, *br.d*	3.94	1H, *d*
3b	3.38	1H, *d*	3.56	1H, *m*	3.39	1H, *ddd*
5a	3.26	1H, *t*	3.48	1H, *m*	3.26	1H, *t*
5b	2.53	1H, *m*	2.78	1H, *m*	2.53	1H, *m*
6a	2.34	1H, *dd*	2.0-2.2	1H, *m*	2.38	1H, *dd*
6b	2.09	1H, *m*			2.15	1H, *m*
7	4.95	1H, *t*	5.58	1H, *t*	5.0	1H, *t*
8	4.24	1H, *m*	4.43	1H, *m*	4.27	1H, *m*
9a	5.39	1H, *d*	5.31	1H, *d*	5.49	1H, *d*
9b	4.01	1H, *d*	4.28	1H, *br.d*	4.09	1H, *d*
14a	2.94	1H, *d*	2.0-2.2	1H, *m*	1.64	1H, *m*
14b	2.74	1H, *d*			2.19	1H, *d*
18a	1.53	3H, *s*			1.73	1H, *m*
18b					3.74	1H, *d*
19a	5.23	1H, *d*	3.76	1H, *dd*	3.62	1H, *d*
19b	5.04	1H, *d*	3.65	1H, *d*	0.85	1H, *d*
20	5.83	1H, *q*	3.50	1H, *q*	5.71	1H, *q*
21	1.87	3H, *dd*	1.40	1H, *d*	1.83	1H, *dd*

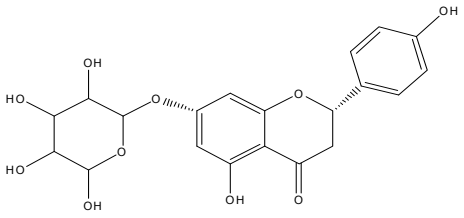
The anti-inflammatory activities of isolated compounds were evaluated using 5-lipoxygenase assay. Nordihydroguareic acid (NDGA) and aspirin were used as positive control. In the class of phenolic compounds the most potent 5-lipoxygenase inhibitors are flavonoids such as quercetin, isoquercitrin, apigenin, luteolin, sideritoflavon, gnaphalin, silibinin, centaureidin, baicalein or rhamnetin, but additional polar groups as in glycosides diminish them (Ammon et al., 1993). In this study, the rank order of the potency of isolated compounds to inhibited 5-lipoxygenase was quercetin 3-O-glucoside, naringenin 7-O-glucoside, 5,4′-di-O-methyl alpinumisoflavone and followed by chrysoeriol (Table 1).

Several natural products, especially from Asteraceae family have been proved to have anti-inflammatory activities. For example, the sesquiterpene inuviscolide from *Inula viscosa* (L.) Aiton, is interferes with leukotriene synthesis and phospholipase A2-induced mastocyte degranulation (Hernandez et al., 2010). Another example is 2-[(2′*E*)-3′,7′-dimethyl-2′,6′-octadienyl]-4-methoxy-6-methylphenol from *Atracty- lodes lancea* (Thunb.) DC. which inhibited the effects of 5-lipoxygenase and cyclooxygenase-1 (Resch et al., 2001). The terpenes *α*-pinene, *β*-caryophyllene, *γ*-terpinene, 1,8-cineole, limonene, linalyl acetate and linalool from *Eriocephalus* L. essential oils inhibited 5-lipoxygenase (Njenga and Viljoen, 2006). The sesquiterpene lactone parthenolide from feverfew (*Tanacetum parthenium* (L.) Sch. Bip) inhibited the pro-inflammatory signalling pathway (Kwok et al., 2001).

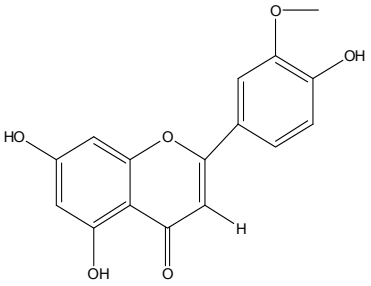
Figure 1 - The isolated compounds from *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm.



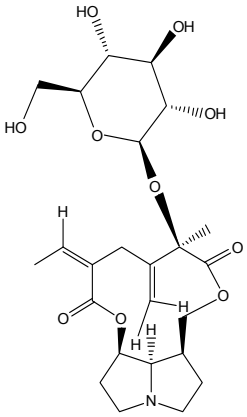
Quercetin-3-O-Glucoside



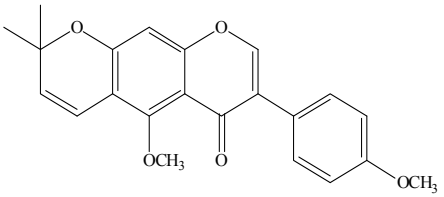
Naringenin-7-O-Glucoside



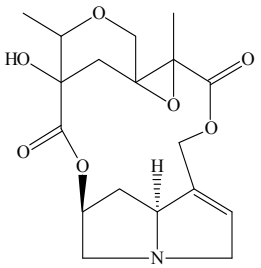
Chrysoeriol



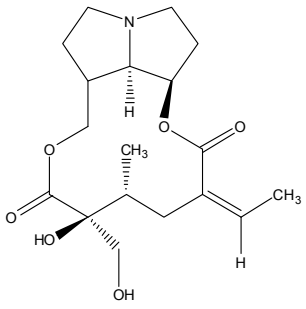
Senecyphylline-O-Glucoside



5,4′-di-O-methyl alpinum isoflavone



Adonifoline



Retrorsine

In spite of lack of antioxidant properties, adonifiline inhibited 5-lipoxygenase even with an IC_{50} value of 26.84 µg/ml superior to that of flavonoids. To date, no literature review supports the evidence of pyrrolizidine alkaloid with 5-lipoxygenase activity. Therefore further research need to be carried on for better understanding about the pyrrolizidine alkaloid and its mechanism action on this assay.

Antioxidants and free radical scavengers have potential to reduce radicals and terminate synthesis of leukotrienes. Therefore, the inhibition of the 5-lipoxygenase enzyme indirectly reduces free radical production (Wagner, 1989). Studies have implicated oxygen free radicals in the process of inflammation to block the cascade process of arachidonic acid metabolism by inhibiting lipoxygenase activity (Srejayan and Rao, 1996; Trouillas et al., 2003).

A combination of anti-inflammatory and antioxidant assay (Choi, 2003) constitutes a good indication on the potential anti-inflammatory activity of a drug (Alitonou et al., 2006), where it is believed that inhibition of the lipoxygenases is due to reaction of the inhibitor with free radicals generated at the active site of the enzyme (Takahama, 1985). Surprisingly, preliminary screening of anti-inflammatory properties of the crude extracts and compounds of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. did not show positive correlations with antioxidant properties (R^2=0.1516). The results from this study substantiate the medicinal use of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm.

4. Conclusions

Strong antioxidant and anti- 5 lipoxygenase activities were displayed by the ethanol and water extracts of *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm., respectively. Phytochemical analysis of the ethanol extract yielded 4 flavonoids and 3 pyrrolizidine alkaloids. Amongst the isolated compounds quercetin 3-O-glucoside elicited strong antioxidant activities and adonifiline was a potent inhibitor of 5-lipoxygenase. The results obtained in the present study demonstrate the potential of the crude extracts and isolated compounds from *Lopholaena coriifolia* (Sond.) E. Phillips & C.A. Sm. as antioxidant and anti-inflammatory agents.

References

Aggarwal, B.B., Harikumar, K.B. 2008. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *The International Journal of Biochemistry and Cell Biology*. 41: 40–59.

Alitonou, G.A., Avlessi, F., Sohounhloue, D.K., Agnaniet, H., Bessiere, J.M., Menut, C. 2006. Investigations on the essential oil of *Cymbopogon giganteus* from Benin for its potential use as an anti-inflammatory agent. *International Journal of Aromatherapy.* 16: 37-41.

Alothman, M., Bhat, R., Karim, A.A. 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chemistry*. 115: 785-788.

Ames, B.N., Shigenaga, M.K., Hagen, T.M. 1990. Oxidants, antioxidants and the degenerative diseases of ageing. *Proceeding of The National Academy of Science*. 90: 7915-7922.

Ammon, H.P.T., Safayhi, H., Mack, T., Sabieraj, J. 1993. Mechanism of anti-inflammatory actions of curcumine and boswellic acids. *Journal of Ethnopharmacology.* 38: 113–119.

Arnous, A., Makris, D.P., Kefalas, P. 2001. Effect of principal polyphenolic components in relation to antioxidant characterictics of aged red wines. *Journal of Agriculture & Food Chemistry*. 49:5736 – 5742.

Baylac, S., Racine, P. 2003. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. *International Journal of Aromatherapy*. 13(2-3): 138-142.

Benzie, I.F.F., Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry*. 239: 70-76.

Bohlmann, F., Wallmeyer, M. 1982. Short reports: Furanoeremophilanes from *Lopholaena* species. *Phytochemistry*. 21(8): 2126-2127.

Cao, L., Si, J.Y., Liu, Y., Sun, H., Jin, W., Li, Z., Zhao, X.H., Pan, R.L. 2009. Essential oil composition, antimicrobial, and antioxidant properties of *Mosla chinensis* Maxim. *Food Chemistry*. 115: 801-815.

Choi, C.W. 2003. Anti-oxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. *Plant Science.* 163: 1161-1168.

Choudhary, M.I., Azizuddin, Jalil, S., Nawaz, S.A., Khan, K.M., Tareen, R.B., Atta-ur-Rahman. 2009. Anti-inflammatory and lipoxygenase inhibitory compounds from *Vitex agnus-castus*. *Phytotheraphy Research*. 23: 1336-1339.

Craig, W.J. 1999. Health promoting properties of common herbs. *American Journal of Clinical and Nutrition*. 70 (3): 491S-499S.

Esposito, E., Rotilio, D., Di Matteo, V., Di Giulio, C., Cacchio, M., Algeri, S. 2002. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. *Neurobiology of Aging*. 23: 719-735.

Flamand, N., Lefebvre, J., Surette, M.E., Picard, S., Borgeat, P. 2006. Arachidonic acid regulates the translocation of 5-lipoxygenase to the nuclear membranes in human neutrophils. *The Journal of Biological Chemistry*. 281: 129-136.

Gelfand, M., Mavi, S., Drummond, R.B., Ndemera, B. 1985. *The traditional medical practitioner in Zimbabwe*. Mambo Press, Zimbabwe.

Grabmann, J. 2000. Antioxidant properties of essential oils. *Arzneinforsch*. 50: 135-139.

Halliwell, B., Gutteridge, J.M., Cross, C.E. 1992. Free radicals, antioxidants, and human disease: where are we now. *Journal of Laboratory and Clinical Medicine*. 119: 598-620.

Hernández, V., Recio, M.C., Máñez, S., Prieto, J.M., Giner, R.M., Ríos, J.L. 2010. A mechanistic approach to the *in vivo* anti-inflammatory activity of sesquiterpenoid compounds isolated from *Inula viscose*. *Planta Medica*. 67(8): 726-731.

Juan-Badaturuge, M., Habtemariam, S., Thomas, M.J.K. 2011. Antioxidant compounds from a South Asian beverage and medicinal plant, *Cassia auriculata*. *Food Chemistry*. 125: 221-225.

Kashif, A., Federica, M., Eva, Z., Martina, R., Young, H.C., Robert, V. 2009. NMR metabolic fingerprinting based identification of Grapevine metabolites associated with Downy Mildew resistance. *Journal of Agricultural and Food Chemistry*. 57: 9599-9606.

Khaled, A.A., Adnan, A.E., Salwa, M.N. 2009. Some pharmacochemical investigations on *Verbena tenuisecta*. *Research Journal of Agriculture & Biological Science*. 5(5): 649-659.

Khan, K.M., Ambreen, N., Mughal, U.R., Jalil, S., Perveen, S., Choudhary, M.I. 2010. 3-Formylchromones: potential antinflammatory agents. *European Journal of Medicinal* Chemistry. 45: 4058-4064.

Koleva, I.I., van Beek, T.A., Linssen, J.P.H., de Groot, A., Evstatieva, L.N. 2002. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochemical Analysis*. 13: 8-17.

Kwok, B.H.B., Koh, B., Ndubuisi, M.I., Elofsson, M., Crews, C.M. 2001. The anti-inflammatory natural product parthenolide from the medicinal herb feverfew directly binds to and inhibits iKB kinase. *Chemistry & Biology*. 8(8): 759-766.

Lourens, A.C.U., Reddy, D., Baser, K.H.C., Viljoen, A.M., van Vuuren, S.F. 2004. *In vitro* biological and essential oil composition of four indigenous South African *Helichrysum* species. *Journal of Ethnopharmacology*. 95: 253-258.

Miller, H.E. 1971. A simplified method for the evaluation of antioxidants. *Journal of American Oil Chemistry Society*. 48: 91-97.

Njenga, E.W., Viljoen, A.M. 2006. *In vitro* 5-lipoxygenase inhibition and antioxidant activity of *Eriocephalus* L. (Asteraceae) species. *South African Journal of Botany*. 72: 637-641.

Pulido, R., Bravo, L., Saura-Calixto, F. 2000. Antioxidant activity of dietray polyphenols as determines by a modified ferric reducing antioxidant power assay. *Journal of Agriculture and Food Chemistry*. 48: 3396-3402.

Ragab, E.A., Hosny, M., Kadry, H.A., Ammar, H.A. 2010. Flavanone glycosides from *Gleditsia caspia*. *Journal of Natural Products*. 3: 35-46.

Resch, M., Heilmann, J., Steigel, A., Bauer, R. 2001. Further phenols and polyacetylenes from the rhizomes of *Atractylodes lancea* and their anti-inflammatory activity. *Planta Medica*. 67(5): 437-442.

Rice-Evans, C., Miller, N., Paganga, G. 1996. Structure-antioxidant activity relationships of flavonoids and phenolic compounds. *Free Radical Biology and Medicine*. 20: 933-956.

Schneider, I., Bucar, F. 2005. Lipoxygenase inhibitors from natural plant sources. Part I: Medicinal Plants with inhibitory activity on arachidonate 5-lipoxygenase and 5-lipoxygenase/cyclooxygenase. *Phytotherapy Research*. 19: 81-102.

Segall, H.J., Dallas, J.L. 1983. ^{1}H NMR spectroscopy of pyrrolizidine alkaloids. *Phytochemistry*. 22: 1271-1273.

Sreejayan, N., Rao, M.N.A. 1996. Free radical scavenging activity of curcuminoids. A*rzneimittelforschung*. 46(2): 169-171.

Stewart, M., Bartholomew, B., Curie, F., Abbiw, D.K., Latif, Z., Sarker, S.D., Nash, R.J. 2000. Pyranoisoflavones from *Rinorea welwitschii*. *Fitoterapia*. 71: 595-597.

Takahama, U. 1985. Inhibition of lipoxygenase-dependent lipid peroxidation by quercetin: mechanism of antioxidative function. *Phytochemistry*. 24: 1443-1446.

Trouillas, P., Calliste, C.A., Allais, D.P., Simon, A., Marfak, A., Delage, C., Duroux, J.L. 2003. Antioxidant, anti-inflammatory and anti-proliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chemistry*. 80: 399-407.

Young, R.N. 1999. Inhibitors of 5-lipoxygenase: a therapeutic potential yet to be fully realized?. *European Journal of Medicinal Chemistry*. 34: 671-685.

Wagner, H. 1989. Search for new plant constituents with potential antiphlogistic and antiallergic activity. *Planta Medica*. 55: 235–241.

Witte, L., Ernst, L., Wray, V., Hartmann, T. 1992. Revised structure of the main alkaloid of *Senecio adonidifolius*. *Phytochemistry*. 31(3): 1027-2018.