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Research Article

In vitro Repression of Cyclooxygenase, Acetylcholinesterase Activities and Bacterial Growth by Trans-phytol and a Glycolipid from the Leaves of *Homalomena sagittifolia*

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Abstract

Background and Objective: The leaf of *Homalomena sagittifolia* was reported to have anti-inflammatory, antimicrobials, narcotic, violent intoxication and hallucinogen effects. This study highlights isolation, identification and biological activities of two compounds from the leaves of *H. sagittifolia*. **Methodology:** Two isolates were investigated for their inhibitory effects against cyclooxygenase and acetylcholinesterase enzymes. They were also tested for antimicrobial effects against five pathogenic bacterial strains using the micro-dilution assay. The structure of the two isolates were elucidated by interpretation of spectroscopic data and previous available reports in literature. They were identified to be trans-phytol (1) and diacylglyceroglycolipid (2). The compounds were investigated for their anti-inflammatory, anticholinergic and antimicrobial effects using the cyclooxygenase, the microplate and the antimicrobial micro-dilution assays, respectively. **Results:** Compound 2 possessed good activity against both COX-1 (IC_{50} = 38) and COX-2 (IC_{50} = 48). The IC_{50} values observed with the indomethacin were 4.1 and 181 μM against COX-1 and COX-2, respectively. The two compounds also inhibited activity of acetylcholinesterase with an IC_{50} values of 8.6 μM (2), 24 μM (1) and 3.3 μM (galanthamine). Compound 2 showed remarkable activity against Gram-negative bacteria *Pseudomonas stutzeri* and *Klebsiella pneumoniae* with an MIC value of 98 μM. The MIC values recorded for tetracycline were 87 and 175 μM against *P. stutzeri* and *K. pneumonia*, respectively. **Conclusion:** These results indicated the potential pharmacological properties of the leaves of *H. sagittifolia* and supported the traditional uses of the plant. Further studies are needed to understand its molecular interactions. This may lead to the development of standardized crude drugs and/or nutraceutical agents.

Key words: *Homalomena sagittifolia*, cyclooxygenase inhibitors, phytol, glycolipids, MIC

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Inflammation is a disorder involving variety of complex mediator molecules. Synthesis of eicosanoid is confirmed to be one of the key factors in the inflammation process. Cyclooxygenase (COX) pathway (prostaglandins) is one of the major pathways of eicosanoid metabolism. The COX enzymes which is known to be in two isoforms (COX-1 and 2) are encoded by different genes located on different chromosomes. It is generally believed that COX-1 is constitutive and COX-2 is inducible. Bioactive molecules with the maximum COX-2 inhibition are still targeted as an ideal therapy accounted for major pharmacological effects of anti-inflammatory drugs. However, evidences have shown that COX-2 selective agents may not simply be the best anti-inflammatory drugs all the time and the mechanism is more complex[1]. Other mechanisms that contribute to the regulation of the inflammatory response are through the effects on the nervous systems through anticholinergic inflammatory pathway, which involves acetylcholinesterase enzyme. Acetylcholine, the principal vague neurotransmitter inhibits inflammation by suppressing the production of pro-inflammatory cytokines through a mechanism depends on the α7 nicotinic acetylcholine receptor subunit. This imply that inhibition of acetylcholinesterase enzyme may contribute to the enhancement of the acetylcholine-dependent macrophage deactivation which represents an essential step for cholinergic inflammatory pathway[1].

Homalomena is a genus within the family Araceae comprising approximately 80-150 species widely distributed in Southern Asia and the Southwestern Pacific[2]. It is intensively used in Chinese traditional medicine as the name *Homalomena* in Chinese is "Qian nian jian" which means "thousand years of health[3]". A number of *Homalomena* species have been used in different traditional practices of medicine to cure various ailments including skin diseases, cuts and wounds, ulcers, swelling, jaundice, fever, headache and back pain. The natives of Papua New Guinea eat the leaves of *Homalomena* species combined with the leaves and bark of *Galbulimima belgraveana* as a narcotic[4,2]. Rhizomes and leaves decoction used for febrifuge. Tuberous roots suspension used to treat diarrhea. Leaves used for skin infections. In Chinese traditional medicine rhizomes used for traumatic injury, stomachache, lumbago and as antibacterial. Stalk powder in Indonesia eaten to treat stomachache. Aqueous extract of the leaves given to treat diarrhea[5]. In Malaysia, Indonesia and Philippine, the *Homalomena* species are used for the treatment of fever, cough and inflammatory pain. Decoction of root and leaves of *H. sagittifolia* is reported to be used for fever and distended stomach. In the village, the leaves are wrapped around heated stones to massage the abdomen of women just after childbirth. Leaves of *Homalomena* species are also reported to have narcotic and violent intoxication and hallucinogen effects and therefore, used for the treatment of certain ailments related to central nervous system disorders[5-9].

Homalomena sagittifolia Jungh.ex Schott is a large herb which can grow up to 60 cm tall. The stem is erect, stout and it is up to 5 cm in diameter. The vegetative parts are strongly aromatic. Leaves are ovate-arrowhead-shaped with spreading lobes up to 30 cm long. The plant is distributed throughout Thailand, Malaysia and Singapore[6].

Previous phytochemical investigation of *Homalomena* sp. had revealed the presence of oplopane, eudesmane and eremophilane-type sesquiterpenoids in the rhizomes[10,11]. Hu *et al.*[12] isolated two sesquiterpenoids, one daucane ester together with five known sesquiterpenoids, oplodiol, oplopanone, homalomenol C, bullatantriol and 1b, 4b, 7a-trihydroxy eudesmane. These isolates also showed remarkable biologiocal activites on osteoblast proliferation, differentiation and mineralization using an *in vitro* model[2]. Zhao *et al.*[13] reported isolation of 6 new sesquiterpenes along with 8 known ones from the rhizomes of *Homalomena occulta*. Antimicrobial activities of 3 new eudesmane-type sesquiterpenoids and 8 known constituents isolated from the aerial parts of *Homalomena occulta* against 6 different bacterial strains were reported by using an agar-diffusion assay[14]. Wong *et al.*[15] reported isolation of essential oils from the leaves and rhizomes of *Homalomena sagittifolia*. The GC-MS analysis applied indicated the presence of monoterpene hydrocarbons and sesquiterpenoids in the oil. Pain sensation and inflammatory related disorders are of complex nature and there is no single test that can be perfect enough to predict efficiency of a potential bioactive agents. Therefore, the use of various experimental models are relevant when screening crude extract or compounds for pharmacological activity[16].

Previous investigation on the rhizome of *H. saittifolia* led to the isolation of a new and known sesquiterpenoids with antibacterial and anti-cholinergic effects[17]. Leaves of the *H. sagittifolia* were frequently mentioned uses in traditional medicine for the treatments of ailments of inflammation nature[5,8].

This study highlights the isolation of trans-phytol and a glycolipid compounds from the leaves of *Homalomena sagittifolia* and determination of their inhibitory effects against cyclooxygenase and acetylcholinesterase enzymes. The study also highlights the antimicrobial properties of the isolates against some pathogenic bacterial strains.

MATERIALS AND METHODS

Plant materials: Leaves of *Homalomena sagittifolia* Jungh.ex Schott was collected from Redang Panjang, Selama, Perak Malaysia. The plant was identified by a taxonomist in the School of Biological Sciences, Universiti Sains Malaysia. The voucher specimen (USM 10126) has been deposited in the University herbarium.

Extraction, fractionation and isolation: The fresh leaves (1.8 kg) were macerated 3 times with methanol (2 L×3) for 14 h at room temperature. The combined filtered extract was evaporated on a rotatory evaporator under reduced pressure to give (45 g) of methanolic crude residue. This crude residue was dissolved in 300 mL of methanol and added 400 mL of water. The aqueous methanolic crude solution was transferred into 2 L separator funnel and defatted with hexane (300 mL×3) which afford (15 g) of fatted extract (crude 1). Then partition was carried out with chloroform (250 mL×3) to obtain (4 g) of chloroform extract (crude 2). The crude 1 was purified with column chromatography over silica gel 60 (0.040-0.063 mm, 230-400 mesh) (Merck, Germany). The column was eluted with gradient solvent system starting from n-hexane-ethyl acetate (7:3-1:4 v/v). A combined fraction of 850 mg was further purified with column chromatography and TLC to yield compound (1) (13 mg). Crude 2 was also subjected to column chromatography over silica gel (100 g, 0.040-0.063 mm, 230-400 mesh, Merck, Germany) using the gradient solvent hexane-ethyl acetate (7:3-2:1 v/v). The eluents were collected by 25 mL each. The eluents were pooled into 17 fractions according to the TLC investigation. Fraction ten (550 mg) was further purified by column chromatography repeatedly and yielded compound (2) (25 mg).

In vitro biological evaluation of the isolated compounds

Anti-inflammatory assessment using cyclooxygenase assays: Anti-inflammatory activity of the isolated compound was determined using both the COX-1 and COX-2 assays. The basic protocol is the same for both assays, allowing a comparison of the inhibitory effects of the isolated compounds on the two enzymes. The COX bioassays was performed according to the methods modified and described earlier[18,19]. In each test assay, four controls were run. Two were background in which the enzyme was inactivated with HCl before the addition of [^{14}C] arachidonic acid and two were solvent blanks. Indomethacin was included in each test assay as a standard (5 μM for the COX-1 assay and 200 μM for the COX-2 assay). The assays were performed in duplicate with double determinations for each sample per assay. The percentage inhibition by the tested compounds were calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank.

Acetylcholinesterase enzyme inhibitory activity: Inhibition of acetylcholinesterase by the isolated compound was investigated using the microplate assay with modifications as it had previously described[20]. The enzyme activity is measured by observing the increase of a yellow colour produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. Acetylthiocholine iodide (ATCl), acetylcholinesterase (AChE) from electric eels (type VI-S lypophilized powder), 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and galanthamine were obtained from Sigma-Aldrich. The following buffers were used; Buffer A: 50 mM tris-HCl, pH 8, buffer B: 50 mM tris-HCl, pH 8, containing 0.1% Bovine Serum Albumin (BSA), buffer C: 50 mM tris-HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_2O$. The assay was performed using an Opsys MR 96-well microplate reader. In the 96-well plates, 25 μL of 15 mM ATCl in water, 125 μL of 3 mM DTNB in buffer C, 50 μL of buffer B and 25 μL of a serially diluted (two-fold) solution of the isolated compound (initial concentration of 60 μM) and galanthamine hydrobromide as positive control (initial concentration of 20 μM) were added. The absorbance was measured at 405 nm every 45 sec (5 times). Then 25 μL of 0.2 U mL^{-1} solution of enzyme were added, the absorbance was measured again every 45 sec (8 times). The rate of reaction was calculated. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the ratio of reaction before adding the enzyme from the rate after adding the enzyme. Percentage of inhibition was calculated by comparing the reaction rates for the sample to the blank (methanol in buffer A). The assay was performed in triplicate.

Micro-dilution antibacterial assay: The serial dilution technique as described by Eloff [21] using 96-well micro-plates was employed to determine the Minimum Inhibitory Concentration (MIC) of the isolated compounds. Two milliliter cultures of two Gram-positive bacterial strains, *Bacillus subtilis* (ATCC6633) and *Staphylococcus aureus* (ATCC12600) and three Gram-negative bacterial strains, *Escherichia coli* (ATCC25922), *Klebsiella pneumoniae* (ATCC13883) and *Pseudomonas stutzeri* (ATCC17588) were prepared and placed in an incubator overnight at 37°C. The overnight cultures were diluted with sterile Mueller-Hinton (MH) broth (1 mL bacteria/50 mL MH) to yield a density of bacterial cells between 10^6 and 10^8 cells mL^{-1}. The tested compounds were re-suspended to a concentration of 10 mg mL^{-1} with ethanol

to yield a final concentration of 2.5 mg mL^{-1} in the assay. For each of the five bacteria used, 100 µL of the test solution were serially diluted two-fold with 100 µL of sterile distilled water in a sterile 96-well micro-plate. A similar two-fold serial dilution of tetracycline (1 mg mL^{-1}) was used as a positive control against each bacterium. One hundred microliters of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37°C. To indicate bacterial growth, 50 µL of 0.2 mg mL^{-1} p-iodonitrotetrazolium violet (INT) was added to each well and the plates were incubated at 37°C for 30 min. Bacterial growth in the wells were indicated by a red colour, whereas clear wells indicated inhibition by the tested substances.

Statistic analysis: Data were expressed as Mean±Standard Deviation (SD). The IC_{50} values were calculated using a logarithmic non-linear regression curve derived from the plotted data using Graph Pad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA).

RESULTS

Identification of the two isolated compounds: Optical rotations were measured on JASCO DIP-370 polarimeter. The IR spectra were recorded on using a Perkim-Elmer system 2000 FT-IR. The ^1H, COSY, NOSY, HMQC, HMBC and ^{13}C-NMR spectra were recorded on Bruker Avance 400 and 300 MHz spectrometer, respectively. Positive-ion HR-MS was performed employing a HP 6890 N coupled to HP 5973 Inert Mass Selective Detector equipped with the Wiley 275 and NIST Library Software. Silica gel 60 (0.040-0.063 mm, 230-400 mesh) (Merck, Germany) was used for column chromatography. The TLC aluminum sheets of silica gel 60 F-254 (20×20, Merck) was used for normal TLC and pre-coated glass plates (20×20×0.5 mm) (Merck, Germany) were for preparative TLC. Five percent sulfuric acid in methanol was used as TLC spray and spot was developed by heating it on hot plate.

Compound 1: The IR bands at 3321 and 1649 indicated the presence of hydroxyl functional groups and double bond. The EI-MS of (1) revealed M+peak at m/z 296. The mass spectral fragments 278 $[M-H_2O]^+$, 263 $[278-CH_3]^+$, 196 $[M-C_6H_{12}O]^+$ and 71 $[M-C1_6H_{33}]^+$ (base peak) were exactly matched with that reported of phytol[22-24]. Thus, the molecular formula, $C_{20}H_{40}O$ was deduced (Fig. 1).

The ^1H-NMR spectrum displayed an oxygenated methylene characteristic [(δH) 4.16 (d, J = 6.8 Hz)] and one olefinic methine proton absorbance was appeared at [(δH) 5.40 (dq, J = 6.8 and 1.2 Hz)], another methylene proton absorbance was revealed at [(δH) 1.98 (t, J = 4.6 Hz)]. The remaining methane and methylene (20H, m) protons were resonated in a rather narrow spectral region, δH 1.0-1.60. Due to overlapping, the identification of the individual multiplets and assignment of their chemical shifts were difficult. Therefore, the above assignments were made with the help of HMQC (Fig. 1). The vinyl methyl proton was revealed at [(δH) 1.70, s] and the four methyl groups resonated at [(δH) 0.85-90, (d, J = 6.5 Hz)].

The ^{13}C-NMR, DEPT 45, DEPT 135 and DEPT 90 spectra confirmed the presence of 20 carbons comprising 5 methyl, 10 methylenes, 4 methanes and one quaternary carbon. The ^{13}C signals at δC 59.82 suggested that compound (1) has one OH functional group. One double bond carbons resonate at

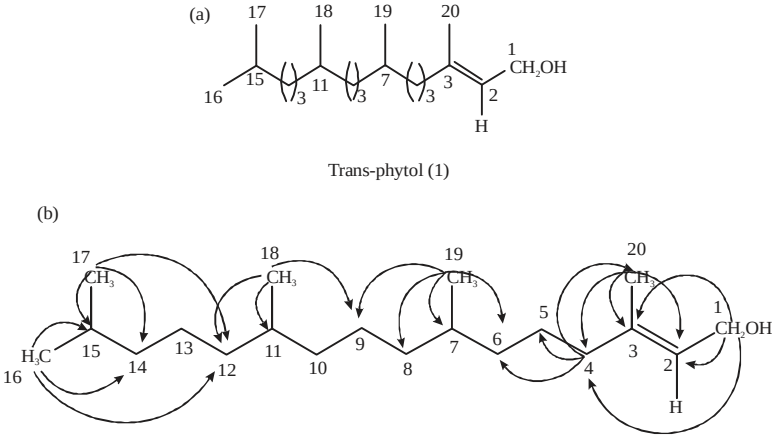

Fig. 1(a-b): (a) Structure of trans-phytol and (b) HMBC correlation of compound (1)

δC 140.72 and 123. 47. The IR spectrum band 3321 and 1649 cm^{-1} further supported that (1) has one OH functional group and one C=C double bond. The 2D HMBC spectrum revealed that H1 correlated with C2, C3 and C4, H_2O with C2, C3 and C4, H4 with C5 and C6, H19 correlated to C6, C7, C8 and C9, H18 with C9, C11 and C12, H17 and H16 correlated C12, C14, respectively. All these values matched with the previous report of Bang *et al.*[24]. The H1 methylene proton appeared at δH 4.16 confirmed compound (1) to be trans-phytol[25].

Compound 2: Through spectral investigation, the structure of compound (2) was determined to be diacylglyceroglycolipid: glycerol 1-linolenyl-2-linoleic-3-O-β-D-glactopyranoside (Fig. 2). It is a class of lipid known as glycolipids. They are carbohydrate-attached lipids and a monosaccharide moiety is attached to position-3 of the glycerol unit by a glycosidic linkage.

The ^{1}H-NMR spectrum of the (2) was assigned with the aid of reference to some previous ^{1}H-NMR studies on glycerides[26]. The ^{1}H-NMR spectrum showed absorptions at three distinctive areas: 0.90-2.90 ppm characteristic of the acyl group of the fatty acid, 3.55-4.45 ppm due to the protons of the glycerol and sugar moieties and 5.30-5.50 ppm due to the vinylic protons of the fatty acids. The presence of poly unsaturated fatty chains was indicted by the overlapping resonances at 2.80 ppm. These singnals arise from the allylic methylene protons with the series of double bonds in the chain [-CH=CH-CH_2-(CH=CH-CH_2-)n][26]. The magnetically not equivalent glycerol Sn1 methylene protons resonated at 4.41 (downfield) and 4.15 ppm (upfield) while Sn3 methylene proton resonated at 3.98 ppm. Coupling between these glycerol backbones protons was confirmed by cross peaks in the 2D COSY spectrum.

The bis-allylic (R 11 and R' 11, 14) and allylic (R 8, 14 and R' 8, 17) protons appears at 2.80 ppm and 2.01-2.11 ppm respectively indicating poly unsaturated nature of the fatty acids[27]. The H-2 and H-3 of the acyl group appeared at 2.30 and 1.60, respectively. The last absorption peak suggested the presence of two acyl groups. The slightly downfield signal of the methyl protons 0.98 was assumed to be the terminal methyl group of linolenic acid. The presence of 10 vinylic protons with chemical shifts between 5.28-5.50 ppm most likely belongs to lenoleic (18:2) and lenolenic (18:3) fatty acids. The four distinct signals for methylene protons of the glycerol moiety (Sn1 and Sn3) suggested that (2) is a 1, 2 diacylglycerol, since in the case of a 1, 3-diacylglycero l24, 27. The chemical shift difference of 0.4 ppm between two acyl groups confirms the attachment of R 1 and R' 1 to the position Sn1 and Sn 2 of the glycerol moiety[28]. Furthermore, linolenic acid carbonyl carbon usually attached to position 1 and lenoleic acid has more marked preference[29] for position 2.

The chemical shift of the protons at C-2 of the glycerol moiety (G2) is overlapped in the vinylic proton region and this was deduced from correlation with G1 and G3 in ^{1}H-COSY. Seven protons 3.53-4.15 ppm were assigned to a pyranose ring attached to position-3 of the glycerol moiety. The large coupling constant of the anomeric proton (S1) (7.5 Hz) indicates a β-glycosidic linkage between the sugar and glycerol moieties[30].

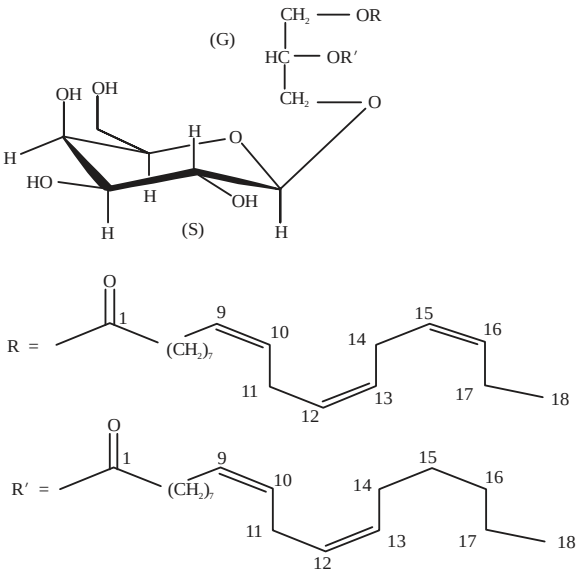

Fig. 2: Structure of compound 2, glycerol 1-linolenyl-2-linoleic-3-O-β-D- glactopyranoside

The ^{13}C signals of glycerides could be classified into four categories: carbonyl carbons (172-174 ppm), unsaturated carbons ranging from (126.8-134.1 ppm), glycerol backbone (61.6-69.1 ppm) and aliphatic carbons (13.9-34.5 ppm), respectively[28]. These features can be seen in the ^{13}C-NMR spectrum of (2). The carbonyl carbons of two acyl groups resonate at 174.2 and 173.8 ppm (assigned to R 1 and R' 1, respectively). Ten signals appear at olefinic carbons region (127. 5-132.3 ppm) were assigned to the lenolenic and lenoleic acids double bonds. All ten olefinic carbons were assigned with reference to the NMR study of glycerol tri-esters in vegetable oils[29] and olive oils[27]. The anomeric carbon atom of galactose resonates at 104.43 ppm, indicating a β-glycosidic linkage between the sugar and glycerol moieties[31]. Three methylene carbons resonate at 62.34, 63.25 and 68. 54 ppm in DEPT 135 spectrum and these were assigned with the aid of HMQC and HMBC to C-6 of the sugar and C-1 and C-3 of the glycerol moiety, respectively. In this region five methane carbons were shown and these were confirmed by DEPT, one of them was assigned to C-2 of the glycerol moiety from its correlation to the downfield H-2 proton of the glycerol moiety. The other four signals were assigned to the sugar moiety and all of them correlate with corresponding protons in HMQC and HMBC. These signals were assigned with the help of their HMQC and HMBC correlations and with reference to the NMR study of glycerol tri-esters in vegetable oils and olive oils[27,29].

Biological activities of the two isolated compounds

Cyclooxygenase enzyme inhibitory activity: Inhibition of prostaglandins biosynthesis (expressed as IC_{50} values) by the isolated compound as detected using COX-1 and COX-2 assays are given in Table 1. Compounds showed inhibition of prostaglandins with IC_{50} values ≤50 μM against COX-1 and/or IC_{50} values ≤200 μM against COX-2 were considered active. Based on this criteria, best inhibition was recorded for compound 2 with IC_{50} values of 38 μM against COX-1 and 48 μM against COX-2. Compound 1 inhibited COX-1 activity with an IC_{50} value of 48 μM. The IC_{50} values obtained for the indomethacin (positive control) were 4 and 181 μM against COX-1 and COX-2, respectively.

Acetylcholinesterase enzyme inhibitory activity: The cholinesterase inhibitory activity of the isolated compound obtained by using the microplate assay and indicated as IC_{50} values are presented in Table 1. Compounds showed inhibitory effects against acetylcholinesterase enzyme with IC_{50} ≤50 μM were considered active. The two compounds tested showed activity against acetylcholinesterase with IC_{50} values of 8.6 and 24 μM for compound 2 and 1, respectively. The IC_{50} for the galanthamine (positive control) was 3.3 μM.

Antibacterial activity: Minimum Inhibitory Concentration (MIC) values of the isolated compounds as detected using the micro-dilution assay are presented Table 2. The two compounds showed antibacterial activity with MIC values (μM) ranging between 98-561 μM against both Gram-positive and Gram-negative bacteria. The MIC values recorded for tetracycline (positive control) was ranging between 42-175 μM. The lowest MIC value (98 μM) was recorded for compound 2 against *Klebsiella pneumoniae* and *Pseudomonas stutzeri* (Gram-negative). The same compound inhibited growth of *Bacillus subtilis*, *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) with an MIC value of 159 μM.

Table 1: Inhibition of cyclooxygenase and acetylcholinesterase activity by the two isolated compounds from the leaves of *Homalomena sagittifolia*

	Cyclooxygenase IC_{50} (μM)		
Samples	COX-1	COX-2	Acetylcholinesterase IC_{50} (μM)
Trans-phytol (1)	49.0±0.7	>200	24±5
Glycolipid (2)	38.7±1.3	48±0.9	8.6±4
Indomethacine	4.1±1.6	181±2.3	nt
Galanthamine	nt	nt	3.3±1.4

Results are expressed as IC_{50} values (μM)±SD, 200 μM was the highest concentration used for the COX assay, nt: Not tested

Table 2: Antibacterial activity of the two isolated compounds from the leaves of *Homalomena sagittifolia* as detected using the minimum inhibitory concentration assay

	MIC values (μM)				
	Bacteria tested				
Compounds	*Bacillus subtills*	*Escherichia coli*	*Klebsiella pneumoniae*	*Pseusomonas stutzeri*	*Staphylococcus aureus*
Trans-phytol (1)	198±2.1	264±4.2	264±2.6	198±2.7	561±3.2
Glycolipid (2)	159±3.7	159±4.1	98±4.0	98±2.5	159±2.4
Tetracycline	42±0.9	175±1.8	175±3.3	87±2.5	87±3.0

Results obtained are presented as MIC values (μM)±SD

DISCUSSION

Pain associated with inflammation is treated with analgesic drugs, including nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors. These broadly used drugs work by inhibiting PGE2 synthesis through inhibition of the activity of COX-2. Selective COX-2 inhibitors therefore are therapeutically targeted as anti-inflammatory compounds. However, evidences have shown that COX-2 isoform can also be constitutive and protective under certain circumstances in some tissues such as central nervous system, kidney, uterus and stomach[32].

It is interesting that compound 2 (a glycolipid) inhibited activities of both COX isoforms with relatively low IC_{50} especially for inhibition against COX-2 compared to indomethacin (the positive control). Plant glycolipids comprise compounds, which can be divided into distinct groups with regard to their hydrophobic a glyca. Glycosyl diacylglycerols usually more abundant followed by glycosylated ceramides and sterols. All these compounds are characteristic lipid constituents of protoplasmic membrane systems, whereas acyl derivatives of glucose and sucrose are extracellular lipids from the surface cover of plants[33]. These class of compounds reported to possess several biological activities, including anti-inflammatory activity, anti-tumor and anti-proliferative activity[34,35]. Ulivi *et al*.[36] reported anti-inflammatory activity of a glycolipid compounds using *in vitro* articluar cartilage. The researchers concluded that the compound had an anti-inflammatory loop triggered by COX-2 and it could be a novel anti-inflammatory agent. Another class of glycolipids were also reported to possess anti-inflammatory properties using different *in vitro* model[37]. The present results of this study obtained by the glycolipid compound against COX-2 is in line with these previous findings and may well support the traditional uses of the plant as crude anti-inflammatory agents. However, inhibition of COX-1 showed by the compound may lead to undesirable side effects that need to be further investigated.

Phytol is a diterpene product of chlorophyll metabolism in plants. Phytol was reported to possess antimicrobial effects against Gram-positive bacteria *Staphylococcus aureus*[38]. However, the trans-phytol isolated in this study showed weak antimicrobial effects against both Gram-positive and Gram-negative bacteria tested. The previously reported activities may be obtained from different isomers or phytol derivatives.

Acetylcholinesterase (AChE) is an enzyme highly concentrated at the neuromuscular junction. It plays an important role in cholinergic transmission by terminating the action of acetylcholine on the post synaptic nicotinic acetylcholine receptors. A common approach to study the role of AChE on neuromuscular transmission is through the use of anticholinesterase agents, which inhibit this enzyme's activity. Due to the its essential biological role, acetylcholinesterase inhibitors have been medicinally targeted in treatments for diseases related to central nervous system disorders including Alzheimer's disease[39,40]. Phytol was reported to possess neuropharmacological properties. Elufioye *et al*.[41] evaluated acetylcholinesterase inhibitory effects of leave extract and phytol derivative compounds isolated from *Pycanthus angolensis* this plant is used traditionally as memory enhancer-the two newly isolated phytol compounds showed inhibitory effects against the enzyme acetylcholinesterase. Another phytol related molecule (Tetramethylhexadec-2-en-1-ol) also showed anxiolytic-like effects on mice during an *in vivo* evaluation model of its neuropharmacological properties. The mechanism of action was believed to be due to interaction with the GABAergic system[42]. Santos *et al*.[43] also reported antinociceptive effects of phytol in the nociception models used, both through its central and peripheral actions beside its antioxidant properties. These results are in line with the observed inhibitory effects of phytol against acetylcholinesterase in this study.

The two compounds isolated in this study showed promising inhibitory effects against acetylcholinesterase enzyme. Compound 2 inhibited acetylcholinesterase activity with a performance comparable to the activity of the galanthamine (positive control). This may indicate the therapeutic potential of the plant in the treatment of cognitive disorders.

Both the isolated compounds in this study showed remarkable inhibitory effects against COX, acetylcholinesterase. Glycolipids and terpenes are rich reservoir of candidate compounds as anti-inflammatory and neuropharmacology properties with different mechanism of actions[42]. Characterization of the cholinergic anti-inflammatory pathway has provided new grounds for understanding and treating inflammatory and neuro inflammatory diseases[16,44]. These study outcomes support the underlying interdependency of the nervous and immune systems suggesting that acetylcholinesterase inhibitors may contribute to the suppression of inflammation symptoms[45].

The two compounds isolated from the leaves of *H. sagittifolia* in this study showed antibacterial effects especially against the Gram-negative *Klebsiella pneumoniae* and *Pseudomonas stutzeri*.

Anti-microbial properties of glycolipids in particular against Gram-positive organisms may be due to their capacity

to break or damage the cell membrane[46]. This action lead to cellular lysis by increasing the permeability of the membrane and metabolite flow due to the changes in the physical structure of the membrane which alters functions such as transport and generation of energy[46]. Another report also highlighted anti-microbial properties of sophorolipids, part of the glycolipid group against several Gram-positive bacteria suggesting that the compounds could be a good potential ingredients in the production of cosmetic, hygienic and pharmacological-dermatological products 46. These previous report are in agreement with this present findings on antimicrobial effects observed by the glycolipid compound against the Gram-positive bacteria tested in this study.

The lower MIC values showed by the glycolipid against *K. pneumoniae* and *P. stutzeri* compared to the positive control tetracycline is very interesting. Both bacterial strains *Pseudomonas* sp. and *Klebsiella* sp. are among the bacteria that readily develop multiple resistance mechanisms to various classes of antibiotics. In addition, they are important nosocomial pathogens affecting both immune competent and immune compromised patients[47]. Antibacterial effects against the two bacterial strains by the glycolipid need close attention and further investigation to understand its mechanism of action.

Naturally occurring bioactive agents occur as multi-component and may therefore not be compatible with the old paradigm one-molecule-one-target dogma of classical pharmacotherapy. This contributes to the general concept that herbal medicine suffers from insufficient modern research owing to lack of clear mapping of mechanism of actions and/or drug body network. This may due in part to, the methodology used in the herbal medicine research which focus mainly on the path of partitioned reductive analysis and the complexity of herbal ingredients with unknown targets in human body and therefore unclear the mechanism of action underlying the herbal medicine[48]. This situation led to acceptance of network pharmacology as a way to treat polygenic diseases, both from target and drug perspectives which will illustrate why botanical drugs can treat many different diseases[49]. Therefore, applying this method in traditional drugs may open up the possibility to understand the explicit targets of active ingredients and the interactions in the context of molecular networks. Liu *et al.*[48] conducted in silico investigation of poly pharmacology network on licorice a well known traditional Chinese herbal drugs. Their results showed that the drug target disease network clearly elucidates mechanisms of action of licorice that exerts various pharmacological effects against diseases including respiratory, cardiovascular and gastrointestinal system diseases.

CONCLUSION

In this study, we investigated pharmacological properties of leaves of *H. sagittifolia* using selected *in vitro* model based on their reputable uses in traditional medicine. The investigation led to the isolation of trans-phytol and a glycolipid compounds. The compounds showed inhibitory effects against cyclooxygenase and acetylcholinesterase enzymes and antimicrobial effects. These pharmacological properties observed are consistent with the indigenous-based uses of the plant as effective crude agents for infectious diseases, inflammations, neuro-inflammation and ailments related to central nervous system disorders. This findings may contribute to the efforts towards scientific evaluation of the efficacy of the *H. sagittifolia* as traditional crude agents. Based on the previous and present finding of the pharmacological properties of *H. sagittifolia*, we recommend that this plant extract should further be subjected to poly pharmacological approaches for better understanding of their interaction and molecular network and could therefore be developed as supplements and/or crude drug.

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