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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Chemical composition, antioxidant, anti-lipooxygenase, antimicrobial, anti-parasite and cytotoxic activities of *Polyalthia longifolia* seed oil

O. Atolani[1] · E. T. Areh[1] · O. S. Oguntoye[1] · M. F. Zubair[2] · O. A. Fabiyi[3] · R. A. Oyegoke[4] · D. E Tarigha[1] · N. Adamu[2] · O. S. Adeyemi[5] · L. Kambizi[6] · G. A. Olatunji[1,2]

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Abstract

This work investigates the chemical compositions of *Polyalthia longifolia* Thw. seed oil with the associated antioxidant, anti-inflammatory, anti-parasite and cytotoxicity potentials. The oil of *P. longifolia* seed obtained by soxhlet extraction was trans-esterified and the fatty acid profile characterized using gas chromatography mass spectrometry (GC–MS). The antioxidant activity was evaluated using DPPH and ABTS assays. The anti-bacterial and anti-fungi properties of the oil were determine on clinical isolates of the organisms using agar diffusion method. The anti-inflammatory activities, cytotoxicity and anti-parasite potential were evaluated using lipooxygenase, mammalian cell and *Toxoplasma gondii* assays respectively. *P. longifolia* seed was observed to contain oleic (30.31%), linoleic acid (19.27%) and palmitic acid (15.11%) as the major fatty acids with low proportion of tricosylic acid (6.10) and stearic acid (5.56%). The oil had significant anti-lipooxygenase activity ($IC_{50} = 0.70 \pm 0.02$ µg/mL) comparable to indomethacin ($IC_{50} = 0.53 \pm 0.07$ µg/mL). The DPPH ($IC_{50} = 55.91 \pm 31.18$ µg/mL) and ABTS ($IC_{50} = 16.89 \pm 15.50$ µg/mL) antioxidant activity of the oil was lower to the ascorbic acid ($IC_{50} = 0.34 \pm 0.04$ and 0.54 ± 0.04 µg/mL). The oil also showed activities against all the tested bacteria and fungi. The highest inhibition was recorded against *S. aureus* (17 ± 1 mm) at concentration 200 mg/mL. Further, the oils showed strong potential to restrict growth of *Toxoplasma gondii* in vitro, but the parasite growth inhibition was mildly abated in the presence of α-tocopherol. The seed oil of the underutilized *P. longifolia* possesses essential fatty acids which could be responsible for the numerous biological potentials which include anti-lipooxygenase, antioxidant, anti-inflammatory, anti-parasite, anti-microbial and cytotoxic activities. The incorporation of the natural oil into pharmaceuticals or cosmetics may enhance antioxidant, anti-inflammatory, antimicrobial and cytotoxicity potential of such products.

Keywords *Polyalthia longifolia* · Oleic acid · Drug discovery · Medicinal chemistry · Medicinal biochemistry · *Toxoplasma gondii*

Introduction

Triglyceride, otherwise known as fixed oil is a class of naturally occurring lipid in both plants and animals. However, plants are major sources of oil used in medicine, cosmetics, for food and source of energy. Seed oils are used for treatment of muscle spasms, dandruff and open wounds (Chivandi et al. 2009; Vermaak et al. 2011). The recent

✉ O. Atolani
atolani.o@unilorin.edu.ng

[1] Department of Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

[2] Department of Industrial Chemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

[3] Department of Crop Protection, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

[4] Department of Biochemistry, University of Ilorin, P.M.B. 1515, Ilorin, Nigeria

[5] Department of Biochemistry, Medicinal Biochemistry and Toxicology Laboratory, Landmark University, Omu-Aran 251101 Kwara State, Nigeria

[6] Department of Horticulture, Cape Peninsula University of Technology, Cape Town, South Africa

upward surge in the industrial demand for seed oil as raw material for pharmaceuticals, cosmetics and greener fuels has unequivocally stimulated the need to further explore non-edible seed oils sources. Obviously, many seed oils are rich in phytochemicals which makes them very beneficial and applicable to human (IFAD 2008; Lautenschläger 2003).

Besides the industrial applications of seed oils, various essential fatty acids such as omega 3 and 6 are derived from seeds of various plants. Many of these fatty acids which exist in ester form as triglyceride in the seeds have not been characterized in order to open up channels of potential applications. Profiling of the fatty acid profile of seed oils involves the trans-esterification of the lipid.

Polyalthia longifolia Thw. of the family Annonaceae, is a tall flowering ornamental tree native to some tropical regions of India, Pakistan, Sri Lanka and part of Africa. Its produces numerous ovoid-shaped fruits which could be up to 1.8–2.0 cm long with one smooth round seed in each fruit. The plant is a versatile plant used for the treatment of conditions such as rheumatism, menorrhagia, scorpion-stung pain, diabetes, skin disease, hypertension and digestive disorder. The bioassay activities of various crude extracts of the plant shows that plant possesses antibacterial, antioxidant, anticancer, antifungal and anti-diabetic activities. While various terpenoids have been isolated from other plant sources (D'Abrosca et al. 2005; Cangiano et al. 2002), terpenoids possessing antibacterial and antifungal properties have been isolated from the hexane extract of the seeds (Marthanda et al. 2005; Satish et al. 2007; Ghosh et al. 2008; Tanna et al. 2009; Katkar et al. 2010; Thenmozhi and Sivaraj 2010; Tripta and Kanika 2011; Subramanion et al. 2012; Sivashanmugam and Chatterjee 2013). There is however no detail pharmacological evaluation of the characterized seeds oil. As a result, this research was undertaken with the primary objective of investigating the physico-chemical properties, fatty acids profile, anti-oxidant, anti-inflammatory, anti-microbial, anti-parasite and cytotoxic activities of the seed oil of the under-explored tropical plant, *P. longifolia*.

Experimental

Chemicals

Reagents and chemicals which include 1,1-diphenyl-2-picryhydrazyl (DPPH), ascorbic acid, trolox, 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid), ABTS were products of Santa Cruz Biotechnology, USA, while fatty acid standards was obtained from Sigma-Aldrich, USA. Potassium hydroxide (KOH), hydrochloric acid (HCl), n-hexane, methanol, dichloromethane and other reagents used were analytical grade excepted otherwise indicated. However, when required, solvents were redistilled before use.

Plant material

P. longifolia seeds were obtained during fruiting season in December 2016 from Ilorin metropolis, Ilorin, Nigeria. The plant sample was submitted for standard identification and authentication at the Herbarium of the Faculty of Life Sciences, University of Ilorin, where voucher number UILH/001/872 was assigned. The seed materials were de-shelled, the endocarp dried at ambient temperature, pulverized and subjected to extraction.

Oil extraction

The pulverized *P. longifolia* seed material was subjected to various batches of soxhlet extractions at 60 °C using *n*-hexane. The combined extract was concentrated via the vacuum rotary evaporator at reduced temperature to obtain the oil.

Trans-esterification of the oils

The trans-esterification process followed standard procedure with slight modification (Atolani et al. 2016). 100 mg of the oil was dissolved in 10 mL hexane and 1 mL of the dilute solution was further mixed with 4 mL hexane as heptadecanoic acid (C-17:0) was added as internal standard (100 µL of 100 ppm). 1 mL 2.5% (v/v) methanolic sulphuric acid solution was added to the solution and the resulting mixture was incubated in the oven for 1 h at 80 °C. The mixture was allowed to cool to room temperature and 1.5 mL 20% (w/v) NaCl was added to the mixture containing the esterified fatty acid and shaken vigorously followed by centrifuging to facilitate separation into two phases. The upper hexane phase containing the fatty acid methyl esters, FAMES was collected into a glass vial and subsequently injected to the gas chromatography. The degree of trans-esterification was estimated by adopting the formula:

$$Percentage\ yield = \frac{Weight\ of\ the\ transesterified\ oil}{Weight\ of\ raw\ oil\ (g)} \times 100\%$$

Spectrometry and spectroscopy characterizations

Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatograph (6890N, Agilent technologies network) coupled to an Agilent technology inert XL EI/CI Mass Selective Detector (MSD) (5975B, Agilent technologies Inc., Palo Alto, CA) was used to separate the FAMEs. Fatty acids standards were injected and the calibration

maintained. A CTC Analytics PAL autosampler was attached to the GC equipped with a non-polar ZB 7 HG-G010-11 capillary column ZB-5MS with size 30 m by 0.25 mm and 0.25 µm film thickness. Helium gas with flow rate of one (1) mL/min was used as the carrier gas with a split mode set at 5:1. The temperature was kept at 250 °C at the injection point while the temperature setting was: 100 °C for 5 min thereafter increased to 180 °C at a rate of 5 °C/min and held isothermally for 5 min and finally raised to terminate at 330 °C at a rate of 8 °C/min while holding for 5 min. The mass spectrometer was operated with the source/quad temperatures set to 230/150 °C in an electron impact mode having ionisation energy 70 eV with scan spanning the range 35–500 m/z. Constituents were identified primarily based on the comparison of retention time with those of the authentic standards and further confirmed by comparison of mass fragmentation pattern with those of NIST library (Atolani et al. 2016; Chamorro et al. 2012).

Fourier transform infrared (FT-IR) spectroscopic analysis

The crude and trans-esterified oils were subjected to infrared spectroscopic analyses separately in order to identify associated functional groups and the extent of esterification. The infrared spectra were recorded on Shimadzu 8400s (Schimadzu Corporation, Kyoto japan) and Nicolet iS5 FT-IR spectrometer using KBr pellet.

Ultraviolet-visible spectroscopic and spectrofluorometric analyses

Beckman Coulter DU 730 Life Science Ultraviolet–Visible Spectrophotometer, (UK) was adopted for the identification of the types of transition in the samples while absorbance measurements for the assays were recorded on a Spectra Max (Plus) UV multiscan spectrophotometer, (US) except otherwise stated. Fluorescence was recorded on spectrofluorometer (Corona Electric, Japan).

Saponification value (SV)

Methanolic potassium hydroxide (25 mL) was added to 1 g of the oil in a 100 mL conical flask. The mixture was placed into the water bath and warmed for 5 min after which phenolphthalein indicator (3 drops) was added and the mixture properly stirred. The resulting mixture was titrated against 0.5 M hydrochloric acid until the disappearance of the pink colour (Atolani et al. 2016). The SV was determined thus:

$$SV = \frac{mL\,of\,(S-B) \times M \times 56.1\,g/mol}{W} = mgKOH/g$$

S—Titre value of sample; B—Titre value blank; M—Molar concentration of potassium hydroxide used.

Acid value (AV)

One gram seed oil was weighed and mixed with 25 mL methanol in a 100 mL conical flask followed by the addition of three drops of indicator (phenolphthalein). The resulting mixture was slightly warmed in a water bath for 5 min and instantly titrated against 0.1 M KOH. The titration was terminated at appearance of the pink colour and the AV determined using the formula (Atolani et al. 2016).

$$AV = \frac{Vol.\,of\,(KOH) \times N \times 56.1\,g/mol}{W} = mgKOH/g$$

Where AV-Acid value; M-Normality of KOH, Weight of sample (g).

Determination of free fatty acids (FFA)

Ethanol (50 mL) was added to 1 g seed oil and the mixture boiled, cooled, 2 drops of phenolphthalein indicator added and instantly titrated against 0.1 N NaOH until pink colour was appeared (Atiku et al. 2014). The FFA was thereafter determined using the equation:

$$\%FFA = \frac{VmL\,OF\,KOH \times N \times 56.1g/mol}{W(g)}$$

Where 56.1 = Molecular mass of KOH; V = Average titre value, N = Normality of KOH.

Iodine value (IV)

One gram seed oil was weighed into 25 mL carbon tetrachloride in a conical flask. 25 mL Wijis solution was added and the flask was stopped and shaken. The resulting mixture was allowed to stand in the dark (1 h) and the liberated iodine was then titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$) using starch indicator. A solution containing no sample was also titrated (blank titration) appropriately (Samuel 2015). The IV was determined using the expression:

$$IV = \frac{V1 - V2 \times M \times 12.69}{W(g)}$$

Where M = Sodium thiosulphate concentration (Mol/L); V1 = Titre value of sodium thiosulphate used in blank; V2 = Titre value of sodium thiosulphate used in sample; W = Weight of sample oil (g).

In vitro DPPH antioxidant assay

The antioxidant activity of *P. longifolia* seed oil was determined using the DPPH free radical antioxidant assay following standard procedure (Adeosun et al. 2015; Atolani et al. 2015). 0.8 mL of 0.1 mM freshly prepared DPPH solution in methanol was added to 2.4 mL of each of the samples prepared in concentrations that range between 10–500 µg/mL and incubated in a dark chamber at room temperature. After 10 mins of incubation, the reduction in the optical density of DPPH was spectrophotometrically at 517 nm, calculated and compared with a blank and standard control drug, α-tocopherol (Atolani and Olatunji 2014).

In vitro ABTS antioxidant assay

ABTS antioxidant potential of *P. longifolia* seed oil was established by reacting 1 mL of various concentrations (10–500 µg/mL) of the sample oil with 2 mL ABTS cations solution following standard assay procedure (Re et al. 1999). $ABTS^{\bullet+}$ radical cations was freshly generated by mixing ABTS standard solution (7 mM) with potassium persulfate (2.45 mM). The resultant solution was kept in darkness for 12 h at ambient temperature for 12 h after which it was further diluted with methanol to reduced optical density of 0.7 ± 0.01 when measure on the UV spectrophotometer at 734 nm. The ABTS solution was allowed to react for 60 s with various concentrations of the oil and the optical density read at the same wavenumber. Ascobic acid standard control as the experiment was carried out in triplicate (Valyova et al. 2012).

Anti-inflammatory activity: lipoxygenase assay

The method of Njenga and Viljoen (2006) was adopted for the determination of the lipoxygenase activity of the sample. Precisely, the assay was carried out by reacting 50 µL aliquots of lipoxygenase solution in potassium phosphate buffer pH 9.0) with 2.0 mL sodium linoleate (100 µM) buffered in standard phosphate solution. 30 µL oil solution prepared in different concentrations were allowed to react with the freshly prepared reagent and the degree of reaction compared to the standard, indomethacin by measuring the formation of 13-hydroperoxyl linoleic acid from the linoleic acid (forming a new conjugated diene) at 234 nm on a multiscan absorbance reader.

Antimicrobial assay

Agar diffusion method was used to determine the antibacterial and antifungal properties of the oil (Atolani et al. 2014). The oil was re-constituted in water at concentration range 62.5–500 mg/mL. Mueller Hinton agar was prepared as per the manufacturer's protocol. The sterile Mueller Hinton agar was poured into sterile Petri dishes and seeded with the six test bacteria (clinical isolates of *Salmonella typhi, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, *Klebsiella pneamananae* and *Staphylococcus aureus*) of Mcfarland standard. Sterile plates were impregnated with each oil solution. The impregnated discs were air-dried at room temperature and thereafter placed on the surface of the inoculated agar plates.

Four fungi (clinical isolates of *Candida albicans, Aspergillus nigar, Penicillium notatum* and *Rhizopus stolonifer*) were inoculated into mycological peptone and then incubated for 1 h and this was then used to swab Sabouraud's Dextrose agar. The plates were incubated for 24 h (for bacteria strains) at 37 °C and 48 h (for fungi strains) at 27 °C. Gentamincin and Tioconazole were used as the positive control for bacterial and fungi respectively while negative controls included disks impregnated with water. The antimicrobial activity of the extracts and compounds tested were evaluated at the end of the inoculated period by measuring the inhibition zone diameter in millimeters. The presence of zones of inhibition around each of well after the incubation period was regarded as the evidence of antimicrobial action while the absence of any measurable zone of inhibition was interpreted as absence of antimicrobial activity.

Minimum inhibitory concentration (MIC)

The MIC values were evaluated according to published procedures (Atolani et al. 2014). MIC was determined by serial dilution of the oil in medium and application on the dish using the disc diffusion method. Dilutions of the sample were determined by incubating at 37 °C and fungi at 27 °C. The zone of inhibition was measured in mm after 24 or 48 h of growth as appropriate. A control experiment was carried out by using equal amount of sterile medium (only). The lowest concentration of the sample solutions that caused complete inhibition of the bacteria was taken as the MIC.

Cytotoxicity of the oil in mammalian cell

The cytotoxicity study utilizes human foreskin fibroblast (ATCC®) cells which were maintained in culture medium containing glutamine-supplemented Dulbecco's Modified Eagle Medium (DMEM, Japan) with 10% (v/v) fetal calf serum, FCS (Invitrogen, UK), and penicillin with streptomycin (Biowhittaker, UK). The Cells were allowed to grow to confluence under normal tissue condition (37 °C, 5% CO_2 atmosphere). At confluence, cells were harvested as per sub-culture protocol, re-suspended and seeded in a 96-well plate (Fisher Scientific, USA) at a concentration of

1×10^5 cells per well. After a 72-h incubation, cells were treated with different concentrations (µg/mL) of the oil. The control well contained only the culture medium without the test sample and staurosporine used as standard control. The plate was incubated for another 72 h and cell viability thereafter determined.

In vitro anti-toxoplasma gondii assay

For the anti-parasite assay, *T. gondii* strain, with ATCC® identification number 50839 used was sustained as previously indicated for the mammalian cell. HFF monolayers infected with *T. gondii* tachyzoites were syringe-released using a 27-guage needle to lyse them. The cells were filtered via 5 µm filter and suspension washed with the freshly prepared medium. The parasite population was thereafter determined and diluted to pre-determined concentration. The purified parasite was introduced into the oil samples constituted in culture medium and incubated in a 96-well plate. The negative control contained the culture medium only while sulfadiazine was included as a positive control. The stability and viability of the RH-2F parasite was checked after a 72 h brooding at 37 °C in a standard 5% carbondioxide condition via measuring the action of galactosidase, using a Beta-Glo luminescent procedure (Promega, Madison, USA) (Adeyemi et al. 2017).

Measurement of intracellular reactive oxygen species (ROS)

This assay is premised on the intracellular oxidation of 2′,7′-dichlorodihydrofluorescein diacetate H_2DCF-DA, to form the fluorescent compound 2′,7′-dichlorofluorescein. In the assay, growing HFF monolayers were treated with various concentrations of the oil sample in the presence or absence of *T. gondii* parasites and incubated for 24 h at 37 °C as hydrogen peroxide served as standard control. Subsequently, the cells were collected, washed, and constituted in bovine serum containing the H_2DCF-DA to make a solution of 100 µM. Suspended cells with dye indicator was subjected to the same incubation condition for approximately 30 min and the fluorescence measured on the spectrofluorometer with excitation/emission programmed to 485/530 nm. The assay follows previous reported procedure of Adeyemi et al. (2017).

Measurement of the mitochondrial membrane potential (MMP)

The MPP assay follows reported procedure (Adeyemi et al. 2017). Various concentrations of the *P. longifolia* oil were introduced to the growing HFF monolayers in the absence/presence of *T. gondii* infection and incubated for 24 h at 37 °C. This was followed by harvesting and purification of the cells, immediate cells staining using 200 nM MitoRed solution (DMT, Japan) and fluorescence measurement at 560 nm excitation with emission at 580 nm.

Statistical evaluations

Results obtained were evaluated on GraphPad Prism 5 (San Diego, CA) using a one-way ANOVA and outcome of each duplicate or triplicate values presented as mean ± Standard Error of Mean, SEM except otherwise indicated. The concentration of the oil showing a 50% inhibition or reduction in parasite and/or cell viability (i.e., EC_{50} and/or IC_{50} values) were also estimated on the GraphPad Prism 5 via a non-linear regression fit.

Results and discussion

Physico-chemical characteristics of *P. longifolia* seed oil

P. longifolia seed oil obtained by soxhlet extraction had the physico-chemical characteristic shown (Table 1).

In this study, saponification value of 147.26 ± 7.01 mgKOH/g was obtained for *P. longifolia* (Table 1). This value falls within the recommended range suggested for edible oil implying that the oil could be good candidate for medicinal and cosmetic or personal care products (Oyedeji et al. 2011; Emmanuel 2012; Ajala and Adeleke 2014). Relatively high saponification value of fats and oils are reportedly due to the predominantly high proportion of shorter carbon chain lengths of the fatty acid (Kirk and Sawyer 1991).

The acid value, 12.62 ± 0.28 mg/KOH/g was obtained for *P. longifolia* (Table 1). This is in tandem with literature report (13.46 mgKOH/g) for *P. longifolia* seed oil (Oyedeji et al. 2011; Paul and Lukman 2014). Acid value is known as a good measure of the breakdown of the triglycerides into

Table 1 Yield and the physico-chemical parameter of *P. longifolia* seed oil

Properties	*P. longifolia* oil
% Oil yield	5.00
% Trans-esterifiable	80
[a]% Free fatty acid	6.486 ± 0.28
[a]Saponification (mgKOH/g)	147.26 ± 7.01
[a]Acid Value (mgKOH/g)	12.62 ± 0.28
[a]Iodine value	94.54 ± 9.52
Colour	brown
Physical state at ambient temperature	Solid

[a]Values are reported as mean ± SEM of duplicate data

free fatty acids, which has an adverse effect on the overall quality of the lipids. Low acid value in oil shows that the oil may find application in cooking while high acid value in oil indicated that the oil may also find application in cosmetic preparations (Bello et al. 2011; Aremu et al. 2015; Samuel 2015). The values obtained for free fatty acid of *P. longifolia* was 6.486 ± 0.28 (Table 1). These values are in agreement with those recorded in the literature (Oyedeji et al. 2011; Emmanuel 2012). Iodine value is used to determine the amount of unsaturation in fatty acids in form of double bonds by reacting with the iodine compounds. The lower the iodine value the harder the soaps and the less the conditioning qualities and vice versa (Afolayan et al. 2014). The iodine value is mostly used for identification of oil or to classify the oil. Oils with iodine value less than 100 gI_2/100 g belongs to the group of non-drying oils. The lower the iodine value the lower the susceptibility of such oil to oxidative rancidity (Aremu et al. 2015; Samuel 2015). *P. longifolia* seed oil had IV of 94.54 ± 9.52 which qualifies it to be classified as a non-drying oil.

Characterizations

FT-IR analysis of raw and transesterified oils

The raw and trans-esterified oils of the *P. longifolia* seed were subjected to infrared analysis to confirm that all the fatty acids were successfully converted to their respective esters. The important peaks obtained from the spectra of both oils are as depicted (Table 2).

The appearance of a broad O–H stretching vibration at 3425–3439 cm^{-1} in the infrared spectrum of the raw oil is indicative of the presence of FFA content. This apparently corroborated the FFA value of 6.486 ± 0.28 mgKOH/g confirmed in the oil. The disappearance of the O–H stretching vibration in the spectrum of the trans-esterified oil confirmed a successful reaction. The reduction in absorption band of O–C stretch of the *P. longifolia oil* from 1259 cm^{-1} (raw oil) to 1204 cm^{-1} (transesterified oil), further strengthen the evidence of a successful trans-esterification. The vibration at 1745–1701 cm^{-1} in the oil corresponds to the carbonyl stretching vibration typical of carboxylic and esters (Rabelo et al. 2015; Oyerinde and Bello 2016). The band at 1465–1377 cm^{-1} was assigned to the C–H bending of methyl groups of the fatty acids and 1602 cm^{-1} ascribed to C=C bending of the fatty acid methyl esters (FAMEs). The peak at 725 cm^{-1} confirms the presence of C=C (alkene group) (Gunstone 2004; Shuit et al. 2010; Hariram et al. 2017). O–CH_3 stretching which is typical of FAMEs was implicated by C–O Stretching at 1204 cm^{-1}.

Table 2 FT-IR data of raw and trans-esterified *P. longifolia* seed oil

Raw oil (cm^{-1})	Trans-esterified oil (cm^{-1})	IR bands
3425.65	–	O-H stretching *(b)*
2964.64	2958.31	sp^2 C–H Stretchings *(w)*
2928.04	2929.02	sp^3 C–H Stretchings *(s)*
2858.60	2856.99	C–H (methylene) *(s)*
1701.27	1767.96	Conj. C=O Stretchings *(s)*
1643.41	1650.66	C=C Stretchings *(w)*
1452.45	1455.77	CH_2 Bending *(w)*
1383.01	1380.76	CH_3 Bending *(w)*
1259.56	1204.00	C–O Stretchings *(w)*
725	725	C=C Bendings (s)

Broad (b), Sharp (s), and Weak (w)

UV–Vis analysis of the raw oils

UV–Visible spectroscopy analysis was used to determine the types of transition and extent of conjugation or unsaturation in the oil. The result of the UV–Visible analysis is as depicted (Table 3). As indicated, the results obtain for both seed oils corroborate the absorption bands observed in the infrared results (Table 2). The correlation is depicted in Table 4.

Fatty-acid profile of *P. longifolia* oil

The prepared FAMEs of the seed oil was subjected to GC-MS analysis for profiling of the fatty acids. The results are shown in Table 5.

The GC-MS analysis revealed the major fatty acids (Table 5) in the oil of *P. longifolia* seed to be oleic acid (30.99%), linoleic acid (19.61%), palmitic acid (15.45%), tricosylic acid (6.10%) and stearic acid (5.68%). Saturated fatty acids were of the lowest proportion (29.47%) in the oil of *P. longifolia*. The highest percentage of unsaturated omega-3 fatty acid eicosenoic acids is 1.37%. Linoleic acid (19.27%) occurs at a highest proportion among the omega-6 FAs obtained in the oil as oleic (30.31%), an omega-9 FA is the most abundant of its kinds.

Essential FAs such as omega-3, 6 and 9 are required for cell manufacture and cell membranes repair thereby, affording human body maximum nutritional benefit whilst expelling toxic materials. Linoleic acid, an omega-3 FA is one of the most applied FA for cosmetic purpose due to its skin moisturizing potential and ability to stimulate recovery process of sunburns as well as other skin conditions. Seed oils rich in linoleic acid and linolenic acid are potent natural

Table 3 UV–Visible spectroscopy analysis of raw *P. longifolia*

Oil samle	Wavelength (nm)	Absorbance	Transition
P. longifolia	320	2.61	C=C band ($\pi \rightarrow \pi^*$)
	391	3.65	C=O band ($n \rightarrow \pi^*$)

anti-inflammatory source used in treatment of acne (Lautenschläger 2003; Kanlayavattanakul and Lourith 2011; Vermaak et al. 2011). Oleic and palmitic acids are known to exhibit skin penetration effect (Kim et al. 2008). Other micronutrients present in seed oils prevent the risk of cardiovascular diseases (Gladine et al. 2011).

Table 4 Comparison of UV and FTIR analysis of raw *P. longifolia*

Oil samle	UV (nm)	IR (cm^{-1})	Remark
P. longifolia	320	1643	C=C bands
	391	1735	C=O bands

Therefore, oils of *P. longifolia* can find application in food industries for the production of unique cooking oil and essential diet composition, in pharmaceutical industries for production of drugs and food supplements as well as in the cosmetic industries for the production of specialized oil-based personal care products.

Anti-lipoxygenase activity

Lipoxygenases (LOX) are important enzymes involved in the conversion of arachidonic, linoleic and other poly-unsaturated FAs into bioactive substances that are

Table 5 Fatty acid profile of *P. longifolia*

Common/Trivial name	Systematic name	Saturation	Concentration (mg/mL)	Composition (%)
Palmitic acid	Hexadecanoic acid	16:0	15.45	15.11
Palmitoleic acid		16:1	1.07	1.04
	cis-10- Heptadecanoic acid	17:1	1.06	1.03
Stearic acid	Octadecanoic acid	18:0	5.68	5.56
Trans oleic acid	trans-9-octadecenoic acid	18:1n9t	2.14	2.09
Oleic acid	cis-9-octadecenoic acid	18:1n9c	30.99	30.31
Linolelaidic acid		18:2n6t	0.99	0.97
Linoleic acid	all-cis-9,12-octadecadienoic acid	18:2n6c	19.69	19.27
Gamma-linolenic acid (GLA)	all-cis-6,9,12-octadecatrienoic acid	18:3n6	2.20	2.15
Alpha linolenic acid (ALA)	all-cis 9,12,15-octadecatrienoic acid	18:3n3	1.01	0.98
Eicosenoic acid	cis-11-eicosenoic	20:1	1.40	1.37
	cis-11, 14-Eicosadienoic acid	20:2	0.98	0.96
Dihomo-gamma-linolenic acid (DGLA)	all-cis-8, 11, 14-Eicosatrienoic acid	20:3n6		
Heneicosylic acid	Heneicosanoic acid	21:0	0.97	0.94
Arachidonic acid	all-cis-5,8,11,14-eicosatetraenoic acid	20:4n6	1.43	1.39
Eicosatrienoic acid (ETE)	all-cis-11, 14, 17-Eicosatrienoic acid	20:3n3	2.81	2.75
Behenic acid	Docosanoic acid	22:0		
	Erucic acid	22:1n9	1.33	1.30
Docosadienoic acid	all-cis-13,16-docosadienoic acid	22:2n6	2.09	2.05
Tricosylic acid	Tricosanoic acid	23:0	6.24	6.10
Lignoceric acid	Tetracosanoic acid	24:0	1.79	1.75
Nervonic acid	cis-15-tetracosenoic acid	24:1	1.64	1.60
Docosahexaenoic acid/ cervonic acid	cis-4,7,10,13,16,19-Docosahexaenoic	22:6n3	1.25	1.22
Total saturated				29.47
Monounsaturated				38.77
Polyunsaturated				31.76
Total unsaturated				70.53

responsible for inflammatory and immune responses. They are enzymes that are key in the biosynthesis of leukotrienes plays vital roles in some inflammation-related conditions including cancer, allergic reactions, asthma, colitis ulcerosa, rheumatoid arthritis, and psoriasis (Catalano and Procopio 2005; Dobrian et al. 2011; Rackova et al. 2007; Eshwarappa et al. 2016). Inhibition of leukotrienes biosynthesis via the LOX pathway with natural products has been reported to be a potential therapeutic means. The anti-LOX activity of the seed oil of *P. longifolia* was determined and compared with a standard drug, indomethacin following standard procedure already described. A dose response activity comparable to the standard drug was obtained (Table 6 and Fig. 1). It was observed that the highest activity (93.88%) was recorded for the oil as against 99.68% recorded for indomethacin at 500 µg/mL. The corresponding IC_{50} values of both the oil and indomethacin were found to be 0.70 ± 0.02 and 0.53 ± 0.07 µg/mL respectively. The seed oil exhibited potent LOX inhibitory activity and it is suggested to possess anti-inflammatory activity.

Antioxidant activities

In order to establish the antioxidant activities of the *P. longifolia* seed oil, two antioxidant bioassays (DPPH and ABTS assays) were adopted. In the DPPH assay, the oil exhibited dose-dependent activities which was however low in comparison with the standard antioxidant, ascorbic acid (Table 7, Fig. 2). The activities recorded for the oil ranges from 18.74 to 24.31% while the ascorbic acid ranges from 87.76 to 98.67% with corresponding IC_{50} values of 55.91 ± 31.18 and 0.34 ± 0.04 µg/mL respectively at concentrations (10 to 500 µg/mL).

ABTS antioxidant potential

The ABTS antioxidant potential of the *P. longifolia* seed oil was examined at various concentrations ranging 10, 50, 100, 200 and 500 µg/mL. ABTS is useful when studying the effect of pH on antioxidant activity of various compounds. Additionally, the solubility of ABTS in organic and aqueous media made it a vital assay for routine assessment of antioxidant activity of samples (Shalaby and Shanab 2013).

The ABTS antioxidant activity of the oil (Table 8, Fig. 3) was lower (16.89 ± 15.5 µg/mL IC_{50}) at all concentrations tested compared to the reference standard, ascorbic acid (0.54 ± 0.04 µg/mL IC_{50}). The ABTS scavenging activity of the oil which was not dose-response peaked at 200 µg/mL when activity was 30.70% and lowest at 100 µg/mL (15.43%) while the ascorbic acid had dose-dependent activity ranging from 76.05 (10 µg/mL) and 96.68% (500 µg/mL).

From the two complementary DPPH and ABTS antioxidant assays (Figs. 2 and 3), the oil exhibited low antioxidant activities. The variation in the antioxidant activities

Table 6 Anti-lipoxygenase activity of *P. longifolia* seed oil and indomethacin (standard) with their corresponding IC_{50} values

Concentration (µg/mL)	Indomethacin (%Inhibition)	*P. longifolia* oil (% Inhibition)
10	79.10 ± 0.20	66.75 ± 0.32
50	87.20 ± 0.13	85.58 ± 0.77
100	90.68 ± 0.27	86.65 ± 1.15
200	99.05 ± 0.07	92.18 ± 0.35
500	99.68 ± 0.023	93.88 ± 0.36
IC_{50}	0.53 ± 0.07	0.70 ± 0.02

Table 7 Anti-oxidant DPPH activities of vitamin C (Control) and oil

Concentration (µg/mL)	Ascorbic acid (% Inhibition)	*P. longifolia* oil (% Inhibition)
10	87.76 ± 0.044	18.74 ± 0.12
50	93.25 ± 0.016	19.41 ± 0.18
100	95.55 ± 0.096	22.49 ± 0.61
200	98.22 ± 0.007	23.91 ± 0.92
500	98.67 ± 0.001	24.31 ± 0.53
IC_{50}	0.34 ± 0.04	55.91 ± 31.18

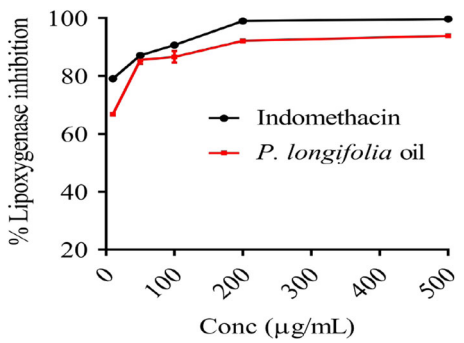

Fig. 1 Lipoxygenase percentage inhibition of Indomethacin (control) and *P. longifolia* oil

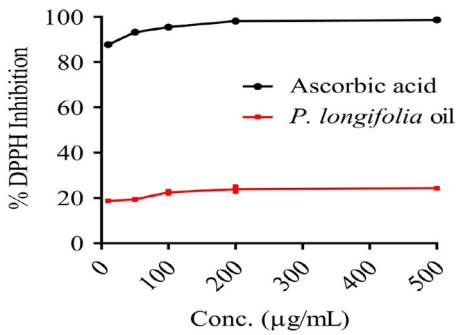

Fig. 2 DPPH anti-oxidant activities of ascorbic acid (control) and *P. longifolia* seed oil

Table 8 ABTS anti-oxidant activities of ascorbic acid (Control) and oil

Concentration (μg/mL)	Ascorbic acid (% Inhibition)	*P. longifolia* oil (% Inhibition)
10	76.05 ± 0.66	17.26 ± 1.75
50	86.04 ± 0.44	21.91 ± 0.52
100	91.49 ± 0.39	15.43 ± 0.27
200	94.43 ± 1.20	30.70 ± 0.36
500	96.68 ± 0.16	24.68 ± 0.33
IC_{50}	0.54 ± 0.04	16.89 ± 15.50

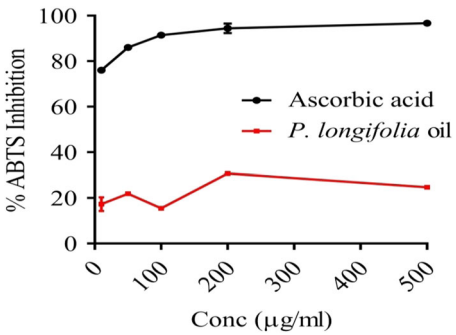

Fig. 3 ABTS percentage inhibition of ascorbic acid (control) and *P. longifolia* oil

on the DPPH and ABTS assay reports is apparently due to differences in pathway of each antioxidant assay.

Anti-microbial activity of the oil

The anti-microbial evaluation of plant extracts and bioactive compounds via in vitro method is well established in literatures (Das et al. 2010; Obi 2014; Balouiri et al. 2016). The *P. longifolia* seed oil in concentrations 12.5, 25, 50, 100 and 200 mg/mL were tested against ten (10) clinical isolates of pathogenic organisms comprising of six bacteria and four fungi. Gentamincin and tioconazole were adopted as the reference anti-bacteria and anti-fungi drugs respectively. At 200 mg/mL, all the organisms namely, *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Klebsiella pneamananae, Candida albicans, Aspergillus nigar, Penicillium notatum* and *Rhizopus stolonifer* had observable sensitivity against oil (Table 9, Fig. 4). The highest microbial inhibition of the *P. longifolia* oil was recorded against *S. aureus* (17 ± 1 mm) at concentration 200 mg/mL while the lowest inhibitory activity at the same concentration was against *R. stolonifer* with 11 ± 1 mm diameter zone of inhibition. All tested organism except *R. stolonifer* had minimum inhibition concentration (MIC) of 50 mg/mL which had (MIC) of 200 mg/mL. No anti-bacterial and anti-fungi activities were recorded at 12.5 and 25 mg/mL concentrations of the oil. The *P. longifolia* seed oil had significant activity which was however lower compared to the standards gentamincin and tioconazole.

Cytotoxicity assay

Cytotoxic compounds affect the cell by causing uncontrolled cell death, preventing cell growth and cell division. The in vitro cytotoxic assay was carried out to evaluate the potential cytotoxicity of the *P. longifolia* oil on mammalian cells using the HFF. The lowest cytotoxic activity (Table 10) was recorded at 250 μg/mL with cell viability (104.83%) while the highest was obtained at 1000 μg/mL (0.03% cell viability). The data depict a dose-dependent cytotoxic action by the *P. longifolia*.

Anti-parasite activity of *P. longifolia*

T. gondii is a common protozoan parasite with low host specificity (Ishiwa et al. 2013; Adeyemi et al. 2017). *T. gondii* is responsible for toxoplasmosis that currently affects more than one third of human population. In healthy individuals, the disease is asymptomatic but could prove fatal in pregnant women as well as in immune-compromised individuals (Choi et al. 2013). The anti-parasite activity of the *P. longifolia* against *T. gondii* was evaluated. A dose-response cytotoxic activity was obtained for the anti-parasite activity of *P. longfolia* seed oil against *T. gondii* (Table 11). From the data, it is revealed that the anti-parasite action of the *P. longfolia* seed oil increases with increasing concentration. At 125 μg/mL, the oil had 80.80% parasite growth and only 2.30% growth at 1000 μg/mL. The oil became highly toxic at concentration above 500 μg/mL.

Luciferase, was measured, and parasite viability was calculated with untreated as a negative control (100% parasite viability) and 1000 μg/mL sulfadiazine as a positive control (0% viability). The experiment was conducted independently thrice and in triplicates.

Selectivity index

The selectivity index of the oil was determined by finding the ratio of the cytotoxicity (IC_{50}) to the anti-parasite activity (EC_{50}). Table 12 shows that the selectivity index (<1) of *P. longifolia* was inferior to that of the reference drug, sulfadiazine (<4), the drug currently used for treatment of toxoplasmosis. Though, *P. longifolia* oil demonstrated good activity against *T. gondii* it failed to show promising selectivity against the parasite and the host cell. The anti-parasite action is therefore suggested to be as a result of general cellular toxicity.

Table 9 Anti-microbial activities of *P. longifolia* oil

	Concentration in (mg/mL)					Positive control	
	200	100	50	25	12.5		MIC (mg/mL)
Tested organism	Diameter zone of inhibition (mm)						
Bacteria						Gentamincin 10 (mg/mL)	
Staphylococcus aureus	17 ± 1	14	10	–	–	34	50
Escherichia coli	15 ± 1	13 ± 1	10	–	–	36	50
Bacillus subtilis	15 ± 1	13 ± 1	10	–	–	36	50
Pseudomonas aeruginosa	14	12	10	–	–	36	50
Salmonella typhi	16	14	10	–	–	36	50
Klebsiella pneamananae	15 ± 1	13 ± 1	10	–	–	34	50
Fungi				–	–	Tioconazole 70%	
Candida albicans	15 ± 1	12	10	–	–	28	50
Aspergillus nigar	15 ± 1	13 ± 1	10	–	–	26	50
Penicillium notatum	14	12	10	–	–	26	50
Rhizopus stolonifer	11 ± 1	–	–	–	–	28	200

MIC minimum inhibition concentration

[a]The Ndata are expressed as Mean ± Standard Error of Mean of duplicate determinations

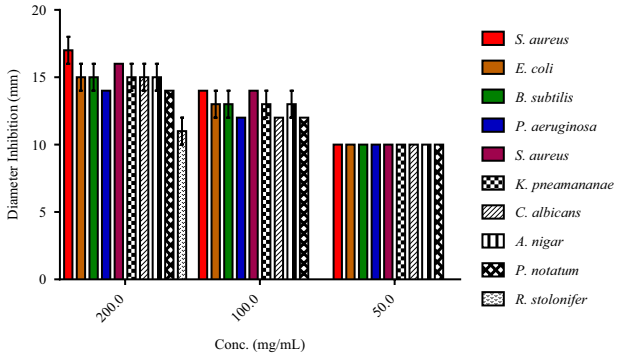

Fig. 4 Comparison anti-microbial activities of *P. longifolia* oil against the tested organisms

Table 10 Cytotoxicity of *P. longifolia* oil

Conc. (μg/mL)	*P. longifolia* (% Viability)
125	96.80 ± 2.97
250	104.83 ± 1.90
500	1.06 ± 0.02
1000	0.03 ± 0.00

Staurosporine (1 μM) served as standard for assay validation and experiment was conducted independently thrice in triplicates

Table 11 Anti-parasite activity of *P. longfolia* seed oil

Conc. (μg/mL)	*P. longifolia* (% parasite growth viability)
125	80.80 ± 4.83
250	62.99 ± 2.86
500	60.69 ± 4.76
1000	2.30 ± 0.68

Table 12 Cytotoxicity IC_{50} and anti-parasite EC_{50}

Samples	Host cell cytotoxicity	Anti-parasite activity	Selectivity index
	IC_{50} (μg/mL)	EC_{50} (μg/mL)	(SI): IC_{50}/EC_{50}
P. longifolia	≤400	≤400	<1
Sulfadiazine	≤500	≤150	<4

Anti-parasite activity in the presence of trolox

Having established the anti-parasite action of the oil extracts, the mode of anti-parasite action was examined via determining if the ROS are culpable in the anti-parasite action of oil extracts by including an antioxidant, α-tocopherol (100 μM) in the anti-parasite screening assay.

From Figs. 5a, b, it could be deduced that the addition of the antioxidant, α-tocopherol reduces the anti-parasite activity appreciably. Data show that the addition of α-tocopherol abated the anti- *T. gondii* potential of the *P. longifolia*. This shows the culpability of oxidative stress in the anti-parasite action of the *P. longifolia*. Further, it could be that the *P. longifolia* predisposes to the generation of ROS, thereby contributing to the action against the *T. gondii*. To further support the fact that ROS production and by extension, oxidative stress might be culpable in mechanistic pathway of *P. longifolia* oil. Results obtained showed that *P. longifolia* promoted ROS production irrespective of the inclusion of elimination of *T. gondii* infection (Figs. 6a, b) respectively. Consequently, the *P. longifolia* oil also reduced cellular mitochondria membrane potential (MMP) when *T. gondii infection* was prevented (Fig. 6c), but not in the inclusion of the *T. gondii* infection

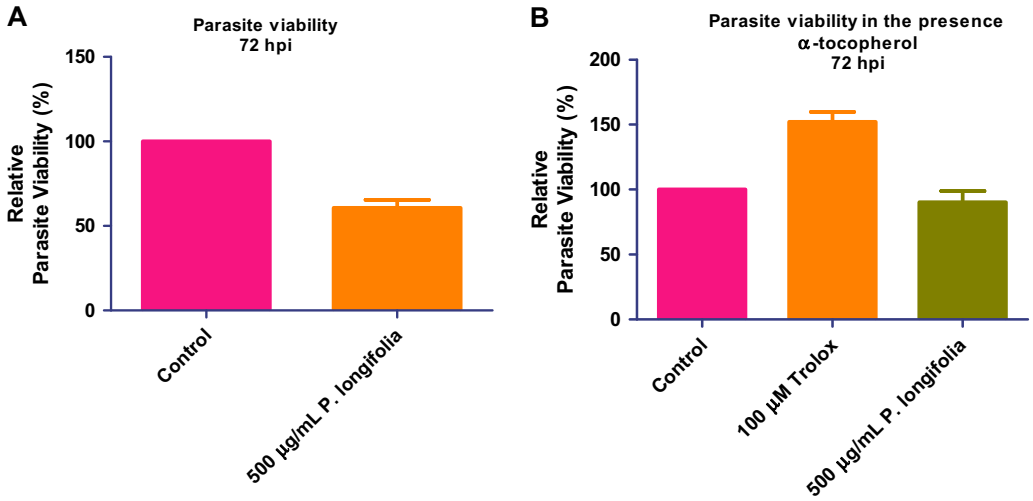

Fig. 5 **a**-**b** Anti-*T. gondii* potential of *P. longifolia* in the absence/presence of α-tocopherol (trolox). The experiment was conducted independently thrice and in triplicates

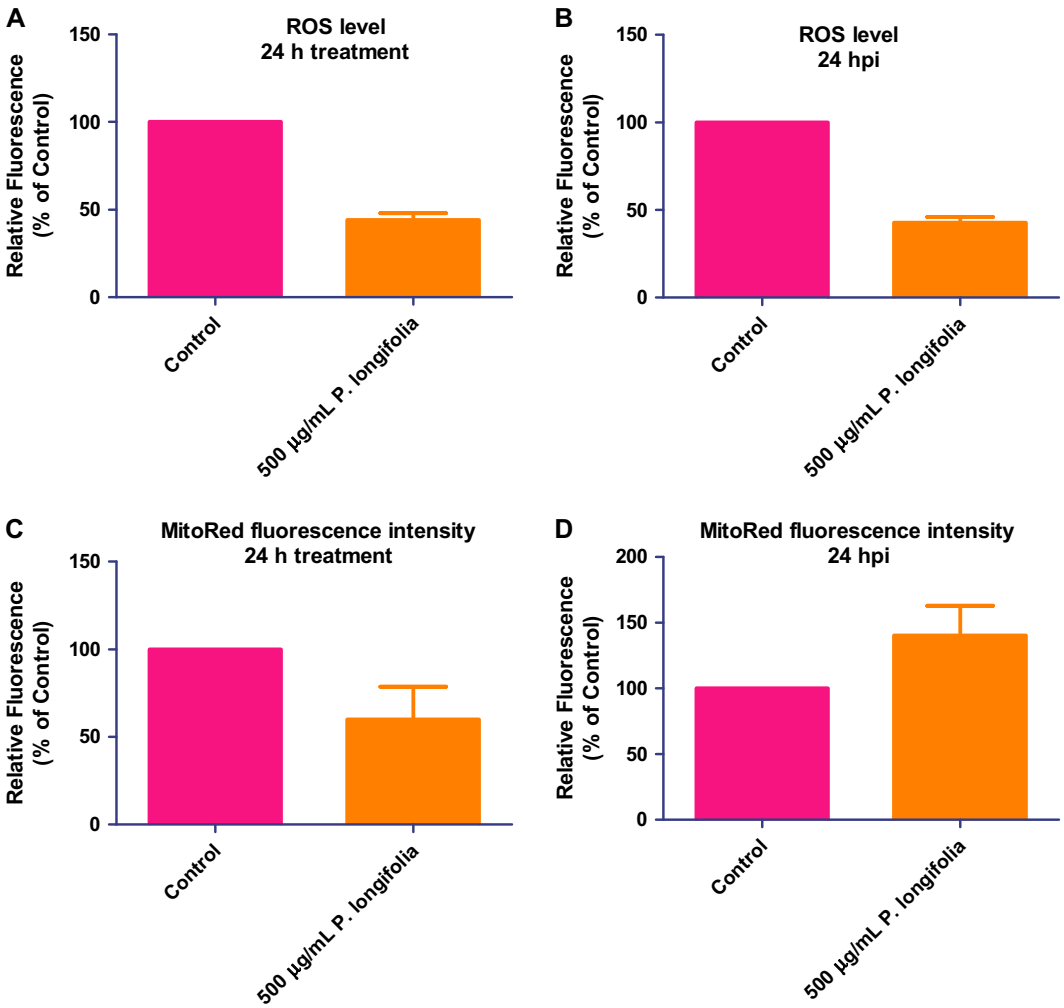

Fig. 6 **a**-**d** ROS level and mitoRed fluorescence intensity following 24 h treatment with *P. longifolia*; **a** without *T. gondii* infection; **b** with *T. gondii* infection; **c** without *T. gondii* infection; **d** with *T. gondii* infection. The experiment was conducted independently thrice and in triplicates

(Fig. 6d). This probably might be due to alteration of physiological status of cells due to *T. gondii* infection.

Conclusions

The oil of the underutilized *P. longifolia* is established to contain oleic acid, linoleic acid and palmitic acid as the major fatty acids. Unsaturated FAs accounted for 70.53% of the total oil composition. The oil exhibited several biological activities which include anti-lipooxygenase, antioxidant, anti-inflammatory, anti-parasite, anti-microbial and cytotoxicity activities. Of particular interest are the antimicrobial, anti-lipooxygenase and anti-parasite activities which were significantly high. The incorporation of the oil into personal care products for topical application may enhance in relieving infection and inflammation-related conditions. These activities could be harnessed for essential applications in the foods, nutraceuticals and cosmetic industries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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