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Evaluation of *Costus afer* Ker Gawl. *in vitro* anti-inflammatory activity and its chemical constituents identified using gas chromatography-mass spectrometry analysis

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PEER REVIEW

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Comments

The research work was interesting. The authors organized the paper logically and data were appropriately presented.
Details on Page 137

ABSTRACT

Objective: To evaluate the anti-inflammatory activity of a tropical African medicinal plant, *Costus afer* (*C. afer*) Ker Gawl. *in vitro* and identify the chemical constituents in the most active fraction.

Methods: Hexane, ethyl acetate, *n*-butanol and aqueous fractions obtained through successive solvent partitioning of 70% methanolic leaf or stem extracts of *C. afer* were subjected to *in vitro* anti-inflammatory screening assays *viz*. anti-denaturation of protein, stabilization of human red blood cell (HRBC) membrane against hypotonicity-induced hemolysis and anti-proteinase activities. Diclofenac sodium was used as a standard drug. The chemical compounds in the most active fraction were determined using quantitative phytochemical and gas chromatography-mass spectrometry (GC/MS) analytical methods, comparing the mass spectra of the GC/MS identified compounds with those of the National Institute of Standards and Technology (NIST) database library.

Results: The hexane fraction of *C. afer* leaf (HFCAL) with an IC_{50} of 33.36 μg/mL, 33 μg/mL and 212.77 μg/mL exhibited the highest anti-denaturation of protein, stabilization of HRBC membrane and anti-proteinase activities respectively when compared with other test fractions. The GC/MS identified compounds in HFCAL known to possess anti-inflammatory property were terpenoids (naphthalene 1,6-dimethyl-; naphthalene 2,3-dimethyl-; phytol), phenol [phenol 2,4-bis(1,1-dimethylethyl)], coumaran [2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4, 4,7atrimethyl and fatty acids (pentadecanoic acid; hexadecanoic acid; *n*-hexadecanoic acid; 9,12-octadecanoic acid-methyl ester; 9,12,15 octadecatrienoic acid and cis-vaccenic acid)].

Conclusion: Therefore, HFCAL could be considered as a potential source of anti-inflammatory agents for herbal formulation or pharmaceutical drug production.

KEYWORDS

Costus afer; Anti-inflammation; GC/MS; Phytochemical

1. Introduction

Costus afer (*C. afer*) Ker Gawl. commonly known as gingerlily or bush cane belongs to the Family of Zingiberaceae now known as Costaceae. It is one of the 150 species of tall, perennial, and rhizomatous herbs[1]. *C. afer* is commonly found in moist or shady

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forests and river banks of tropical West Africa. It is often used as a medicinal herb throughout tropical Africa, especially in treating inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic, miscarriages, epileptic attack and hemorrhoids. Also, it is used as laxative, diuretics, and had served as an antidote for poison[2-4]. In Nigeria, the plant extract is used as medicament to treat goats with retained placenta[5]. In the western part of Nigeria, it is called different names such as; "ireke omode", "ireke ogun" while it is called "okpete" or "okpoto" in Igboland, "Kakizawa" in Hausa and "Mbritem" in Efik and anglophone Cameroon calls it "Monkey sugar cane"[2].

The rhizomes of *C. afer* contain several steroidal sapogenins (diosgenin), aferosides, dioscin and paryphyllin C and flavonoid glycoside kaempferol 3-O- -L-rhamnopyranoside[1]. Sesquiterpenoids are the most abundant group of volatile compounds in the essential oil of the leaf[6]. The chloroform and methanol extracts from the aerial parts reduced carrageenan*n*-induced rat paw oedema[1], while extracts of *C. afer* stem and leaves exhibited antioxidant and hepatoprotective activities[7,8].

The search for an alternative source of anti-inflammatory drugs is of paramount interest to research institutes, pharmaceutical companies and academia[9]. This is the undesirable side effects experienced by rheumatoid arthritis patient after the use of either synthetic steroidal or no*n*-steroidal anti-inflammatory drugs (NSAIDs). In addition, the World Health Organization had reported that these drugs are often associated with drug-induced toxic effects or secondary adverse effects on long term use[10,11]. Furthermore, alternative sources of anti-inflammatory drugs of plant origin are readily accessible, available and affordable and contain bioactive compounds of therapeutic value.

To the best of our knowledge no attempts have been made to identify the chemical compounds present in *C. afer* plant used by locals in the treatment of several inflammatory diseases. Therefore, this study aimed to isolate the most active anti-inflammatory fraction of *C. afer* using *in vitro* screening methods and identify its chemical compounds.

2. Materials and methods

2.1. Collection of plant materials

C. afer plants were obtained from a farm land at Irolu in Ikenne Local Government Area, Ogun State, Nigeria. The plant was identified and authenticated by Professor Denton, O.A., a crop scientist in the Department of Agronomy and Landscape Design, School of Agriculture and Industrial Technology, Babcock University, Ilisa*n*-Remo, Ogun State, Nigeria. A voucher sample with number of FHI-108001 has been deposited at Forestry Herbarium Ibadan (FHI).

2.2. Plant processing, extraction and solvent partitioning

The leaves and stem were separated from the root which was discarded. The leaves and chopped stem pitches were air-dried under room temperature and pulverized using mechanical grinder. Three hundred grams powdered leaves and stem samples were extracted using 1 800 mL of 70% methanol at 28 ℃ with intermittent shaking for 48 h. The extracts were filtered using Whatman No.1 filter paper and the filtrates were subsequently concentrated using rotary evaporator at 30 ℃ (Buchi Rotavapor RE; Switzerland). The concentrates obtained were reconstituted in water (2:1 w/v) and subsequently partitioned by sequential solvent fractionation method (1:1 v/v) successively in the following order: hexane, *n*-butanol, ethyl acetate to obtain corresponding fractions while the remaining portion was considered as the aqueous fraction. The fractions obtained were concentrated again using rotary evaporator at 30 ℃, percentage yields were calculated and further subjected to three standard *in vitro* anti-inflammatory screening methods.

2.3. Anti-inflammatory assays (in vitro methods)

2.3.1. Anti-denaturation studies

The effects of the *C. afer* fractions on protein denaturation were studied using a modified method of Sakat *et al.*[12]. The reaction mixtures contained 50 μL of various studied concentrations of standard drug (Diclofenac sodium), test fractions and methanol as control in test tubes. Bovine serum albumin (BSA) (450 μL, 5% w/v) was added to the above test tubes and subsequently incubated at 37 ℃ for 20 min and then heated at 57 ℃ for 3 min. After cooling the test tubes, 2.5 mL phosphate buffered saline (pH 6.3) was added to each tube. The absorbance of these solutions was measured using double beam UV-Visible spectrophotometer (T80 model PG Instrument, UK) at wavelength of 660 nm. The fifty percent inhibitory concentration (IC_{50}) was estimated using linear regression equation. IC_{50} is the concentration sufficient to obtain 50% inhibition of BSA denaturation.

2.3.2. Erythrocyte stabilization assay

Human blood (5 mL) was obtained from a subject by venipuncture of the arm vein using 5 mL hypodermal syringe and the blood was immediately transferred to an anti-coagulant (ethylene diaminetetraacetic acid) bottle. The whole blood was centrifuged for 10 min at 3 000 revolution per minute and supernatant (plasma and leucocytes) was carefully removed while the packed red blood cells were washed in freshly prepared 0.9% w/v NaCl. The process of washing and centrifugation was repeated until the supernatant became clear.10% human red blood cell (HRBC) was prepared in 0.9% w/v NaCl. The assay mixture contained 1 mL sodium phosphate buffer (pH 7.4, 0.15 mol/L), 2 mL 0.36 % w/v NaCl, 0.5 mL stock HRBC suspension (10%, v/v) with 0.5 mL of standard drug diclofenac sodium or plant fractions of varying concentrations in test tubes. For the control, distilled water replaced NaCl (0.36%, w/v) to induce 100% hemolysis. The different test tubes were incubated at 56 ℃ in a water bath (Uniscope, SM801A England) for 30 min and then centrifuged at 5 000 r/min[13,14]. The hemoglobin content in each tube was estimated using T80 double beam UV-Visible spectrophotometer (T80 model PG Instrument, UK) at 560 nm. The fifty percent inhibitory concentration (IC_{50}) was estimated using linear regression equation.

2.3.3. In vitro anti-proteinase activity

This assay was performed according to the method described by

Oyedapo and Famurewa[15], as modified by Sakat *et al.*[12]. A stock solution of 10 mg/mL *C. afer* leaf and stem fractions and standard diclofenac sodium were prepared. From this stock solution 5 different concentrations of 50-500 μg/mL were prepared. The reaction mixture contained 100 μL of trypsin (0.6 mg/mL), 350 μL 25 mmol/L Tris-HCl buffer (pH 7.4) and 50 μL of test and standard solutions (50-500 μg/mL). The mixtures were incubated at 37°C for 5 min, followed by the addition of 500 μL of 2% casein. The mixtures were incubated again at 37 °C for 20 min. Two millilitres of 5% TCA was added to terminate the reaction. For the control tests, 50 μL of buffer instead of test solution was used. The cloudy suspension obtained was centrifuged at 5000 r/min for 5 min. The absorbance of the supernatant was read at 280 nm using a double beam UV-Visible spectrophotometer (T80 model PG Instrument, UK). The fifty percent inhibitory concentration (IC_{50}) was estimated using linear regression equation.

2.4. Quantitative determination of the phytochemical constituents

Total phenol content was determined by the Folin-Ciocalteu method as described by Zovko *et al.*[16]. Total flavonoid content was by the method described by Ordonez *et al.*[17]. Total flavonol content was determined by a method described by Karunakaran and Kumaran[18]. Total proanthocyanidin content was by the procedure reported by Ashafa *et al.*[19]. Tannin content using vanillin-HCl methanol method as described by Noha *et al.*[20]. Saponin determination as used by Okwu and Josiah[21]. Alkaloids content was determined using the method described by Onyilagha and Islam[22].

2.5. Gas chromatography-mass spectrometry (GC/MS) analysis

The hexane fraction of *C. afer* leaf which exhibited the most active anti-inflammatory property was subjected to GC-MS analytical method. This was carried out at the Department of Chemistry, University of Lagos, Akoka. The GC-MS Specification was: Agilent Technologies model 7890A GC-MS, MSD=5975C (detector) Agilent Technologies, Injector: 7683B series, initial temperature=100 °C held for 2 min, final temperature=270 °C at the rate of 10 °C/min, 1 μL of 0.2 g/mL fraction was injected. Temperature of heater was 250 °C, pressure was 3.2652psi, mode type splitless, column type (HP5MS: 30 mol/L 320 μmol/L 0.25 μmol/L) and carrier gas (helium, 99.9999% purity, flow rate=1.4963 mL/min; average velocity=45.618 cm/sec). The constituent compounds were determined by comparing the retention times and mass spectrum of the authentic samples obtained by GC with the mass spectra from the National Institute of Standards and Technology (NIST) Version 2.0 MS database library.

2.6. Statistical analysis

Statistical analysis was carried out with the aid of SPSS for Windows; SPSS Inc., Chicago, Standard version 17.0. Linear regression was performed to determine fifty percent inhibitory concentration (IC_{50}) values for the fractions. Values were reported as mean±standard deviation as three replicate analysis.

3. Results

Table 1 showed that ethyl acetate fraction (28.98%) of the 70% methanolic leaf extract had the highest yield compared to other leaf fractions while *n*-butanol fraction (17.99%) of the 70% methanolic stem extract had the highest yield compared to other stem fractions.

Table 1
Percentage yield of different fractions of *C. afer* leaf and stem from 70% methanolic extract.

C. afer	Weight of sample (g)	*C. afer* extract	Solvent used in partitioning	Yield (%)
Leaf	300	70% Methanol	Aqueous	12.51
			n-Butanol	15.71
			Ethyl acetate	28.98*
			Hexane	2.76
Stem	300	70% Methanol	Aqueous	13.98
			n-Butanol	17.99*
			Ethyl acetate	12.16
			Hexane	7.83

*: highest yield of solvent fraction.

The data in Figure 1 showed that that the diclofenac sodium, aqueous, *n*-butanol, ethyl acetate and hexane leaf and stem fractions inhibited thermally induced protein denaturation in a reverse concentration dependent manner. It also showed that hexane leaf fraction (IC_{50}=33.36 μg/mL) had the highest antidenaturation of protein activity while aqueous stem (IC_{50}=316.46 μg/mL) fractions had the lowest anti-denaturation of protein activity (Table 2).

Furthermore, diclofenac sodium, aqueous, *n*-butanol, ethyl acetate and hexane leaf and stem fractions stabilized erythrocyte membrane against hypotonicity induced hemolysis in a concentration dependent manner (Figure 2). Hexane leaf fraction (IC_{50}=33.00 μg/mL) had the highest effect on stabilizing erythrocyte membrane while aqueous stem (IC_{50}=58.14 μg/mL) had the lowest effect on erythrocyte membrane stabilization (Table 2).

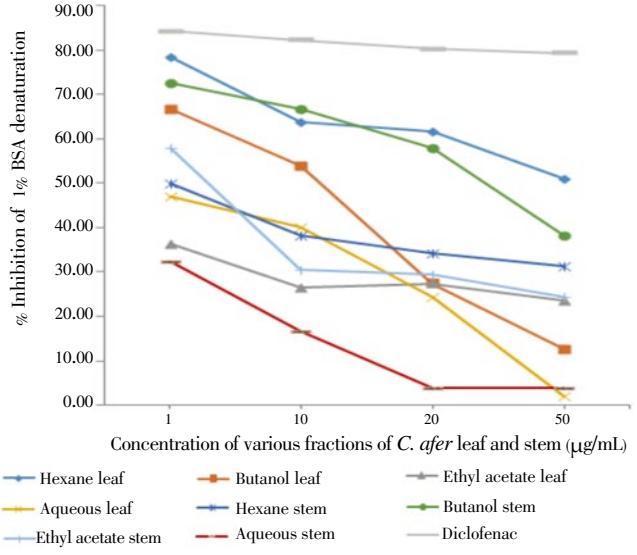

Figure 1. Percentage inhibition of thermally induced protein denaturation by different fractions of *C. afer* leaf and stem.

Table 2

Fifty percent inhibitory concentration (IC_{50}) of *C. afer* leaf and stem fractions on BSA denaturation, HRBC membrane stabilization and anti-proteinase activity.

Fractions of *C. afer* (μg/mL)	IC_{50} (μg/mL)		
	Protein denaturation assay (1-50 μg/mL)	Stabilization of HRBC assay (1-50 μg/mL)	Anti-proteinase assay (50-500 μg/mL)
Diclofenac sodium	23.14	27.17	193.05
Hexane leaf	33.36	33.00	212.77
n-Butanol stem	39.40	52.52	284.09
Hexane stem	55.87	40.49	298.02
Ethyl acetate stem	68.97	54.47	409.84
Ethyl acetate leaf	74.07	53.53	335.57
n-Butanol leaf	83.75	56.82	279.33
Aqueous leaf	144.51	44.96	326.80
Aqueous stem	316.46	58.14	328.85

BSA: Bovine serum albumin; HRBC: Human red blood cell.

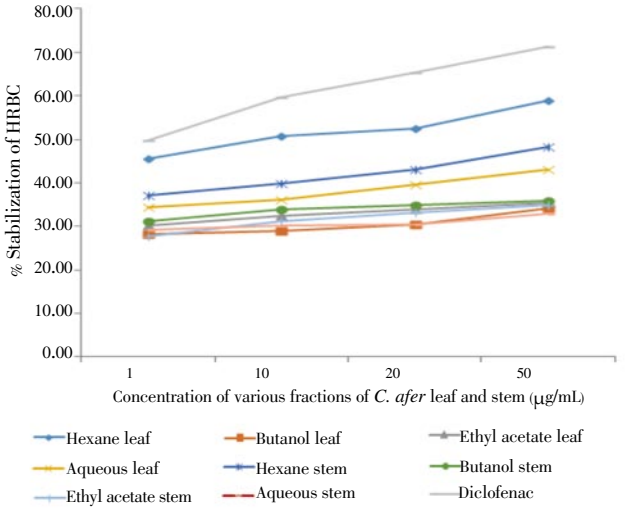

Figure 2. Percentage stabilization of HRBC at concentrations between by different fractions of *C. afer* leaf and stem.

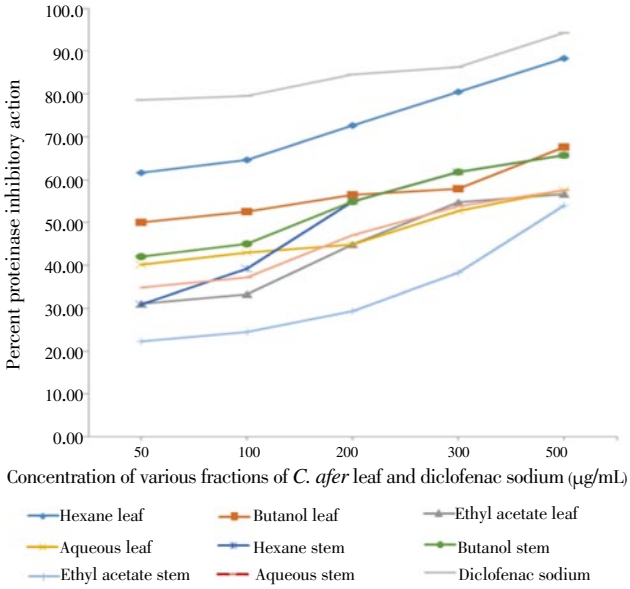

Figure 3. Percent proteinase inhibitory action of different concentrations of *C. afer* stem and leaf.

Figure 3 showed that diclofenac sodium, aqueous, *n*-butanol, ethyl acetate and hexane leaf and stem fractions inhibited trypsin activity in a concentration dependent manner. Hexane leaf fraction (IC_{50}=212.77 μg/mL) exhibited the highest anti-proteinase activity while ethyl acetate stem (IC_{50}=409.84 μg/mL) had the lowest anti-proteinase effect (Table 2).

The data in Table 3 revealed that the most potent anti-inflammatory fraction (hexane leaf fraction of *C. afer*) had a total phenol content of (0.19±0.01) mg Gallic acid equivalent/g; total flavonoid content was (0.56±0.06) mg quercetin equivalent/g, total flavonol content was (0.58±0.02) mg quercetin equivalent/g, proanthocyanidin content were (2.17±0.17) mg catechin equivalent/g; total tannin, saponin and alkaloid contents were 2.90±0.18%, 52.00±2.82% and 2.75± 0.35% respectively.

Table 3

Quantitative phytochemical evaluation of hexane leaf fraction of *C. afer*.

Hexane fractions of *C. afer*	Quantity present
Total phenol	(0.19±0.01) mg GAE/g
Total flavonoid	(0.56±0.06) mg QUE/g
Total flavonol	(0.58±0.02) mg GAE/g
Total proanthocyanidins	(2.17±0.17) mg CAE/g
Total tannin	(2.90±0.18)%
Saponin	(52.00±2.82)%
Alkaloid	(2.75±0.35)%

GAE: Gallic acid equivalent; QUE: Quercetin equivalent; CAE: Cathechin equivalent.

The GC/MS data and spectrum of the chemical constituents in hexane leaf fraction of *C. afer* are shown in Table 4 and Figure 4 respectively. The chromatogram showed that hexane leaf fraction contained 19 compounds and they are naphthalene, 1,6-dimethyl- (0.360%), naphthalene, 2,3-dimethyl(0.153%), phenol, 2,4-bis(1,1-dimethylethyl)- (0.763%), 2(4H)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7atrimethyl (0.836%), benzene, (1-pentylhrptyl) (0.153%), 1-naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl (0.963%), pentadecanoic acid, methyl ester (0.282%), tricyclo[5.2.1.0(2,6)]decane, 3-methylene-4-phenyl (0.299%), 2-pentadecanone, 6,10,14-trimethyl (0.703%), hexadecanoic acid, methyl ester (7.656%), *n*-hexadecanoic acid (1.766%), heptadecanoic acid, methyl ester (0.868%), 9,12-octadecadienoic acid, methyl ester (3.930%), 9,12,15-octadecatrienoic acid, methyl ester (4.485%), phytol (4.79%), methyl 16-methyl-heptadecanoate (1.350%), Cis-vaccenic acid (8.639%), 9-octadecenal (0.793%) and 1,2-benzenedicarboxylix acid, mono (2-ethylhexyl) ester (1.109%).

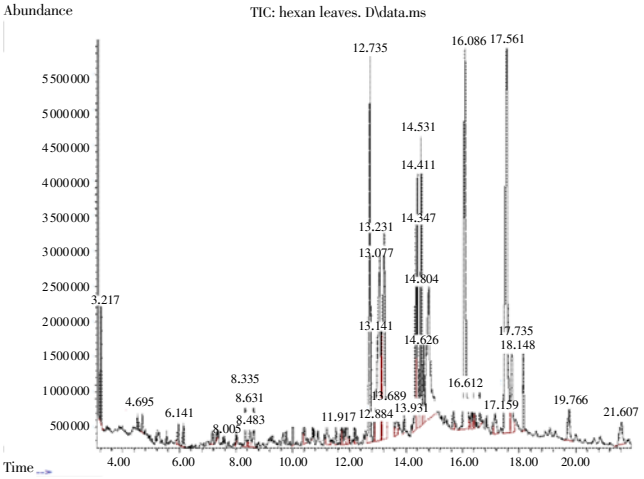

Figure 4. GC/MS chromatogram of hexane fraction of *C. afer* leaf.

Table 4
GC/MS analysis of hexane fraction of *C. afer* leaves.

Peak No.	Retention time	Library ID	Percent of total	Bioactivity
5	7.172	Naphthalene, 1,6-dimethyl-	0.360	Anti-inflammatory
6	7.327	Naphthalene, 2,3-dimethyl	0.153	Anti-inflammatory
8	8.334	Phenol, 2,4-bis(1,1-dimethylethyl)-	0.763	Anti-inflammatory, antioxidant, antibacterial for eye infection
10	8.631	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7atrimethyl	0.836	Antifungal, antioxidant, antibacterial
13	10.709	Benzene, (1-pentylhrptyl)	0.153	Plasticizer
14	11.218	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl	0.963	No report
16	11.710	Pentadecanoic acid, methyl ester	0.282	Anti-inflammatory, antibacterial
17	11.744	Tricyclo [5.2.1.0(2,6)] decane, 3-methylene-4-phenyl	0.299	Not reported
19	11.916	2-Pentadecanone, 6,10,14-trimethyl (hexahydrofarnesyl acetone)	0.703	Antimalarial
21	12.734	Hexadecanoic acid, methyl ester (palmitate)	7.656	Anti-inflammatory antioxidant
24	13.140	*n*-Hexadecanoic acid (paimitic acid)	1.766	Anti-inflammatory antioxidant
26	13.690	Heptadecanoic acid, methyl ester	0.868	Antioxidant
28	14.348	9,12-Octadecadienoic acid, methyl ester (linoleic acid)	3.930	Anti-inflammatory hepatoprotective hypocholesterolemic anti-arthritic antihistamine
29	14.411	9,12,15-Octadecatrienoic acid, methyl ester (-linolenic acid)	4.485	Anti-inflammatory
30	14.531	Phytol (diterpernes)	4.790	Anti-inflammatory
31	14.628	Methyl 16-methyl-heptadecanoate	1.350	Antioxidant
32	14.805	Cis-Vaccenic acid	8.639	Anti-inflammatory hypolipidemic antioxidant
38	17.157	9-Octadecenal	0.793	No report
41	18.147	1,2-Benzenedicarboxylix acid, mono (2-ethylhexyl) ester (phthalate)	1.109	Antimicrobial, antifouling

4. Discussion

The inherent problems associated with the use of animals at the initial stage of drug discovery such as lack of rationale for their use when other suitable methods are available, ethical issues and difficulty with cross species extrapolation led us to adopt *in vitro* methods such as anti-denaturation of protein, stabilization of erythrocyte membrane and anti-proteinase bioassays for the assessment of anti-inflammatory property of *C. afer*.

In this study, data showed that all test fractions and standard drug diclofenac sodium (2-[(2,6-Dichlorophenyl)amino] benzene acetic acid sodium salt) inhibited heat induced denaturation of protein in a reverse concentration dependent manner. This is in agreement with the statement made by Williams and his colleagues that the anti-denaturation properties of plant extracts are usually exhibited at low concentrations. It also suggests that *C. afer* may possess anti-inflammatory activity[23]. The HFCAL exhibited the highest anti-denaturation of protein activity compared with the other test fractions. This suggests that the anti-inflammatory compound(s) present in HFCAL are probably no*n*-polar. Protein denaturation has been implicated as the cause of rheumatoid arthritis[23,24]. More so, the mechanism of protein denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure. It has also been reported that compounds capable of preventing these changes and inhibit thermally-induced protein denaturation could be of a potential therapeutic value as anti-inflammatory agent[25].

The stabilization of HRBC membrane against hypotonicity-induced hemolysis study showed that all test fractions stabilized human erythrocyte membrane against hypotonic induced hemolysis. HFCAL exhibited the highest stabilization effect on erythrocyte membrane. This suggests that HFCAL may possess a higher anti-inflammatory activity. Research has shown that the composition of erythrocyte membrane is similar to that of lysosomal membrane; hence, any agent that could stabilize the membrane from hemolysis would provide a good insight into the anti-inflammatory mechanism of action[26]. It has been reported that the stabilization of lysosomal membrane is important in limiting the cascades of inflammatory response by preventing the release of lysosomal constituent such as activated neutrophils, bactericidal enzymes and proteases[27]. Furthermore, HFCAL could prevent the physical interaction of membranes with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the hemolysis of red blood cells[28].

HFCAL also had the highest anti-proteinase activity when compared with other test fractions. This further supports the previous finding that identified HFCAL as the most active anti-inflammatory fraction. Previous studies have shown that neutrophils contain serine proteinases in their lysosomal granules and these proteinases have been implicated in chronic inflammations especially in arthritis[29]. Proteinase inhibitors confer significant protection against tissue damage during inflammation[30].

Quantitative phytochemical study indicated an appreciable amount of polyphenols, flavonoids, flavonols, proanthocyanidins, tannins, saponins and alkaloids. The observed inhibition of thermally-induced denaturation of protein and stabilization effect on hypotonicity-induced hemolysis of erythrocyte membrane by *C. afer* may be attributed to the presence of these phytochemicals. Furthermore, saponins and flavonoids have been reported to exert profound stabilizing effect on lysosomal membrane while tannins and saponins bind cations, thereby stabilizing erythrocyte membranes and other biological macromolecules[14,31]. Alkaloids are known anti-inflammatory effects[32]. Flavonoid and phenolic compounds are potent antioxidants which prevent oxidative cell damage and also possess anti-inflammatory, anti-allergic and anti-thrombotic[33,34]. Proanthocyanidins are a type of bioflavonoid that has been shown to have very potent antioxidant activity[35]. Previous studies had also shown that plant extracts possessing anti-inflammatory properties may contain phytochemicals with antioxidant activity against deleterious chain reactions triggered by reactive oxygen species associated with inflammations[36,37].

Naphthalene 2,3-dimethyl- and naphthalene 1,6-dimethyl- are compounds with naphthalene moiety known to exhibit anti-

inflammatory property[38]; phenol, 2,4-bis (1,1-dimethylethyl) is a good antioxidant, anti-inflammatory and antibacterial agent[39]; Coumara*n*- benzofuran, 2,3-dihydro had been reported to possess anti-inflammatory and antioxidant activity[40]; hexadecanoic acid, *n*-hexadecanoic acid, pentadecanoic acid, 9,12-octadecadienoic acid, methyl ester, 9,12,15-octadecatrienoic acid, methyl ester, cis-vaccenic acid have been reported to exert anti-inflammatory effect through inhibition of cyclooxygenase and 5-lipooxygenase enzymes[41]. Phytol is a promising pharmaceutical agent used in the treatment of rheumatoid arthritis and possibly other chronic inflammation[42,43]. It can therefore be concluded that the anti-inflammatory activity of hexane fraction *C. afer* leaf may be due to the presence of naphthalene derivatives (terpenoids), phenols, phytols (diterpenes) and poly unsaturated fatty acids.

Conflict of interest statement

The authors declare no conflict of interest and are solely responsible for the writing and content of this work.

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Comments

Background

C. afer is used for the treatment of inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic, miscarriages, epileptic attack and hemorrhoids. Also, it is used as laxative, diuretics, and had served as an antidote for poison. However, there has not been any report on the chemical constituent of *C. afer*.

Research frontiers

The present work showed that the hexane fraction of *C. afer* has anti-inflammatory activity and for the first time, a chemical profile of the plant was carried out.

Related reports

Compounds detected in this plant have been reported to have anti-inflammatory effect.

Innovations and breakthroughs

Narrowing the study of the anti-inflammatory effect to the nonpolar compounds makes it easier for further study.

Applications

Further studies need to be conducted to isolate the compounds responsible for the anti-inflammatory effect of the plant.

Peer review

The research work was interesting. The authors organized the paper logically and data were appropriately presented.

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