Original Article

Identification of Gingerols in Ginger (*Zingiber officinale* Roscoe) by High Performance Liquid Chromatography-Tandem Mass Spectrometry and Pharmacologic Studies of its Aqueous Extract on the Rabbit Isolated Duodenum Contractility

Ahui M. L. B., *Konan A. B., Zannou-Tchoko V. J., Amonkan A. K., Kati-Coulibaly S. and Offoumou M. A.

Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire.

Abstract

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a plant used in traditional medicine in Côte d'Ivoire, notably for asthma attacks. In various medical traditions, rhizome of the plant is indicated as a cough-drop, antipyretic, anti-emetic, anti-inflammatory and physiologic functions stimulant. Gingerols and related compounds were identified as being its major active compounds. In this study, liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) coupled with diode array detection (DAD) was used to identify gingerol-related compounds from crude extracts of ginger rhizoma from Côte d'Ivoire. The isometric contractile force of rabbit duodenum strips was recorded in the presence of aqueous extract. 27 gingerol-related compounds were identified by LC-ESI-MS/MS-DAD in the methanolic crude extracts of ginger rhizoma. Short treatments (24 hours) of mice with the the aqueous extract of ginger (EZo) gave LD_{50} values of 1242.54 ± 8 mg/kg of b.w. and of 1089.6 ± 12 mg/kg of b.w., respectively by the method of Miller and Tainter and the method of Dragstedt and Lang. Our observation regarding the isolated duodenum of rabbit revealed that EZo was decreased the basal tonus and contractile force. For low concentration of EZo, the amplitude of this smooth muscle was increased but at higher concentration the amplitude was decreased. Chemical analysis allowed us to identify [8]-gingerol as the major phenyl-propanoid

*Corresponding author: Laboratory of Nutrition and Pharmacology, UFR-Biosciences, Félix Houphouët-Boigny University, 22 BP 582 Abidjan 22, Côte d'Ivoire.

Received on: 03 Feb 2013
Revised on: 16 Feb 2013
Accepted on: 20 Feb 2013
Online Published on: 28 Feb 2013

in the studied batch. Pharmacologic study confirmed that ginger aqueous extract was lightly toxic substance by intraperitoneal administration and evidenced that it had relaxing and antihistaminic activity on the duodenum.

Keywords: *Zingiber officinale*, Chromatography, Acute toxicity, Histamine, Duodenum.

Abbreviations

HPLC/ESI-MS/MS	Liquid Chromatography Electrospray -tandem Mass Spectrometry
HPLC/DAD	High Performance Liquid Chromatography-Diode Array Detection
EZo	Aqueous extract of rhizome of *Zingiber officinale* Roscoe
MeOH	Methanol
MZo	Methanolic extract of *Zingiber officinale* Roscoe
LD_{50}	Lethal dose 50
DP	Declustering Potential
FP	Focalising Potential
CAD	Collision gaz pressure

Introduction

Ginger (*Zingiber officinale* Roscoe) belongs to the Zingiberaceae family and is widely used in foods as a spice around the world (Afzal et al., 2001). Numerous chemical investigations of this plant material led to the isolation and identification of a large number of bioactive compounds, such as gingerols, gingerones and shogaols (Uehara et al., 1987; Kikuzaki et al., 1991 and 1992; Yu et al., 1998).

Ginger (*Z. officinale*) is one of the traditional medicinal plant that has been widely used in Chinese medicines for several thousand years (Wu et al., 2002; Wang and Wang, 2005; Rong et al., 2009). In the Tibb and Ayurvedic systems of medicine preventive and ameliorative effects of ginger have been described in the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes (Chrubasik et al., 2005; Ali et al., 2008). Extracts were reported to have effects as an anti-inflammatory (Chang et al., 1995), antioxidant (Jeyakumar et al., 1999), antithrombotic (Thomson et al., 2002; Chrubasik et al., 2005) and anticancer activities (Surh, 2002). Ginger extracts was also reported to be effective at reducing the symptoms of arthritis in humans (Altman and Marcussen, 2001).

In previous studies, we showed that 6-gingerol, a major constituent of ginger, was able to suppress eosinophilia in a model of inflammation. An aqueous extract of rhizoma suppressed Th2-mediated immune responses and might thus provide a possible therapeutic application in allergic asthma (Ahui et al., 2008). To identify known and unknown gingerol-related compounds in ginger rhizoma, He et al. (1998) and Jiang et al. (2005) used HPLC/DAD-ESI-MS and MS/MS: Both negative and positive ionization modes provided useful data, with typical fragmentation patterns. We here applied a similar method to a methanolic extract of ivorian ginger.

On the pharmacological viewpoint, we here provide an insight on the smooth muscle relaxation and antihistaminic activities of a traditionally used aqueous extract, so as to challenge its claimed benefit in allergy and nauseous. We investigated the *in vitro* effect of this medicinal plant on the rabbit duodenum contractions in the presence or absence exogenous histamine.

Materials and Methods

Ethical Considerations

Experimental procedures and protocols used in this study were approved by Ethics Committee of Félix Houphouët-Boigny University. These guidelines were in accordance with the internationally accepted principles for laboratory use and care (Mosihuzzaman and Choudhary, 2008).

Sample Preparations of Ginger

Rhizoma of *Zingiber officinale* Roscoe were collected in the market of Abidjan (Côte d'Ivoire) and dried at 70 °C. The plant was authenticated by a botanist, Professor AKE-Assi Laurent of the "Centre National de Floristique de Côte d'Ivoire" at Félix Houphouët-Boigny University in Abidjan. Plant material was ground into a fine powder using a pestle and mortar and the powder was soaked in

distilled water (1L) for 24 h at room temperature (27 ± 3 °C). The resulting solution was filtered and freeze dried. From a 50 g sample of ginger dried rhizome, 5 g of solid material (EZo) was obtained (yield: 10 %). Extract was stored at 4°C until use. The solid material was subsequently reconstituted in a known volume of distilled water and then serially diluted.

A methanolic (MeOH) total extract (MZo), was also obtained, as follows: The powder of ginger (30 g) was extracted in methanol (600 ml) in a Soxhlet apparatus for 2 days. MeOH was evaporated under reduced pressure, to give a brown extract (yield: 11 %).

Animals

Mice *Mus musculus* (12-20 weeks old), and rabbits *Oryctolagus cumuculus* (2.0 ± 0.4 kg) were used in our experiments. These animals were obtained from the Animal House of the Laboratory of Nutrition and Pharmacology of UFR-Biosciences at Félix Houphouët-Boigny University in Abidjan (Côte d'Ivoire). They were housed in a constant temperature rooms with a light/dark cycle of 14/10 hours. All animals were fed and given water *ad libitum*.

Chemical Used

Histamine (Hist) was purchased from Sigma Chemical Company (USA). All drugs (EZo and Hist) were dissolved and/or diluted in distilled water on each day of our experiments (Chiwororo and Ojewole, 2009; Konan et al., 2011 and 2012a). Drugs concentrations quoted in the text refer to final organ-bath concentration.

Chromatographic analysis of ginger extracts

High performance liquid chromatography-Diode array detector (HPLC/DAD) profiles of aqueous (EZo) and MeOH total (MZo) extracts were compared. High performance liquid chromatography–tandem mass spectrometry (HPLC-DAD-MS/MS) analysis MZo extracts was thus conducted. All analyses were performed at 30°C with a C_{18} Sunfire column (WATERS, 5 µm, 4.6×150 mm) equipped with a C_{18} Sunfire pre-column (5 µm). HPLC-DAD-MS/MS was performed on a HPLC Agilent system 1100 series coupled to an electrospray (ESI)-quadripole-time-of-flight (TOF) spectrometer (PE SCIEX QSTAR PULSAR, PE Sciex Instruments). A gradient of H_2O (A) and CH_3CN (B) was used (1→40 min: 20 % B→100 % B, 40→55 min: 100 % B), at a flow rate of 1 ml/min. The acquisition parameters for positive and negative modes were respectively: GS1 (nebulizing gas): 50 psi, GS2 (drying gas): 55 psi, curtain gas: 35 psi, ionspray voltage: 5500 V or −4500 V, DP (declustering potentiel): 30 V or −30 V, FP (focalising potential): 180 V or −180 V, DP2: 20 V or −20 V, CAD (collision gaz pressure): 2 mTorr (N2). For MS/MS experiments, collision energy was set at 35 eV or −35 eV. Mass range measured was *m/z* 100–800.

Acute Toxicity Study

According to Özbek et al. (2004), Néné-Bi et al. (2008) and Konan et al. (2012b), male and female mice were randomly gathered in 9 groups with 10 animals in each group. First group was treated with normal saline (NS) and considered as control and the other 8 groups were treated with *Z. officinale* rhizome aqueous extract (EZo) given intraperitoneally (i.p.) in increasing dosages ranging from 233 mg/kg b.w. to 11680 mg/kg b.w. Maximum volume of extract administered to mice was kept below 0.5 ml. To study behavioural changes, the 8 treated groups were observed every 30 min for a period of 2 hours (Mandal et al., 2001, Konan et al., 2012b). Mortality rate was monitored on a 24 hours period. Both methods of Miller and Tainter (1944) and of Dragstedt and Lang (1957) were used to determine the LD_{50} of EZo.

Recording of the Contractile Activity of Rabbit Isolated Duodenum

After sacrifice of animals, by cervical dislocation, a median laparotomy was practised. The duodenum was taken and split up in 2 cm length scraps. These strips (2 cm) were transferred in a Petri dish containing a normal Mac Ewen solution with following composition (mM): NaCl, 130; KCL, 2.5; $CaCl_2$, 2.4; NaH_2PO_4, 1.18; $NaHCO_3$, 11.9; $MgCL_2$, 0.24; glucose, 2.2). The solution (pH of 7.4 and gassed with 95% O_2 + 5% CO_2) was kept at a temperature of 35°C (Datté et al., 1998; Konan et al., 2011 and 2012a).

A selected fragment was put in an isolated organ bath containing the oxygenated physiological solution of Mac Ewen, thermostated at a temperature of 37°C. Using a cotton yarn, one end of the duodenum strip was vertically attached to a hook and the other was connected to a stylet inscriptor which transmitted the movements of the duodenum strips on moving paper (speed 0.1 cm/s). After two hours, the time necessary for stabilization of the contractile movement, the concentrations to be tested were added directly into the organ bath.

Statistical Analysis

Data were expressed as means ± sem obtained from *n* separate experiments. Statistical analyses were carried out and graphics were built using the softwares Microsoft Excel 2007 and GraphPad Prism 5 (San Diego, California, USA), respectively. Statistical analysis of the results was determined by using the unpaired Student's *t*-test. $P < 0.05$ was considered as indicative of significance.

Results and Discussion

Traditional medicine plays an important role in primary health care in Africa (Pousset, 1989). However, this medicine which remains relatively empirical causes concerns (Astin, 1998; Mashour *et al.*, 1998). Therefore, the rational evaluation and scientific studies of drugs commonly used in traditional medicine would guarantee a best use, reduce the risks of accidents and permit the establishment of specific treatments of these intoxications (Gies, 1993). This study should be done according to several research areas. We can mention the determination of the chemical composition of these natural substances. Various techniques are used.

Comparison of HPLC-DAD profiles of aqueous (EZo) and methanolic (MZo) extracts of *Z. officinale* rhizome were similar. Methanolic extract (MZo) of dried ginger rhizoma was directly analyzed, without further sample cleanup. Retention times, UV spectra, molecular ions and fragmentation patterns were compared with that of the literature. (+)ESI-MS allowed detection of gingerol-related compounds as $[2M+Na]^+$, $[M+Na]^+$ and / or $[M+H]^+$ adducts. (-)ESI-MS allowed discrimination of phenol-bearing derivatives as $[M-H]^-$ ions. 27 Gingerol-related compounds were thus characterized, according to UV spectra, molecular mass, and mass of fragments (Table 1). Elution order was consistent to that observed by others. Among other aryl-alcanones, [8]- and [6]-gingerols appeared as major representatives (Fig. 1). Identified active ingredients in ginger are aryl-alcanones such as gingerols and gingerol analogues (shogaols and paradols, among others) (Jiang et al., 2005) and most activities of ginger were attributed to [6]-gingerol (Ahui et al., 2008). The chromatographic profile obtained for MZo prepared from our batch of Ivorian ginger (Table 1) evidenced slight qualitative and quantitative discrepancies with that .described in the litterature (He et al., 1998; Jiang et al., 2005), [6]-gingerol (2) not being the major representative, as described in the literature (He et al., 1998). Indeed, as evidenced in our previous research (Ahui et al., 2008), [8]-gingerol (10) is the most abundant aryl-alcanone of the extract. It is also noteworthy that non methylated catechol-bearing compounds were observed (nor-[12]-paradol (8), nor-[8]-gingerdione (12)), consistently with proposed biosynthetic pathway (Ramirez-Ahumada et al., 2006).

The study of the acute toxicity of *Z. officinalle* was also made. For Lüllmann et al. (1998), the treatment of a disease by a plant extract implies the administration of various molecules. The molecule can exert therapeutic action but also toxic effects. Intoxications following the use of traditional drugs are severe (Binlin-Dadié et al., 1997).

The effects of EZo in low i.p. doses ranging between 233 mg/kg and 467 mg/kg b.w. on mice behaviour during a 24 hours period were hardly perceptible. According to injected amount, some mice presented locomotor difficulties but recovered mobility at an hour. At the dose 467 mg/kg b.w., the extract induced death at eighteen hours. On the over land, beyond 934 mg/kg b.w., death was observed five minutes after injection of EZo. Determination of 50 % lethal dose (LD_{50}) at of EZo was performed by the graphical method of Miller and Tainter (1944): With the last nine (9) groups of mice, various doses were administered. After 24 hours, the percentage of mortality raised. The percentages of mortality were converted to probits (Table 2).

Repetition of this experiment (n = 3) showed a LD_{50}-value of 1242.54 ± 8 mg/kg b.w. (Fig. 2). By calculation method of Dragsted and Lang (1957), LD_{50}-value of EZo was 1089.6 mg/kg b.w. The repetition of this experiment (n = 3) gave a LD_{50} of 1084.2 ± 12 mg/kg b.w. The LD_{50}-value of aqueous extract of *Z. officinale* determined according to the method of Miller and Tainter (1944) and that of Dragsted & Lang (1957) were 1242.54 ± 8 mg/kg b.w. and 1089.6 ± 12 mg/kg b.w., respectively. These values were statistically very close. According to the classification of Diezi (1989), EZo was lightly toxic substance. It had toxicity appreciably equal to that of *Erythrina senegalensis* (Fabaceae) the LD_{50}-value was 1663 mg/kg b.w. (Traoré et al., 2002). On the other hand, the toxicity caused by EZo was less important than those of other medicinal plants such as *Sesamum radiatum* (Pedaliaceae), *Securidaca longepedunculata* (Polygalaceae), and *Swartzia madagascariesis* (Ceasalpiniceae) having LD50-values respectively equal to 169.9 ± 15 mg/kg b.w., 64 mg/kg b.w. and 5.99 mg/kg b.w. (Traoré et al., 2002, Konan et al., 2012b). Ginger is considered as a safe herbal medicine. An acute 64 days experimental study suggested that a patented ginger extract (25-100 mg/kg) with a high content of gingerols and shogaols did not induce significant changes in blood glucose, blood coagulation, blood pressure and heart rate in normal male rats (Weidner and Sigwart, 2000). Rong et al. (2009) study demonstrated minimal toxic effects of a ginger aqueous extract in rats. However, this toxicity could not be a barrier to its use because all pharmacodynamic substances are toxic when the administered doses are supraliminal (Lüllmann et al., 1998).

Ginger is marketed for treating diseases of the digestive tract including dyspepsia and diarrhea. A systematic review concluded that ginger is a promising anti-emetic herbal remedy, although the clinical data were considered insufficient to draw firm conclusions (Ernst and Pittler, 2000). In contrast to most anti-emetic drugs that act on the central nervous system, the anti-emetic effect of ginger is thought to be due to local gastrointestinal actions (Mowrey and Clayson, 1982). In the present study we found that ginger possesses inhibitory effects on rabbit duodenum motility and antihistaminic effect.

As shown in figures 3 and 4, EZo applied in the organ bath induced relaxation of this smooth muscle. The concentrations ranging from 0 to 10 µg/ml increased the contractile force in a concentration-dependent way up to 26.1 %. However, at highest concentrations ranging from 10 µg/ml to 100 µg/ml, a decrease of the contractile force down to 78.4 % was observed, with significant relaxation of duodenum (1050 ± 183.7 mg to 1350 ± 199.2 mg).

Figures 5 and 6 illustrate the effect of histamine on the contractility of duodenum isolated from rabbit. Histamine caused contraction in a concentration dependant manner. At concentrations of 5.10^{-3} µg/ml and 0.5 µg/ml, the contractile force was increased to 1612.5 ± 259 mg and 2200 ± 321 mg, respectively. The basal tonus of duodenum was increased too: at concentrations of 0.5 µg/ml, it attained 1066.6 ± 106 mg.

Pre-treatment of the duodenum with histamine (10 µg/ml) increased the contractile force at the value 1375± 97 mg and the basal tone too. At this concentration the basal tonus was evaluated to 572.75 ± 9.8 mg. The effect of contractile activity induced by histamine on duodenum isolated from rabbit was affected by addition of EZo (Fig. 7 and 8) : At EZo (10^5 µg/ml) both basal tone and contractile force decreased to 125 ± 68 mg and 154 ± 0 mg respectively.

We found that ginger possesses inhibitory effects on rabbit duodenum motility and antihistaminic effect. Effects which could explain anti vomiting and anti nausea effect attributed to ginger (Borrelli et al., 2004). The effect of ginger on intestinal motility in our study is similar to that of Borrelli et al. (2004), who showed that ginger reduced contractility of the rat isolated ileum and directly inhibited smooth muscle activity. Ginger (0.01-1000 µg/ml) inhibited both EFS (electrical stimulation) - and acetylcholine-evoked contractions, being more potent in inhibiting the contractions induced by EFS (Borrelli et al., 2004).

Our data show that histamine induces significant increase of contractile force and basal tonus of rabbit's duodenum. But ginger is able to reduce Histamine-induced contractions in the rabbit

isolated duodenum. The ability of ginger to reduce the contractions spontaneous indicates a direct antispasmodic effect.

Table 1: Chromatographic and mass spectral characteristics of gingerol-related compounds detected by LC-ESI-MS in extracts from ginger rhizome.

N°	Tr	Compounds	Positive ESI (*m/z*)	Negative ESI (*m/z*)
1	21.6	[4]-gingerol	555: $[2M+Na]^+$; 289: $[M+Na]^+$; 249: $[M+H-H_2O]^+$; 177	266: $[M-H]^-$
2	27.7	[6]-gingerol	611: $[2M+Na]^+$; 317: $[M+Na]^+$; 295: $[M+H]^+$; 277: $[M+H-H_2O]^+$; 177	293: $[M-H]^-$
3	28.5	[6]-gingerdiol	615: $[2M+Na]^+$; 319: $[M+Na]^+$; 261: $[M+H-2H_2O]^+$; 177	296: $[M-H]^-$
4	29.9	Me-[6]-gingerol	639: $[2M+Na]^+$; 331: $[M+Na]^+$; 291: $[M+H-H_2O]^+$; 191	n.d.
5	30.1	Me-[8] gingerdiol	$[2M+Na]^+$: n.d. ; 361: $[M+Na]^+$; 317: $[M+H-H_2O]^+$; 191	n.d.
6	31.0	OAc-[6]-gingerdiol *	699: $[2M+Na]^+$; 361: $[M+Na]^+$; 301: $[M+Na-AcOH]^+$; 261: $[M+H-AcOH-H_2O]^+$; 177 ; 163 $^{\$}$	337: $[M-H]^-$
7	31.2	OAc-[4]-gingerol *	639: $[2M+Na]^+$; 331: $[M+Na]^+$; 309: $[M+H]^+$; 291: $[M+H-H_2O]^+$; 231: $[M+H-H_2O-AcOH]^+$	307: $[M-H]^-$
8	31.4	nor-[8]-gingerdione	635 $[2M+Na]^+$; 329 : $[M+Na]^+$; 275: $[M+H-H_2O]^+$; 205 **	305: $[M-H]^-$
9	32.0	[6]-shogaol	575: $[2M+Na]^+$; 299: $[M+Na]^+$; 277 $[M+H]^+$	276: $[M-H]^-$
10	32.1	[8]-gingerol	667 $[2M+Na]^+$; 345: $[M+Na]^+$; 305: $[M+H-H_2O]^+$; 177	321: $[M-H]^-$
11	32.7	OAc-[8]-Me-gingerdiol *	727: $[2M+Na]^+$; 375: $[M+Na]^+$; 275: $[M+H-AcOH-H_2O]^+$; 177	n.d.
12	34.0	nor-[12]-paradol	719: $[2M+Na]^+$; 371: $[M+Na]^+$	347: $[M-H]^-$
13	34.6	[8]-paradol	635: $[2M+Na]^+$; 329: $[M+Na]^+$; 177	305: $[M-H]^-$
14	35.0	[8]-dehydrogingerdione	659: $[2M+Na]^+$; 341: $[M+Na]^+$; 319; 177	n.d.
15	35,1	Me-[8]-gingerol	695: $[2M+Na]^+$; 359: $[M+Na]^+$; 337: $[M+H]^+$; 319: $[M+H-H_2O]^+$	n.d.
16	35.3	OAc-[8]-gingerol *	417: $[M+Na]^+$; 395 $[M+H]^+$; 357: $[M+Na-H_2O]^+$; 335 : $[M+H-AcOH]^+$; 317: $[M+H-AcOH-H_2O]^+$, 377: $[M+H-H_2O]^+$	393: $[M-H]^-$
17	35.7	[10]-gingerol	723: $[2M+Na]^+$; 373: $[M+Na]^+$: 333: $[M+H-H_2O]^+$; 177	349: $[M-H]^-$
18	36.0	[10]-gingerdione	719: $[2M+Na]^+$; 371: $[M+Na]^+$; 331: $[M+H-H_2O]^+$	347: $[M-H]^-$
19	37.8	Me-[10]-gingerol	751: $[2M+Na]^+$; 387: $[M+Na]^+$; 347: $[M+H-H_2O]^+$	n.d.
20	38.0	Me-OAc-[8] gingerol	779: $[2M+Na]^+$; 401 : $[M+Na]^+$; 301: $[M+H-AcOH-H_2O]^+$	n.d.
21	38.3	[10]-shogaol	687: $[2M+Na]^+$; 355: $[M+Na]^+$; 333: $[M+H]^+$	329: $[M-H]^-$
22	38.9	Me-[8]-paradol	663: $[2M+Na]^+$; 343: $[M+Na]^+$	n.d.
23	40.1	[12]-paradol	747: $[2M+Na]^+$; 385: $[M+Na]^+$	n.d.
24	41.1	Me-[10]-shogaol	369: $[M+Na]^+$	n.d.
25	41.6	[12]-shogaol	779: $[2M+Na]^+$; 383: $[M+Na]^+$	n.d.
26	43.9	[14]-dehydrogingerdione	425: $[M+H-H_2O]^+$	n.d.
27	46.0	Me-[12]-gingerol	415: $[M+Na]^+$; 393: $[M+H]^+$	n.d.

* Acetyl position was not determined; n.d: not detected.

Table 2: Result of acute toxicity study in mice of *Zingiber officinale* Roscoe rhizome aqueous extract.

Groups	Dose of EZo (mg/kg b.w.)	Effectif of mice	Mortality (%)	Mortality(Probits)
1	NS	10	0	1.9
2	233	10	0	1.9
3	467	10	30	4.47
4	934	10	60	5.25
5	1401	10	60	5.25
6	1869	10	60	5.25
7	2330	10	80	5.84
8	4673	10	80	5.84
9	11680	10	100	8.7

Group 1 was treated with normal saline (NS) and considered as control and the other 8 groups (Group 2-9) were treated with *Zingiber officinale* Roscoe rhizome aqueous extract (EZo) administered intraperitoneally (i.p.). The mortality rate raised in the 24 hours of experimentation. The percentages of mortality were converted to probits.

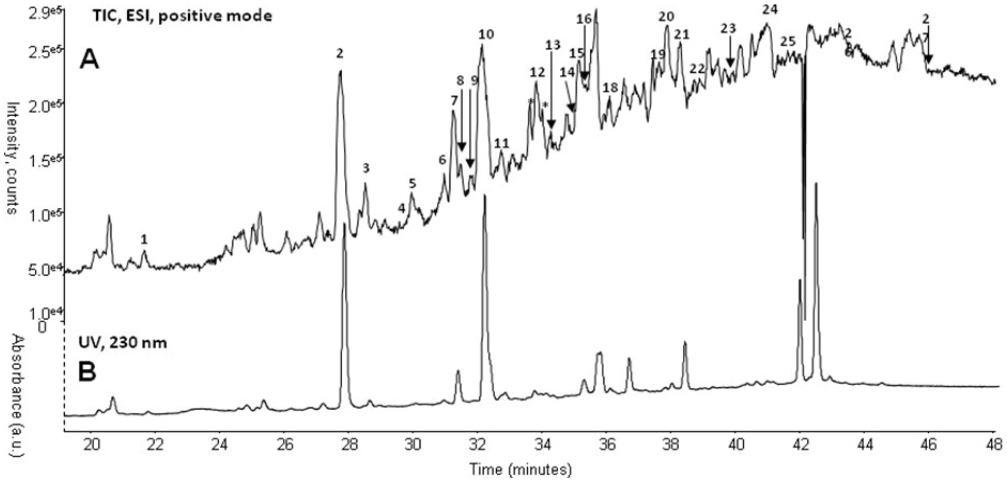

Fig. 1: LC/MS analysis of ginger rhizome extract EZo. (A) Total ion current (TIC) chromatogram from positive ion ESI-HPLC-MS; (B) HPLC-DAD chromatogram set at 230 nm of the crude ginger rhizome extract.

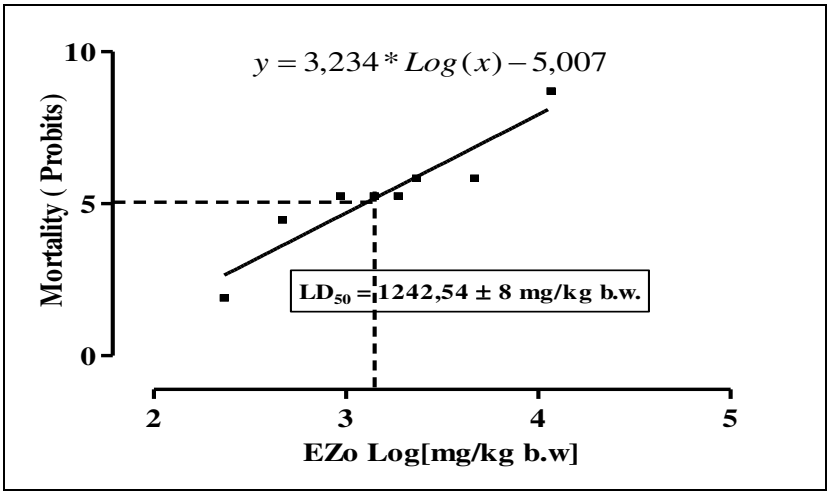

Fig. 2: Curve of acute toxicity of the rhizomes aqueous extract of *Zingiber officinale* Roscoe (EZo). The animals were treated by intraperitoneal (i.p.) administration of EZo. Horizontal scale: EZo log [mg/kg b.w.], vertical scale: mortality (Probits).

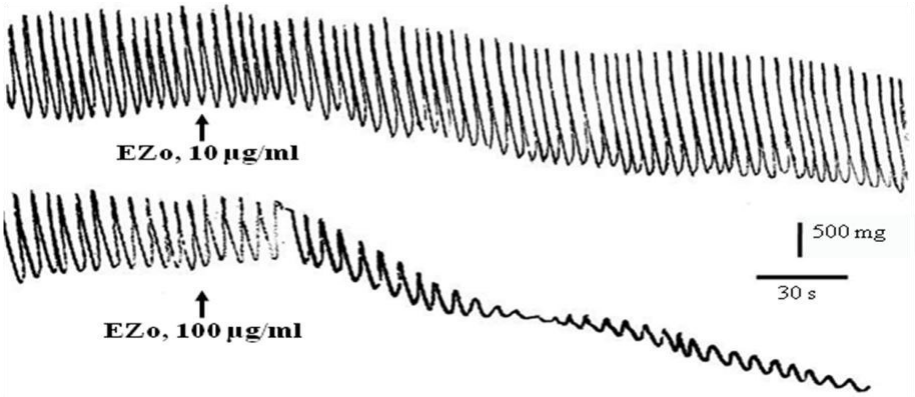

Fig. 3: Original tracing showing the effect of two concentrations (10 µg/ml and 100 µg/ml) of the aqueous extract of *Zingiber officinale* Roscoe on the contractile activity of duodenum isolated from rabbit. The arrows indicate administration of EZo. Horizontal scale: 30 s, vertical scale 500 mg.

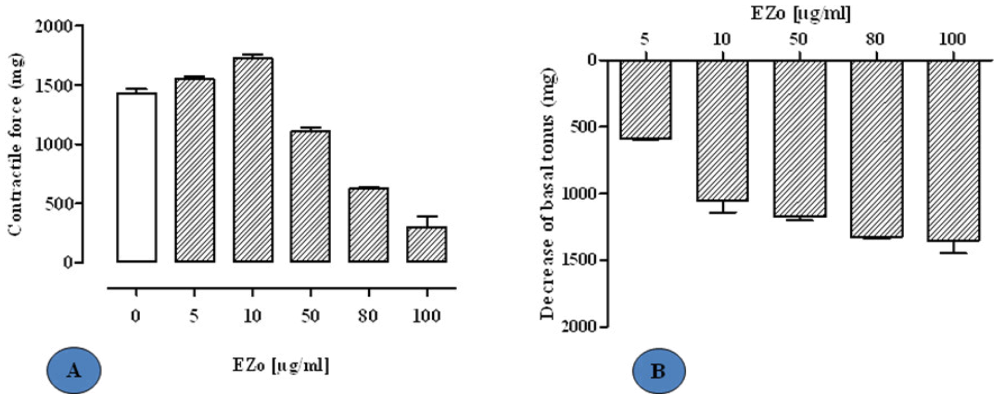

Fig. 4: Concentration-reponse curve for aqueous extract of *Zingiber officinale* Roscoe (EZo) on the rabbit duodenum contractility. A: EZo applied in a concentration ranging from 5 µg/ml to 10 µg/ml increased the contractile force of duodenum strips in concentration dependent manner. But the highest concentrations (10 µg/ml to 100 µg/ml) decreased the contractile force. B: In the same time, EZo (5 µg/ml to 100 µg/ml) decreased the basal tone in concentration-dependent manner. Horizontal scale: concentration [µg/ml], vertical scale variation of contractile activity (mg). (Mean ± sem, n = 4-6, $p < 0.05$).

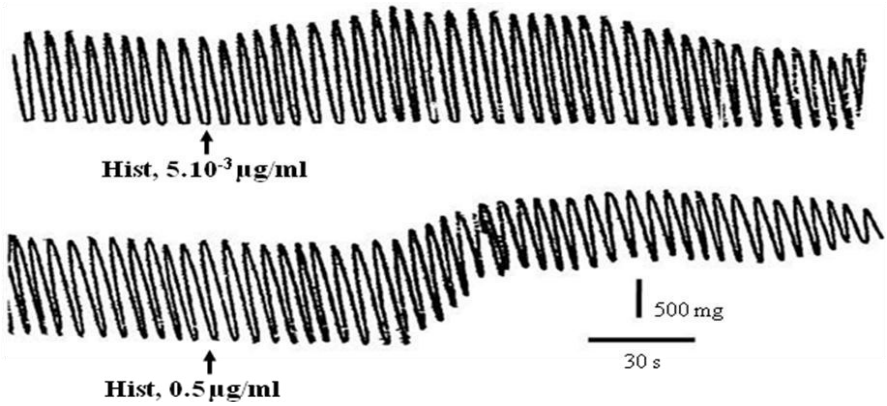

Fig. 5: Original tracing showing the effect of two concentrations (5×10^{-3} µg/ml and 0.5 µg/ml) of the histamine of EZo on the contractile activity of duodenum isolated from rabbit. The arrows indicate administration of EZo. Horizontal scale: 30 s, vertical scale: 500 mg.

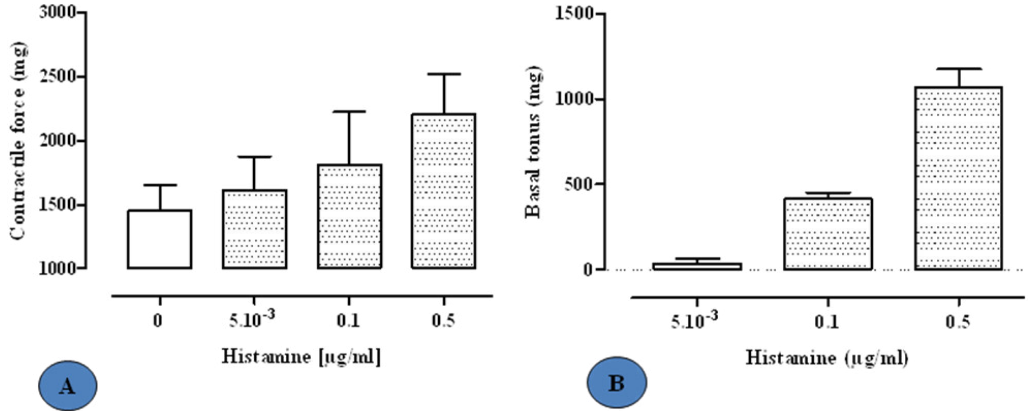

Fig. 6: Concentration-reponse curve for Histamine on the rabbit duodenum contractility. **A**: Histamine (5×10^{-3} µg/ml to 0.5 µg/ml) increased the contractile force (A) and the basal tone (B) of duodenum strips in concentration dependent manner. Horizontal scale: concentration [µg/ml], vertical scale: variation of contractile activity (mg). (Mean ± sem, n = 4-6, $p < 0.05$).

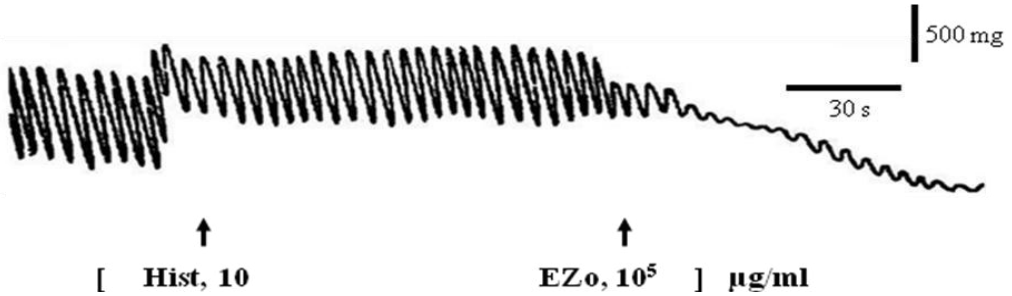

Fig. 7: Original tracing showing the effect of EZo (10^5 µg/ml) on the contractile activity of rabbit isolated duodenum pretreated with histamine (10 µg/ml). The arrows indicate administration of the drugs. Horizontal scale: 30 s, vertical scale: 500 mg.

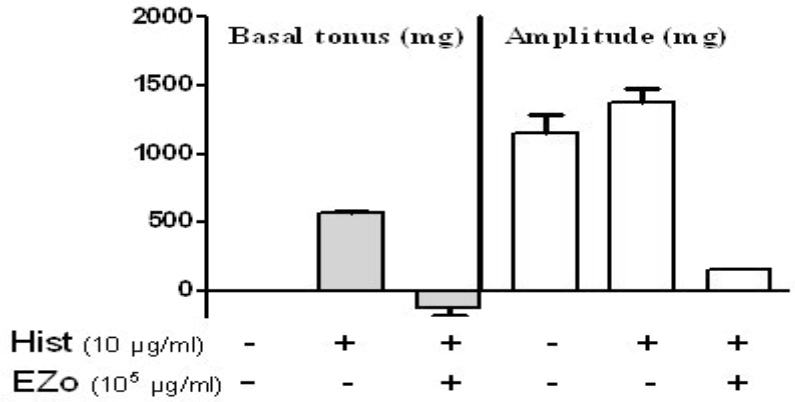

Fig. 8: Concentration-reponse curve for the aqueous extract of EZo (10^5 µg/ml) on the rabbit duodenum contractility in the presence of Histamine (10 µg/ml). Histamine (10 µg/ml) increased the contractile force and the basal tone of duodenum strips. After addition of EZo (10^5 µg/ml) both of basal tonus and contractile force decreased. Horizontal scale: concentration [µg/ml], vertical scale: variation of contractile activity (mg). (Mean ± sem, n = 4-6, $p < 0.05$).

In addition, Borrelli et al. (2004) showed that the different potency of ginger (*Z. officinale*) in inhibiting contractions could indicate that ginger possesses inhibitory effect on excitatory transmission throughout a prejunctional site of action.

Aqueous extract of *Z. officinale* is a nonspecific antihistaminic but it involves local histaminic receptors and the mediators. This result indicates that the antihistaminic effect of ginger might be mediated by its peripheral effect.

Antiemetics are frequently used to treat nausea induced by vestibular or central nervous system causes. Unlike forms of nausea, which tend to be mediated by dopamine and serotonin, vestibular system–induced nausea is mediated primarily by histamine and acetylcholine (Flake et al., 2004). Consequently, the American Gastroenterological Association (AGA) recommends the use of antihistaminics and anticholinergics in the treatment of nausea secondary to vertigo and motion sickness (Quigley et al., 2001). Oral ginger probably is effective and is thought to be safe in treatment of patients with pregnancy-induced nausea (Vutyavanich et al., 2001).

Conclusion

In Conclusion: 1. The *Z. Officinale* rhizome aqueous extract has a low toxicity which permits its uses by populations. 2. As shown by pharmacologic and the phytochemical studies of the rhizome, this plant could have many pharmacological properties justifying its traditional use to treat many diseases, including diarrhoea and childbirth asthma. We have shown ginger, an herbal remedy mainly promoted for the treatment of diarrhoea and reduced contractility of the rabbit isolated duodenum.

Ginger directly inhibits smooth muscle activity and reduces contractility induced by histamine.

Acknowledgements

The authors are indebted to Professor Aké-Assi Laurent (Laboratory of Botany, Unité de Formation et de Recherche – Biosciences, Félix Houphouët-Boigny University of at Abidjan, Côte d'Ivoire) for botanical identification of *Zingiber officinale Roscoe* (Zingiberaceae). We are grateful to Dr P. Champy for phytochemical analysis; to Dr. I. Schmitz-Afonso and to Pr. O. Laprévote for their help with MS/MS studies.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

Authors' contributions

All authors contributed equally in the study. They made substantial contributions to the design of the study, the collection of the data as well as the preparation and analysis of the data. They also drafted the manuscript and gave final approval for its submission to the journal for consideration of publication.

Declaration of Interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References

Afzal M, Al-Hadidi D, Menon M, Pesek J, Dhami M (2001). Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabol. Drug Interact., 18(3-4): 159-190.

Ahui MLB, Champy P, Ramadan A, Pham VL, Araujo L, Konan BA, Diem S, Damotte D, Kati-Coulibaly S, Offoumou AM, Dy M, Thieblemont N, Herbelin A (2008). Ginger prevents Th2-mediated immune responses in a mouse model of airway inflammation. Int. Immunopharmacol., 8(12): 1626-1632.

Ali BH, Blunden G, Tanira MO, Nemmar A (2008). Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food. Chem. Toxicol., 46(2): 409-420.

Altman RD, Marcussen KC (2001). Effects of a ginger extract on knee pain in patients with osteoarthritis. Arthritis Rheum., 44(11): 2531-2538.

Astin JA (1998). Why patients use alternative medicine ? Results of a national study. JAMA, 279(19): 1548-1553.

Binlin-Dadié R, Soro S, N'Dri KD (1997). Complications after traditional drugs use (clinical symptomatology). Méd. Afr. Noire, 44(3): 128-130.

Borrelli F, Capasso R, Pinto A, Izzo AA (2004). Inhibitory effect of ginger (*Zingiber officinale*) on rat ileal motility *in vitro*. Life Sci., 74(23): 2889-2896.

Chang CP, Chang JY, Wang FY, Chang JG (1995). The effect of Chinese medicinal herb Zingiberis rhizome extract on cytokine secretion by human peripheral blood mononuclear cells. J. Ethnopharmacol.. 48(1): 13-19.

Chiwororo WDH, Ojewole JAO (2009). Spasmolytic effect of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extract on rat isolated uterine horns. J. Smooth muscle Res., 45(1): 31-38.

Chrubasik S, Pittler MH, Roufogalis BD (2005). *Zingiberis rhizoma*: a comprehensive review on the effect and efficacy profiles. Phytomedicine, 12(19): 684-701.

Datté JY, Traoré A, Offoumou AM, Ziegler A (1998). Effects of leaf extract of *Caesalpinia bonduc* (Caesalpiniaceae) on the contractile activity of uterine smooth muscle of pregnant rats. J. Ethnopharmacol., 60(2): 149-155.

Diezi J (1989). Toxicologie. Principes de base et répercussions clinique, in: Pharmacologie des concepts fondamentaux aux applications thérapeutiques (ed). Slatkine-Genève; 33-44.

Dragstedt A, Lang B (1957). Etude de la toxicité par administration unique d'un nouveau médicament. Annales Pharmaceutiques français, p. 11.

Ernst E, Pittler MH (2000). Efficacy of ginger for nausea and vomiting: a systematic review of randomized clinical trials. Br. J. Anaesth., 84(3): 367-371.

Flake ZA, Scalley RD, Bailey AG (2004). Practical Selection of Antiemetics. Am. Fam. Physician., 69(5): 1169-1174.

Gies JP (1993). Bases de pharmacologie moléculaire. Edition Marketing, Paris, France, 5-6.

He X, Bernart MW, Lian L, Lin L (1998). High-performance liquid chromatography-electrospray mass spectrometric analysis of pungent constituents of ginger. J. Chromatogr. A., 796(2): 327-334.

Jeyakumar SM, Nalini N, Venugopal MP (1999). Antioxidant activity of ginger (*Zingiber officinale* Roscoe) in rats fed a high fat diet. Med. Sci. Res., 27: 341-344.

Jiang H, Solyom AM, Timmermann BN, Gang DR (2005). Characterization of gingerol related compounds in ginger rhizome (*Zingiber officinale* Rosc.) by high performance liquid chromatography/electrospray ionization mass spectrometry. Rapid Commun. Mass Spectr., 19(20): 2957-2964.

Kikuzaki H, Kobayashi M, Nakatani NA (1991). Diarylheptanoids from rhizomes of *Zingiber officinale*. Phytochemistry, 30(11): 3647-3651.

Kikuzaki H, Tsai SM, Nakatani N (1992). Gingerdiol related

compounds from the rhizomes of *Zingiber officinale*. Phytochemistry. 31(5): 1783-1786.

Konan BA, Amonkan AK, Ahui MLB, Bouafou KGM, Kouakou LK, Kpahé FZ, Datté JY (2011). Myostimulating effects of *Sesamum radiatum* aqueous leaf extract in isolated guinea-pig *Taenia caeci* contractile activity. Afr. J. Tradit. Complement. Altern. Med., 8(4): 377-385.

Konan BA, Bouafou KGM, Kouakou LK, Bléyéré MN, Amonkan KA, Zannou-Tchoko JV, Ahui MLB, Datté JY (2012a). Effects of *Sesamum radiatum* aqueous leaf extract on rhythmic contractions of uterine smooth muscle bundles from pregnant rats. LSMR, 2(4): 27-36.

Konan BA, Bouafou KGM, Bléyéré NM, Zannou-Tchoko V, Amonkan KA, Oussou KR, Datté JY (2012b). Acute toxicity study and effects of sesame (*Sesamum radiatum*) aqueous leaf extract on rabbit's electrocardiogram. Int. J. Biomol. & Biomed., 2(1): 17-27.

Lüllmann H, Mohr K, Ziegler A (1998)**.** Atlas de poche de Pharmacology. Ed Flammarion Médecine-Sciences, Paris, France, 7-10.

Mashour NH, Lin GL, Frishman WH (1998). Herbal medicine for the treatment of cardiovascular disease. Arch. Intern. Med., 158(20)**:** 2225-2234.

Mandal SC, Dhara AK, Kumar ACK, Maiti BC (2001). Neuropharmacological activity of *Xanthium strumarium* Linn. Extract. J. Herbs Spices Med. Plants, 8(1): 69-77.

Miller LC, Tainter MC (1944). Estimation of ED_{50} and its Error by means of logarithmic-Probit Graph Paper. Proc. Soc. Exp. Biol. Med., 57(2): 261-264.

Mosihuzzaman M, Choudhary MI (2008). Protocols on safety, efficacy, standardization and documentation of herbal medicine. Pure Appl. Chem., 80(10): 2195-2230,.

Mowrey DB, Clayson DE (1982). Motion sickness, ginger, and psychophysics. Lancet, 319(8273): 655-657.

Néné-Bi SA, Traoré F, Zahoui OS, Soro TY (2008). Phytochemical composition, pharmacological and toxicological studies of an aqueous extract of *Bridelia ferruginea* Benth. (euphorbiaceae) in mammalians. Afrique Science, 4(2): 287-305. (Published in French).

Özbek H, Öztürk M, Öztürk A, Ceylan E, Yener Z (2004). Determination of lethal doses of volatile and fixed oils of several plants. East. J. Med., 9(1): 4-6.

Pousset JL (1989). African medicinal plants. Practical use. A.C.C.T., Ed. Ellipses, 2-12. (Published in French).

Quigley EM, Hasler WL, Parkman HP (2001). AGA technical review on nausea and vomiting. Gastroenterology, 120(1): 263-286.

Ramirez-Ahumada MDC, Timmermann BN, Gang DR (2006). Biosynthesis of curcuminoids and gingerols in turmeric (*Curcuma longa*) and ginger (*Zingiber officinale*): Identification of curcuminoid synthase and hydroxycinnamoyl-CoA thioesterases. Phytochemistry, 67(18): 2017-2029.

Rong X, Peng G, Suzuki T, Yang Q, Yamahara J, Li Y (2009). A 35-day gavage safety assessment of ginger in rats. Regul. Toxicol. Pharmacol., 54(2): 118-123.

Surh Y-J (2002). Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities. a short review. Food Chem. Toxicol.. 40(8): 1091-1097.

Thomson M, Al-Qattan KK, Al-Sawan SM, Alnaqeeb MA, Khan I, Ali M (2002). The use of ginger (*Zingiber officinale* Rosc.) as a potential anti-inflammatory and antithrombotic agent. Prostaglandins Leukot. Essent. Fatty Acids, 67(6): 475-478.

Traoré F, Soro TY, Néné-Bi SA, Souza A (2002). Toxicological studies of *Swartzia madagascariensis* (Caesalpiniaceae) and *Erythrina senegalensis* (Fabaceae). Rev. Iv. Sci. Tech., 3: 141-151. (Published in French).

Uehara S-I, Yasuda I, Akiyama K, Morita H, Takeya K, Itokawa H (1987). Diarylheptanoids from rhizomes of *Curcuma xanthorrhiza* and *Alpinia officinarum*. Chem. Pharm. Bull., 35(8): 3298-3304.

Vutyavanich T, Kraisarin T, Ruangsri R (2001). Ginger for nausea and vomiting in pregnancy: randomized, double-masked, placebo-controlled trial. Obstet. Gynecol., 97(4): 577-582.

Wang WH, Wang ZM (2005). Studies of commonly used traditional medicine ginger. ZHongguo Zhong Yao Za Zhi., 30(20): 1569-1573. (Published in Chinese).

Weidner MS, Sigwart K (2000). The safety of a ginger extract in the rat. J. Ethnopharmacol*.,* 73(3): 513-520.

Wu Y, Zulin D, Liu D, Gao Z (2002). The desease and its control on zingiberous plants in South China Botanical Garden. Zhong Yao Cai., 25(11): 773-775. (Published in Chinese).

Yu Z, Wu H, Ding J (1998). The volatile chemical components of fresh *Zingiber officinale*. Acta Botanica Yunnanica, 20(1): 113-118. (Published in Chinese).