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Phytochemical composition of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils and their anti-inflammatory and analgesic properties on Wistar rats

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Abstract *Cymbopogon citratus* and *Eucalyptus citriodora* are widely used herbs/plants as a source of ethnomedicines in tropical regions of the world. In this work, we studied the anti-inflammatory and gastroprotective effects of *C. citratus* and *E. citriodora* essential oils on formol-induced edema, and acetic acid induced abdominal cramps in Wistar rats. To fully understand the chemically induced anti-inflammatory properties of these plants, we first analyzed the chemical composition of the essential oils. A total of 16 chemical constituents accounting for 93.69 % of the oil, were identified in C. *citratus* among which, Geranial (27.04 %), neral (19.93 %) and myrcene (27.04 %) were the major constituents. For *E. citriodora*, 19 compounds representing 97.2 % of the extracted oil were identified. The dominant compound of *E. citriodora* essential oil was citronellal (83.50 %). In vivo analysis and histological assay showed that the two essential oils displayed significant dose dependent edema inhibition effect over time. They displayed strong analgesic and antipyretic properties similar to that induced by 50 mg/kg of acetylsalicylate of lysine. However, the *E. citriodora* essential oil was more effective than that of *C. citratus*. We identified significant numbers of aldehyde molecules in both essential oils mediating antioxidant activity that may contribute to the anti-inflammatory effects observed on the rats. Altogether, this work demonstrates the anti-inflammatory property of *C. citratus* and *E. citriodora* suggesting their potential role as adjuvant therapeutic alternatives in dealing with inflammatory-related diseases.

Keywords Essential oil · *Cymbopogon citratus* · *Eucalyptus citriodora* · Anti-inflammation · Wister rats

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Introduction

Plants are well-known for the medicinal properties. Phytomedicines have shown great promise in the treatment of various human and animal diseases [1–3]. Most of the developing countries rely generally on the use of traditional medicines as primary source of health care. In Africa for instance, a wide range of indigenous plant species are often used as the main constituents of traditional medicines [4, 5]. Despite the huge amount of medicinal plant varieties available worldwide, only a small number have been phytochemically and pharmacologically analyzed for their

potential use in medicine. In addition, a single medicinal plant species may contain up to several hundreds of health promoting chemical constituents that are still underexploited. More than 80 % of world population uses herbal medicines to deal with their daily medicinal issues. Therefore, the study of medicinal plant constituents has great pharmaceutical potential.

Cymbopogon citratus and *Eucalyptus citriodora* are two medicinal plant species belonging to the family of Poaceae and Myrtaceae respectively [6]. They are both aromatic herbs/plants known for their essential oils often used in chemistry, medicine, soap industry, cosmetics and agribusiness [7–10]. As part of our ongoing research acterization and cataloguing phytochemical constituents that promote human health, we here studied the anti-inflammatory and analgesic properties of *C. citratus* and *E. citriodora* essential oils. Natural essential oils are more environmentally friendly than synthetic products due to biodegradation and environmental safety issues [11, 12]. *C. citratus* is being used for treatment of nervous and gastrointestinal disturbances, and as an antispasmodic, analgesic, anti-inflammatory, antipyretic, diuretic and sedative [13]. Recent studies have only demonstrated the use of *C. citrates* and *E. citriodora* essential oils in mycotoxin inhibition and anti-microbial activity [14–16]. The full medicinal potential of these plants is still not exhaustively studied.

In this study, we first established the phytochemical composition of *C. citratus* and *E. citriodora* essential oils, and then evaluate their anti-inflammatory and analgesic activities on Wistar rats with the ultimate goal to fully harnessing the medicinal properties of these plants. Generally the early phase of acute inflammation involves cellular influx associated with the release of mediators such as histamine and serotonin followed by the production of bradykinin and prostaglandins [17], which ultimately lead to inflammation [18]. Phytochemical and pharmacological tests were preliminary carried out to establish the subchronic lethal dose of the oils on the animals, assessing the gastric tolerance and the muscular activity of the rats. We then investigated the anti edema, analgesic and antipyretic activities of *C. citratus* and *E. citriodora* extracts on the rats. Our data indicate that the *C. citratus* and *E. citriodora* essential oils contain substantial phytochemicals with great anti-inflammatory effects.

Materials and methods

Ethics statement

The Wistar rats used in this study were handled according to the Institutional animal safety guidelines (Animal facility, Faculty of Health Science, University of Abomey Calavi, Benin). The experiments were performed according to the Institutional Animal Ethics No. 084 MS/DC/SG/DFRS/CNPERS/SA (University of Abomey Calavi, Benin).

Plant material

The leaves of *C. citratus* and *E. citriodora* used in this study were collected from the institutional botanical garden (University of Abomey Calavi, Benin). The plants as depicted in Fig. 1a, b were identified at the institutional herbarium center (University of Abomey Calavi, Benin) with the following accession numbers: *C. citratus* (AAC 173/HNB) and *E. citriodora* (AAC 181/HNB).

Fig. 1 Field representation of *C. citratus* (**a**) and *E. citriodora* (**b**) plants. The *arrow heads* show the leave of *E. citriodora* (used in this study) in the botanical field background

Animal specimen, growth and feeding conditions

Three month old Wistar rats (both male and female) with an average weight of 163 ± 12 g were kept in living conditions as recommended by the breeders (Animal facility, Faculty of Health Science, University of Abomey Calavi, Benin). The rats were kept in sets of six per feeding device in standard wire mesh cages with stainless steel tray floor, in a room illuminated at 12 h light, 12 h dark at 25–30 °C with relative humidity of 70–80 %. Rats were fed with diet composed of 53 % crushed maize, 19 % fish meal, 20 % wheat bran, 5 % groundnut oil, 1.5 % vitamin complex (Olivitasol), and 1.5 % NaCl. The chemical analysis of the diet was 16.1 % crude protein, 12.9 % crude fiber and 2.6 % crude fat. The diet and drinking water were provided ad libitum. Periodic feeding times were set and the animals were fed with food mixed with essential oils before 9:00 am. The mixed food material was composed of corn (as above detailed) and essential oils extracted from *C. citratus* and *E. citriodora* leaves respectively. A total of 6 animals were considered per test throughout this work.

Extraction and chemical analysis of essential oils

The essential oils were extracted by a steam distillation using "Clevenger" type equipment as previously described [19]. The chemical composition of the essential oils was analyzed on a Trace gas chromatographic (GC) Thermo Quest system equipped with FID and a DB-5 column under the following analytical conditions: the GC oven temperature was maintained at 50 °C for 5 min and programmed to reach 300 °C at a rate of 5 °C/min [19]. An aliquot (1 µl at 5 % v/v dilution with pentane) of essential oil was injected into the column, while the injection temperature was 240 °C. Hydrogen served as carrier gas at a flow rate of 35 ml/min. The GC–MS analysis was carried out using a Hewlett Packard 5970 GC fitted with a DB-1 column (25 m × 0.23 mm i.d.) with ionization energy of 70 eV and Helium used as carrier gas at a flow rate of 0.9 ml/min. In order to identify the essential oil constituents, we used the Kovats retention indices calculation method with the mixture of C8–C26 Alcan chain molecules. Oil constituents were identified on the basis of their Kovats retention indices by analyzing the mass spectral fragmentation according to Adams [20].

In vivo tests for anti-inflammatory activity

In order to assess the anti-inflammatory activity of essential oils, we first checked the subchronic lethal doses of the essential oils, investigated the gastric tolerance, and observed the weight variation of the animals after treatment [21, 22]. The animals were orally fed with *C. citratus* essential oil at a dose ranging from 600 to 4,000 mg/kg. They were similarly fed with *E. citriodora* oil at dose varying from 600 to 2,600 mg/kg. Upon satisfactory non-lethal doses, we proceeded to the anti-inflammatory activity. The anti-inflammatory test was carried out by injecting 0.1 ml of 1 % (v/v) formol in the left foot aponevrosis of the rats to induce an edema [23, 24]. The antalgic and analgesic activities were then assessed using tail-flick and tail immersion Koster method respectively [24]. The antipyretic activity was determined by recording the variation of the anal temperature after intra-peritoneal injection (IP) of Brewer's yeast in the abdomen of the rats as previously described [24]. The motor behaviour of the rats was analyzed by a modified method of Martin [25]. Two most significant doses of essential oils were tested in this anti-inflammatory assay. For this test, we consider 2,000 and 3,000 mg/kg for *Cymbopogon citrates* and 1,700, 1200 and 1800 mg/kg for *E. citriodora*.

Results and discussion

Essential oil extraction: phytochemical compound characterization

The present study was carried out to characterize the anti-inflammatory and analgesic activity of *C. citratus* (Fig. 1a) and *E. citriodora* (Fig. 1b) on Wistar rats under laboratory conditions. To do so, we first analyzed the chemical composition of the extracted oils. The essential oils extraction yield was 0.90 % (v/w) and 3.24 % (v/w) for *C. citratus* and *E. citriodora* respectively. Using high performance GC chromatography, the largest detected peaks in *C. citratus* were for Myrcene, Neral and Geranial (Fig. 2a), while in *E. citriodora*, the largest detected peaks were for Citronnellal, Isopulegol, Citronnellol and Methyleugenol molecules (Fig. 2b). There were many other small peaks in the first 20 min of the gas chromatogram, where mono- and sesquiterpenes are eluted (Fig. 2). The presence of terpenoid volatiles supports the physico-chemical aromatic characterization of these herbs/plants [26]. In addition, the phytochemical investigation revealed the presence of aldehydes, oxygenated/non-oxygenated compounds, alcohols, esters and ketones (Table 1). A detail chemical composition of the two essential oils is presented in Table 2. We confidently identified 93.69 % of *C. citratus* chemical constituents and 97.2 % of that of *E. citriodora*. The two essential oils analyzed do not have the same chemical composition (Table 2). In *C. citratus*, 60.53 % of the identified compounds are oxygenated compounds; and 49.75 % are aldehyde molecules. On the other hand, 84.26 % of

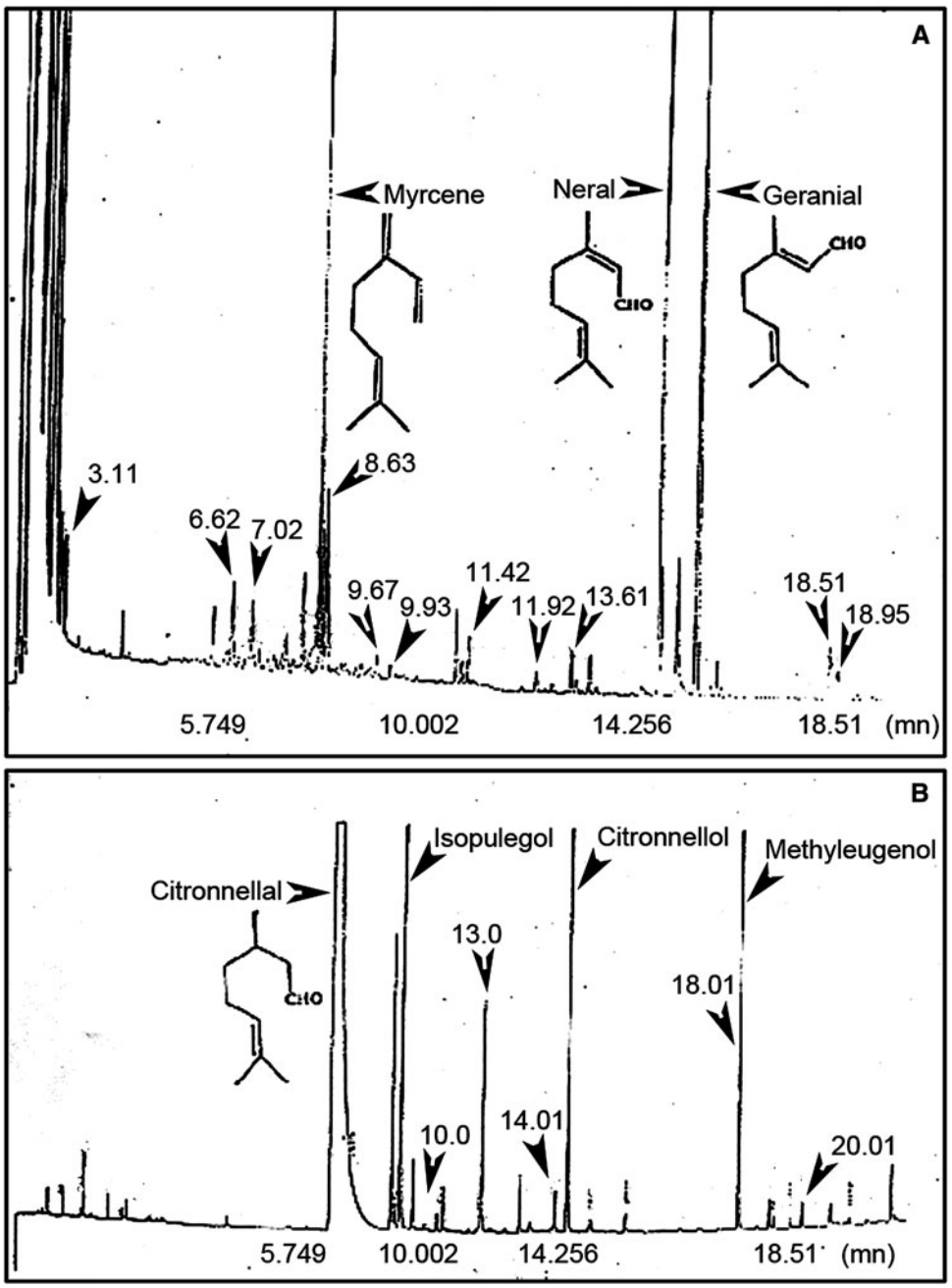

Fig. 2 Complete gas chromatogram profile of *C. citratus* (**a**) and *E. citriodora* (**b**) essential oils

E. citriodora identified oil compounds were oxygenated compounds and 83.5 % of them are aldehyde molecules. Interestingly, the majority of phytochemical composites identified in *C. citratus* essential oil were the myrcene (27.83 %), the geranial (27.04 %), the neral (19.93 %) and the geraniol (4.33 %), while citronellal (83.50 %) was the major phytochemical compound of *E. citriodora* essential oil (Table 2). In summary, the chromatographic analysis of the essential oils revealed that citrals are the dominant chemical compounds of *C. citratus*, while citronellal compound was found to be the predominant product of *E. citriodora*.

Sub-chronic lethal dose investigation of the essential oils

In order to ascertain the biologically acceptable dose of the essential oil to administer to the animals in this study, we first investigated the sub-chronic lethal dose of the essential oils. To carry out this test, the animals were administered with increasing doses of the essential oils, after which we checked the gastric tolerance and histological architecture of different organs under a dose dependent manner. Our results indicated that $\leq$ 3,000 and 2,500 mg/kg of *C. citratus* and *E. citriodora* essential oils respectively were not

Table 1 Major components of *C. citratus* and *E. citriodora* essential oils

Chemical component characterization	Essential oils	
	C. citratus (in %)	*E. citriodora* (in %)
Total identified components	93.69	97.2
Total non identified components	6.31	2.8
Oxygenated components	60.53	84.26
Aldehydes	49.75	83.50
Ketones	2.45	–
Alcohols	6.54	10.81
Esters	1.28	1.51
Extraction yield	0.90	3.24

Table 2 Detail chemical components of *C. citratus* and *E. citriodora* essential oil extracts

Major chemical components	Kovats indices	Essential oils	
		C. citratus Percentage	*E. citriodora* Percentage
Heptanal*	911	2.03	–
α-Thujene	930	2.29	–
α-Pinene	939	0.46	0.13
Camphene	954	1.18	
Sabinene	975	1.10	
β-Pinene	978	–	0.30
6-Methylhepta-5-en-2-one*	986	2.45	–
Myrcene	991	27.83	0.11
Limonene	1,029		0.09
1,8-cineole*	1,035	0.51	0.17
Cis β-ocimene	1,049	0.30	
Linalool*	1,110	1.22	0.17
Neo-isopulegol*	1,142	–	1.90
Isopulegol*	1,151	–	4.40
Citronellal*	1,158	0.75	83.50
Iso-isopulegol*	1,161	–	Trace (< 0.01)
Neo-iso-isopulegol*	1,171	–	Trace (< 0.01)
Terpinen-4-ol*	1,177	0.99	–
Phenylethyl acetate*	1,182	–	0.11
Citronnellol*	1,235	–	1.85
Neral (Citral B)*	1,247	19.93	–
Geraniol*	1,260	4.33	–
Geranial (Citral A)*	1,283	27.04	–
p-Mentha-3,8-diol*	1,317	–	0.08
Citronellyl acetate*	1,360	–	1.10
Methyleugenol*	1,380	–	2.20
Geranyl acetate*	1,387	1.28	0.30
γ-elemene	1,400	–	0.25
Trans-caryophyllene	1,427	–	0.50

* The asterisk indicates the oxygenated compounds

lethal to the animals, but concentrations higher than these were lethal to the animals. Under non-lethal dose, we followed the gastric tolerance and the histological architecture of the stomach organs at different days after the animals were treated with *C. citratus* and *E. citriodora* essential oils. We found that the stomach organ structures were not altered under *C. citratus* treatment as indicated by the normal biological stomach tissue texture clearly observed in Fig. 3a). However, we observed significant erosion zones of the gastric partition of the stomach under *E. citriodora* essential oil treatment as clearly shown in Fig. 3b illustrating the strong alteration of the spongy structure of the stomach. This effect was more pronounced under increased concentrations of *E. citriodora* essential oil (Fig. 3c).

We still wanted to rule out the trivial explanation that the effect of the oils on the gastric organs (Fig. 3) was not due to artifacts. We hypothesized that the oils will have the same positive correlative effect (as observed in Fig. 3) on other sensitive organs of the animals if the results observed in Fig. 3 were real. To test our hypothesis, we checked the structural architecture of the liver under the essential oil treatment. As hypothesized, *C. citratus* treatment did not affect the structural architecture of the liver (Fig. 4a). However, we have observed an aqueous overload of the liver after the animals have been treated with *E. citriodora* essential oil as expected (Fig. 4b, c), confirming that the above essential oils concentrations mediating organ structural alterations were not artifacts but indeed sub-chronic lethal doses of the oil extracts. Interestingly, most of the rats gained weight upon treatment with all the different doses of *C. citratus*. However, we also noticed rare cases where rats lost weight under the same treatment. For example, more than half of the rat population lost weight after 4 days of *E. citriodora* essential oil treatment. In summary, *C. citratus* essential oil was found to have no adverse effect on structural membrane of the rat organs when given orally. However, *E. citriodora* essential oil caused structural destruction of the organs at dose higher than 2,200 mg/kg, indicating that *E. citriodora* essential oil might be toxic when used at sub-chronic dose (dose higher than 2,200 mg/kg) under the same oral administration condition.

Anti-inflammatory and analgesic activity of the essential oils

The anti-inflammatory effect of the essential oils was investigated on formol-induced edema in the animals. Treatments with *C. citratus* essential oil reduced the edema over time in a dose dependent manner. We also observed a reduced volume of edema when rats were treated with *E. citriodora* essential oil. *C. citratus* essential oil was

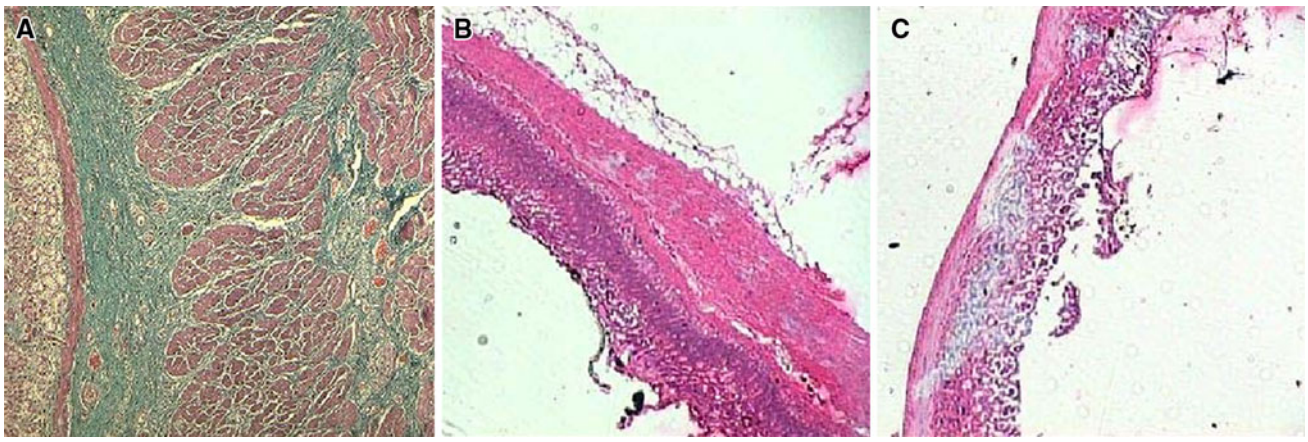

Fig. 3 Histological structure of rat stomach after administration of different doses of essential oils. The structure was normal in animal receiving *C. citratus* essential oil at 3,000 mg/kg body weight (**a**); but the stomach structure was significantly altered at different doses (**b**, **c**) of *E. citriodora* essential oil. **b** 1,800 mg/kg, and **c** 2,400 mg/kg of *E. citriodora* essential oil respectively

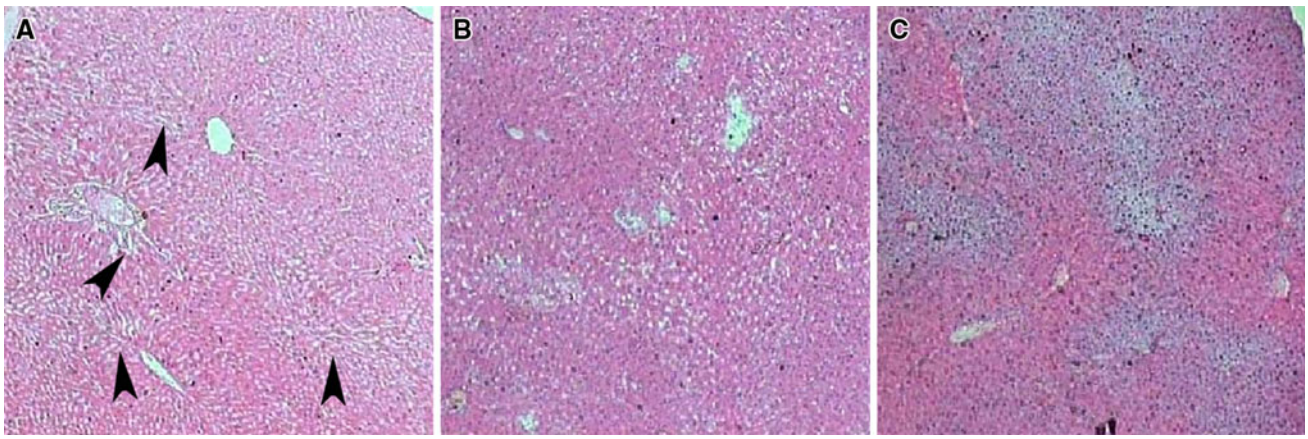

Fig. 4 Histological structure of rat liver after administration of different doses of essential oils. The structure of the liver was normal (as indicated by *arrow heads* pointing to the fine micro-indentations characteristic of a normal liver) with animals receiving *C. citratus* essential oil at 3,000 mg/kg body weight (**a**); but it was significantly altered at different doses (**b**, **c**) of *E. citriodora* essential oil. **b** 1,800 mg/kg and **c** 2,400 mg/kg of *E. citriodora* essential oil respectively. The fine micro-indentations characteristic of a normal liver are progressively altered and disappeared in (**b**, **c**)

Table 3 Tail-flick test mediating the staying period of animal tails in warm (50 °C) water after essential oil treatment

Treatment	Dose (mg/kg)	Time (s)
C. citratus essential oil	3,000	8.44 ± 2.22
C. citratus essential oil	2,000	5.96 ± 1.28
E. citriodora essential oil	1,800	9.43 ± 0.96
E. citriodora essential oil	1,200	5.44 ± 0.28
Aspegic	50	7.20 ± 1.59
Aspegic	25	4.99 ± 1.3
Control	–	4.75 ± 0.96

Table 4 The essential oils decrease the number of abdominal cramps induced by acetic acid

Treatment	Dose (mg/kg)	Number of Cramps
C. citratus essential oil	3,000	30.33 ± 1.66
C. citratus essential oil	2,000	39.00 ± 3.00
E. citriodora essential oil	1,800	34.33 ± 1.66
E. citriodora essential oil	1,200	29.38 ± 4.66
Aspegic	50	33.67 ± 3.33
Control	–	55.00 ± 3.00

found to have a preventive effect at 3,000 mg/kg of animal weight, while *E. citriodora* essential oil was found to have a curative effect at 1,800 mg/kg of animal weight. We next tested the analgesic activities of the essential oils by tail immersion test. The essential oil treated animal tails were immersed in hot water kept at 50 °C and the retention time of the tails in the water bath was recorded and compared to

Table 5 Reduction effects of *C. citratus* and *E. citriodora* essential oils on rat hyperthermia

Treatment	Dose (mg/kg)	Before induction	After induction	Time (min)					
				60	120	180	240	300	360
C. citratus	3,000	36.03 ± 0.00	37.03 ± 0.20	35.83 ± 0.00	35.83 ± 0.13	35.70 ± 0.33	35.73 ± 0.07	35.66 ± 0.33	36.00 ± 0.03
C. citratus	2,000	36.20 ± 0.14	37.00 ± 0.30	36.66 ± 0.00	36.66 ± 0.16	36.50 ± 0.22	36.28 ± 0.15	36.13 ± 0.03	36.10 ± 0.10
E. citriodora	1,800	36.43 ± 0.40	37.53 ± 1.00	36.10 ± 1.30	35.90 ± 1.60	35.73 ± 1.80	35.86 ± 1.80	35.70 ± 1.60	35.95 ± 1.70
E. citriodora	1,200	35.03 ± 0.10	37.86 ± 0.00	37.20 ± 0.00	36.76 ± 0.00	36.73 ± 0.70	36.28 ± 0.90	36.01 ± 0.10	36.06 ± 0.20
Aspegic	50	36.46 ± 0.30	37.16 ± 0.01	36.15 ± 0.05	36.10 ± 0.40	36.01 ± 0.09	36.18 ± 0.08	36.30 ± 0.12	36.30 ± 0.08
Aspegic	25	36.30 ± 0.24	37.06 ± 0.22	36.50 ± 0.07	36.43 ± 0.07	36.36 ± 0.10	36.46 ± 0.04	36.50 ± 0.04	36.46 ± 0.04
Control	–	35.06 ± 0.04	37.10 ± 0.10	37.20 ± 0.39	36.98 ± 0.17	36.90 ± 0.08	36.73 ± 0.15	36.58 ± 0.00	36.58 ± 0.07

the untreated animals. Animals treated with essential oils were able to keep their tails longer in a hot water bath (50 °C) than the untreated animals (Table 3), demonstrating the analgesic activity of the essential oils. In addition, the essential oil treated animals had reduced number of acetic acid induced abdominal cramps compared to untreated animals (Table 4). We therefore expected that the essential oils will be able to reduce hyperthermia. Indeed, the two essential oils significantly reduced the hyperthermia (Table 4), although the reduced hyperthermia effect of *E. citriodora* essential oil was better than that of *C. citratus* (Table 5). This analytical data coupled with the histological (Figs. 2, 3, 4) and biochemical data support the anti-inflammatory and analgesic properties of *C. citratus* and *E. citriodora* on Wistar rats.

Understanding the role of phytochemical compounds in human disease control is a long-standing goal in traditional medicine [27]. *C. citratus* and *E. citriodora* have been used in various traditional medicines, especially in developing countries [19, 28]. The ability of *C. citratus* and *E. citriodora* essential oils to reduced acetic acid induced abdominal cramps, edema and hyperthermia in Wistar rats is here demonstrated.

Injection of formol to female rats is known to induce tissue lesion mediated local inflammation, an effect induced by prostaglandin and histamine by-products of formol metabolism [29]. The results of our antipyretic test show that the hyperthermia is a complex immuno-inflammatory mechanism leading to a release of endogenous pyrogens and prostaglandins. The essential oils are thought to inhibit prostaglandin biosynthesis such as salicylate drugs and non-steroid anti-inflammatory drugs [29, 30]. The essential oils are suggested to block the excitation of neuronal ends induced by pro-inflammatory substances. In our extracts, these anti-inflammatory effects are suggested to be carried out by the aldehyde and Ketone molecules [29] representing more than 55.72 % of *C. citratus* identified components and 83.5 % of all identified *E. citriodora* components.

In summary, *C. citratus* and *E. citriodora* essential oils have been shown to have anti-inflammatory, analgesic and antipyretic effects when orally administered to rats. These effects are however time and dose dependent. We found that preventive test was more efficient than curative test with *C. citratus* essential oil, while curative test was more efficient than preventive test with *E. citriodora* essential oil. Thus, our study indicates that *C. citratus* and *E. citriodora* essential oils could be used as promising anti-inflammatory agents.

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