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Chemical composition and spasmolytic activity of *Cymbopogon schoenanthus* (L.) Spreng. (Poaceae) essential oil from Sudan

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Abstract: The plants of genus *Cymbopogon* are well known for their use in traditional medicine and for their high content of essential oils that are widely used as flavoring agents, fragrances, cosmetics, and pharmaceuticals. Essential oils isolated from the dried stems and inflorescence of cultivated *Cymbopogon schoenanthus* (L.) Spreng. (Poaceae) collected from Khartoum state (Sudan) in two periods were studied. The results of chemical analysis of *C. schoenanthus* revealed that this plant is rich in essential oil which is a valuable source of the commercially important monoterpene, piperitone (47.7-71.5%). Other abundant constituents of the oils were intermedeol (6.1-17.3%), δ-2-carene (4.5-10.0%) and elemol (2.7-9.0%). The essential oil from the inflorescence was tested for spasmolytic activity using three different experimental models: against spontaneous contractions, contractions induced with acetylcholine and contractions induced with potassium chloride. The oil exhibited strong, significant and dose-dependent spasmolytic activity, indicating the possibility for further investigations of this essential oil for its medicinal purposes or application in food industry.

Key words: *Cymbopogon schoenanthus*; essential oil composition; piperitone; spasmolytic activity.

INTRODUCTION

Essential oils and aromatic plants have been used by many cultures around the world for centuries. Their uses vary between cultures, from religious to healing purposes. Nowadays essential oils have many applications in medicine, pharmacy, cosmetics and the food industry. Interest in aromatic plants and essential oils has increased substantially in the last decades due to their high medicinal and economic value [1].

Cymbopogon Spreng. (Poaceae) is a genus of about 180 species, subspecies, varieties, and subvarieties that inhabit warm and tropical regions of the Old World and Oceania [2]. Species of this genus are well known for their use in traditional medicine as well as for their remarkably high content of essential oils with pleasant aroma and wide usage for flavoring, fragrances, cosmetics, pharmaceuticals and perfumery [3]. Additionally, the oils possess numerous biological activities, including antimicrobial, anticancer, pesticidal, mosquito repellent, antiinflammatory and hypoglycemic activity [4]. The best-known species of this genus in terms of commercially important essential oils are: *C. citratus* Stapf (East Indian lemongrass) and *C. flexuosus* Stapf (West Indian lemongrass) that produce lemongrass oil, *C. martini* Stapf (palmarosa oil source), *C. nardus* (L.) Rendle and *C. winterianus* Jowitt, which give citronella oil [5].

Cymbopogon schoenanthus (L.) Spreng. (camel grass) is an aromatic herb, common in northern Africa [6]. This plant is used as a culinary herb in salads and traditional meat dishes. Because of its pleasant aroma, it is also consumed as a refreshing beverage prepared by steeping the aerial parts in hot water [7]. Its medicinal properties were considered helpful in the treatment of gout, prostate inflammation, kidney disorders, stomach pain, fever and rheumatism [6,8]. Furthermore, *C. schoenanthus* is traditionally used as a digestive, for treating intestinal spasms as well as for treating anorexia, i.e. for increasing appetite [7,9].

Previous studies examining biological activities showed that *C. schoenanthus* oil exhibits insecticidal [10,11], antitrypanosomal [8], antioxidant and acetylcholinesterase activities [9]. In addition, *C. citratus* Stapf and *C. martinii* Stapf., which are traditionally used as antispasmodics, were previously investigated to validate their use in folk medicine. The results have shown that extracts of these plants exhibit strong and significant spasmolytic activity *in vitro* [12,13].

Bearing in mind the abovementioned uses of *C. schoenanthus*, our aim was to investigate the spasmolytic activity of its inflorescence oil to contribute to the evaluation of the traditional use of this plant. Moreover, a comprehensive analysis of the essential oils from the stems and inflorescence of cultivated *C. schoenanthus* collected in two different periods was carried out in order to assess variability in the chemical composition of the essential oil.

MATERIALS AND METHODS

Chemicals

Alverine (alverine citrate salt), atropine (atropine sulfate salt monohydrate), potassium chloride (KCl), 1,1-diphenyl 2-picryl-hydrazyl (DPPH) and acetylcholine (ACh, acetylcholine iodide) were obtained from Sigma Chemical Corp. (USA).

Plant material and essential oil isolation

The aerial parts of *C. schoenanthus* were collected from the experimental field of the Medicinal & Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, in November 2013 and in February 2015 (Table 1). The voucher specimens are deposited at MAPRI, Khartoum, Sudan. The essential oils were isolated from the air-dried and powdered stems and inflorescences by hydrodistillation for 2 h, using a Clevenger-type apparatus.

GC and GC-MS analyses

GC and GC-MS analyses were carried out using an Agilent 6890N Gas Chromatograph equipped with a split/splitless injector (200°C), a HP-5MS capillary column (30 m x 0.25 mm; film thickness 0.25 μm) and flame ionization detector (FID), and coupled with an Agilent 5975 MS Detector (MSD) operating in the electron impact (EI) mode at 70 eV. The FID and transfer line temperatures were set at 300°C and 250°C, respectively. The carrier gas was He (1.0 mL/min), and the oven temperature was programmed from 60 to 280°C at a rate of 3°C/min. The injected volume was 1 μl and the split ratio 10:1.

Identification of the compounds was based on a comparison of their retention indices (KI), retention times (RT) and mass spectra with those from the NIST/NBS, Wiley libraries and the literature [14]. The linear KIs were determined in relation to a homologues series of *n*-alkanes (C8-C40) run under the same operating conditions [15]. Relative percentages of compounds were calculated based on the peak areas from the FID data.

Spasmolytic activity

The essential oil from inflorescence of *C. schoenanthus* collected in February 2015 (sample CI-2) was tested for spasmolytic activity.

Isolation of ratileum

Six- to eight-week-old Wistar rats (160-200 g) of both sexes were used in this study. All experimental procedures with animals where conducted in compliance with Directive 2010/63/EU of the European Parliament and of the Council of Europe from 22 September 2010 and approved by Ethical Committee of the University of Niš – Faculty of Medicine. Rats were killed by cervical dislocation and exsanguination. The ileum portions were isolated out, the mesenteries were removed and 2-cm-long preparations were mounted in 10-mL tissue baths containing Tyrode's solution (NaCl 136.9; KCl 2.68; $CaCl_2$ 1.8; $MgCl_2$ 1.05; $NaHCO_3$ 11.9; NaH_2PO_4 0.42 and glucose 5.55 mM), maintained at 37°C and aerated with a mixture of 5% carbon dioxide in oxygen.

Contraction recording

One end of the ileum segment was attached to the bottom of the bath and the other to an isotonic force transducer (TSZ-04-E, Experimetria Ltd, Budapest,

Hungary). The data were recorded and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd, Budapest, Hungary). The segments were suspended under 1-g tension and allowed to equilibrate for 30 min. Under these experimental conditions, the segments exhibited spontaneous rhythmic contractions. The investigated essential oil (CI-2) (dissolved in 0.5% carboxymethyl cellulose) and the control drugs were added directly to the organ bath in volumes not exceeding 1% of the bath volume [16].

Experimental procedure

The effects of essential oil on spontaneous contractions and contractions induced with ACh and KCl were evaluated as described previously [16]. In the first series of experiments, the spasmolytic effect of atropine on spontaneous contractions of isolated ileum was investigated. Then the effect of the essential oil on spontaneous contractions was investigated, and the results are presented as % of maximal effect obtained with atropine. Atropine and essential oil were added to the water bath cumulatively and concentration response curves were obtained.

In the second series of experiments, the effects of the essential oil on ACh-induced contractions was investigated using a single dose regimen with a contact time of 30 s and time cycle of 5 min. Increasing concentrations of ACh were added to the organ bath cumulatively and a concentration-effect curve was generated in the absence and then in the presence of four different concentrations of essential oil (30, 60, 90 and 120 μg/mL), while atropine was used as a control substance.

In the third experimental series, sustained, tonic contraction of isolated rat ileum was induced with 80 mM KCl. Essential oil was then added to the water bath and a concentration-response curve was obtained by the cumulative addition of different concentrations of essential oil at 5 min intervals after the addition of 80 mM KCl. For this experimental model, alverine citrate, an agent known to inhibit the sensitivity of contractile proteins to Ca^{2+}, was used as a control substance. Experiments were also conducted in parallel with controls using the tissue from the same animal and adding an equivalent volume of vehicle (0.5% carboxymethyl cellulose) instead of essential oil.

Table1. Samples and oil yield of *C. schoenanthus* essential oils

Sample	Collection date	Plant part	Oil Yield (% v/w)
CS-1	November 2013	stems	0.6
CI-1		inflorescence	2.0
CS-2	February 2015	stems	0.2
CI-2		inflorescence	1.9

Measurements and statistical analysis

Contractions were measured as the area under the curve produced by tissue contraction at 5 min intervals just before the addition of the next concentration of the tested sample, and the results were expressed as the percentage of control or maximum induced response for each tissue. Mean and standard error values were calculated for each group of results ($n>4$ for each set of experiments) and the significance of differences between the means was determined by the Mann-Whitney *U*-test using SPSS 11.5 software. A probability value of $p<0.05$ was considered signficant.

RESULTS AND DISCUSSION

Chemical analysis of the essential oil

The inflorescence of *C. schoenanthus* was rich in essential oil, yielding 1.9-2.0% (v/w) of oil, whereas the content of essential oil in the stems was 0.2-0.6% (v/w), calculated on a dry weight basis (Table 1). The obtained essential oils were fragrant and yellowish.

The results of GC and GC-MS analyses of the essential oils are summarized in Table 2. More than 45 compounds were identified in each oil, representing 98.8-99.4% of the total oils. The oils from stems and inflorescence, as well as the oils originating from different periods of plant material collection, were similar in qualitative composition with some quantitative differences. All the investigated essential oils were characterized by a high content of oxygenated monoterpenes (50.8-75.5%). The dominant compound was piperitone (47.7-71.5%). The other abundant constituents were intermedeol (6.1-17.3%), δ-2-carene (4.5-10.0%) and elemol (5.2-9.0%), except in the oil from stems collected in November 2013.

The results of our study are in agreement with previous investigations into *C. schoenanthus* oils of

Table 2. Chemical composition of essential oils from stem and inflorescence of *C. schoenanthus* cultivated in Khartoum, Sudan

		November 2013		February 2015	
Compound	**KI exp**	**Stems CS-1 (%)**	**Inflorescence CI-1 (%)**	**Stems CS-2 (%)**	**Inflorescence CI-2 (%)**
Verbenene	965	0.1	0.2	0.2	0.3
dehydro-1,8-Cineole	992	0.1	tr	tr	tr
δ-2-Carene	1004	6.3	10.0	4.5	9.7
α-Phellandrene	1006	tr	tr	tr	tr
α-Terpinene	1017	tr	tr	tr	tr
p-Cymene	1024	0.1	tr	tr	tr
Limonene	1028	1.5	1.9	1.1	1.8
(*Z*)-β-Ocimene	1036	tr	tr	tr	tr
(*E*)-β-Ocimene	1049	tr	tr	tr	tr
Fenchone	1087	0.1	tr	tr	tr
cis-*p*-Menth-2-en-1-ol	1123	0.8	0.6	tr	tr
cis-*p*-Mentha-2,8-dien-1-ol	1137	tr	tr	tr	tr
trans-*p*-Menth-2-en-ol	1140	0.6	0.4	tr	tr
p-Mentha-1,5-dien-8-ol	1171	0.5	0.4	0.2	0.4
p-Methyl acetophenone	1183	tr	tr	tr	0.5
p-Cymen-8-ol	1184	0.2	tr	tr	tr
α-Terpineol	1191	0.9	0.8	0.7	0.8
cis-Piperitol	1199	tr	tr	tr	tr
trans-Piperitol	1210	0.2	tr	tr	tr
cis-*p*-Mentha-1(7),8-dien-2-ol	1232	-	-	0.3	tr
Carvotanacetone	1248	0.2	0.9	tr	-
Piperitone	1253	71.5	47.7	52.9	58.7
(*E*)-Cinnamaldehyde	1270	0.4	-	-	-
α-Terpinen-7-al	1288	0.1	tr	tr	tr
Thymol	1294	tr	tr	tr	tr
β-Elemene	1394	0.2	0.5	0.3	0.2
α-Barbatene	1410	tr	tr	tr	tr
(*E*)-Caryophyllene	1422	1.1	2.1	0.6	1.1
Isobazzanene	1440	tr	tr	tr	tr
β-Barbatene	1445	0.1	tr	tr	tr
α-Humulene	1455	0.1	tr	tr	tr
β-Chamigrene	1480	0.1	0.2	tr	tr
α-Selinene	1501	0.2	0.3	0.6	0.2
Valencene	1502	0.3	0.4	0.7	tr
β-Dihydro-agarofuran	1506	-	-	tr	0.6
α-Chamigrene	1508	0.1	0.1	0.2	0.2
Cuparene	1509	0.5	0.5	0.7	0.5
β-Bazzanene	1523	0.1	tr	tr	tr
(7)-*epi*-α-Selinene	1524	0.1	tr	0.2	tr
Kessane	1532	-	-	tr	0.4
(*E*)-γ-Bisabolene	1533	0.4	0.9	0.4	0.4
Elemol	1551	2.7	9.0	5.2	5.3
Caryophyllene oxide	1585	0.8	0.3	1.3	0.7
γ-Eudesmol	1633	0.6	1.8	1.6	0.5
Hinesol	1642	tr	tr	1.6	-
β-Eudesmol	1652	1.1	1.8	3.6	1.3
α-Eudesmol	1655	1.2	2.1	2.7	1.1
Intermedeol	1668	6.1	14.4	17.3	13.1

Table 2. continued

Bulnesol	1672	tr	tr	0.5	0.7
α-Bisabolol	1688	tr	tr	tr	tr
Hexadecanoic acid	1962	-	-	0.4	-
Methyl linoleate	2098	-	0.2	-	-
Oleic acid	2144	-	0.7	-	-
Identified		99.4	98.2	97.8	98.5
Monoterpene hydrocarbons		8.1	12.1	5.8	11.8
Oxygenated monoterpenes		75.5	50.8	54.1	59.9
Sesquiterpene hydrocarbons		3.3	5.0	3.7	3.2
Oxygenated sesquiterpenes		12.5	29.4	33.8	23.1
Others		tr	0.9	0.4	0.5

[a] KI exp – Relative retention indices calculated against *n*-alkane on HP-5MS column.
[b] % – Relative area percentage.
[c] tr – trace (<0.05%).

different origin. The leaf essential oils of *C. schoenanthus* from Benin [8], Burkina Faso [17] and Togo [18] were also dominated by the presence of piperitone (42.0-69.01%), followed by δ-2-carene (8.2-16.9%) and elemol (4.9-6.2%).

The essential oils of *C. schoenanthus* from Sudan analyzed in this study differentiate from previously analyzed oils in the presence of sesquiterpene intermedeol (6.1-17.3%), which was not detected in the previously analyzed oils.

A high occurrence of piperitone also characterizes the essential oils of some other *Cymbopogon* species such as *C. parkeri* Stapf (80.8%) and *C. olivieri* (Boiss) Bor (72.8%) from Iran [3] and *C. jwarancusa* (Jones) Schultz (79.0%) from India [19]. Piperitone is an important raw material for conversion into menthol and thymol [19], which are well known for their medicinal properties and use as flavoring agents.

Spasmolytic activity

Essential oil from the inflorescence of *C. schoenanthus* exhibited strong and concentration-dependent spasmolytic activity. The oil (10-130 μg/mL) concentration-dependently inhibited spontaneous contractions of isolated rat ileum. The effect was strong and in the concentration of 130 μg/mL comparable (105.23±29.56%) to the maximal relaxant effect of atropine obtained in a concentration of 6.4 μM in a previous series of experiments. The results are presented in Fig. 1.

Essential oil exhibited a concentration-dependent effect in the second series of experiments where the effect on contractions induced by ACh was investigated. The spasmolytic effect of the oil was assessed for four concentrations (30, 60, 90 and 120 μg/mL). In a concentration of 30 μg/mL, the oil exhibited a weak effect (data not shown). At 60 μg/mL, the oil exhibited a strong and significant spasmolytic effect on contractions induced with lower concentrations of ACh (0.01-0.44 μg/mL). The effect on contractions induced with higher concentrations was weaker, inhibiting the maximal effect of ACh to 77.71±13.85%.

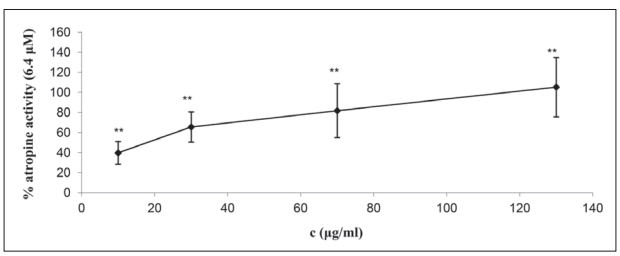

Fig. 1. Effect of *C. schoenanthus* inflorescence essential oil on spontaneous contractions of the isolated rat ileum compared with atropine. Activity is presented as the percentage of maximal spasmolytic effect achieved with atropine at a concentration of 6.4 μM (4.44 μg/mL). The concentrations presented on the x-axis are the final cumulative concentrations; and each point represents means±SEM of five or more experiments. Stars show statistically significant differences in comparison with the control, the vehicle (Na-CMC)-treated group (**p<0.01).

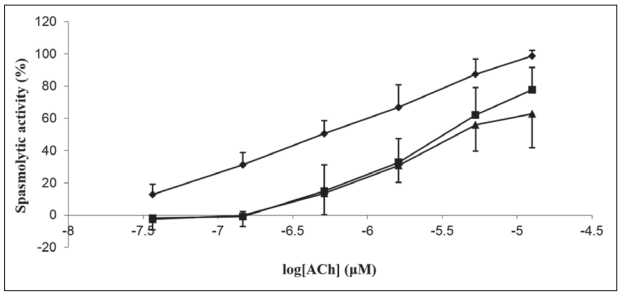

Fig. 2. Concentration-response curves of ACh in the absence (rhombus) and presence of 60 μg/mL (square) and 90 μg/mL (triangle) of *C. schoenanthus* inflorescence essential oil in isolated rat ileum. The values presented on the y-axis represent responses expressed as percentages of the maximum response to ACh. Each point represents means±SEM of seven or more experiments. Stars show statistically significant differences in comparison with the control, the vehicle (Na-CMC)-treated group (*p<0.05, **p<0.01).

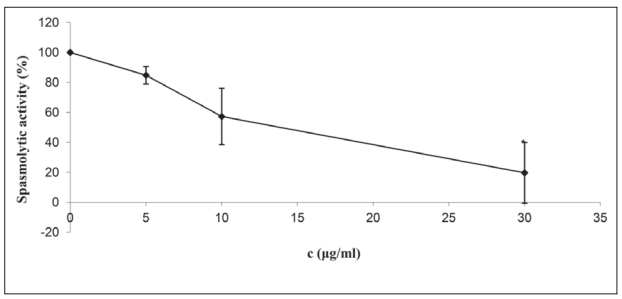

Fig. 3. Concentration-related inhibitory effects of *C. schoenanthus* inflorescence essential oil on contractions induced with KCl (80 mM) in isolated rat ileum. The spasmolytic effect of the essential oil is represented by the decrease (values on the y-axis) of KCl contractile effect (100%). Each point represents means±SEM of four or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*p<0.05).

In the concentration of 90 μg/mL, the oil exhibited similar activity at lower concentrations of ACh (0.01-0.44 μg/mL), but the effect on contractions induced with higher concentrations was stronger and significant, with an inhibition of the maximal effect of ACh to 62.76±21.00%. At 120 μg/mL, the oil exhibited the strongest relaxant activity and completely abolished the contractile effect of ACh (data not shown). The results are shown in Fig. 2. Atropine, used as a reference drug in this model, exhibited stronger activity and in a concentration of 0.14 μM completely inhibited the spasmogenic effect of ACh.

The oil demonstrated the strongest activity against tonic contractions induced with KCl (80 mM). At 30 μg/mL, the oil inhibited the contractile effect of KCl to 19.67±20.26%. The results are presented in Fig. 3.

Piperitone, the main metabolite of the investigated essential oil (58.7%), was previously shown to exhibit spasmolytic activity. Tested in a range of 1-100 μg/mL, piperitone concentration-dependently inhibited contractions of isolated rat uterus contracted with KCl (60 mM) with a calculated EC_{50}=10.73±1.27 μg/mL [20]. It was previously shown that limonene, a minor component of this oil, exhibits spasmolytic activity as well [21]. Relaxant activity was previously demonstrated for *β*-eudesmol, another minor metabolite of *C. schoenanthus* essential oil (1.3%). This sesquiterpene inhibited histamine- and barium chloride-induced contractions of guinea-pig ileum [22]. Additionally, the essential oil of *Perovskia abrotanoides* Kar. exhibited a relaxant effect on spontaneous and KCl (80 mM)-induced contractions of isolated rabbit jejunum. The main metabolite of this oil was δ-3-carene [23]. Therefore, *δ*-2-carene present in *C. schoenanthus* essential oil (9.7%) might contribute to the demonstrated activity of the oil. It could be postulated that the strong spasmolytic activity of *C. schoenanthus* inflorescence essential oil demonstrated in our experiments could be partly due to the presence of piperitone.

CONCLUSION

A high essential oil content and stable composition makes *C. schoenanthus* from Sudan a valuable source of the commercially important monoterpene, piperitone. Significant spasmolytic activity of inflorescence essential oil was observed, emphasizing its beneficial properties, especially for gastrointestinal complaints. Our data represent a good basis for further investigations of this essential oil for its medicinal purposes, as well as for application in the food industry.

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Authors' contribution: E.O.: sampling of the plant material and essential oil isolation N.K., M.R., S.B., M.D. and I.P.: conceived and designed the experiments. I.P., E.O., M.D. and S.B.: performed the experiments and analyzed the data. N.K. was the main supervisor of the research project. All authors contributed to manuscript preparation. All authors read and approved the final manuscript.

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

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