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FACULTY OF PHARMACY

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Comparative analysis of chemical composition, antimicrobial, antioxidant and spasmolytic activity of essential oils of *Cymbopogon nervatus* (Hochst.) Chiov. and *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) from Sudan

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Uporedna analiza hemijskog sastava, antimikrobne, antioksidantne i spazmolitičke aktivnosti etarskog ulja *Cymbopogon nervatus* (Hochst.) Chiov. i *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) iz Sudana

Doktorska disertacija

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DEDICATION

I dedicate this work

To my lovely parents,

To my wife

To my lovely kids

To my brothers and sisters

To all whom I love

With my deepest love and

Respect

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Firstly I deeply thank my God upon completion of this work successfully.

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ABSTRACT

Comparative analysis of chemical composition, antimicrobial, antioxidant and spasmolytic activity of essential oils of *Cymbopogon nervatus* (Hochst.) Chiov. and *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) from Sudan

This PhD dissertation deals with investigation of two species from genus *Cymbopogon*, *Cymbopogon nervatus* (Hochst.) Chiov. and *Cymbopogon schoenanthus* (L.) Spreng. collected in Sudan. They were investigated in terms of content and detailed chemical analysis of essential oils obtained from different plant parts and collected in different time periods. In addition, essential oils from both species were investigated for antimicrobial, antioxidant and spasmolytic activities.

For analyses of essential oils, obtained by steam distillation from dried plant material, GC and GC-MS were applied. The most dominant constituents in the oils of *C. nervatus* were *p*-menthadienols: *trans*-*p*-mentha-1(7),8-dien-2-ol (19.9-32.6%), *cis*-*p*-mentha-1(7),8-dien-2-ol (18.9-23.3%), *trans*-*p*-mentha-2,8-dien-1-ol (10.6-21.0%) and *cis*-*p*-mentha-2,8-dien-1-ol (8.1-10.4%). The major constituents in the oils of *C. schoenanthus* were piperitone (47.7-71.5%), intermedeol (6.1-17.3%), δ-2-carene (4.5-10.0%) and elemol (2.7-9.0%).

The antimicrobial activities of the essential oils of both plants were tested using broth microdilution method against seven standard strains of bacteria and two strains of fungi. MIC values obtained for the essential oils of *C. nervatus* were in the range of 783-1060 μg/ml and those for *C. schoenanthus* were 771-1300 μg/ml. It was concluded that investigated essential oils exhibited weak antimicrobial activity.

The total antioxidant capacity of the investigated essential oils estimated by Ferric Reducing Antioxidant Power (FRAP) test was in the range of 2.03-2.88 nmol Fe/mg EO for *C. nervatus* essential oils and for *C. schoenanthus* in the range of 2.49-2.96 nmol Fe/mg and weaker than the capacity of rutin (5.17 μmol Fe/mg) and ascorbic acid (10.40 μmol Fe/mg) used as the referent antioxidants.

In the DPPH radical scavenging assay, essential oil of *C. nervatus* showed moderate activity with SC_{50} value of 23.8 μl/ml. DPPH scavenging activity of essential

oils of *C. schoenanthus* represented by percentage (%) of inhibition was 62.8-65.1% when using 40 µl of essential oil per mL of test solution. In comparison with reference substances (rutin and ascorbic acid) the essential oils exhibited a weaker activity.

The essential oils of inflorescences from both plants were tested for spasmolytic activity using three different experimental models: models of spontaneous contractions and model in which contractions were induced with ACh or KCl. The essential oils exhibited strong, significant and concentration-dependent spasmolytic activity. Applied in concentration of 200 µg/ml essential oil of *C. nervatus* showed 88.44 ±16.78% of maximal spasmolytic effect of atropine (6.4 µM) against spontaneous contraction. Essential oil (90 µg/ml) inhibited ACh-induced contractions as well, and reduced the effect of the highest applied concentration of ACh to 37.29±16.16%. Additionally, the oil exhibited strong activity against contractions induced with KCl (80 mM) and in concentration of 200 µg/ml completely abolished contractile effect of KCl. Essential oil of *C. schoenanthus* applied in concentration of 130 µg/ml, exhibited 105.23±29.56% of maximal spasmolytic effect of atropine (6.4 µM) against spontaneous contraction. Also, essential oil (90 µg/ml) inhibited ACh-induced contractions as well and reduced the effect of the highest applied concentration of ACh to 62.76±21.00%, while in concentration of 120 µg/ml oil completely abolished contractile effect of ACh. Furthermore, the essential oil of *C. schoenanthus* exhibited strong activity against contractions induced with KCl (80 mM) and in concentration of 30 µg/ml inhibited contractile effect of KCl to 19.67±20.26%.

Keywords: *Cymbopogon*, essential oil, chemical composition, antimicrobial activity, antioxidant activity, spasmolytic activity
Academic expertise: Pharmacy
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REZIME

Uporedna analiza hemijskog sastava, antimikrobne, antioksidantne i spazmolitičke aktivnosti etarskog ulja *Cymbopogon nervatus* (Hochst.) Chiov. i *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) iz Sudana

Glavni cilj istraživanja u okviru ove doktorske disertacije je ispitivanje dve biljne vrste koje pripadaju rodu *Cymbopogon* (Poaceae), a koje su zastupljene u flori Sudana.

Jedna je *Cymbopogon nervatus* (Hochst.) Chiov.. Ova vrsta je najzastupljeniji predstavnik ovog roda koja se javlja u spontanoj flori Sudana. Veoma je popularna u narodnoj medicine i često se koristi. Drugi vrsta, koja je bila predmet ispitivanja u okviru ove disertacije, jeste *Cymbopogon schoenanthus* (L.) Spreng. Pošto je veoma cenjena kao lekovita vrsta u Sudanu, ali i u širem region, često se gaji, pa su uzorci za potrebe ovog rada i uzeti iz organizovane proizvodnje.

Istraživanja su podeljena u dve celine. U okviru prvog dela urađeno je određivanje sadržaja i detaljna hemijska analiza etarskih ulja izolovanih destilacijom vodenom parom iz različitih delova vrste *C. nervatus*, sakupljenih na lokalitetima u istočnom i zapadnom delu Sudana u različitim vremenskim periodima, kao i iz uzoraka gajenog *C. schoenanthus* (The Medicinal & Aromatic Plants Research Institute - MAPRI, Khartoum state, Sudan), sakupljenih u različitim vremenskim periodima.

Drugu celinu je predstavlja farmakološki skrining etarskih ulja dobijenih iz ispitivanih *Cymbopogon* vrsta. U okviru ovog dela, urađeno je ispitivanje antimikrobne, antioksidativne i spazmolitičke aktivnosti.

Dobijeni rezultati hemijskog ispitivanja, kao i rezultati farmakoloških testova upoređivani su međusobno, kao i sa literaturnim podacima koji su dostupni za obe ispitivane biljne vrste, odnosno za druge vrste roda *Cymbopogon*.

Utvrđeno je da je prinos etarskog ulja u uzorcima vrste *C. nervatus*, koji su prikupljeni u regionu zapadnog i istočnog Sudana u maju 2014. godine (letnji period) i februaru 2015. godine (zimski period), bio veći u cvasti (0.6%-2.1% v/v) nego u stabljici (0.1%-0.2% v/v). Takođe, sadržaj ulja bio je veći u biljnom materijalu prikupljenom tokom zime (februar 2015. godine).

Svi uzorci etarskog ulja *C. nervatus* bili su slični u pogledu kvalitativnog sastava uz postojanje razlika u odnosu sastojaka. Glavni sastojci ulja pripadaju grupi

oksidovanih monoterpena (81.1-94.6%), dok su aciklični i biciklični monoterpeni potpuno odsutni.

Dominantni sastojci etarskog ulja *C. nervatus* su *p*-mentadienoli (64.0-80.3%): *trans*-*p*-menta-1(7),8-dien-2-ol (19.9-32.6%), *cis*-*p*-menta-1(7),8-dien-2-ol (18.9-23.3%), *trans*-*p*-menta-2,8-dien-1-ol (10.6-21.0%) i *cis*-*p*-mentha-2,8-dien-1-ol (8.1-10.4%).

Rezultati hemijske analize uzoraka sa različitih lokaliteta, sakupljenih u različitim periodima, kao i poređenje sa podacima iz literature ukazuju na to da je, suštinski, sastav etarskog ulje biljne vrste *C. nervatus* prilično stabilan, odnosno da deo biljke, poreklo i vreme sakupljanja ne utiču značajno na sastav ovog etarskog ulja.

Cvast vrste *C. schoenanthus* bogata je etarskim uljem (1.9-2.0%, v/v), dok je sadržaj etarskog ulja u uzorcima stabljika manji (0.2-0.6%; v/v). Etarska ulja dobijena iz različitih delova biljke (stabljike i cvasti), odnosno iz uzoraka sakupljanih u različitom periodu, bila su slična po kvalitativnom sastavu uz postojanje određenih razlika u odnosu između sastojaka.

I uzorke etarskog ulja vrste *C. schoenanthus* karakteriše visok sadržaj oksidovanih monoterpena (50.8-75.5%). Dominantan sastojak ovog ulja je piperiton (47.7-71.5%). Drugi sastojak po zastupljenosti je intermedeol (6.1-17.3%), a zatim slede *δ*-2-karen (4.5-10.0%) i elemol (5.2-9.0%) (osim u etarskom ulje dobijenom iz stabljike prikupljene u novembru 2013. godine, gde je elemol prisutan u manjoj količini od 2.7%).

Ispitivanje antimikrobne aktivnosti etarskih ulja *C. nervatus* i *C. schoenathus* rađeno je mikrodilucionom metodom na šest standardnih sojeva bakterija (*S. aureus, S. epidermidis, E. faecalis, E. coli, K.pneumoniae* i *P. aeruginosa*) i na dva soja gljivice *C. albicans*. Osetljivost testiranih mikroorganizama na ispitivana etarska ulja je bila slična. U ispitivanim koncentracijama, etarska ulja su pokazala aktivnost protiv sojeva *S. aureus, S. epidermidis* i *C. albicans*, a nisu inhibirala rast Gram (-) bakterija. Generalni zaključak je da je antimikrobna aktivnost uzoraka etarskih ulja *C. nervatus* i *C schoenathus*, testiranih u ovoj studiji, bila slaba.

Antioksidativna aktivnost etarkih ulja izolovanih iz cvasti vrsta *C. nervatus* i *C. schoenanthus,* procenjivana je primenom FRAP testa za određivanje ukupnog

antioksidativnog kapaciteta i DPPH testa za određivanje sposobnosti neutralizacije slobodnih radikala.

The total antioxidant capacity of the investigated essential oils estimated by Ferric Reducing Antioxidant Power (FRAP) test was in the range of 2.03-2.88 nmol Fe/mg EO for *C. nervatus* essential oils and for *C. schoenanthus* in the range of 2.49-2.96 nmol Fe/mg and weaker than the capacity of rutin (5.17 μmol Fe/mg) and ascorbic acid (10.40 μmol Fe/mg) used as the referent antioxidants.

In the DPPH radical scavenging assay, essential oil of *C. nervatus* showed moderate activity with SC_{50} value of 23.8 μl/ml. DPPH scavenging activity of essential oils of *C. schoenanthus* represented by percentage (%) of inhibition was 62.8-65.1% when using 40 μl of essential oil per mL of test solution. In comparison with reference substances (rutin and ascorbic acid) the essential oils exhibited a weaker activity.

Ukupni antioksidativni kapacitet etarskih ulja obe ispitivane vrste (FRAP vrednosti 2.03-2.96 nmol Fe/mg EO) bio je sličan i slabiji kapaciteta rutina i askorbinska kiseline, supstanci koje su korišćene kao referentni antioksidansi. U DPPH testu etarsko ulja cvasti *C. nervatus* pokazalo je umerenu aktivnost (SC_{50} 23.8 μl/ml), dok su etarska ulje *C. schoenanthus* ispoljila slabu aktivnost (primenjena u koncentraciji 40 μl etarskog ulja/ml ispitivanog rastvora ostvarila su 62.8-65.1% inhibicije DPPH radikala).

Etarska ulja cvasti *C. nervatus* i *C. schoenanthus* su testirana na spazmolitičku aktivnost korišćenjem tri različita eksperimentalna modela: model spontanih kontrakcija i model kontrakcija indukovanih dodatkom Ach i KCl. Oba ispitivana etarska ulja ispoljila su jaku, signifikantnu i dozno zavisnu spazmolitičku aktivnost u svim eksperimentalnim modelima.

Etarsko ulje cvasti *C. nervatus* primenjeno u koncentraciji od 200 μg/ml, ispoljilo je 88.44±16.78% maksimalnog spazmolitičkog efekta atropina (6.4 uM) kod spontanih kontrakcija. Etarsko ulje (90 ug/mL) inhibiralo je Ach-indukovane kontrakcije i umanjilo efekat najviše primenjene koncentracije Ach do 37.29±16.16%. Takođe, ulje je pokazalo snažno dejstvo na kontrakcije koje su indukovane KCl (80 mM) i u koncentraciji od 200 μg/ml u potpunosti otklonilo kontrakcije izazvane KCl.

Etarsko ulja cvasti *C. schoenanthus* primenjeno u koncentraciji od 130 μg/ml ispoljilo je 105.23±29.56% maksimalnog spazmolitičkog efekta atropina (6.4 uM)

protiv spontanih kontrakcija. Pored toga, etarsko ulje (90 μg/ml) je inhibiralo kontrakcije indukovane Ach i umanjilo efekat najvišeg primenjene koncentracije Ach do 62.76±21.00%, dok je u koncentraciji od 120 μg/ml ulje u potpunosti otklanilo kontrakcije izazvane Ach. Takođe, ulje je ispoljilo snažno dejstvo na kontrakcije koje su indukovane KCl (80 mM) i u koncentraciji od 30 μg/ml umanjilo kontrakcije izazvane KCl do 19.67 ± 20.26%.

Rezultati dobijeni i prezentovani u ovoj doktorskoj disertaciji pokazali su da cvasti i stabljike obe ispitivane biljne vrste, *C. nervatus* i C. *schoenanthus* koje su uzorkovane u Sudanu, sadrže značajnu količinu etarskog ulja i da ova ulja imaju stabilan i ujednačen sastav. Etarska ulja cvasti su ispoljila i značajnu spazmolitičku aktivnost, što se može smatrati korisnim svojstvom i osnovom za njihovu primenu kod gastrointestinalnih problema.

Podaci dobijeni u okviru ove disertacije mogu predstavljati dobru osnovu za dalja istraživanja ovih etarskih ulja za medicinske svrhe, kao i za primenu u prehrambenoj industriji. Pored toga, visok sadržaj etarskog ulja i njegov prilično stabilan sastav čini *C. schoenanthus* iz Sudana vrednim izvorom komercijalno važnog monoterpena piperitona.

Ključne reči: *Cymbopogon*, etarsko ulje, hemijski sastav, antimikrobna aktivnost, antioksidantna aktivnost, spazmolitička aktivnost
Naučna oblast: Farmacija
Uža naučna oblast: Farmakognozija
UDK broj:

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I Introduction

1. Genus *Cymbopogon* Spreng.

The genus of *Cymbopogon* (family Poaceae) comprised about 180 species, subspecies, varieties, and subvarieties. The plants of this genus grow in warm temperate, and tropical regions of the Old World and Oceania.

The name of this genus was introduced by Sprengel in 1815 and at that time the genus consisted of a few species, which were then moved to the genus *Andropogon*. Both *Cymbopogon* and *Andropogon* belong to the tribe *Andropogoneae,* a monophyletic tribe that includes 85 genera (Bertea and Maffei, 2010). The limited difference in the plant traits between *Andropogon* and *Cymbopogon,* has debated the possibility that species belonging to *Cymbopogon* might be a subgenus of *Andropogon*. Most of *Andropogoneae* have pairs of spikelets in the inflorescence, one sessile and one on a pedicel, although in somespecies one or the other of these spikelets appear to be suppressed. The inflorescence form is also highly variable morphologically, the main difference in the genus *Cymbopogon* is the presence of some pair of spikelets, for each spike, with unisexual male flowers, whereas in the *Andropogon* spikelets are usually sessile and often sterile (Mathews et al., 2002).

1.1. Description

Plants of *Cymbopogon* genus are perennial, tall (up to and above 1 m) with narrow and long leaves that are mostly characterized by the presence of silica thorns aligned on the leaf edges (Figure 1).

Figure 1. Lemongrass with narrow and long leaves (www.theida.com/lemongrass-cymbopogon-citratus/lemongrass-3)

Culm nodes are glabrous and culm internodes are solid, smooth and distally glabrous (Figure2).

Figure 2. Culm nodes in *Cymbopogon* species (www.tuninst.net/MMPD/TIL/famP/pix-poaceae/opp-p149-300.jpg)

Inflorescence: Paniculate (decompound, leafy); Non-digitate. Rachides hollowed, flattened, winged, or neither flattened, nor hollowed not winged. A complex of 'partial inflorescences' and intervening foliar organs. *Spikelet-bearing axes* 'racemes' paired with very slender rachides; densely long-hairy to somewhat hairy (Metcalfe, 1960).

Spikelets: paired (or with a terminal triplet) not secund; sessile and pedicellate consistently in 'long-and-short' combinations. Pedicels of the 'pedicellate' have spikelets free of the rachis or discernible, but fused with the rachis and free of the rachis, sometimes the pedicel of the homogamous pair being swollen and more or less fused with the internode. The 'shorter' spikelets hermaphrodite. The 'longer' spikelets male-only (usually), or sterile. Pedicellate spikelets its floret usually male but occasionally sterile, or suppressed (Figure 3). The lemmas awnless. Female-fertile spikelets 3-7 mm long; compressed laterally or not noticeably compressed or compressed dorsiventrally; falling with the glumes. Rachilla terminated by a female-fertile floret. Hairy callus present. *Callus* short; blunt (Metcalfe, 1960).

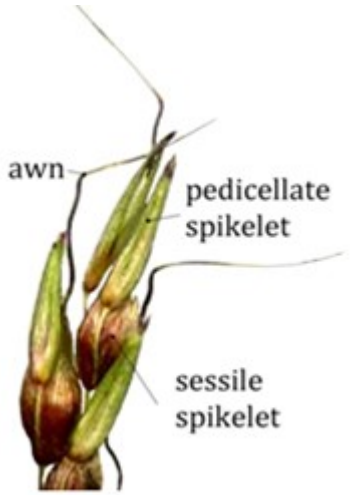


Figure 3. Spikelets paired (pedicellate/sessile combinations)
(http://www.aecos.com/GRAPHICS/Sorghum_spikelets.jpg)

Glumes: Two; more or less equal; long relative to the adjacent lemmas; awnless; very dissimilar, the lower (first glume) bicarinate, the upper (second glume) naviculate. Lower glume two-keeled (the keels sometimes winged apically); flattened on the back to sulcate on the back; not pitted; relatively smooth; 1-5 nerved. Upper glume 1-5 nerved (Figure 4).

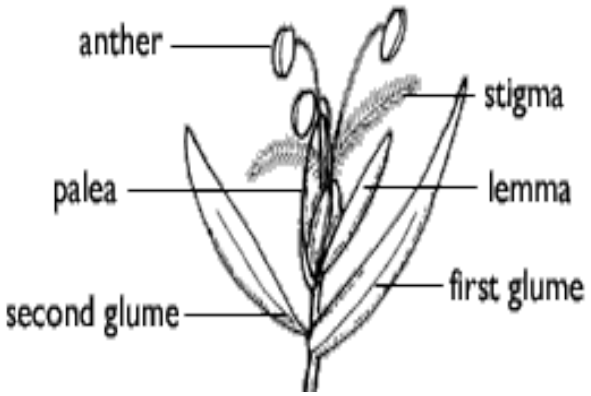


Figure 4. Lower and upper glumes on spikelet
(http://www.desertmuseum.org/books/images/nhsd_grass_spikelet.gif)

Spikelets: With incomplete florets. The incomplete florets proximal to the female-fertile florets.
Lemmas: Hairless; non-carinate; nerved. *Palea* absent. *Lodicules* present; fleshy; glabrous.
Stamens, *Anthers*: penicillate.
Ovary glabrous. Styles free to their bases.
Stigmas: red pigmented (Metcalfe, 1960).

1.2. Distribution

Species of this genus are wildely distributed in the tropical and subtropical Africa, Asia and Australia. Mesophytic to xerophytic. Species of open habitats; West African Rainforest; Namib-Karoo; Indian, Indo-Chinese, Malesian and Papuan; North and East Australian, South-West Australian and Central Australian; Sahelo-Sudanian, Somalo-Ethiopian, South Tropical African and Kalaharian (Soenarko, 1977).

Among the species belonging to the genus *Cymbopogon* the most important, in terms of essential oil production are *C. citratus* (common name: West Indian lemongrass) and *C. flexuosus* (common name: East Indian lemongrass), *C. martini* also known as palmarosa; *C. nardus* from Sri Lanka and *C. winterianus* from Java (produce the famous citronella oil). Also known for essential oil production are *C. schoenanthus* or camel grass primarily native to East Africa; *C. caesius* or inchi/kachi grass of

deciduous savanna bush land and wooded grassland; abundant throughout the region and, in general, over all of tropical Africa (Bertea and Maffei, 2010).

Table 1. The most important *Cymbopogon* species and their distribution

***Cymbopogon* species**	**Common name**	**Native**
C. martini (Roxb.) Wats. *C. citrates* (D.C.) Stapf	Palmarosa West Indian lemongrass	India, Kumaon Himalayan West Indian
C. flexuous (Steud.) Wats.	East Indian lemongrass	India, Sri Lanka, Burma, and Thailand
C. nardus (L.) Rendle	Citronella grass	Sri Lanka and Java
C. winterianus Jowitt	Citronella grass	Java, Haiti, Honduras, Taiwan, Guatemala, and China
C. schoenanthus (L.) Spreng subsp. *proximus* Hochst.	Camel grass	East Africa
C. caesius (Nees) Stapf	Inchi/kachi grass	All of tropical Africa, northeast India
C. densiflorus (Steud.) Stapf	Capim-caboclo por	Central tropical Africa and Brazil
C. nervatus (Hochest.) Chiov.	Naal grass	Western and eastern Sudan
C. pendulus (Nees ex Steud.) Wats.	North Indian lemongrass	North India
C. jwarancusa (Jones) Schult.	Khavi grass	Indian Thar desert, Kumaon Himalayan
C. confertiflorus (Steud.) Stapf	Ceylon citronella	India

2. Chemical constituents of the plants of genus *Cymbopogon*

The genus *Cymbopogon* is well-known for its high content of essential oils. Besides, some other pharmacologically active secondary metabolites including flavonoids, phenolic acids, non-volatile terpenoids and tannins are present in the plants of this genus (Avoseh et al., 2015).

2.1. Essential oils

Aromatic grasses are one of the chief sources of essential oils. The genus *Cymbopogon* comprises a large number of species, out of which lemongrass, citronella, palmarosa and few others produce oil of commercial importance (Akhila, 2010). Several *Cymbopogon* species, such as *C. flexuosus*, *C. citratus*, *C. pendulus, C. martinii*, *C. winterianus* are cultivated on a large scale in different parts of the world, especially in tropics and subtropics (Pandey, 2010). The ability of *Cymbopogon* species to grow in moderate and harsh climatic conditions and high essential oil content contribute to their commercial value (Avoseh et al., 2015). The *Cymbopogon* species have a great prospects for producing quality essential oils and they have direct relevance to the perfumery industry with economic benefit to humankind. However, the actual potential of Cymbopogons has not been exploited to the fullest. Despite tremendous work that has been done regarding *Cymbopogon* chemistry, a lot more needs to be done to make use of the major and minor constituents present in its essential oils, particularly the mono- and sesquiterpenes (Akhila, 2010).

The trading of essential oils depends on the knowledge regarding its quality. The components present in essential oils and the odor value provided to it are of immense value. These have been important criteria since ancient and medieval times. During the last two decades, the methods for analysis of oils and determination of their chemical composition have been improved many fold. Consequently, the data regarding major and minor constituents found in essential oils has also multiplied and helped in evaluating the quality of the essential oils besides providing information on the constituents that could be isolated and used in pure form.

2.1.1. Lemongrass essential oil

Lemongrass oil is distilled from two morphologically different species of lemongrass, *C. flexuosus* (common name: East Indian lemongrass) and *C. citratus* (common name: West Indian lemongrass). The chemical composition of these oils is very similar with citral (mixture of geranial and neral) as the most dominant compound, though the percentage of citral and other major monoterpenes vary to some extent. A high yield of citral has been reported in *C. pendulus* (common name: North Indian lemongrass), which is another wild growing species, under limited cultivation (Akhila, 2010).

Cymbopogon flexuosus **(Steud.) Wats.**

East Indian lemongrass, Cochin or Malabar grass (*C. flexuosus*) is indigenous to South India, found in the Malabar and Cochin regions, Malay Peninsula, Vietnam, Sri lanka, Burma. It's essential oil is commonly called Malabar lemongrass oil (Akhila, 2010).

The essential oil of *C. flexuosus* is consisted mainly of citral (75-85%) which is mixture of geranial (citral a) and neral (citral b). Other compounds present in prominent amounts reported in numerous previous studies are: citronellal (0.37%-8.04%), citronellol (0.44%-4.58%), citronellyl acetate (1.2%–3.6%), geraniol (1.73%-40.0%), geranyl acetate (1.95%-5.1%), limonene (2.4%-3.7%), methyl eugenol (20.0%) and myrcene (0.1%-14.2%) (Ganjewala, 2009; Akhila, 2010).

Cymbopogon citratus **(D.C.) Stapf.**

The West Indian lemongrass (*C. citratus*) essential oil is also mainly composed of citral reaching up to 90% of the oil (Tajidin et al., 2011 2012; Pinto et al., 2015). The West Indian lemongrass oil differs from the East Indian (*C. flexuosus*) type by the occurrence of substantial quantities of myrcene, which is present in higher amounts in latter one.

***Cymbopogon pendulus* (Nees ex Steud.) Wats.**

The North Indian lemongrass essential oil (*C. pendulus*) occurs in wild areas of northern India such as Saharanpur (in the state of Uttar Pradesh). The major compound of *C. pendulus* essential oil is citral (geranial 30-50% and neral 20-35%) and other constituents present are: geranyl acetate (3%–5%), β-caryophyllene (2.1%), elemol (2.2%), geraniol (2%–6%), and linalool (3.0%). This is also a major source of lemongrass oil (Akhila, 2010). In one of the studies, elemicin content (53.7%) was found to be very high in the essential oil obtained from this plant (Shahi et al., 1997). Some examples of chemical structures of lemongrass oil components are shown in Figure 5.

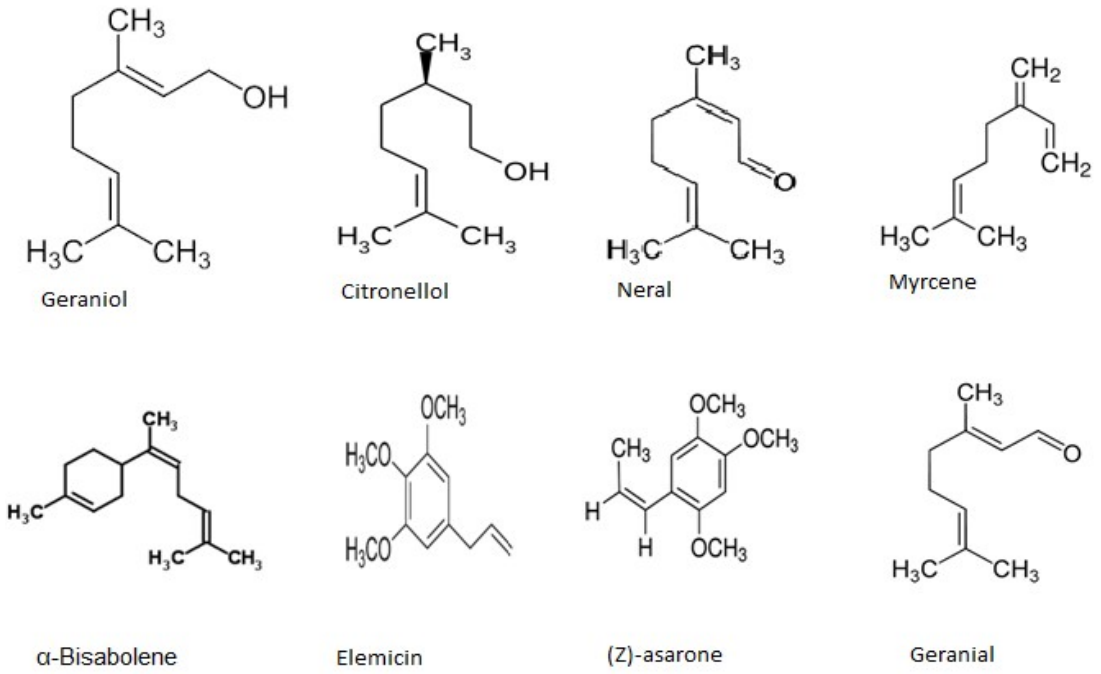


Figure 5. Chemical structures of some major components obtained from lemongrass essential oils

2.1.2. Citronella essential oil

Citronella essential oil is produced from *C. winterianus* and *C. nardus* which are cultivated on a large scale and these are closely related to each other in various aspects.

Both species are distinguished morphologically by the shape and length of their leaves. The chemical composition of the essential oil obtained from them also differs considerably (Wijesekara et al., 1973).

***Cymbopogon winterianus* Jowitt.**

The Java citronella *(C. winterianus*) is grown mainly in Java, Haiti, Honduras, Taiwan, Guatemala, and China, and is highly priced in comparison to the Ceylon type because its oil contains higher percentages of monoterpene alcohols and their esters. Phytochemical analysis of *C. winterianus* essential oils revealed presence of citronellal, citronellol, and geraniol as the major constituents (Quintans-Junior et al., 2008; Simic et al., 2008). Java citronella oil is one of the most important essential oils because of the high content of citronellal and is mainly used for the isolation of citronellal, which is converted into citronellol. Citronellol is further converted into citronellol esters, hydroxy citronellal, and synthetic menthol (Dev Kumar et al., 1977).

***Cymbopogon nardus* (L.) Rendle.**

Citronella essential oil derived from *C. nardus* and is also called "Lanabatu oil". The grass is mostly cultivated in Sri Lanka, and hence the oil obtained from it is also known as Ceylon citronella oil. This essential oil is similar in composition with *C. winterianus* essential oil with citronellol, citronellal and geraniol as the major components (Nakahara et al., 2003; De Toledo et al., 2016). However, the presence of phenolic derivatives (methyl eugenol and methyl isoeugenol) is the most significant difference between the Ceylon-type and Java-type oils. The wild varieties of citronella growing in Sri Lanka contain phenylpropanoids in abundance, whereas phenyl propanoids are present in traces in the Java-type oil (Akhila, 2010). Some examples of chemical structure of some components are given on Figure 6.

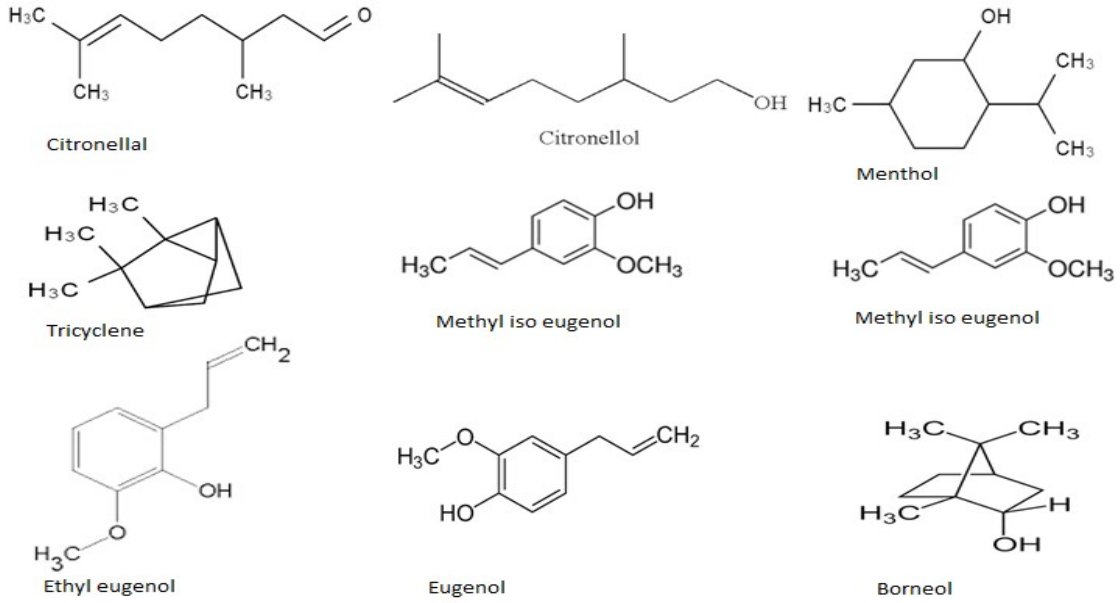


Figure 6. Chemical structures of some major components obtained from citronella essential oil or "Lanabatu oil."

2.1.3. Palmarosa essential oil and Gingergrass essential oil

Palmarosa essential oil is obtained from *C. martinii* var. *motia*, which yields an oil of better quality having more geraniol and which is commercially much more important than the oil obtained from *C. martinii* var. *sofia* which is known as gingergrass oil. (Akhila, 2010).

Cymbopogon martinii (Roxb.) Wats.

C. martinii is widely distributed in India. Palmarosa essential oil, distilled from variety *motia*, has geraniol (67-85%) as the major component. In addition, geranyl acetate and geranial are present in the essential oil (Raina et al., 2003). Chemical structures of some components are given in Figure 7.

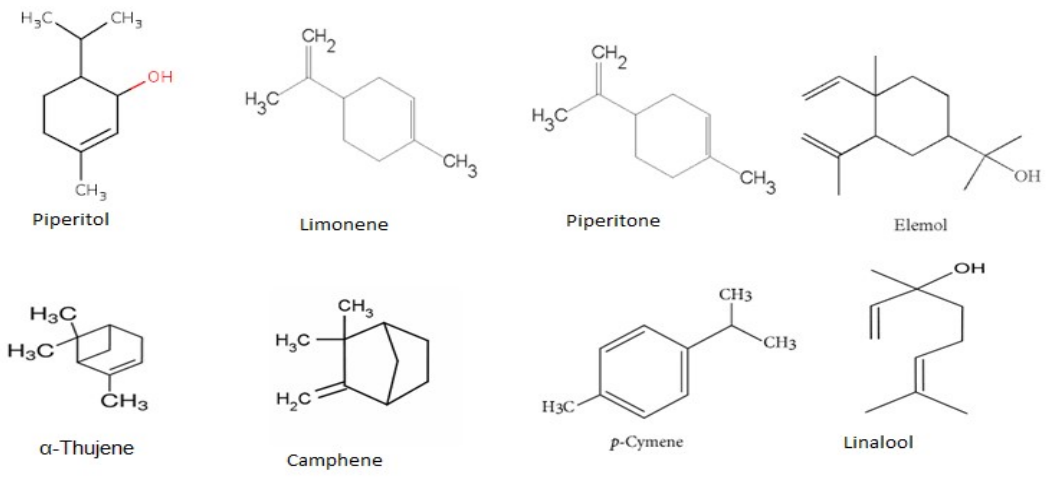


Figure 7. Chemical structure of some major components obtained from palmarosa and gingergrass essential oil

2.1.4. Essential oils of other *Cymbopogon* species

The essential oils of numerous wild growing *Cymbopogon* species have been chemically examined. The obtained results reveal that some of them can be used as a source of valuable essential oils.

Volatile constituents of the essential oil of *C. caesius* (Nees) Stapf, species of deciduous savanna bushland and wooded grasslandand, over all of tropical Africa, were studied by Kanjilal et al. (1995). The main constituents were perillyl alcohol (25.61%), geraniol (19.80%) and limonene (7.26%).

The essential oil of *C. coloratus* (Hook. f.) Stapf. from the vegetative stage was found to be rich in camphene (20.4%), limonene (18.9%), borneol (4.9%) and caryophyllene oxide (4.4%), whereas the oil from the flowering plants was rich in β-caryophyllene (5.6%), γ-cadinene (5.0%), δ-cadinene (4.1%), caryophyllene oxide (4.4%) β-bisabolol (27.2%) (Mallavarapu et al., 1998).

Leaves and inflorescence of *C. distans* (Steud.) Wats., a perennial aromatic grass wildly growing in xerophytic grasslands of upper Himalayan region contain essential oils that are dominated by oxygenated monoterpenes with geranial (26.3–38.2 %), geranyl acetate (22.6–25.6 %), neral (16.0–18.9 %), geraniol (4.8–12.3 %), limonene (1.0–1.7 %), linalool (0.8–1.3 %), and citronellal (1.0–1.2 %) as the main components (Verma et al., 2013).

2.2. Flavonoids, phenolic acids and phenylpropanoids

Isolation and identification of phenolic compounds with different biological activities from *Cymbopogon* species have been reported.

Isoorientin and tricin were isolated from the dichloromethane extract of *C. parkeri* (Rizk et al., 1995), luteolin, luteolin 7-*O*-glucoside (cynaroside), isoscoparin and 2"-*O*-rhamnosyl isoorientin from the leaves and rhizomes of *C. citratus*, quercetin, kaempferol and apigenin chlorogenic acid, caffeic acid from the aerial parts of *C. citratus* (Cheel et al., 2005).

Phenylpropanoids such as eugenol (4-allyl-2-methoxyphenol), elemicin (5-allyl-1,2,3-trimethoxybenzene), eugenol methylether (4-allyl-1,2-dimethoxybenzene) and *trans*-iso-elemicin (1,2,3-trimethoxy-5-(1-propenyl) benzene) were isolated from Australian species *C. ambiguus* (Grice et al., 2011).

2.3. Non-Volatile terpenoids

Bottini *et al*. (1987) isolated a novel bis-monoterpenoid named cymbodiacetal from *C. martinii*. The triterpenoids cymbopogone and cymbopogonol were also reported from the leaves of *C. citratus* (Hanson et al., 1976).

2.4. Tannins

A literature search on the phytochemical screening of *C. citratus* also reveals the presence of tannins. However, very little effort has been made in the isolation of these compounds despite the appreciable amounts reported through quantitative phytochemical tests (Avoseh et al., 2015).

3. Pharmacological investigations of *Cymbopogon* species

Essential oils and extracts of *Cymbopogon* species were subjects of various studies concerning investigation of their biological activity. Several bioassays have confirmed the potency of *Cymbopogon* species for their uses as pharmacologicaly active natyral products.

3.1. Anticonvulsant activity and peripheral analgesic activity

The essential oil from fresh leaves of *C. winterianus* showed anticonvulsant effects in different models of epilepsy in rodents (Quintans-Júnior et al., 2008).

When it is administate orally to the rats, infusion of *C. citratus* fresh leaves produces a dose-dependent analgesia for the hyperalgesia induced by subplanted infections of either caragenin or prostaglandin E2. The use of infusion did not affect hyperalgesia which was induced by dibutyryl cyclic AMP. Myrcene was identified as the active constituent responsible for this activity (Lorenzetti et al., 1991).

3.2. Activity against malignancy

Lemongrass essential oil variety of *C. flexuosus* and its major chemical constituent sesquiterpene isointermedeol were investigated for their ability to induce apoptosis in human leukemia HL-60 cells because dysregulation of apoptosis is the hallmark of cancer cells. Essential oil and isointermedeol inhibited cell proliferation with 48 h IC50 of ~30 and 20 μg/mL, respectively (Kumar et al., 2007).

Induction of increased Glutathione *S*-transferase activity (GST activity), which is believed to be a major mechanism for chemical carcinogen detoxification, has been recognized as one of the characteristics of the action of anticarcinogens. *d*-Limonene from lemongrass (*C. citratus*) oil increased GST activity two to three fold than controls in the mouse liver and the mucosa of the small and large intestines. Also, geraniol from Lemongrass oil showed high GST inducing activity in the mucosa of the small and large intestines, which was about 2.5 fold greater than controls (Zheng et al., 1993).

3.3. Antimale sex hormone agent

The antimale sex hormone agent (5-reductase inhibitor that converts testosterone to active dihydrotestosterone) is extracted from the leaves, stems, rhizomes, roots or whole plant of *C. flexuosus.* This agent is especially useful as hair growth stimulants (Kisaki et al., 1998).

3.4. Anthelmintic activity

The essential oil of *C. martinii* var. *motia* have shown in *in vitro* tests excellent anthelmintic activity against tapeworms, round worms, and earthworms (Sangwan et al., 1985).

3.5. Hypoglycemic and hypolipidemic effect

The fresh leaf aqueous extract of *C. citratus* was investigated for the hypoglycemic and hypolipidemic effects. The single daily oral dosing of 125-500 mg/kg of in normal male Wistar rats for 42 days, caused lowering of fasting plasma glucose and lipid parameters dose dependently, while raising the plasma HDL-c level in same dose-related fashion but with no effect on plasma triglycerides level (Adeneye and Agbaje, 2007).

In another report, *C. proximus* herb was assessed for hypoglycemic and hyperinsulinemic action on alloxan diabetic rats. The results revealed that considerable hypoglycemic effect was exerted after 16 days. The level of serum insulin was also increased in diabetic rats (Eskandar and Won Jun,1995).

3.6. Repellent and larvicidal activity

Lemongrass essential oil in different classes of base and the oil in ointment, cream, liquid, and paraffin solution have been evaluated for mosquito repellency in a topical application. Repellency of mosquito was tested by determining the bite deterrence of product samples applied on an experimental bird's skin against a 2-day-

starved culture of *Aedes aegypti* L. mosquitoes. It was confirmed that 1% v/v solution and 15% v/w cream and ointment preparations of the oil exhibited ≥50% repellency lasting for 2–3 h, which may be attributed to citral, a major oil constituent (Oyedele et al., 2002).

3.7. Antiinflammatory activity

C. giganteus is widely used in traditional medicine against several diseases. The essential oil from leaves of *C. giganteus* from Benin exhibited inhibitory effect on 5-lipoxygenase *in vitro*, and has been found useful as an antiinfl-ammatory agent (Alitonou et al., 2006).

The essential oil from *C. martinii* leaves produced dose-dependent inhibition of carrageenan-induced paw edema in experimental male albino rats when administed orally (Akhila, 2010).

3.8. Antioxidant activity

Essential oils of various *Cymbopogon* species were investigated for antioxidant properties using different in vitro assays (Ganjewala, 2009). The essential oils of *Cymbopogon* species showed some antioxidant potential but activity was mainly weak to moderate.

3.9. Pesticid activity

The effect of different concentrations (0.2%–0.8%) of the essential oil of *C. citratus* on *Spodoptera litura* larvae has been studied in relation to host plant resistance in peanut. When treated with the oil before feeding larvae showed significant higher mortality on the diet containing resistant pods than on that containing susceptible pods (Rajapakse and Jayasena, 1991).

The essential oils of *C. martinii* var. *motia, C. flexuosus* and *C. winterianus* are reported to possess insect-repellant, nematicidal, and insect-attractant properties (Ahmad et al., 1993). Also, essential oils of citronella (*C. winterianus*) and palmarosa

(*C. martinii*) showed pesticidal activity against the stored grain insect *Tribolium castaneum* (Naik et al., 1995). The essential oil of *C. schoenanthus* and its major constituent piperitone exhibited insecticidal activity against *Callosobruchus maculatus* (Ketoh et al., 2006).

3.10. Antimicrobial activity

Due to rapidly devoloping resistance of pathogenic micoorganisms to antibiotics, plant products as well as essential oils as potential antimicrobial agents come into focus of research for its antimicrobial activity.

Essential oils from *Cymbopogon* species have been studied for its *in vitro* antimicrobial properties using different methods against different microorganisms (Khunkitti, 2010; Ganjewala, 2009). Bearing in mind that many factors affect the results of testing microbial susceptibility to essential oils (solubility of oil, presence of solubilizers, vehicles, method and conditions of testing) the reports available in the literature are rarely directly comparable (Janssen et al., 1987).

Lemongrass (*Cymbopogon citratus*) essential oil as one of the most important *Cymbopogon* essential oils was the subject of many previous investigations concernig antimicrobial activity (Helal et al., 2006; Hammer et al., 1999; Kalemba and Kunicka, 2003; Wannissorn et al., 1996).

Lemongrass (*C. citratus*) oil from Thailand was active against dermatophytes such as *Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum* and *Microsporum gypseum* with MIC values 115-235 μg/ml (Wannissorn et al., 1996). Also, a commercial sample of essential oil of *C. citratus* exhibited good antimicrobial activity against *Candida tropicalis* (MIC 16 μg/ml), *Escherichia coli* and *Candida albicans* (MICs 63 μg/ml), *Staphylococcus aureus* and *Enterococcus faecalis* (MICs 125 μg/ml) and moderate activity against *Moraxella catarrhalis* (MIC 250 μg/ml.

The essential oil of *C. citratus* cultivated in India was investigated for antimicrobial activity against some pathogenic bacterias (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) by broth dilution method. The oil was not effective against

Pseudomonas aeruginosa, whereas MIC values for other microorganisms were in the range of 0.06-0.50% (v/v) (Naik et al., 2010).

The essential oil of *C. flexuosus*, with citral as the main component, demonstrated moderate antimicrobial activity (MIC values 125-1000 µg/ml) (Ahmad and Viljoen, 2015).

Hammer and co-workers (1999) investigated antimicrobial activity of *C. citratus*, *C. martini* and *C. nardus* essential oils against *Staphylococcus aureus*, *Escerichia coli* and *Candida albicans* by broth microdilution method (Hammer et al., 1999). All of the oils were more active against *S. aureus* and *C. albicans* than against *E. coli*.

The essential oils of *C. martini*, *C. winterianus* and *C. nardus* were investigated for antimicrobial properties by microdillution method against four bacterial and two strains of fungi. The best activity was demonstrated towards *Candida tropicals* (MICs 32-63 µg/ml). Generally, the most active was *C. martini* essential oil (MICs 32-125 µg/ml) with geraniol as the most dominant constituent (79.7%) (Ahmad and Viljoen, 2015).

4. The uses of *Cymbopogon* species

In the previous section, different chemical constituents isolated from various species of the genus *Cymbopogon* have been listed. However, the most important product derived from wild growing and cultivated *Cymbopogon* plants are essential oils. The volatiles of different *Cymbopogon* species were the subject of previous investigations, but the most important commercially product are three types of essential oils, namely lemongrass, citronella and palmarosa essential oils. These oils are directly relevant for perfumery industry and they have an important economic benefit for their producers. Below, some more details about implementation and commercial importance of these three essential oils, their pharmacological activities as well as uses in traditional medicine are present.

4.1. The use of Lemongrass essential oil

The Lemongrass essential oil (produced from *C. flexuosus* and *C. citratus*) is widely used for the production of soaps and detergents. Further, it can be used as aditive for the culinary purposes (Opdyke, 1976).

This essential oil can be used as a row material for the production of citral. Citral is the major component of the lemongrass esential oil, and it can be used as flavors for cosmetics and perfumes.

The ionones (group of compounds known as rose ketones) are very aromatic with strong and lasting odor. They can be synthesized from citral and can be used as fragrances for perfumery. Further, ionones can be used in the manufacture of synthetic vitamin A (Akhila, 2010). These facts give a new importance and usefulness of lemongrass oil.

The leftover residue from lemongrass leaves has been successfully utilized as a source of raw material for cellulose pulp and paper production (Ciaramello et al., 1972; Siddique-Ullah et al., 1979).

Lemongrass is commonly used in folk medicine in many countries for the treatment of nervous, different type of gastrointestinal disturbances and for treatment of fewer (Tangpu and Yadav, 2006; Shah et al., 2011).

Concerning approved pharmacological activities, the most literature data can be found about antimicrobial and antioxidant activity of lemongrass oil (Chalchat et al., 1997; Handique and Singh, 1990; Moris et al., 1979; Mehmood et al., 1997; Orafidiya, 1993; Onawunmi et al., 1984; Shadab-Qamar et al., 1992; Singh et al., 1978; Wannissorn et al., 1996; Yadav and Dubey, 1994).

There are some reports about allergic contact dermatitis that can be seen after usage of lemongrass oil (Selvag et al., 1995). Some other authors reported that this oil can be used as preservative of sensitization reactions (Opdyke, 1976; Arora and Pandey, 1977).

The other important use of the lemongrass oil has been in the preparation of an insect-repellent complex (Sukari et al., 1992). Those preeparation has been tested for insect repellent/attractant and nematicidal activities (Ansari and Razdan, 1995).

Also *C. citratus* oil has been tested for anticarcinogenic activities (Zheng et al., 1993).

The source of North Indian lemongrass essential oil is a *C. pendulus.* The main constituent of oil obtained from this species is elemicin (53.7%). It is interesting that this compound is the basis for the synthesis of the antimalarial drug trimethoxyprim (Shahi et al., 1997).

4.2. The use of Citronella essential oil

Java Citronella oil (*C. winterianus)* is usually employed for the production of soaps and all kinds of technical preparations as well as for the isolation of some aromatic compounds. This essential oil can be use for the production of a formulation which can be used as mosquito repellent. Simmilary, this formulation can be effective as housefly repellent (Osmani et al., 1972).

Java citronella oil is one of the most important source of citronellal. After purification, citronellal can be converted into citronellol. Further, citronellol can be converted into citronellol esters, hydroxy citronellal and synthetic menthol (Dev Kumar et al., 1977).

The Ceylon citronella oil (*C. nardus*) posses very strong antimicrobial activity against bacteria and fungi (Akhila, 2010).

4.3. The use of Palmarosa essential oil and Gingergrass essential oil

The palmarosa essential oil is obtained from the *C. martini* var. *motia.* The gingergrass essential oil is obtained from the same species but different variety (*C. martinii* var. *sofia*). As the dominant constituent, palmarosa oil contains geraniol. Because of this constituent, this oil is commercially much more important than the oil of gingergrass (Shylaraj and Thomas, 1992).

More than any other *Cymbopogon* essential oils, palmarosa oil is used for the production of soaps, because of its rose-like prominent and lasting odor. Further, this oil can be employed in the flavoring of tobacco and other mouth fresheners (Akhila, 2010).

The oil of gingergrass is used in low-cost perfume formulations and for scenting of soaps and cosmetics.

4.4. The use of *Cymbopogon* species in folk medicines

It is known that traditional medicine and traditional methods of healing are integral part of primary health care in many developing countries from Africa, Asia and from the rest of the world. Thus, species of *Cymbopogon* genus are the integral part of traditional, folk medicine in countries where they are growing wild. Beside medical application, these species are used for insects' control and for every day using in the food preparation or for the production of common cosmetics. It is interesting and it has to be noted that national, local names of *Cymbopogon* species often show some of their properties, functions or methods of administration.

Different *Cymbopogon* species are used as herbal tea for the infusion preparation or in the form of classical pharmaceutical forms.

In the India, the herb and leaves of *C. nardus* are used as insect repellent. In the same area the species *C. flexuosus* and *C. pendulus* are used as antiseptic and for the treatment of fever (Avoseh et al., 2015). Both species are used in cosmetic industry and for perfumes production (Desai et al., 2012).

The herb and leaves of *C. parkeri* from Pakistan are used as antiseptic and as stomachic (Bagheri et al., 2007). In the same country, species *C. olivieri* is known as putar. This herb is used against fewer as antipyretic, for the treatment of malaria and for

rheumatism. Further, it is used as vomitory agent and diuretic (Abbas, 2003; Mahboubi et al., 2012).

In the Egypt, species *C. proximus* is known as halfa bar and it is used for expulsion of renal and ureteric calculi (El-Askary et al., 2003).

The species *C. excavatus* is native to South Africa. The national name of this species is bread-leavened or turpentine grass and it is used as insecticides (Govere et al., 2000).

In the Eastern and Southern Africa the species *C. validus* is known as African bluegrass. This herb is used as skin toner and for anti-ageing treatment in men. Further, it is used as fumigant and for rodent control (Avoseh et al., 2015). In the South Africa, species *C. marginatus* is known as lemon-scented grass and it is used as moth repellent (Secoy and Smith, 1983).

C. giganteus from Cameroon is known as tsauri grass. The leaves and flower of this species are used as decoctions for the treatment of cough and arterial hypertension (Jirovetz et al., 2007).

The leaves and rhizome of *C. densiflorus*, known in Congo as lemongrass, are used for the production of extracts which are used against asthma, epilepsy, abdominal cramps and pains.

C. ambiguous is native in Australia and it is known as a lemon grass. The leaves and stems of this species are used for the treatment of different conditions, headache remedy, chest infections, muscle cramp and scabies (Grice, 2011).

Another species native in Australia is *C. procerus* and it is known as a scent grass. The leaves and stems of this species are used as medicinal body wash as antiseptic. It is also use for headache (Smith, 1991).

The species C. *refractus* from Australia, locally is known as barbed wire grass. The leaves of this species are used for feeding of animals (Beeftalk, 2011).

The species *C. obtectus* from central Australia, the local named silky-heads, is traditionally used for treatment of cold and flu, fever and sore throat as well as for headaches.

C. winterianus in Brazil is used for treatment of epilepsy and anxiety (Leite et al., 2011).

5. *Cymbopogon nervatus* (Hochst.) Chiov.

C. nervatus (syn. *Andropogon nervatus* Hochst.) is one of the most important species among nine species of the genus *Cymbopogon* found in the flora of Sudan. Common names of this plant are Nal or Naal.

Figure 8. *C. nervatus*- Nal
(http://www.virboga.de/pics/big/001495.jpg)

"HABIT: Annual; culms solitary, or caespitose. Culms erect, or geniculately ascending; 50–180 cm long; 1.5–5 mm diam.; without nodal roots, or with prop roots. Ligule an eciliate membrane; 3 mm long. Leaf-blade base broadly rounded. Leaf-blades 15–30 cm long; 6–10 mm wide; flaccid; glaucous; aromatic.

INFLORESCENCE: Synflorescence compound; linear; 10–15 cm long; dense. Inflorescence composed of racemes; terminal and axillary; subtended by a spatheole; enclosed. Spatheole lanceolate; 1.2–1.6 cm long. Peduncle 0.4–0.8 cm long.

Racemes 2; paired; deflexed; 1.2–1.6 cm long. Rhachis fragile at the nodes; semiterete; glabrous on surface; ciliate on margins. Rhachis internodes linear; 2 mm long. Rhachis internode tip transverse; cupuliform. Raceme-bases flattened; subequal.

Spikelets in pairs. Fertile spikelets sessile; 1 in the cluster. Companion sterile spikelets pedicelled; 1 in the cluster. Pedicels linear; semiterete; 2 mm long; ciliate; hairy on margins.

STERILE SPIKELETS: Basal sterile spikelets well-developed; 2 in number (lower raceme); 0 in upper raceme; with swollen internode in lower raceme; sessile and pedicelled. Basal sterile spikelet pedicels free; swollen in lower raceme. Basal sterile spikelets equalling fertile.

Companion sterile spikelets well-developed; male; oblong; 4–5 mm long; as long as fertile; deciduous with the fertile. Companion sterile spikelet glumes chartaceous; winged on keels; distinctly veined; 7 -veined; acute; muticous. Companion sterile spikelet lemmas 2; enclosed by glumes.

FERTILE SPIKELETS: Spikelets comprising 1 basal sterile florets; 1 fertile florets; without rhachilla extension. Spikelets lanceolate; dorsally compressed; 4–5 mm long; falling entire; deciduous with accessory branch structures. Spikelet callus base obtuse; inserted.

GLUMES: Glumes dissimilar; exceeding apex of florets; firmer than fertile lemma. Lower glume lanceolate; 1 length of spikelet; membranous; with oil streaks; 2-keeled; keeled all along; keeled laterally; winged on keel; winged narrowly. Lower glume intercarinal veins distinct; 2 in number. Lower glume surface with V-shaped depression. Lower glume apex emarginate. Upper glume lanceolate; 1-keeled; winged on keel. Upper glume apex acute.

FLORETS: Basal sterile florets barren; without significant palea. Lemma of lower sterile floret hyaline. Fertile lemma lanceolate; 4 mm long; hyaline; without keel. Lemma apex lobed; 2 -fid; incised 0.5 of lemma length; awned; 1 -awned. Principal lemma awn from a sinus; geniculate; 8–16 mm long overall; with twisted column. Column of lemma awn glabrous. Palea absent or minute.

FLOWER: Anthers 3; 2 mm long." (Clayton WD, Vorontsova MS, Harman KT. and Williamson H. (2006 onwards). GrassBase - The Online World Grass Flora. http://www.kew.org/data/grasses-db.html, last access 30 October 2016).

Distribution

Africa: northeast tropical, Asia-temperate: Arabia, Asia tropical: Indo-China (Clayton WD, Vorontsova MS, Harman KT. and Williamson H. (2006 onwards). GrassBase - The Online World Grass Flora. http://www.kew.org/data/grasses-db.html, last access 30 October 2016).

It constitutes an important proportion of savannah grass in Western and Eastern Sudan (Modawi et al., 1984).

5.1. Chemical constituents

There are a few previous studies concerning essential oil of *C. nervatus*. The essential oil from aerial parts of *C. nervatus* from Sudan (Butana) was characterized by presence of mentadienols: *trans*-*p*-mentha-1(7),8-dien-2-ol (21.25%), *cis*-*p*-mentha-1(7),8-dien-2-ol (9,83%) and *trans*-*p*-mentha-2,8-dien-1-ol (9,83%) (Abushama et al., 2013).

In the essential oil from inflorsecence of *C. nervatus* originated from Eastern Sudan *cis*-*p*-menta-1(7),8-dien-2-ol (25.2%) and *trans*-*p*-menta-1(7),8-dien-2-ol (22.9%) were the most dominant components (El-Kamali et al., 2005).

Banthorpe et al. (1976) reported on the composition of essential oil obtained from leaves of *C. nervatus* from Sudan (Abu Naama-loam plain, Blue Nile Prov.) collected after flowering, where the main constituents were also *p*-menthadienols (up to 89 %).

5.2. Use in folk medicines

In Sudanese traditional medicine, the inflorescence of *C. nervatus* is used as decoction to treat kidney pains and urethritis (El-Kamali et al., 2005). Traditionally, the leaves are used to treat indigestion and also as a carminative and tonic (El-Kamali and El-Khalifa, 1999).

6. *Cymbopogon schoenanthus* (L.) Spreng.

C. schoenanthus is a perennial herb, native in Africa and Asia. Common names of this plant are Camel grass and Maharaib in Northern and Eastern Sudan, and Izkhir in Arabia (Hashim et al., 2016).

Figure 9. *C. schoenanthus* - Maharaib
(http://www.ville-e.ch/musinfo/bd/cjb/africa/images/data/images/POA_Cym_sch3.JPG)

"HABIT Perennial; caespitose. Butt sheaths persistent and investing base of culm. Culms 30–120 cm long. Ligule an eciliate membrane. Leaf-blades filiform, or linear; flat, or involute; 10–35 cm long; 1–4 mm wide; aromatic. Leaf-blade surface scaberulous.

INFLORESCENCE Synflorescence compound; paniculate; 5–40 cm long; dense. Inflorescence composed of racemes; terminal and axillary; subtended by a spatheole; enclosed.

Racemes 2; paired; deflexed; 1–3 cm long. Rhachis fragile at the nodes; semiterete; villous on margins. Rhachis hairs 2–4 mm long. Rhachis internodes linear. Rhachis internode tip transverse; cupuliform. Raceme-bases flattened; subequal.

Spikelets in pairs. Fertile spikelets sessile; 1 in the cluster. Companion sterile spikelets pedicelled; 1 in the cluster. Pedicels linear; semiterete; villous; with 2–4 mm long hairs.

STERILE SPIKELETS Basal sterile spikelets well-developed; 2 in number (lower raceme); 0 in upper raceme; sessile and pedicelled. Basal sterile spikelet pedicels fused to internode in lower raceme; swollen in lower raceme. Basal sterile spikelets equalling fertile.

Companion sterile spikelets well-developed; male; lanceolate; 4–7 mm long; as long as fertile; deciduous with the fertile. Companion sterile spikelet glumes chartaceous; acute; muticous. Companion sterile spikelet lemmas 2; enclosed by glumes.

FERTILE SPIKELETS Spikelets comprising 1 basal sterile florets; 1 fertile florets; without rhachilla extension. Spikelets lanceolate; dorsally compressed; 4–7 mm long; falling entire; deciduous with accessory branch structures. Spikelet callus pilose; base obtuse; inserted.

GLUMES Glumes dissimilar; exceeding apex of florets; firmer than fertile lemma. Lower glume linear; 1 length of spikelet; chartaceous; 2-keeled; keeled all along; keeled laterally; wingless. Lower glume intercarinal veins absent. Lower glume surface concave. Lower glume apex emarginate. Upper glume lanceolate; 1-keeled. Upper glume apex acute.

FLORETS Basal sterile florets barren; without significant palea. Lemma of lower sterile floret hyaline. Fertile lemma lanceolate; hyaline; without keel. Lemma apex dentate; 2 -fid; awned; 1 -awned. Principal lemma awn from a sinus; straight; 5–9 mm long overall; with a straight or slightly twisted column. Column of lemma awn glabrous. Palea absent or minute.

FLOWER Anthers 3." (Clayton WD, Vorontsova MS, Harman KT. and Williamson H. (2006 onwards). GrassBase - The Online World Grass Flora. http://www.kew.org/data/grasses-db.html, last access 30 October 2016).

Distribution

C. schoenanthus grows wild in tropical and temperate regions of Africa and Asia.

- East Tropical Africa: Kenya
- Northeast Tropical Africa: Chad; Djibouti; Ethiopia; Somalia; Sudan
- Northern Africa: Algeria; Egypt; Libya; Morocco

- West Tropical Africa: Benin; Burkina Faso; Cote D'Ivoire; Ghana; Guinea; Mali; Mauritania; Niger; Nigeria; Senegal; Togo
- Arabian Peninsula: Oman; Saudi Arabia; Yemen
- Western Asia: Egypt - Sinai (http://e-monocot.org/taxon/urn:kew.org:wcs:taxon:477146)

It grows on dry stony ground of sub-desert requiring minimum amount of water (El-Olemyl and Fraid Fattah, 1994). It is found in central, northern and western Sudan and also cultivated in Khartoum state.

6.1. Chemical constituents

Monoterpene piperitone (up to 80%) has been identified as the main compound of the essential oil of *C. schoenanthus*. Further, elemol, eudesmol as well as *cis*-carveol, citral-a, citral-b, dihydrocarveol, limonene and linalool were reported as the constituents of essential oil. The minor constituents of this essential oil are: α-pinene, β-elemene, β-selinene, calamenene and cadalene (Modawi et al., 1984; Yentéma et al., 2007; Khadri et al., 2008).

6.2. Use in folk medicines and pharmacological investigations

C. schoenanthus has no known toxicity or carcinogenicity. It has a long history of safe use and is Generally Recognized As Safe (GRAS) by the US Food & Drug Administration.

In Africa *C. schoenanthus* 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 (Ben Othman et al., 2013). Its medicinal properties were considered to be helpful in the treatment of gout, prostate inflammation, kidney disorders, stomach pain, fever, and reumatism (Eltahir and AbueReish, 2010; Khadri et al., 2008). Furthermore, *C. schoenanthus* is traditionally used as a digestive, for treating intestinal spasms as well as for anorexa i.e. for bringing back the appetite (Ben Othman et al., 2013; Kpoviessi et al., 2014).

Previous studies concerning biological activities showed that *C. schoenanthus* oil exhibits insecticidal (Ketoh et al., 2006; Bossou et al., 2013) antitrypanosomal (Khadri et al., 2008), antioxidant and acetylcholinesterase activities (Kpoviessi et al., 2014).

For the the insecticidal activity of the essential oil, piperitone has been recognized as main compound. It was assessed in different developmental stages of *Callosobruchus maculatus* Piperitone inhibited the development of newly laid eggs and neonate larvae, but was less toxic than the crude extract to individuals developing inside the seeds (Ketoh et al., 2006).

II Aim of research

The main objective of research in the framework of this doctoral dissertation is investigation of two species belonging to the genus *Cymbopogon* (Poaceae) from the flora of Sudan.

The first species is *Cymbopogon nervatus* (Hochst.) Chiov., the most abundant wild growing species of this genus in the flora of Sudan. This species is very popular and often used herbal drug in the traditional medicine of this country.

The second investigated species is *Cymbopogon schoenanthus* (L.) Spreng., sample from the field production. The herb of this species is one of the most valuable traditional herbal drug in the region and because of that it is cultivated at the different location in the east part of Africa.

The research will be separated in a two parts:

1. Determination of content and detailed chemical analysis of essential oils obtained by hydrodistillation from different plant parts of *C. nervatus* and *C. schoenanthus*, collected in different periods.

2. Pharmacological screening of essential oils obtained from two *Cymbopogon* species which will comprise investigation of antimicrobial, antioxidant and spasmolytic activities.

The results obtained by chemical analysis as well as from pharmacological tests of two investigated essential oils will be compared with each other and with the literature data available for other species of the genus *Cymbopogon*.

This study should contribute to a better knowledge of the studied species. It should also serve to define the quality of plant raw materials and provide additional informations concerning the pharmacological effects. Based on this information, it may be possible to justify the application of *C. nervatus* and *C. schoenanthus* in traditional medicine.

III Experimental part

1. Plant material

The aerial parts of *Cymbopogon nervatus* (Hochst.) Chiov. were collected from two different states in Sudan (Umrwaba in Western and Algadarif in Eastern Sudan) in May 2014 and February 2015 (Table 2).

The aerial parts of cultivated *Cymbopogon schoenanthus* (L.) Spreng. were collected from the experimental field of The Medicinal & Aromatic Plants Research Institute (MAPRI), Khartoum state (Shambat), Sudan, in November 2013 and in February 2015 (Table 3).

The plant material was identified by a botany specialist Prof. Awatif Ahmed Mohammed Siribel, from the Medicinal and Aromatic Plants Research Institute (MAPRI), National Centre for Research, Khartoum, Sudan, and voucher specimens are deposited at MAPRI.

Stems and inflorescences were separated and dried in shade and open area.

Table 2. Origin, time of collection of plant material and samples of essential oils of *C. nervatus*

Origin	**Collection date**	**Plant part**	**Essential oil sample**
Western Sudan, Umrwaba	May 2014	stems	WS-1
		inflorescence	WF-1
	February 2015	stems	WS-2
		inflorescence	WF-2
Eastern Sudan, Gadarif	May 2014	stems	ES- 1
		inflorescence	EF- 1
	February 2015	stems	ES- 2
		inflorescence	EF- 2

Table 3. Origin, time of collection of plant material and samples of essential oils of *C. schoenanthus*

Origin	**Collection date**	**Plant part**	**Essential oil sample**
Experimental field of MAPRI	November 2013	stems	CS-1
		inflorescence	CI-1
	February 2015	stems	CS-2
		inflorescence	CI-2

2. Isolation and chemical analysis of essential oils

2.1. Isolation of essential oils

The essential oils were isolated separately from stems and inflorescence of *C. nervatus* and *C. schoenanthus* by hydrodistillation.

Hydrodistillation is one of the simplest, oldest and primitive process known to man for obtaining essential oils from plants. Hydrodistillation is a simple form of steam distillation which is often used to isolate non-water soluble, high boiling natural products. The advantage of this technique is that the desired material distills at a temperature below 100 °C.

Air dried stems and inflorescences of *C. nervatus* and *C. schoenanthus* were separated, grinded or squashed in coarse powder and measured before hydrodistillation. Chopping the grass gives a higher yield of oil than with uncuted grass. The plant material is almost entirely covered with water as suspension in the round bottom flask which is placed on a mantel heat. Plant material was hydrodistilled for 2-3 h in a Clevenger type apparatus. After distillation, the volume of oils was measured and oil yield calculated as % (v/w). The oils were kept at - 4° until they were analyzed.

2.2. GC and GC-MS analyses of essential oils

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-ionisation 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 °C to 280 °C at a rate of 3 °C/min. 10 μl of each essential oil was mixed with 990μl of hexane, and 1μl volume was injected in the split ratio 10:1 (Figure 10).

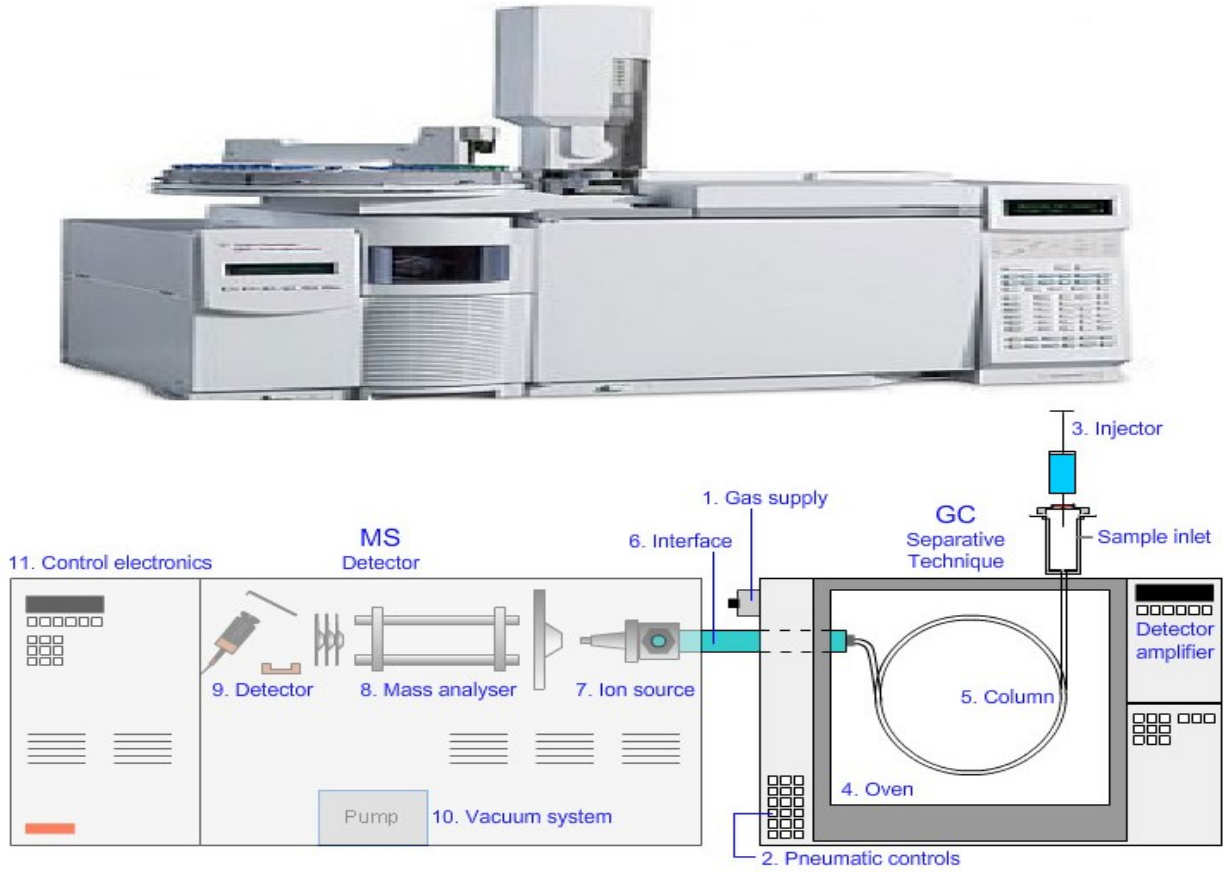


Figure 10. GC-MS Apparatus

The identification of the compounds was based on comparison of their retention indices (KI), their retention times (RT) and mass spectra with those from the NIST/NBS, Wiley libraries and the literature (Adams, 2007). The linear KIs were determined in relation to a homologues series of *n*-alkanes (C8–C40) run under the same operating conditions (Van den Dool and Kratz, 1963).

Relative percentages of compounds were calculated based on the peak areas from the FID data.

3. Investigation of antimicrobial activity of essential oils

The antimicrobial activity of the *C. nervatus* and *C. schoenanthus* essential oils were examined using the broth-microdilution method (Candan et al., 2003), using an appropriate range of dilutions of the essential oils in DMSO and appropriate solutions of the standard antibiotics ampicillin and amikacin as positive control of the antibacterial and nystatin as positive control of the antifungal effect.

The minimal inhibitory concentrations (MICs) were determined according to the Clinical and Laboratory Standards Institute (CLSI 2007). The MIC value was defined as the lowest concentration of essential oil at which the microorganism did not demonstrate visible growth.

3.1. Standard strains of microorganisms and culture conditions

The microbial growth inhibitory properties of the isolated essential oil were determined using the broth microdilution method against three Gram (+) bacteria: *Staphylococcus aureus ATCC* 25923*, S. epidermidis ATCC* 12228*, Enterococcus faecalis ATCC* 29121 and three Gram (–) bacteria*: Escherichia coli ATCC* 25922*, Klebsiella pneumoniae NCIMB* 9111 and *Pseudomonas aeruginosa ATCC* 27853 as well as against two strains of *Candida albicans (ATCC* 10259 *and* 24433*).* The MICs of standard antibiotics Ampicillin, Amikacin and Nystatin were determined in parallel experiments.

All tests were performed in Mueller Hinton broth for the bacterial strains because Mueller-Hinton Broth (MHB) is recommended as the medium of choice for susceptibility testing of commonly isolated, rapidly growing aerobic or facultative organisms. Additionally, Sabouraud dextrose broth was used for the *C. albicans* strains. Overnight broth cultures of each strain were prepared at a final concentration of 5105 microorganisms/ml in a 96-well microtiter plate.

To prepare growth microorganism solution or culture three standard gram positive bacteria, three standard gram negative bacteria and two standard fungi were selected in these studies. To prepare broth culture, the top of each colony have been touched with a loop or sterile swab and transferred the growth into a tube containing 4

to 5 mL of a suitable broth medium as Muller-Hinton Broth for bacteria and Sabouraud dextrose broth for fungi. The broth culture was incubated at 35 ± 2°C for bacteria and 26°C for fungi until it achieves or exceeds the turbidity of the 0.5 McFarland standard (two to six hours). The turbidity of the actively growing broth culture has been adjusted with sterile saline to achieve a turbidity equivalent to that of a 0.5 McFarland standard. Photometric device has been used to perform this step accurately. 0.05% of (TTC) Triphenyl tetrazolium chloride was added to the culture medium as a coloring growth indicator.

3.2. Broth microdilution test

The microdilution test was conducted in 96-well plates according to the CLSI (2007). A dilution series of the each essential oil were obtained from stock solution of essential oil in DMSO (20 mg/ml), using appropriate medium as the solvent. Each well received 100 µl of the specific concentration of the essential oil and 100 µl of Muller-Hinton Broth for bacteria and Sabouraud dextrose broth for fungi inoculated with the test microorganism (1.5×10^4 CFU/ml). The final volume in each well was 200 µl.

The microplates were covered and incubated in a bacteriological oven for at 37°C for 24 hours for bacteria and 48 hours at 30 °C for fungi. The bacterial growth was indicated by the presence of a pink colour or precipitate, whereas growth of fungi was monitored by the appearance of turbidity and/or precipitate.

The MIC values were defined as the lowest concentration of essential oil that completely inhibits growth of the microorganism in microdilution wells as detected by the unaided eye. The tests were performed two times against each microorganism. The MIC of Ampicillin, Amikacin and Nystatin were determined in parallel experiments.

4. Investigation of antioxidant activity *in vitro*

The antioxidative properties of inflorescence essential oils of *C. nervatus* and *C. schoenanthus* were estimated by Ferric Reducing Antioxidant Power (FRAP) assay for determination of total antioxidant capacity and by DPPH test for determination of free radical scavenging ability.

4.1. FRAP Assay (Ferric Reducing Antioxidant Power)

The total antioxidant capacity of essential oils was determined by FRAP assay, i.e. the ability of essential oils to reduce Fe^{3+} to Fe^{2+}. FRAP assay is based on ability of analyte to reduce the ferric tripyridyltriazine [Fe (III)-TPTZ] complex to the ferrous tripyridyltriazine [Fe (II)-TPTZ] which has an intense blue colour and can be monitored by measuring the change in absorption at 593 nm. FRAP values are obtained by comparing the absorbance change at 593 nm in test reaction mixtures with those containing ferrous ions in known concentration (Szőllősi and Szőllősi, 2002).

Procedure

10, 20, 30 40 and 50µl of essential oils were mixed with absolute ethanol to form 100µl of sample solution, and then dissolved with 3.0 mL of freshly prepared FRAP reagent. FRAP reagent was consisted of 300 mM acetate buffer (pH 3,6), 10 mM TPTZ solution in 40 mM HCL and 20 mM $FeCl_3 \cdot 6H_2O$ (ratio 10:1:1).

Samples were incubated for 30 min and the absorbance was recorded at 593 nm. Calibration curve of ferrous sulfate was used (100-1000 µmol/mL) and the results are expressed as FRAP value, i.e. mmol/L Fe^{2+} in investigated sample. The Fe^{3+} reducing ability was also determined for rutin and ascorbic acid.

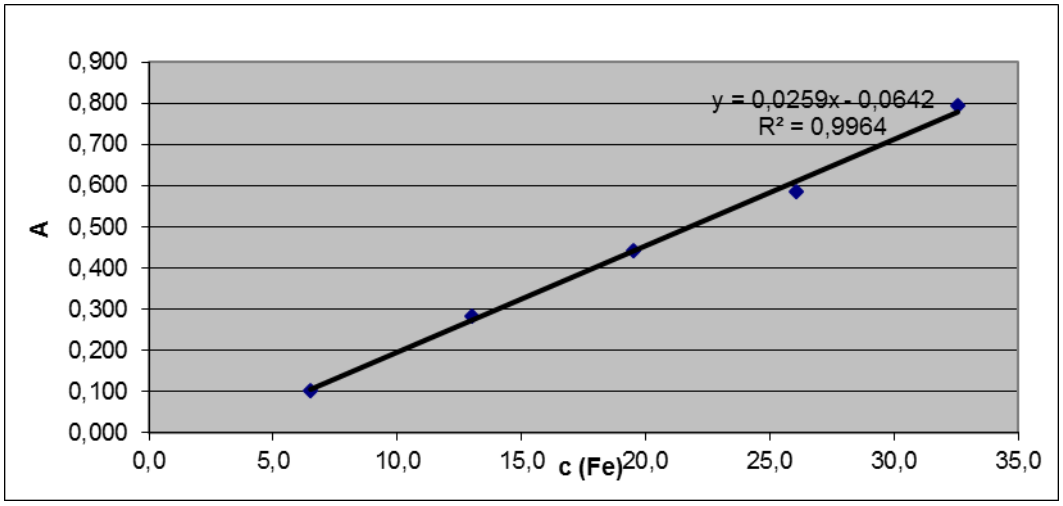
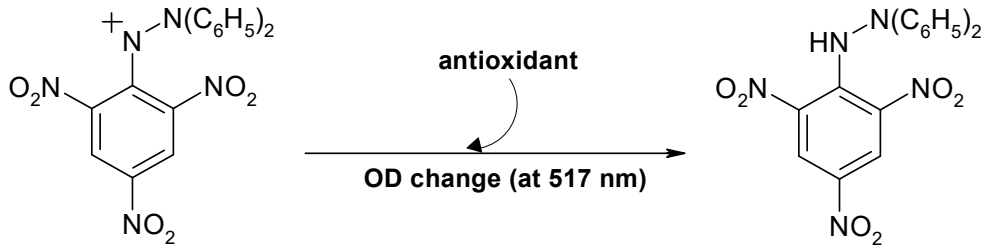


Figure 11. Calibration curve of ferrous sulfate

4.2. DPPH Assay (DPPH radical scavenging activity)

The radical scavenging activity of *C. nervatus* and *C. schoenantus* inflorescences essential oils were determined by a DPPH test (Cuendet et al. 1997).

In the reaction with molecules of each sample which cannot fire a hydrogen atom, a stable purple colored DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is reduced to a yellow colored DPPH-H.



Reducing the color intensity was monitored by measuring the change in absorption spectrophotometrically at 517 nm on Evolution UV-VIS 300 (Thermo Fisher).

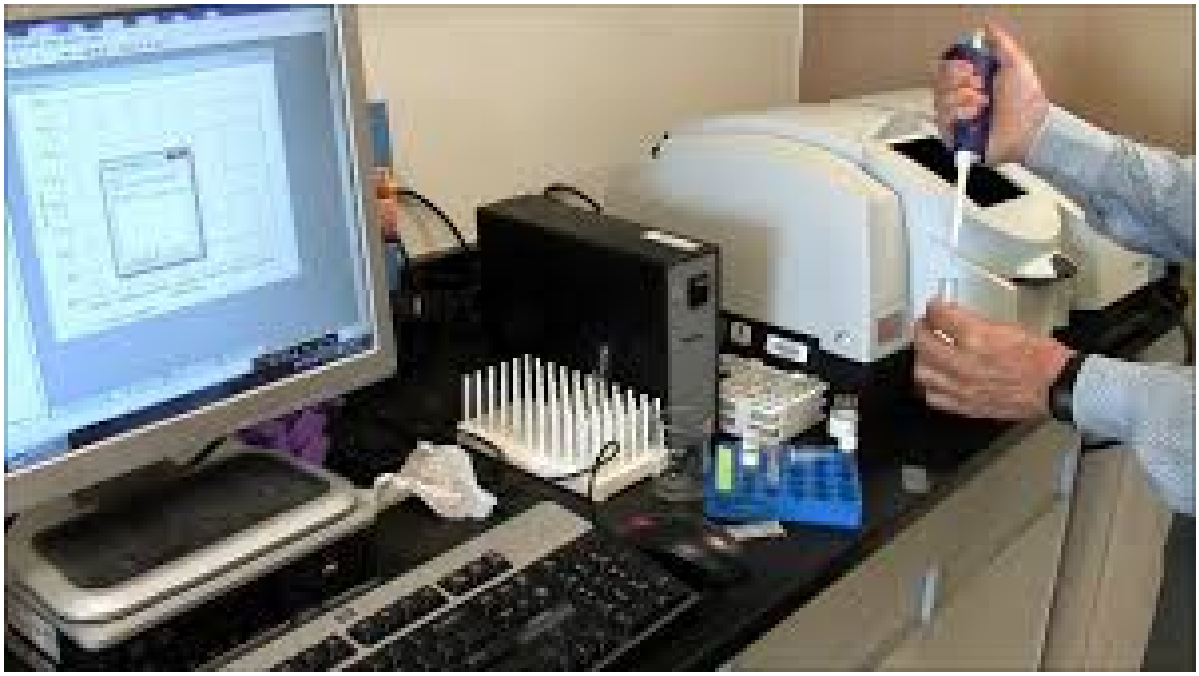


Figure 12. Spectrophotometer Evolution UV-VIS 300.

Procedure

In brief, 0.0086 g of DPPH was added into 50 ml volumetric flask and dissolved with sufficient amount of ethanol and the volume completed with ethanol to form 0.5 mM DPPH. Different aliquots of oil (10-100 µl) in test tubes were mixed with 500 µl of 0.5 mM DPPH radical in absolute ethanol, and final volume was adjusted to 2.5 ml by ethanol. Mixtures were vigorously shaken in sonicator, left for 30 min in the dark place, preferably in the refrigerator, protected by aluminum foil. 0.5 ml of 0.5 mM DPPH mixed with 2.0 ml of absolute ethanol was used as control.

Absorbance was measured at 517 nm using absolute ethanol as blank.

Scavenging of DPPH radical was calculated using the equation:

$$SC_{50}(\%) = 100 \times (A_0 - A_s)/A_0$$

where A_0 is the absorbance of the control, and A_s is the absorbance of tested sample.

The results are expressed as a SC_{50} value (µl/ml). The SC_{50} value represented the concentration of the essential oil that caused 50% of DPPH radical scavenging. Results were compared with the activity of L-ascorbic acid and rutin.

For the samples of oils of *C. schoenanthus* that couldn't achieve SC_{50} value in the experimental conditions, the results are expressed as percentage of scavenging of DPPH radical by 40 µl of essential oil per mL of test solution.

5. Investigation of spasmolytic activity

The essential oil from inflorescence of *C. nervatus*, collected in February 2015 from Western Sudan (sample WF-2) and the inflorescence essential oil of *C. schoenanthus*, collected in February 2015 (sample CI-2) were tested for spasmolytic activity using three different experimental models: against spontaneous contractions, and contractions induced with ACh and KCl.

Isolation of rat-ileum and recording of the contractions

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 University of Niš – Faculty of Medicine. Rats were sacrificed by cervical dislocation and exsanguinations. The ileum portions were isolated out, cleaned off mesenteries and two centimeters 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 ºC and aerated with a mixture of 5% carbon dioxide in oxygen. One end of the segment was attached to the bath bottom 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 tensions and allowed to equilibrate for 30 min. Under these experimental conditions, the segments exhibited spontaneous rhythmic contractions. Investigated essential oils dissolved in 0.5% Na-carboxymethyl cellulose (Na-CMC), and the control drugs were added directly to the organ bath in volumes not exceeding 1% of the bath volume (Pavlović et al., 2012).

Procedure

The effects of essential oil on spontaneous contractions, contractions induced with ACh and KCl were evaluated as described previously (Pavlović et al. 2012). In the first series of experiments the spasmolytic effect of atropine on spontaneous contractions of isolated ileum was investigated. Then, the effect of 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 in water bath cumulatively and concentration response curves were obtained.

In the second series of experiments the effects of essential oil on ACh induced contra-ctions was investigated. The effects on ACh induced contractions were studied 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 absence and then in presence of three different concentrations of essential oil (30, 60 and 90 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 into the water bath and concentration-response curve was obtained by cumulative addition of different concentrations of essential oil at 5 min intervals after addition of 80 mM KCl. For this experimental model alverine citrate, agent known to inhibit 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% Na-carboxymethyl cellulose) instead of essential oil.

Measurements and statistical analysis

Contractions were measured as area under the curve produced by tissue contraction at 5 min intervals just before addition of the next concentration of the tested sample and the results were expressed as 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 significance of differences between the

means was determined by the Mann-Whitney *U*-test using SPSS 11.5 software. A probability value of $p < 0.05$ or less was noted as indicative of significance.

IV Results and Discussion

1. Essential oils analysis

1.1. Essential oils of *C. nervatus*

The essential oils were isolated separately from stems and inflorescence of *C. nervatus*, collected from Western and Eastern Sudan in May 2014 (summer) and February 2015 (winter). The yield of oils, calculated on a dry weight basis varied between 0.1% and 0.2% *v/w* in the stems and 0.6% and 2.1% in the inflorescence (Table 4). The obtained essential oils were fragrant and yellowish. The content of essential oil was higher in inflorescence than in stems, and also was higher in plant material collected during the winter (February 2015).

Table 4. Provenance and oil yield of *C. nervatus* essential oils

Origin	**Collection period**	**Plant part**	**Sample**	**Oil Yield [% *(v/w)*]**
Western Sudan, Umrwaba	May 2014	stems	WS-1	0.1
		inflorescence	WF-1	1.0
	February 2015	stems	WS-2	0.2
		inflorescence	WF-2	2.1
Eastern Sudan, Gadarif	May 2014	stems	ES- 1	0.1
		inflorescence	EF- 1	0.6
	February 2015	stems	ES- 2	0.1
		inflorescence	EF- 2	1.2

The results of GC and GC-MS analyses of essential oils are summarized in Table 5. The GC-MS chromatograms of essential oils are shown in Figures 13-20.

In each oil twenty or twenty-one constituents were identified, representing 88.0-97.5% of the samples investigated. The oils were similar regarding qualitative pattern with some quantitative differences. No significant difference in qualitative composition was observed between the oils from different plant parts i.e. stems and inflorescence, as well as between the samples collected from different localities and in different periods.

All the oils were composed mainly of oxygenated monoterpenes (81.1-94.6%) and among them *p*-menthadienols (64.0-80.3%) were predominant. The major constituents were *trans*-*p*-mentha-1(7),8-dien-2-ol (19.9-32.6%), *cis*-*p*-mentha-1(7),8-dien-2-ol (18.9-23.3%), *trans*-*p*-mentha-2,8-dien-1-ol (10.6-21.0%) and *cis*-*p*-mentha-2,8-dien-1-ol (8.1-10.4%). *cis*-Carveol (5.2-8.7%) was abundant in all samples, whereas *trans*-carveol was present in prominent amounts (4.6-9.2%) in the samples collected in May 2014 and in the oil of inflorescence collected in February 2015 (6.6%). Monoterpene hydrocarbons were present in small amounts (0.5-6.2%), whereas sesquiterpene compounds were not detected in either of investigated *C. nervatus* oils.

The results of our study are in agreement with previous investigations concerning essential oil composition of *C. nervatus*. Previous analysis of essential oil from aerial parts of *C. nervatus* from Sudan (Butana) revealed that the main compounds were also mentadienols: *trans*-*p*-mentha-1(7),8-dien-2-ol (21.25%), *cis*-*p*-mentha-1(7),8-dien-2-ol (9,83%) and *trans*-*p*-mentha-2,8-dien-1-ol (9,83%) (Abushama et al., 2013).

The essential oil from inflorsecence of *C. nervatus* originated from Eastern Sudan was similar in composition, with *cis*-*p*-menta-1(7),8-dien-2-ol (25.2%) and *trans*-*p*-menta-1(7),8-dien-2-ol (22.9%) being the most dominant components (El-Kamali et al., 2005).

Also, Banthorpe et al. (1976) reported on the composition of essential oil obtained from leaves of *C. nervatus* from Sudan (Abu Naama- loam plain, Blue Nile Prov.) collected after flowering, where the main constituents were also *p*-menthadienols (up to 89 %).

Compounds with *p*-menthadiene skeleton (*cis*- and *trans*-*p*-mentha-2,8-dien-1-ol) were the major components in essential oils of inflorescence, leaves and stems of *C. giganteus* Chiov. from Cameroon (Jirovetz et al., 2007), Benin (Kpoviessi et al., 2014) and in the essential oil of leaves from Ivory coast (Boti et al., 2006).

The results of the chemical analysis of samples collected from different localities and in different time periods obtained in this study, and their comparison with literature data, suggest that essential oil composition of *C. nervatus* is rather stable, i.e that plant part, origin and time of collection do not affect significantly the composition of the essential oil of *C. nervatus*.

Table 5. Chemical composition of essential oils from stem and inflorescence of *C. nervatus*

Compound	KI Exp[a]	Western Sudan				Eastern Sudan			
		May 2014		February 2015		May 2014		February 2015	
		WS-1	WF-1	WS-2	WF-2	ES-1	EF-1	ES-2	EF-2
		%[b]							
dehydro-1,8-Cineole	992	-	Tr[c]	Tr	Tr	-	Tr	Tr	Tr
p-Cymene	1024	0.3	0.5	0.2	0.2	1.2	0.2	0.2	0.4
Limonene	1028	Tr	1.6	0.7	5.7	Tr	0.2	0.1	2.4
p-Cymenene	1093	0.3	0.5	0.2	0.3	0.6	0.3	0.2	0.3
n-Nonanal	1104	Tr	Tr	-	0.1	0.1	Tr	0.2	Tr
trans-*p*-Mentha-2,8-dien-1-ol	1124	14.4	18.5	20.3	16.5	10.6	14.8	21.0	14.3
cis-Limonene oxide	1136	-	-	Tr	0.5	-	-	Tr	Tr
cis-*p*-Mentha-2,8-dien-1-ol	1137	10.0	10.4	8.6	8.1	8.7	9.2	9.4	9.3
trans-Limonene oxide	1140	Tr	0.6	0.4	1.5	0.2	0.3	0.4	0.7
p-methyl-Acetophenone	1185	0.9	0.7	0.3	0.6	1.4	1.0	0.6	0.7
trans-*p*-Mentha-1(7),8-dien-2-ol	1191	28.9	24.9	23.4	19.9	32.6	29.0	26.6	22.0
trans-4-Caranone	1200	0.2	0.7	1.7	1.2	0.2	0.3	1.5	1.3
cis-4-Caranone	1203	-	-	1.4	1.2	-	-	0.9	0.6
trans-Carveol	1218	6.4	4.6	1.2	2.2	9.2	4.7	1.6	6.6

Table 5. Chemical composition of essential oils from stem and inflorescence of *C. nervatus* (continued)

cis-Carveol	1229	8.7	8.0	5.2	6.3	8.0	8.7	6.0	8.5
cis-*p*-Mentha-1(7),8-dien-2-ol	1232	21.2	22.4	22.4	19.5	18.9	23.2	23.3	22.0
Carvone	1244	3.5	3.6	3.6	3.8	3.3	3.7	3.4	3.9
Perilla aldehyde	1274	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.4
trans-Carvone oxide	1277	0.2	0.2	Tr	Tr	0.2	0.3	0.2	0.2
Perilla alcohol	1296	Tr	Tr	Tr	Tr	Tr	Tr	Tr	0.2
Dodecanoic acid	1566	Tr	-	-	-	0.3	Tr	-	-
Hexahydrofarnesyl acetone	1845	Tr	Tr	Tr	Tr	0.2	Tr	0.4	Tr
Hexadecanoic acid	1960	0.8	-	-	-	-	-	-	-
Heptacosane	2700	Tr	Tr	-	-	0.3	Tr	-	-
Grouped components									
Monoterpene hydrocarbons		0.6	2.6	1.1	6.2	1.8	0.7	0.5	3.1
Oxygenated monoterpenes		93.9	94.0	88.6	81.1	92.3	94.2	94.6	90.0
Sesquiterpene hydrocarbons		-	-	-	-	-	-	-	-
Oxygenated sesquiterpenes		-	-	-	-	-	-	-	-
Others		1.7	0.7	0.3	0.7	2.3	1.0	0.8	0.7
Identified		96.1	97.5	90.0	88.0	96.3	96.2	96.3	93.8

[a] KI exp- Relative retention indices calculated against *n*-alkane on HP-5MS column.

[b] %, Relative area percentage.

[c] Tr, trace (< 0.05 %).

WS-1, essential oil from stems collected in May 2014 from Western Sudan.

WF-1, essential oil from inflorescence collected in May 2014 from Western Sudan.

WS-2, essential oil from stems collected in February 2015 from Western Sudan.

WF-2, essential oil from inflorescence collected in February 2015 from Western Sudan.

ES-1, essential oil from stems collected in May 2014 from Eastern Sudan.

EF-1, essential oil from inflorescence collected in May 2014 from Eastern Sudan.

ES-2, essential oil from stems collected in February 2015 from Eastern Sudan.

EF-2, essential oil from inflorescence collected in February 2015 from Eastern Sudan.

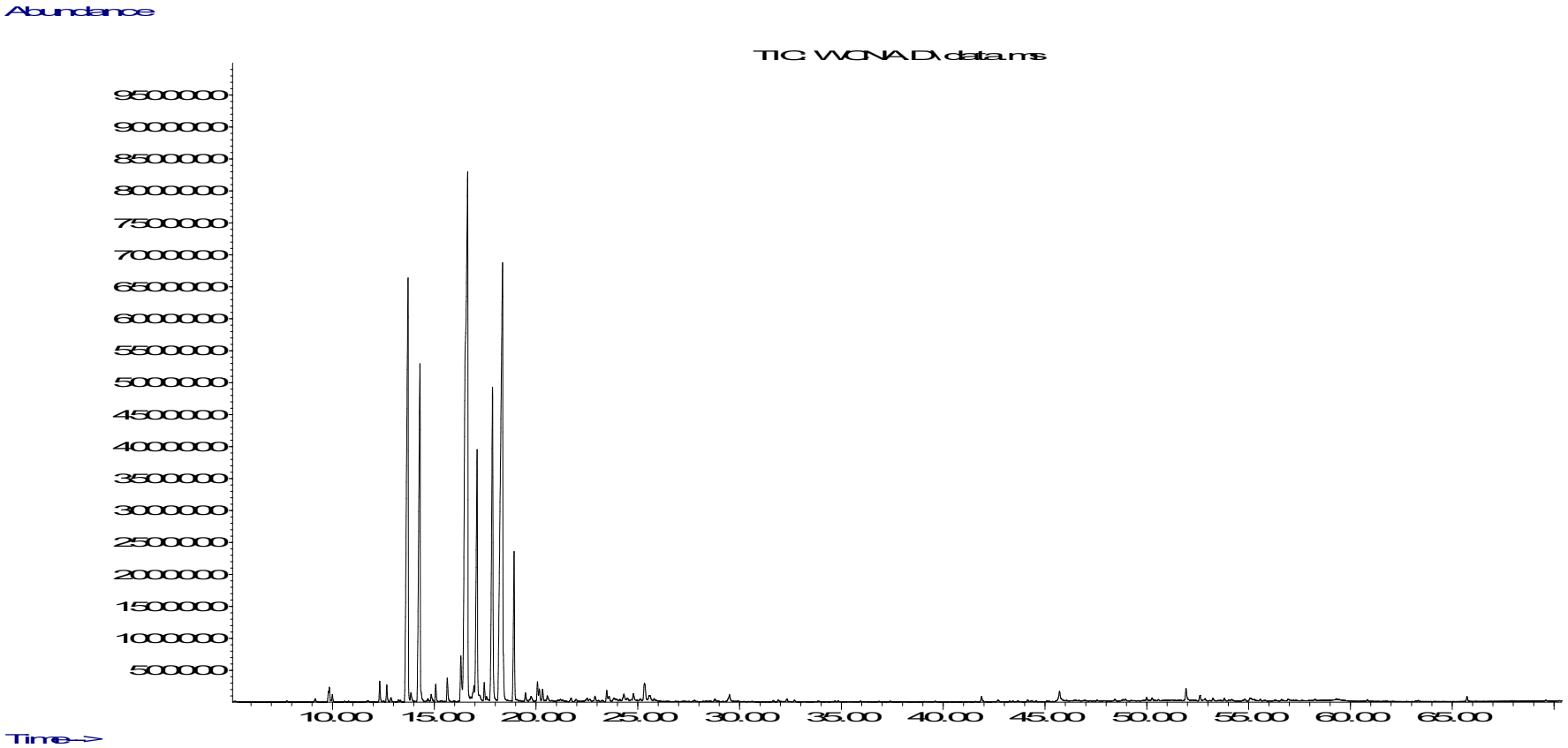


Figure 13. GC-MS chromatogram of essential oil from stems of *C. nervatus* collected in Western Sudan in May 2014 (sample WS-1)

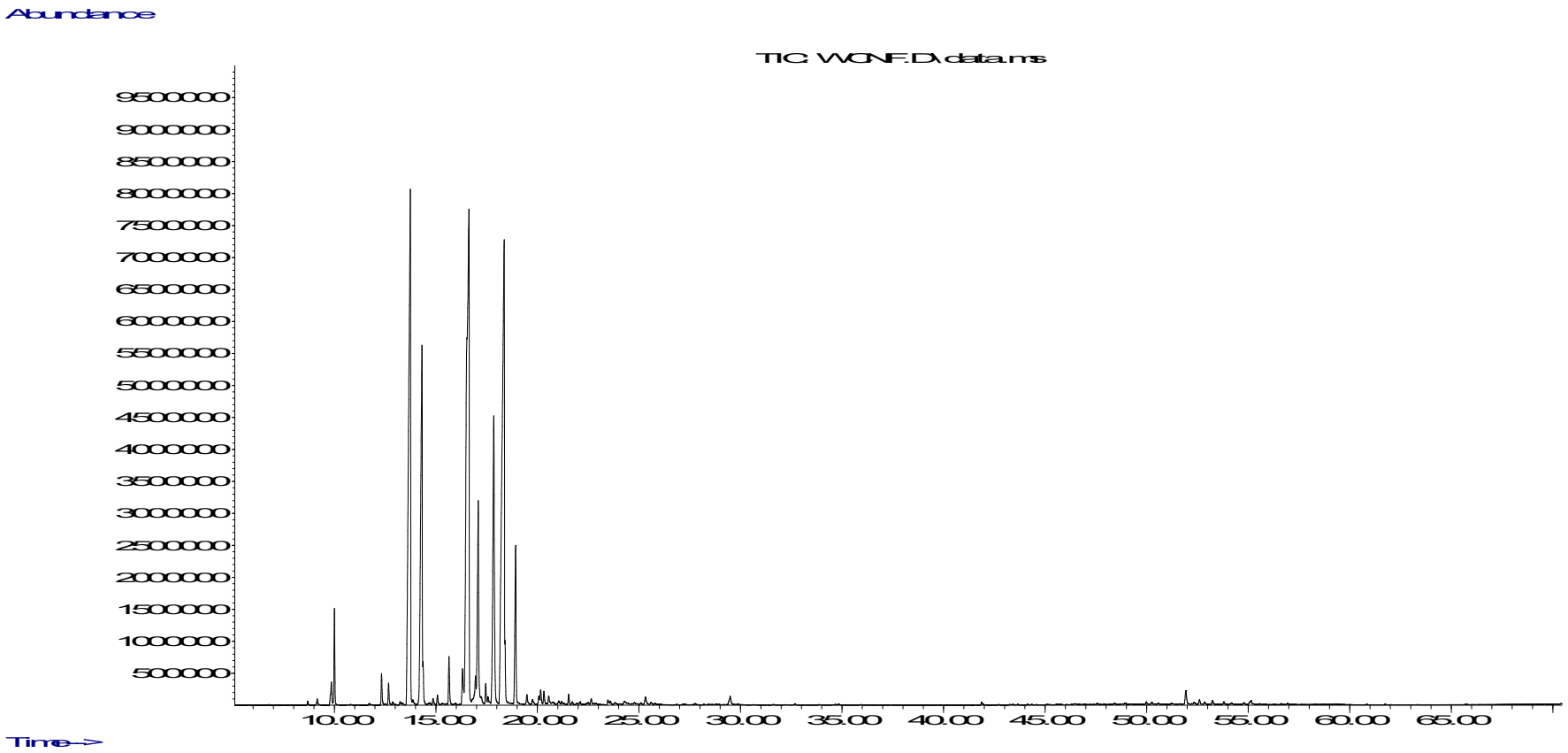


Figure 14. GC-MS chromatogram of essential oil from inflorescence of *C. nervatus* collected in Western Sudan in May 2014 (sample WF-1)

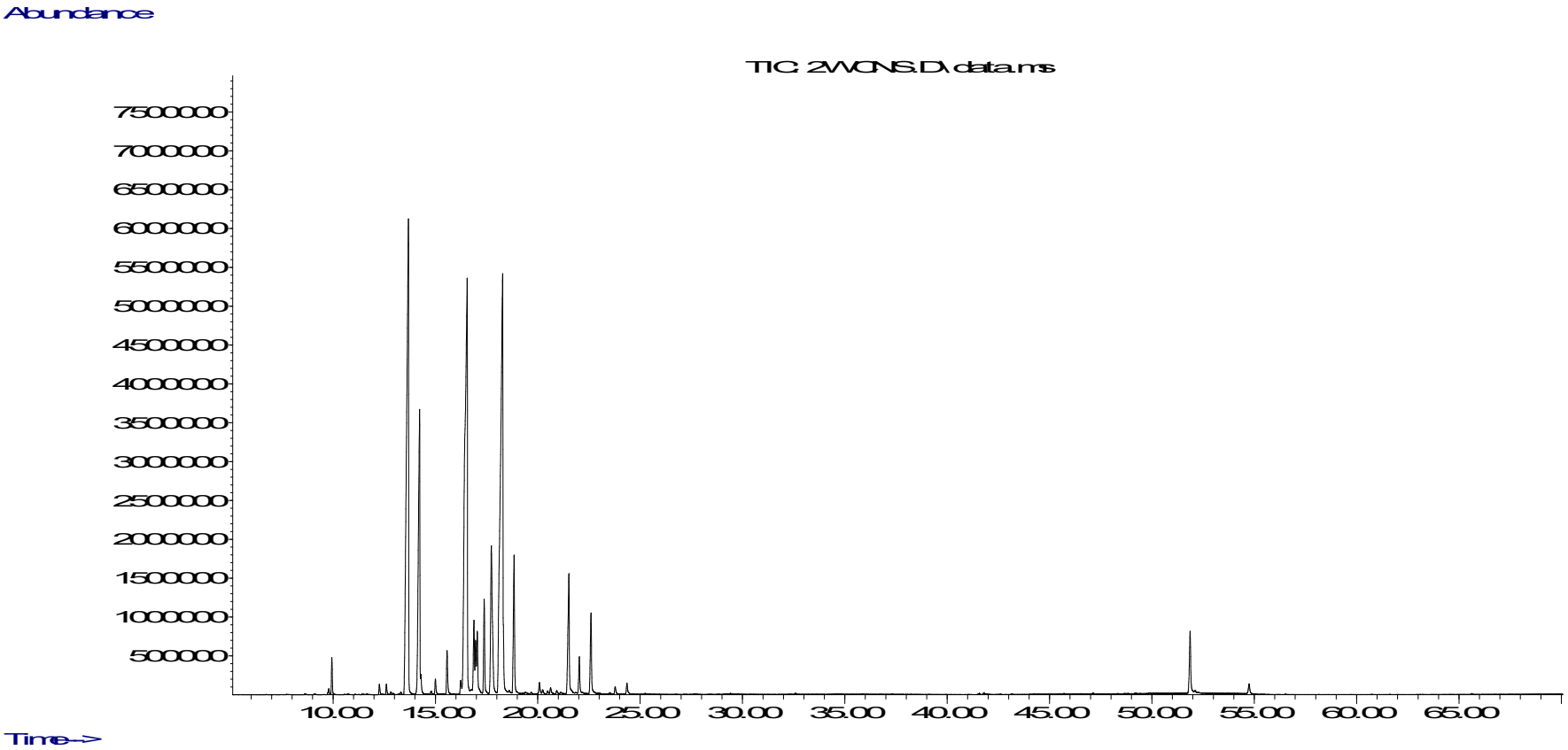


Figure 15. GC-MS chromatogram of essential oil from stems of *C. nervatus* collected in Western Sudan in February 2015 (sample WS-2)

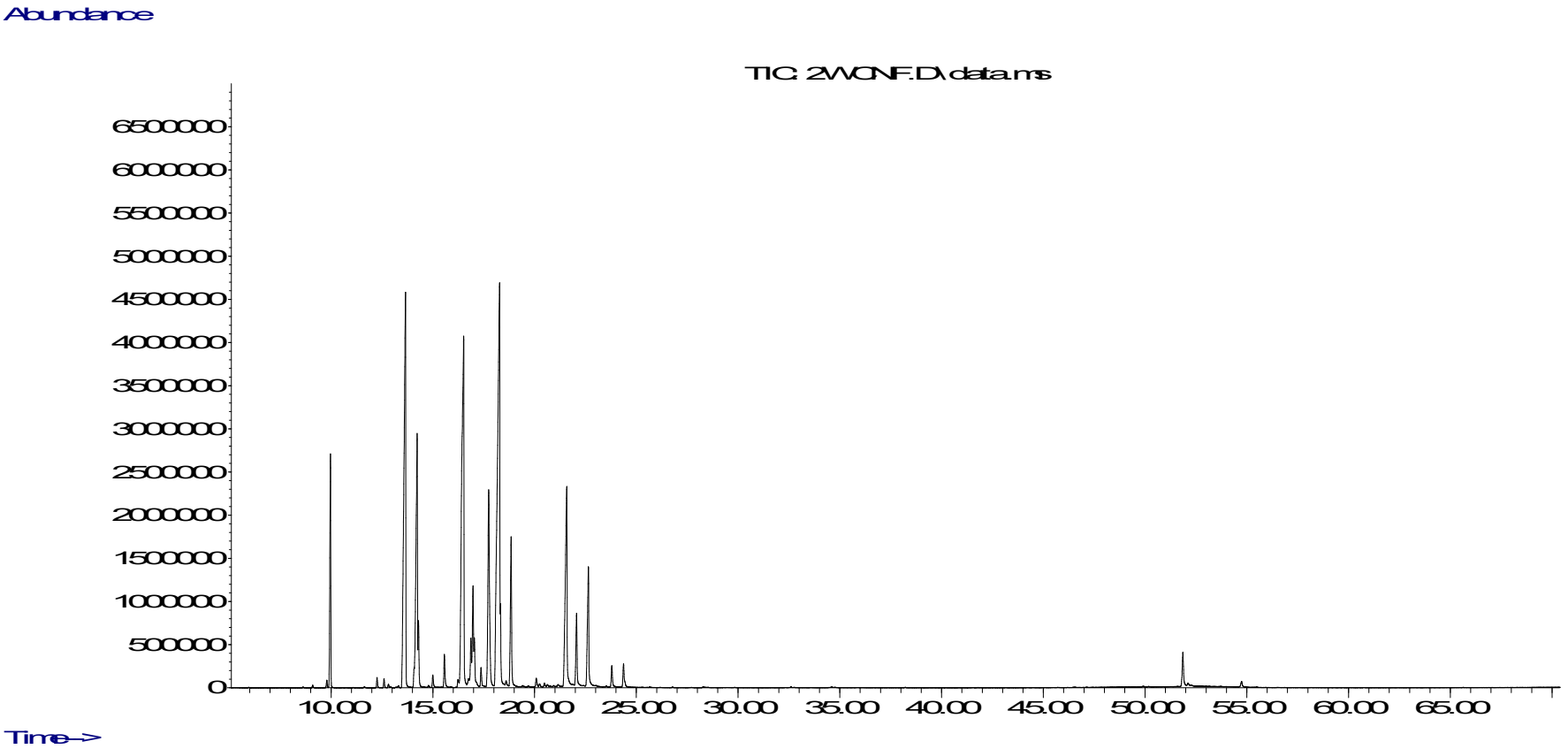


Figure 16. GC-MS chromatogram of essential oil from inflorescence of *C. nervatus* collected in Western Sudan in February 2015 (sample WF-2)

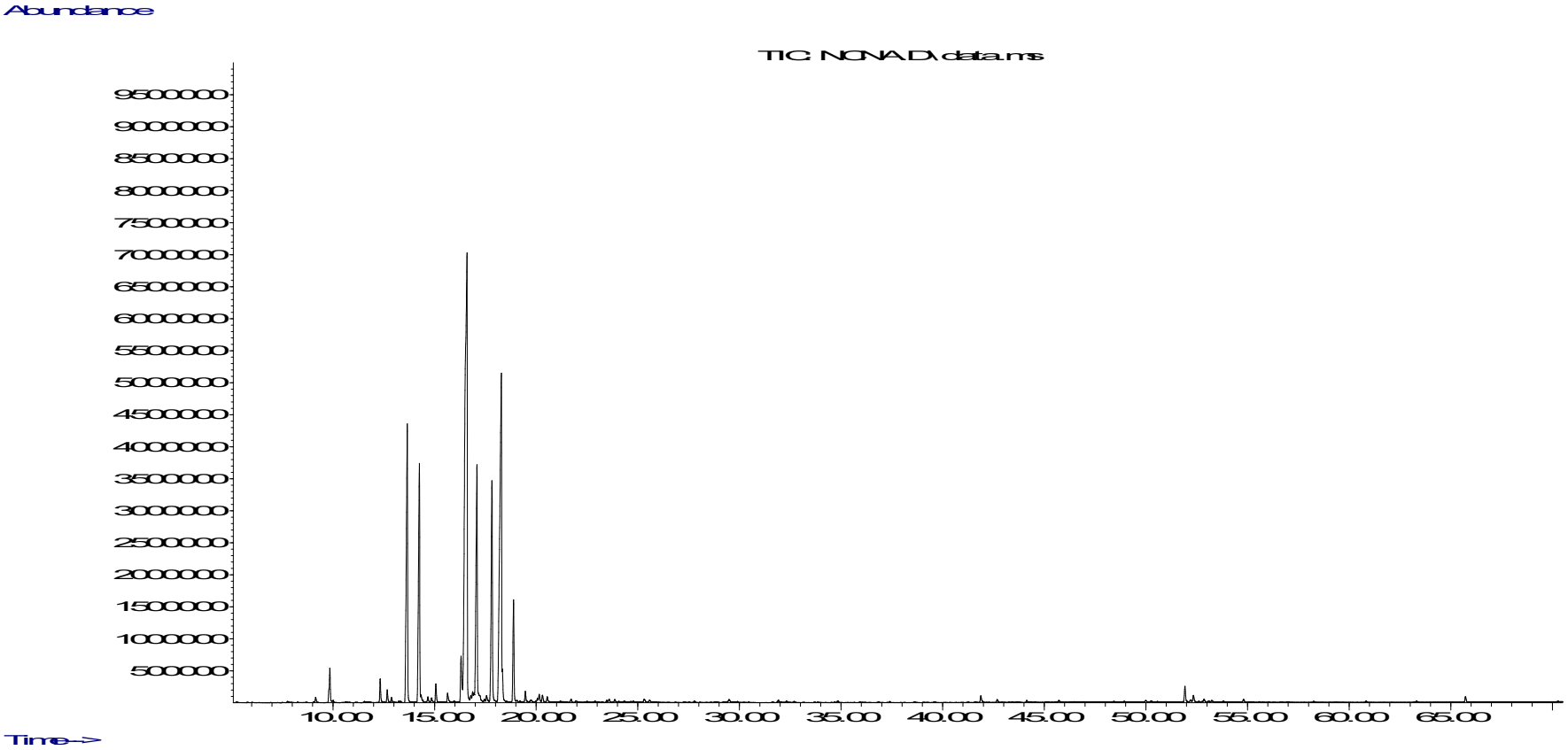


Figure 17. GC-MS chromatogram of essential oil from stems of *C. nervatus* collected in Eastern Sudan in May 2014 (sample ES-1)

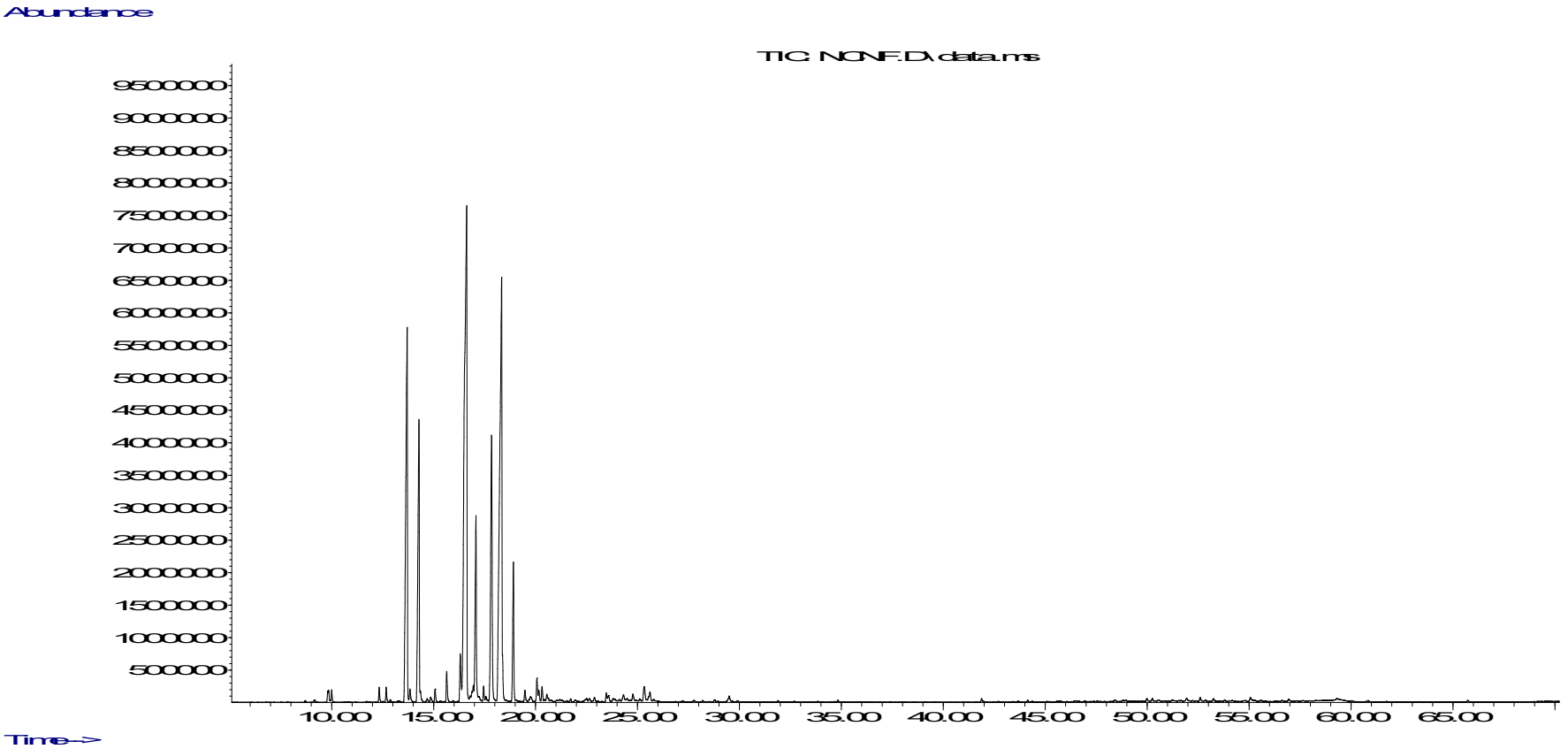


Figure 18. GC-MS chromatogram of essential oil from inflorescence of *C. nervatus* collected in Eastern Sudan in May 2014 (sample EF-1)

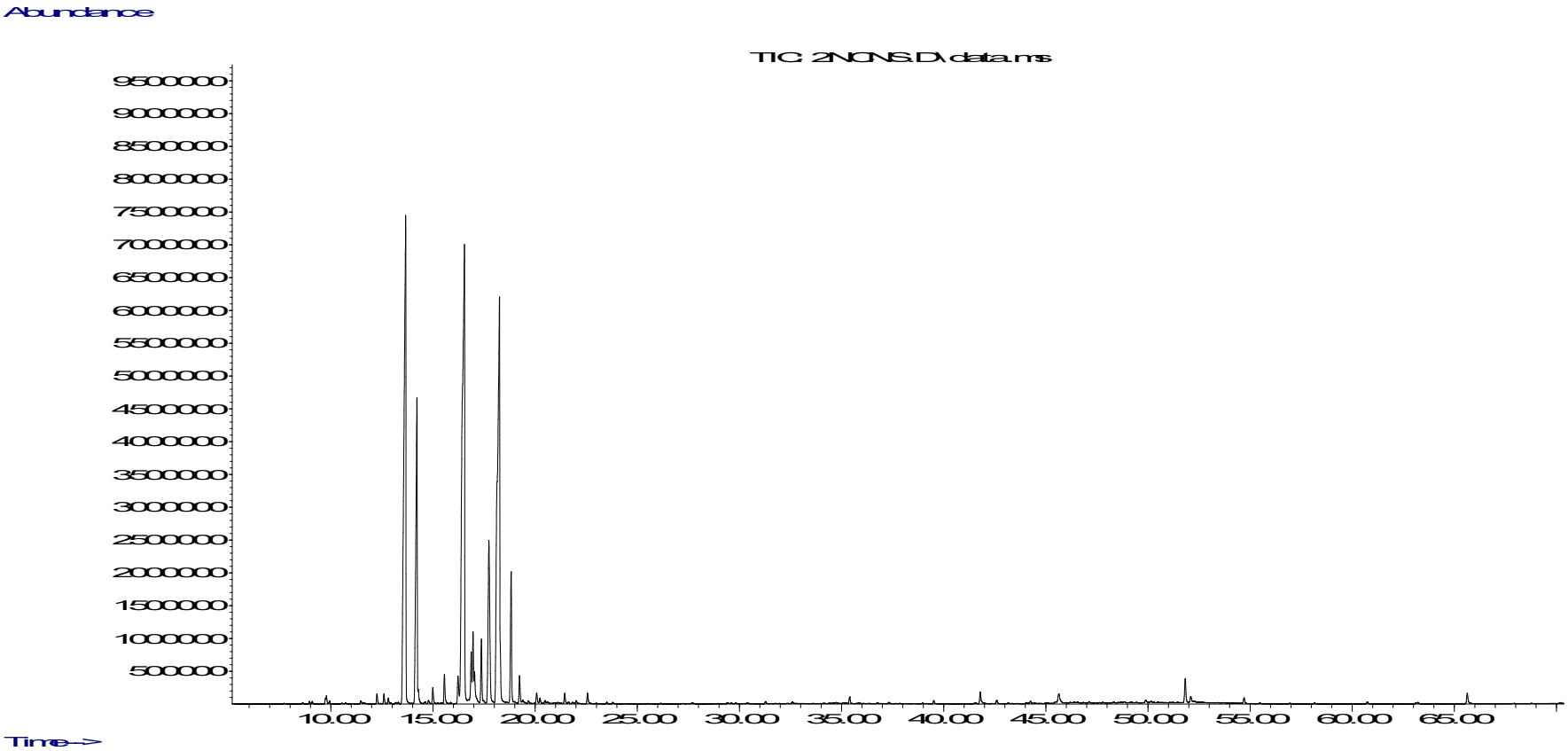


Figure 19. GC-MS chromatogram of essential oil from stems of *C. nervatus* collected in Eastern Sudan in February 2015 (sample ES-2)

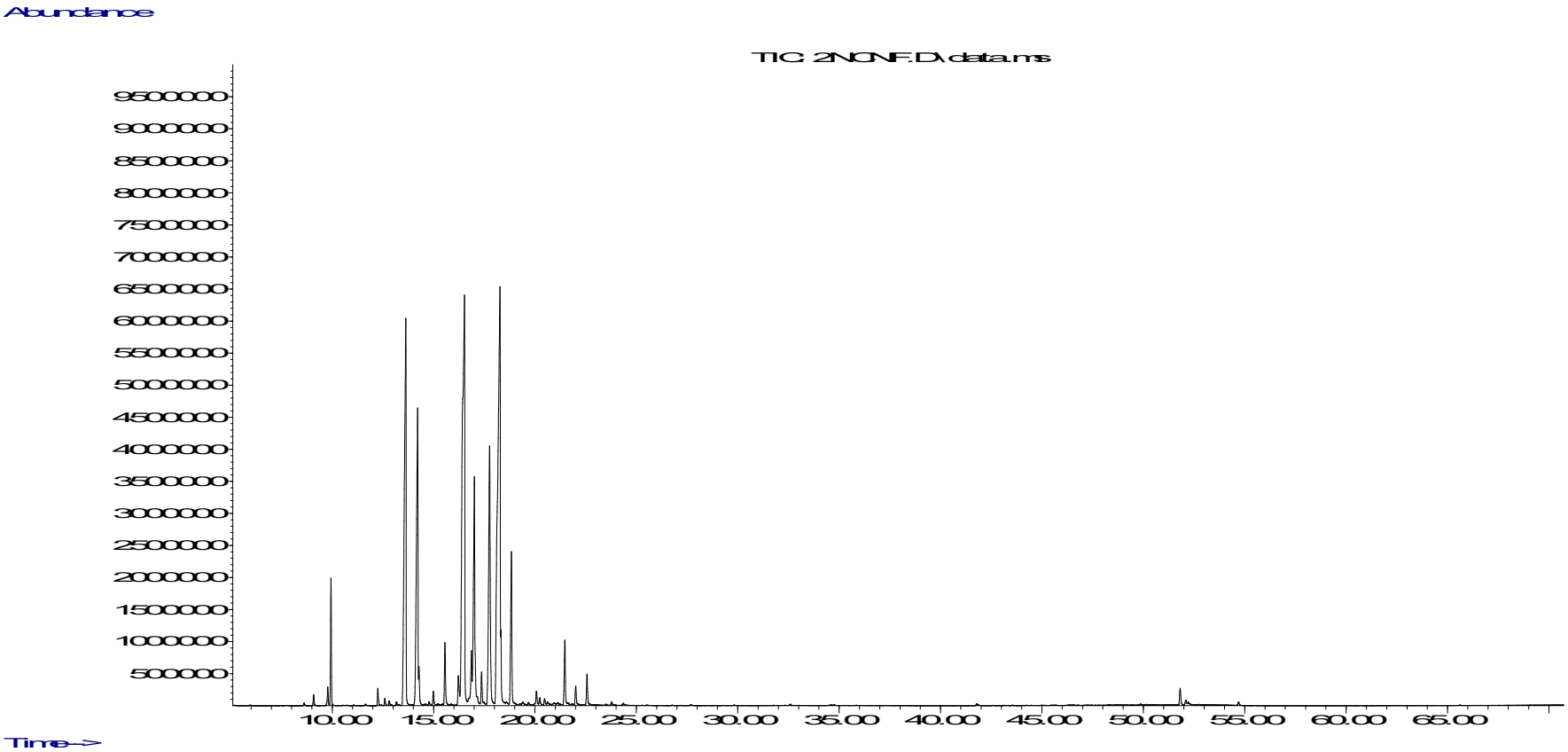


Figure 20. GC-MS chromatogram of essential oil from inflorescence of *C. nervatus* collected in Eastern Sudan in February 2015 (sample EF-2)

1.2. Essential oils of *C. schoenanthus*

The essential oils were isolated from stems and inflorescence of cultivated *C. schoenanthus,* collected from the experimental field of The Medicinal & Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan, in November 2013 and in February 2015 (Table 6).

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 6). The obtained essential oils were fragrant and yellowish.

The results of GC and GC-MS analyses of essential oils are summarized in Table 7. The GC chromatograms of analysed essential oils are given in Figures 21-24.

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 originated from different periods of plant material collection, were similar in qualitative composition with some quantitative differences. All investigated essential oils were characterised by high content of oxygenated monoterpenes (50.8-75.5%).

The most 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).

Table 6. Samples and oil yield of *C. schoenanthus* essential oils

Origin	**Collection period**	**Plant part**	**Sample**	**Oil Yield [% *(v/w)*]**
Khartoum state (Shambat)	November 2013	stems	CS-1	0.6
		inflorescence	CI-1	2.0
	February 2015	stems	CS- 2	0.2
		inflorescence	CI- 2	1.9

The results of our study are in agreement with previous investigations on the *C. schoenanthus* oils of different origin. The leaf essential oils of *C. schoenanthus* from Benin (Kpoviessi et al., 2014), Burkina Faso (Yentéma et al., 2007) and Togo (Ketoh et al., 2005) were also dominated by 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 analysed in this study distinquish from the previously analyzed oils by the presence of sesquiterpene intermedeol (6.1-17.3%) which was not detected in previously analyzed oils.

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 (Avoseh et al., 2015), *C. jawarancusa* (Jones) Schultz (79.0%) from India (Dhar et al., 1981).

Piperitone is an important raw material for conversion into menthol and thymol (Dhar et al., 1981) which are well known for its medicinal properties as well as flavouring agents.

The insecticidal activity of the crude essential oil extracted from *C. schoenanthus* and its main constituent, piperitone, was assessed in different developmental stages of *Callosobruchus maculatus*. Piperitone was more toxic to adults with a LC50 value of 1.6 µl/l vs. 2.7 µl/l obtained with essential oil. Also, piperitone inhibited the development of newly laid eggs and of neonate larvae, but was less toxic than the crude extract to individuals developing inside the seeds. (Ketoh et al., 2006).

Table 7. Chemical composition of essential oils from stem and inflorescence of *C. schoenanthus* cultivated in Khartoum, Sudan

		November 2013		February 2015	
Compound	**KI exp**[a]	**Stems CS-1 (%)**[b]	**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 [c]	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

Table 7. Chemical composition of essential oils from stem and inflorescence of *C. schoenanthus* cultivated in Khartoum, Sudan (continued)

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

Table 7. Chemical composition of essential oils from stem and inflorescence of *C. schoenanthus* cultivated in Khartoum, Sudan (continued)

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
Bulnesol	1672	tr	tr	0.5	0.7
α-Bisabolol	1688	tr	tr	tr	tr

Table 7. Chemical composition of essential oils from stem and inflorescence of *C. schoenanthus* cultivated in Khartoum, Sudan (continued)

Hexadecanoic acid	1962	-	-	0.4	-
Methyl linoleate	2098	-	0.2	-	-
Oleic acid	2144	-	0.7	-	-
Grouped components					
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
Identified		99.4	98.2	97.8	98.5

[a] KI exp- Relative retention indices calculated against *n*-alkane on HP-5MS column.

[b] %, Relative area percentage.

[c] tr, trace (< 0.05 %).

KS-1, essential oil from stems collected in November 2013 from Khartoum

KF-1, essential oil from inflorescence collected in November 2013 from Khartoum

KS-2, essential oil from stems collected in February 2015 from Khartoum

KF-2, essential oil from inflorescence collected in February 2015 from Khartoum

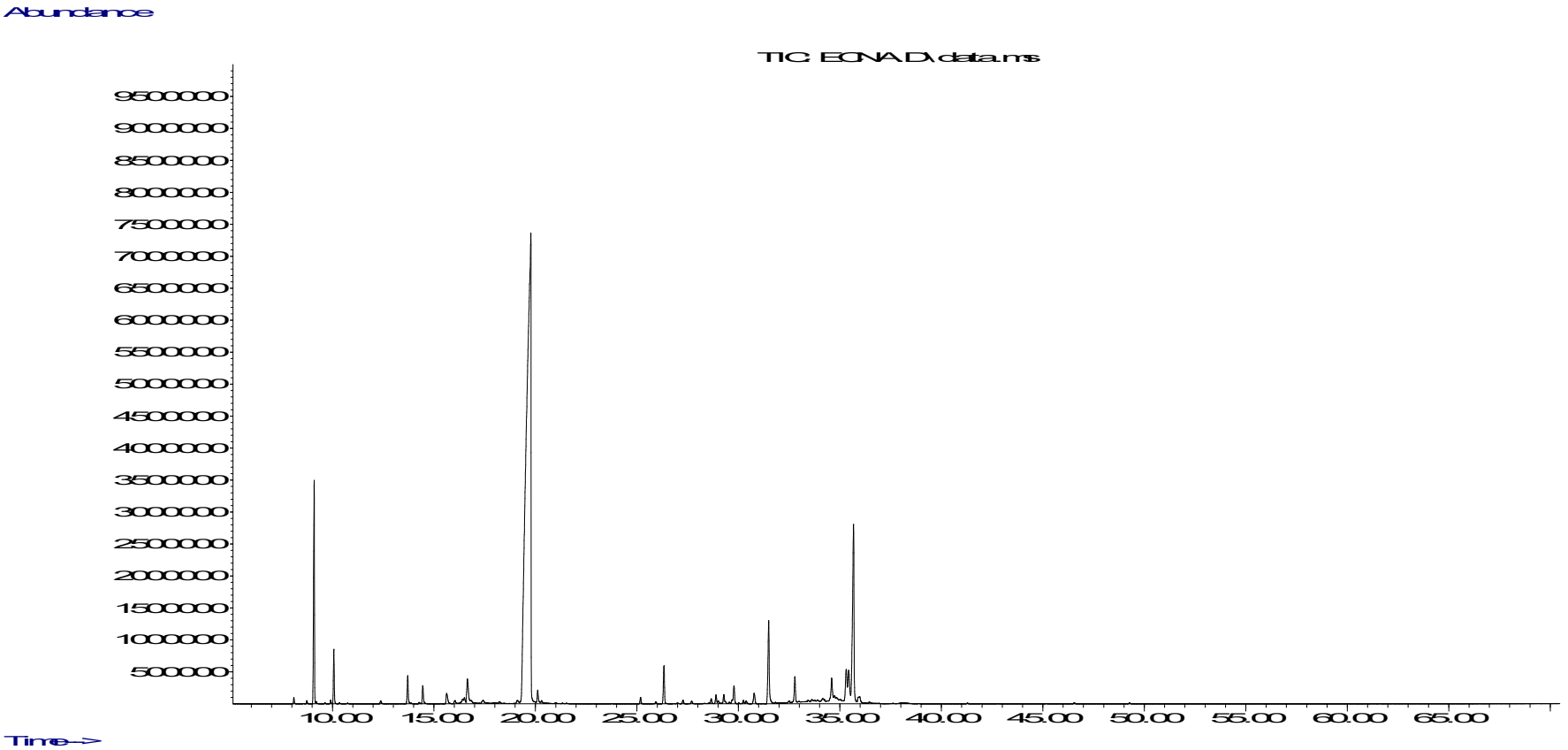


Figure 21. GC-MS chromatogram of essential oil from stems of *C. schoenanthus* collected in November 2013 (sample CS-1)

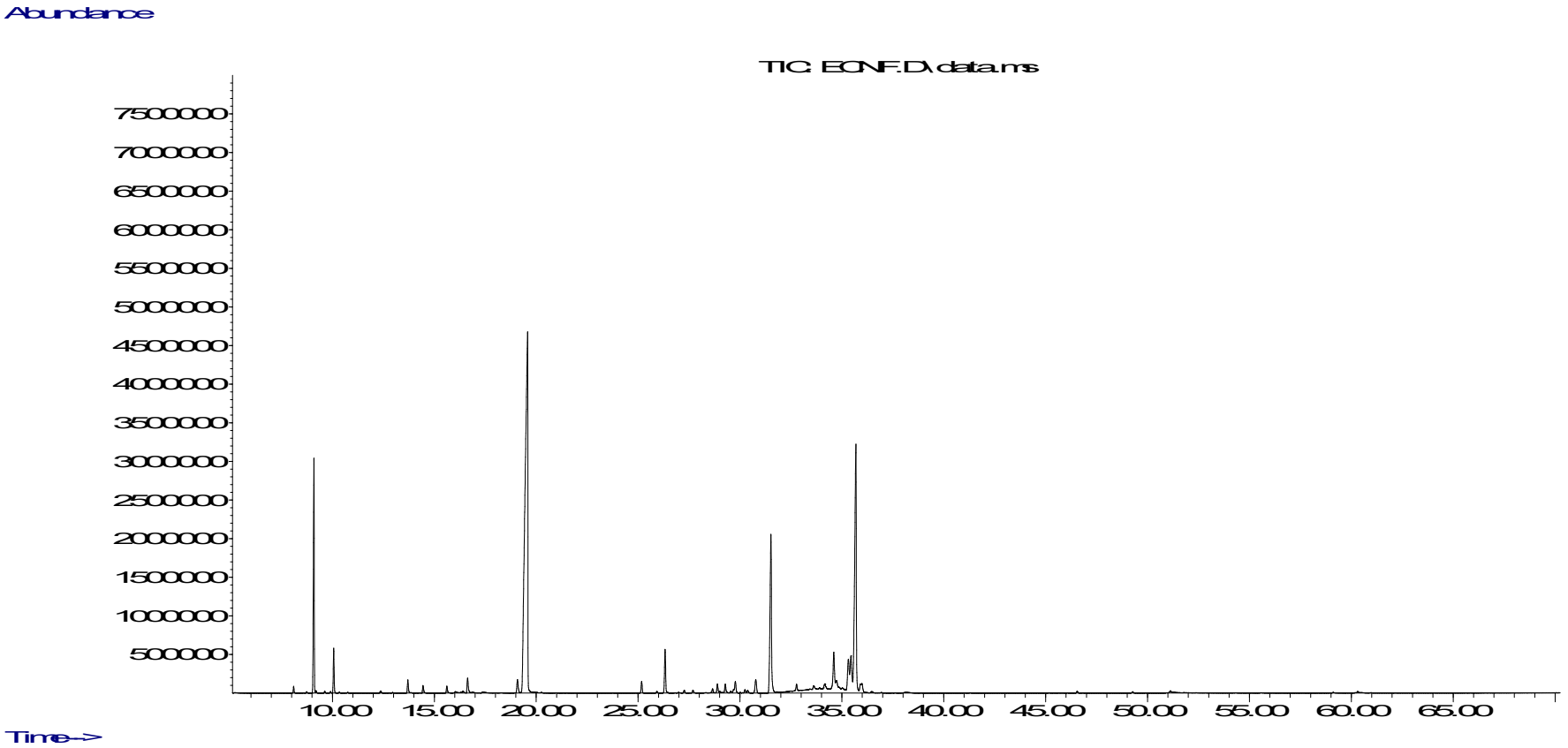


Figure 22. GC-MS chromatogram of essential oil from inflorescence of *C. schoenanthus* collected in November 2013 (sample CI-1)

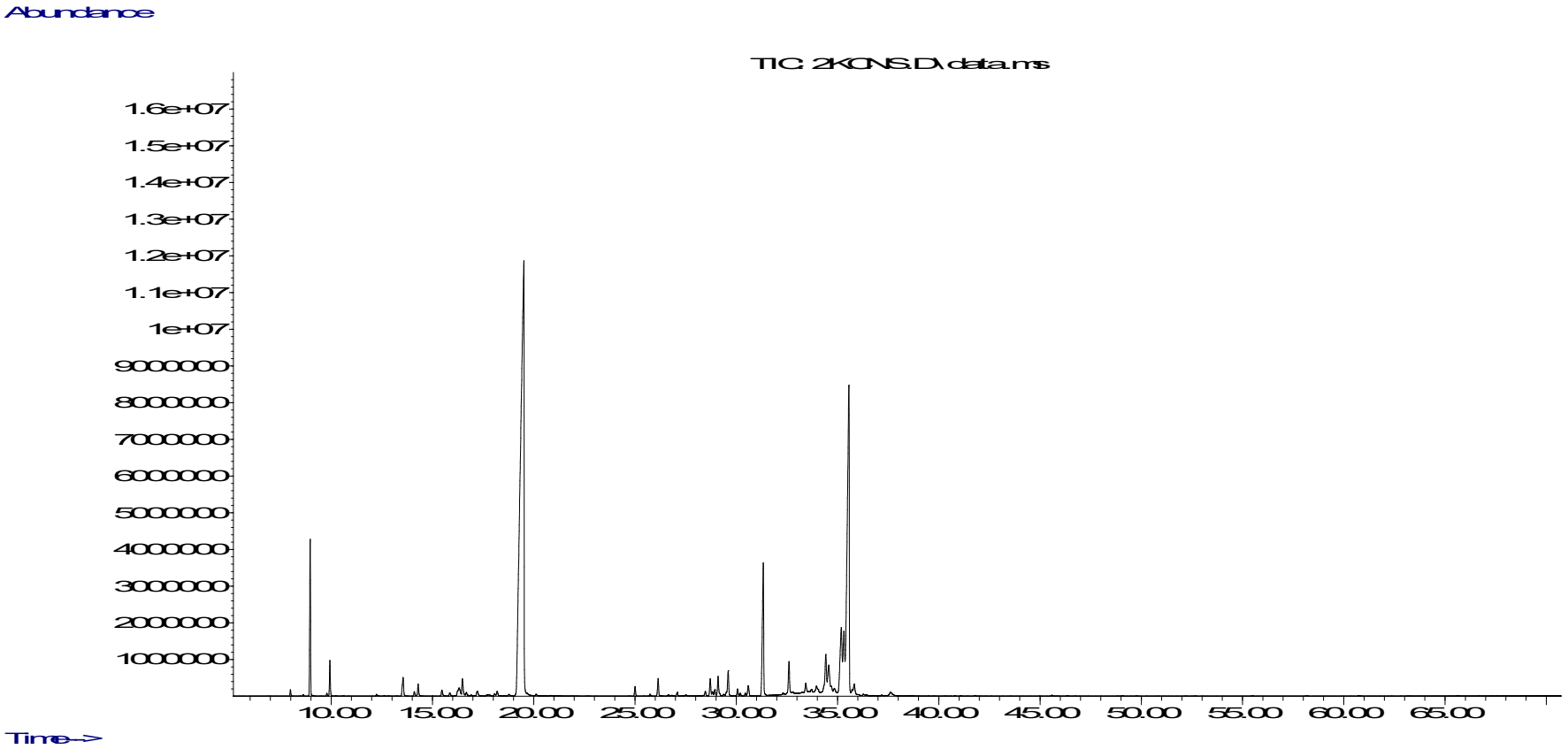


Figure 23. GC-MS chromatogram of essential oil from stems of *C. schoenanthus* collected in February 2015 (sample CS-2)

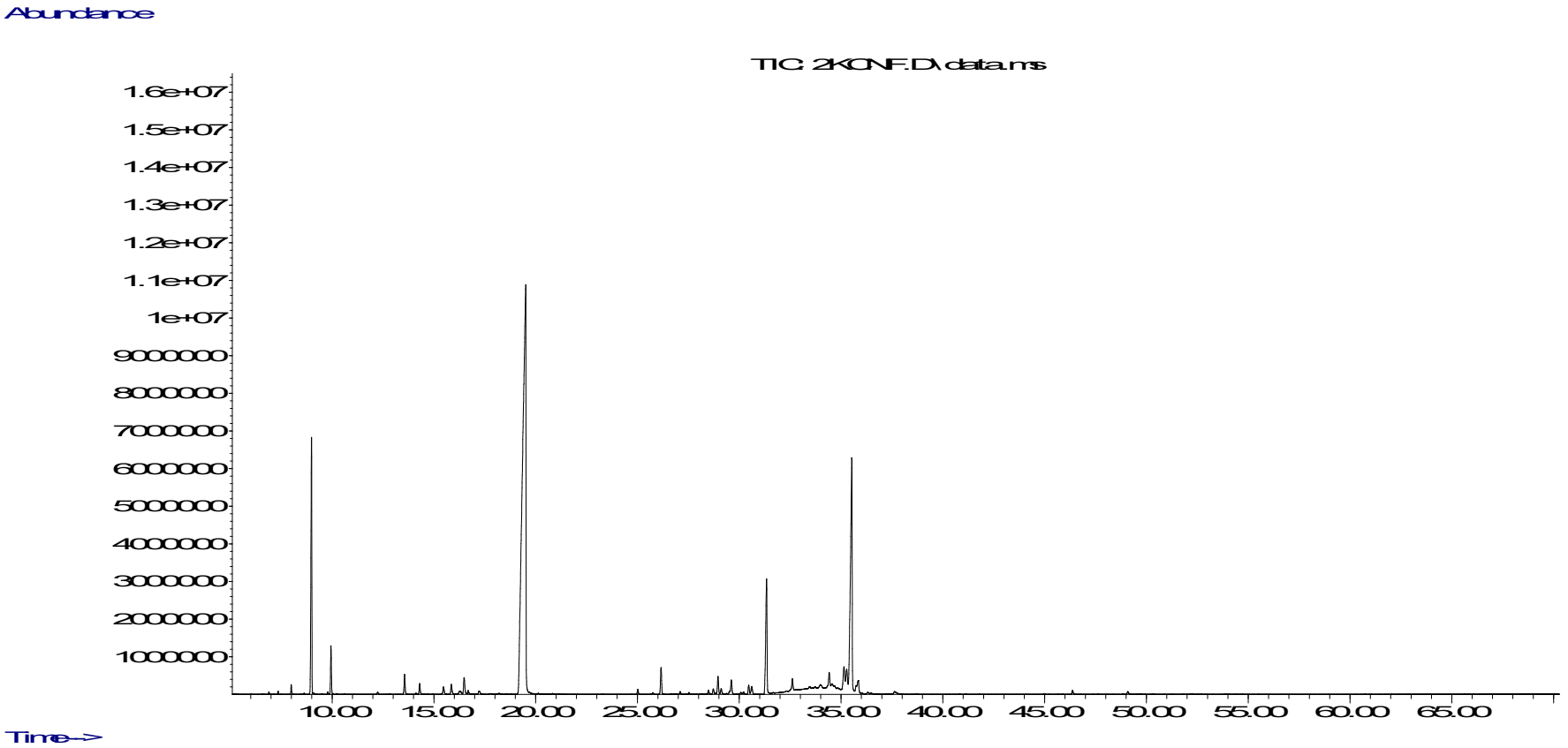


Figure 24. GC-MS chromatogram of essential oil from inflorescence of *C. schoenanthus* collected in February 2015 (sample CI-2)

2. Antimicrobial activity of essential oils

2.1 Antimicrobial activity of essential oils of *C. nervatus*

The antimicrobial activity of the essential oils of *C. nervatus* were tested using broth microdilution method against six standard strains of bacteria (*S. aureus, S. epidermidis*, *E. faecalis, E. coli*, *K. pneumonia*, *P. aeruginosa)* and two strains of fungi *C. albicans*. The results of antimicrobial activity are presented in Table 8.

Similar antimicrobial profile was observed for all the tested samples of essential oils of *C. nervatus* (*i.e.* the oils of stems and inflorescence originating from plants collected in eastern and western Sudan), which could be explained by the similar qualitative and quantitative composition of essential oils.

All the oils were active against the strains of *S. aureus* (MIC 857 – 1060 µg/ml), *S. epidermidis* (MIC 783 – 1060 µg/ml) and against both tested *C. albicans* strains (MIC 783-907 µg/ml). Other tested microorganisms, i.e. *E. faecalis* and all strains of Gram (-) bacteria, were not susceptible to *C. nervatus* essential oils in the tested concentrations.

Antistaphylococcal potential differed in respect to the plant part as well as the geographical origin: stem oil from Western Sudan was more active than the corresponding inflorescence oil, whereas Eastern Sudan inflorescence oil exhibited better activity than the stem oil. On the other hand, inflorescence oil of Western Sudan plants and stem oil of Eastern Sudan plants showed better anticandidal activity than the other oils.

However, the overall antimicrobial activity of *C. nervatus* oils was weak since relatively high concentrations were necessary for the growth inhibition of susceptible organisms. It is generally accepted that antimicrobial activity with MIC values higher than 100 µg/ml should not be considered as significant (Rios and Recio, 2005).

Table 8. The results of antimicrobial activity of essential oils of *C. nervatus* and standard antibiotics

Microorganism	Minimal inhibitory concentrations MIC (µg/ml)						
	C. nervatus				**Antibiotics**		
	Western Sudan, May 2014		**Eastern Sudan, May 2014**				
	Stems	**Inflorescence**	**Stems**	**Inflorescence**	**Ampicilin**	**Amikacin**	**Nystatin**
	WS-1	**WF-1**	**ES-1**	**EF-1**			
S. aureus ATCC 25923	857	1020	1060	992	1.0	2.0	nt
S. epidermidis ATCC 12228	783	931	1060	907	0.25	nt	nt
E. faecalis ATCC 29212	>591	>639	>700	>636	0.9	nt	nt
E. coli ATCC 25922	>591	>639	>700	>636	3.6	7.4	nt
K. pneumoniae NCIMB 9111	>591	>639	>700	>636	4.2	7.3	nt
P. aeruginosa ATCC 27853	>591	>639	>700	>636	3.0	2.0	nt
C. albicans ATCC 10259	783	639	700	907	nt	nt	3.8
C. albicans ATCC 24433	783	639	700	907	nt	nt	6.1

2.2. Antimicrobial activity of essential oils of *C. schoenathus*

The antimicrobial activity of the essential oils of *C. schoenathus* was tested using broth microdilution method against six standard strains of bacteria (*S. aureus, S. epidermidis*, *E. faecalis, E. coli*, *K. pneumonia*, *P. aeruginosa)* and two strains of fungi *C. albicans*. The results of antimicrobial activity are presented in Table 9.

The susceptibility of tested microorganisms to the essential oils of stems and inflorescences of C. *schoenanthus* were similar. The observed antimicrobial spectrum was similar to that of *C. nervatus* oils, the oils being active against the strains of *S. aureus*, *S. epidermidis* and *C. albicans* and without inhibition of growth of Gram (-) bacteria in tested concentrations. Furthermore, it can be noticed that stem oil acted stronger than the inflorescence oil.

Considering the above mentioned recommendations regarding preferred MIC values (bellow 100 μg/ml), the overall antimicrobial activity of *C. nervatus* and *C. schoenathus* oils, tested in this study, was weak.

In the current study lack of the sensitivity of Gram-negative bacteria to the presence of *C. nervatus* and *C. schoenantus* EOs could be observed. Probably the main reason for this difference in sensitivity is the composition of the cell wall and outer membrane arrangement (Kalemba and Kunicka, 2003). In Gram-positive bacteria the wall is mainly composed of peptidoglycan, which forms a thick, fibrous layer. The action of essential oils against Gram-positive bacteria and fungi appears to be similar. The oil components destroy the bacterial and fungal cell wall and cytoplasmic membrane, causing a leakage of cytoplasm and coagulation. They also inhibit the synthesis of DNA, RNA, proteins, and polysaccharides in fungal and bacterial cells (Himejima and Kubo 1993; Zani et al. 1991).

On the other hand, due to presence of hydrophilic lipopolysaccharide in the cell envelope, Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *Klebsiella* sp.) are resistant to a wide variety of essential oils and their components which are mainly hydrophobic. Thus, essential oil constituents are unable to penetrate the membrane barrier (Khunkitti, 2010).

Table 9. The results of antimicrobial activity of essential oils of *C. schoenathus* and standard antibiotics

	Minimal inhibitory concentrations MIC (µg/ml)				
	C. schoenanthus				
	November 2013		**Antibiotics**		
Microorganism	Stems	Inflorescence	Ampicilin	Amikacin	Nystatin
	CS-1	CI-1			
S. aureus ATCC 25923	970	1300	1.0	2.0	nt
S. epidermidis ATCC 12228	970	1300	0.25	nt	nt
E. faecalis ATCC 29212	>591	>633	0.9	nt	nt
E. coli ATCC 25922	>591	>633	3.6	7.4	nt
K. pneumoniae NCIMB 9111	>591	>633	4.2	7.3	nt
P. aeruginosa ATCC 27853	>591	>633	3.0	2.0	nt
C. albicans ATCC 10259	771	1314	nt	nt	3.8
C. albicans ATCC 24433	771	857	nt	nt	6.1

Previosly, essential oils of a number *Cymbopogon* species were tested for *in vitro* antimicrobial activity against different microorganisms and by various methods. Having in mind that many factors affect the results of testing microbial susceptibility to essential oils (solubility of oil, presence of solubilizers, vehicles, method and conditions of testing) the reports available in the literature are rarely directly comparable (Janssen et al., 1987).

Previously, the essential oil of *C. nervatus* inflorescence from Sudan was investigated for antimicrobial activity against twelve bacterial strains including *Staphylococus aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa, Shigella dysenteriae* and *Klebsiella pneumoniae* by agar well-diffusion method (El-Kamali et al., 2005). The obtained zones of inhibition suggested that the most sensitive were *Shigella dysentriae* and *Klebsiella pneumoniae* and that oil was inactive against *Salmonella typhi*, whereas our oil was effective only against strains of *S. aureus* and *S. epidermidis*. However, no direct comparison could be made bearing in mind different testing methods.

The essential oil of C. *schoenanthus*, originated from Algeria inhibited growth of *Candida albicans*, and the bacteria *Escherichia coli, Salmonella typhimurium, Staphylococus aureus, Enterococcus feacium and Streptococcus agalactiae* in variable degree in the agar diffusion method (Hellali et al., 2016). The obtained results could not be compared with our results of antimicrobial activity for *C. schoenanthus* essential oils originated from Sudan, due to different methodology used.

The essential oil of *C. schoenanthus* from Tunisia was evaluated for antimicrobial activity against the pathogenic strains of gram positive (*B. cereus, B. subtilis, E. faecium, E. faecalis, L. monocytogens*, *S. aureus*,) and gram negative bacteria (*A. hydrophila, E. coli, E. coli O157:H7, P. aeruginosae, S. typhimurium, K. pneumoniae, P. mirabilis*). The oil was inactive against *P. aeruginosa*. The minimum inhibiting concentrations of oil for Gram negative bacteria ranged from 112 to 182 mg/ml and 108 to 217 mg/ml for the Gram positive bacteria, indicating weak antimicrobial activity (Khadri et al., 2011).

The essential oil of *C. giganteus*, which was characterized by the presence of some compounds (*cis-p*-mentha-2,8-dien-1-ol (21.3%), 1,3,8-p-menthatriene (17,8%), *trans-p*-mentha-1(7),8-dien-2-ol (16.2%), and carveol (8,8%)) found in prominent amounts in prsently studied *C. nervatus* oils, exhibited weak antimicrobial activity

against *Escherichia coli* (MIC 1000 μg/ml), *Staphylococcus aureus* and *Moraxella catarrhalis* (MICs 500 μg/ml) and *Enterococcus faecalis* (MIC 250 μg/ml), as well as good activity against *Candida albicans* and *C. tropicalis* (MICs 125 μg/ml) (Ahmad and Viljoen, 2015).

Lemongrass (*Cymbopogon citratus*) essential oil was investigated for antimicrobial activity against some pathogenic bacterias (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) by broth dilution method. The oil was not effective against *Pseudomonas aeruginosa*, whereas MIC values for other microorganisms were in the range of 0.06-0.50% (v/v) (Naik et al., 2010).

Lemongrass (*C. citratus*) oil was active against dermatophytes such as *Trichophyton mentagrophytes, T. rubrum, Epidermophyton floccosum* and *Microsporum gypseum* with MIC values 115-235 μg/ml (Wannissorn et al., 1996).

In another study, essential oil of *C. citratus* with citral as dominant compound (72.6%) exhibited good antimicrobial activity against *Candida tropicalis* (MIC 16 μg/ml), *Escherichia coli* and *Candida albicans* (MICs 63 μg/ml), *Staphylococcus aureus* and *Enterococcus faecalis* (MICs 125 μg/ml) and moderate activity against *Moraxella catarrhalis* (MIC 250 μg/ml. On the other hand, the essential oil of *C. flexuosus*, also with citral as the main component, in the same study demonstrated generally weaker activity (MIC values 125-1000 μg/ml) (Ahmad and Viljoen, 2015).

Ahmad and Viljoen (2015) investigated antimicrobial properties of essential oils of *C. martini*, *C. winterianus* and *C. nardus* by microdillution method against four bacterial and two strains of fungi. The best activity was demonstrated towards *Candida tropicals* (MICs 32-63 μg/ml). Generally, the most active was *C. martini* essential oil (MICs 32-125 μg/ml) with geraniol as the most dominant constituent (79.7%).

The essential oils of *C. citratus*, *C. martini* and *C. nardus* were also the subject of another study on antimicrobial activity against *Staphylococcus aureus*, *Escerichia coli* and *Candida albicans* by broth microdilution method (Hammer et al., 1999). All of the oils were more active against *S. aureus* and *C. albicans* than against *E. coli*. The obtained MIC values were in the range comparable to the MICs obtained for the oils of *C. nervatus* and *C. schoenanthus* towards *S. aureus* and *C. albicans* in this study.

3. Antioxidant activity of essential oils

The antioxidative properties of essential oils of *C. nervatus* and *C. schoenanthus* were estimated by Ferric Reducing Antioxidant Power (FRAP) assay for determination of total antioxidant capacity and by DPPH test for determination of free radical scavenging ability.

3.1. Total antioxidant activity of essential oils (FRAP test)

The total antioxidant capacity of essential oils of inflorescence of *C. nervatus* collected from Western and Eastern Sudan in February 2015, and as well as that of inflorescence essential oils of *C. schoenanthus* from two different periods of collection and that of *C. schoenanthus* stems essential oil was measured through FRAP test. The obtained FRAP values that represent nmol Fe^{2+} per mg of essential oils and referent antioxidant substances (rutin and ascorbic acid) are given in Table 10.

The obtained FRAP values were similar for different essential oils. The total antioxidant capacity of the essential oils was in the range of 2.03- 2.96 (nmol Fe/mg EO) and weaker than the capacity of rutin (5.17 μmol Fe/mg) and ascorbic acid (10.40 μmol Fe/mg) used as the referent antioxidants.

Table 10. The total antioxidant activity of essential oils of *C. nervatus* and *C. schoenanthus* and reference compounds

Species	Plant part	Origin and collection period	Sample	FRAP (nmol Fe/mg EO)
C. nervatus	inflorescence	Western Sudan (Umrwaba), February 2015	WF-2	2.03
	inflorescence	Eastern Sudan (Gadarif), February 2015	EF-2	2.88
C. schoenanthus	Inflorescence	MAPRI November 2013	CI-1	2.96
	Inflorescence	MAPRI February 2015	CI-2	2.49
	Stems	MAPRI November 2013	CS-1	2.72
FRAP values of rutin and ascorbic acid used as positive control				
Rutin		5.17 (µmol Fe/mg)		
Ascorbic acid (vitamin C)		10.40 (µmol Fe/mg)		

3.2. DPPH radical scavenging activity

In the DPPH radical scavenging assay, essential oil from inflorescence of *C. nervatus* collected in Western Sudan in February 2015 (sample WF-2) showed moderate activity with SC_{50} value of 23.8 µl/ml.

Scavenging activity of essential oils from inflorescence of *C. schoenanthus*, collected in two periods (samples CI-1 and CI-2), measured by the DPPH test and represented by percentage (%) of inhibition was 62.8% for sample CI-1 and 65.1% for sample CI-2, when using 40 µl of essential oil per mL of test solution. In comparison with reference substances (rutin and ascorbic acid) the essential oils exhibited a significantly weaker activity.

The results given in the literature suggest that essential oils from some *Cymbopogon* species have also a weak to moderate antioxidant activity in the FRAP and DPPH assay as well as some other antioxidant tests used.

The essential oil from leaves of *C. giganteus* of Benin, which was characterized by the presence of menthadienols as well as essential oils of *C. nervatus* from Sudan, showed weak antiradical DPPH activity (SC_{50} 1.18 g/l) in comparison to commercial antioxidant butylated hydroxytoluene (BHT) (SC_{50} 8.8 mg/l) (Alitonou et al., 2006).

The essential oil of *C. schoenanthus* from Algeria, with similar major constituents as the oil investigated in this study (piperitone (63.35%), β-eudesmol (9.305%) and elemol (6.915%)) was tested for radical-scavenging ability using the DPPH radical, the 2,2'-azino-bis (ABTS) radical and for reducing power ability with a test based on the reduction of ferric cations (FRAP). In all tests, the essential oil did not show a prominent antioxidant activity (Hellali et al., 2016).

On the other hand, the antioxidant capacity of the essential oils of *Cymbopogon schoenanthus* (L.) Spreng. ssp. *laniger* from Tunisia determined by DPPH radical scavenging method, was better compared to that of our samples of *C. schoenanthus*, which could be explained by different chemical composition of the oils. Namely, in the volatile oils of *Cymbopogon schoenanthus* (L.) Spreng. ssp. *laniger* from Tunisia The major components were limonene (10.5-27.3%), β-phellandrene (8.2–16.3%),

δ-terpinene (4.3-21.2%) and α-terpineol (6.8-11.0%), whereas in our samples those were was piperitone (47.7-71.5%), intermedeol (6.1-17.3%) and *δ*-2-carene (4.5-10.0%) (Khadri et al., 2008).

The essential oil of *C. proximus* with piperitone as the main component exhibited weak DPPH radical scavenging activity (SC_{50} 998.47 µg/ml) in comparison with BHT (SC_{50} 17.58 µg/ml) and ascorbic acid (SC_{50} 14.7 µg/ml) as standard antioxidant reagents (Selim, 2011).

In the previous investigation, *C. citratus* essential oil showed also a lower anti-DPPH activity than BHT used as positive control (Adesegun et al., 2013).

The essential oils from leaves of *C. nardus* (Citronella grass) and *C. citratus* (Lemongrass), which were dominated by geraniol and citral, respectively, exhibited low antioxidant activity in DPPH test. as compared to α-tocopherol standard (Jumepaeng et al., 2013).

4. Spasmolytic activity of essential oils

The essential oil of *C. nervatus* inflorescence from Western Sudan, collected in February 2015 (sample WF-2) and essential oil from inflorescence of *C. schoenanthus*, collected in February 2015 (sample CI-2) were tested for spasmolytic activity using three different experimental models, i.e. against spontaneous contractions, contractions induced with acetylcholine (ACh) and contractions induced with potassium chloride (KCl).

4.1. Spasmolytic activity of essential oil of *C. nervatus*

Essential oil of *C. nervatus* inflorescence from Western Sudan, collected in Febrauary 2015 (sample WF-2) exhibited strong, significant and concentration-dependent antispasmodic activity in all experimental models.

Spasmolytic activity against spontaneous contractions

Essential oil (10-200 μg/ml) dose-dependently relaxed spontaneous contractions of isolated ileum and in concentration of 200 μg/ml exhibited 88.43% of maximal relaxant effect of atropine (6,4 μM). The results are presented in Figure 25.

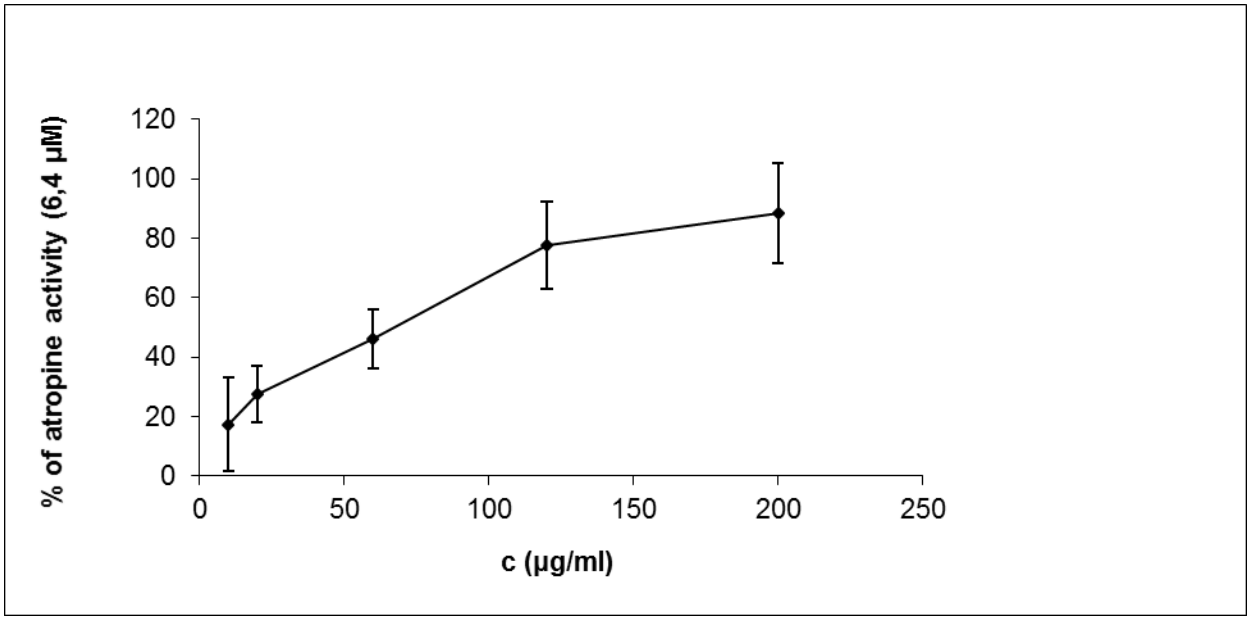


Figure 25. Effect of the essential oil WF-2 on spontaneous contractions of the isolated rat ileum compared with atropine. Activity is presented as % of maximal spasmolytic effect achieved with atropine (4.44 µg/ml). The concentrations presented on abscissa scale are the final cumulative concentrations and each point represents mean ± S.E.M. of six or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*$p < 0.05$).

Spasmolytic activity against contractions induced with Ach

Essential oil exhibited dose-dependent effect on contractions induced with ACh as well. The effect of oil was investigated in three concentrations: 30, 60 and 90 µg/ml. In concentration of 30 µg/ml oil exhibited no effect (results not shown). In concentration of 60 µg/ml oil exhibited dose-dependent and significant spasmolytic activity and lowered maximal effect of ACh to 65.23±22.95%. In concentration of 90 µg/ml oil almost completely abolished contractile effect of ACh and lead to reduction of the effect of the highest concentration of ACh to 34.16±16.16%. The results are shown in Figure 26.

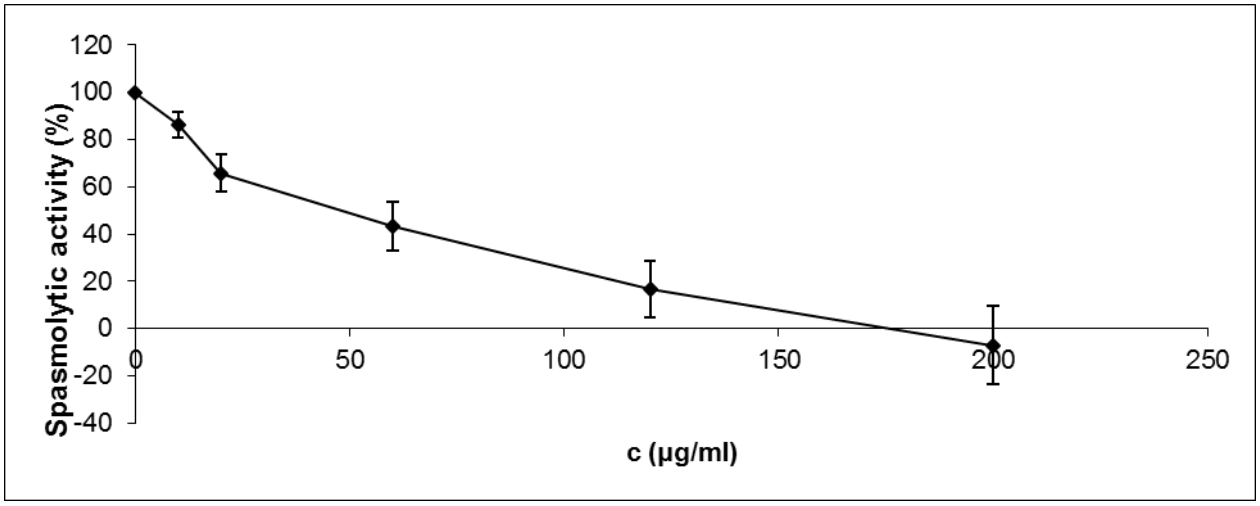


Figure 26. Concentration-response curves of ACh in the absence (rhombus) and presence of 60 µg/ml (square) and 90 µg/ml (triangle) of the essential oil in isolated rat ileum. The values presented on ordinate scale represent responses expressed as % of the maximum response to ACh. Each point represents mean + or - S.E.M. of five or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*$p < 0.05$, **$p < 0.01$).

It was previously demonstrated that the extract of *C. citratus* exhibited inhibitory activity on ACh induced contractions of isolated rabbit and guinea-pig ileum as well. The effective concentrations were higher compared to effective concentrations of tested *C. nervatus* oil, but it should be noted that in these experiments different experimental animals were used (Devi et al., 2011).

Spasmolytic activity against tonic contractions induced with KCl

The oil demonstrated significant, strong and dose-dependent activity against tonic contractions of isolated ileum induced with KCl (80 mM). In highest applied concentration (200 µg/ml) oil completely abolished contractile effect of KCl and the results are presented in Figure 27.

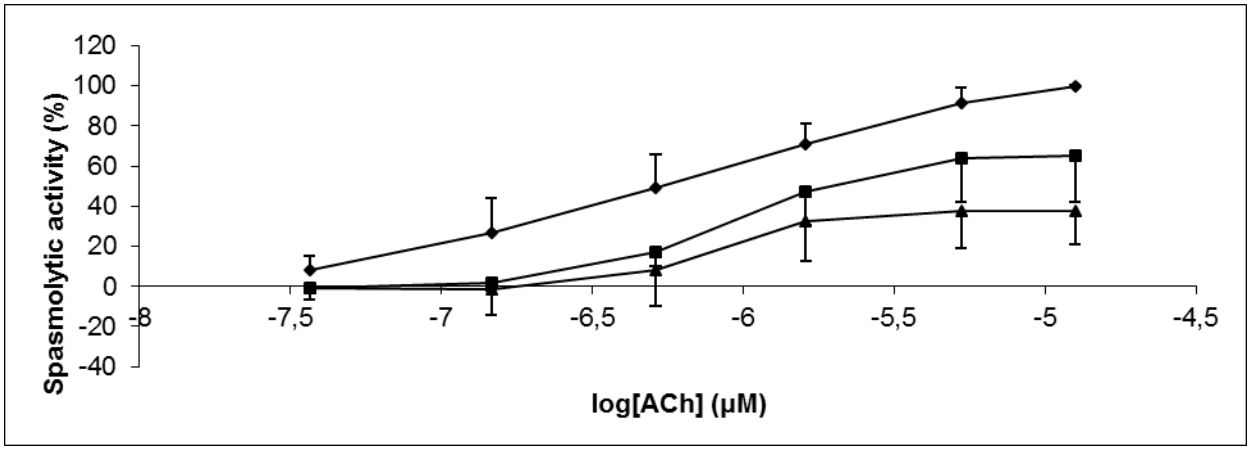


Figure 27. Concentration-related inhibitory effects of the essential oil WF-2 on contraction induced with KCl (80 mM) in isolated rat ileum. Each point represents mean ± S.E.M. of nine experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*$p < 0.05$)

Devi et al. (2011) previously showed that MeOH extract of leaves of *C. citratus* exhibit relaxant acitivity on KCl (80 mM) induced contractions of isolated rabbit ileum. The inhibitory effect was weaker compared with tested essential oil WF-2 ranging from 1.49-43.88% (with significant effects obtained at concentrations of 0.1, 0.3 and 1 mg/ml). However, the different experimental animal was used (Devi et al., 2011).

Limonene, a monoterpene which was present in the tested oil in amount of 5.7%, previously exhibited vasorelaxant activity in isolated rings of rat superior mesenteric artery with functional endothelium pre-contracted with phenylephrine (10 µM) (Cardoso Lima et al., 2012). Spasmolytic activity was also previously demonstrated for some other minor constituents of tested *C. nervatus* oil such as limonene oxides, as well as carvone. These compounds were found to exhibit *in vitro* relaxant effect on KCl induced contractions of isolated guinea-pig ileum (De Sousa et al., 2008). To our knowledge there are no data considering the relaxant activity of *p*-menthadienols, the main constituents of oil WF-2. However, some plants with high amount of these compounds (such as *Artemisia sieberi* Besser) are similarly to *Cymbopogon* species traditionally used as muscle relaxants (Yousefzadeh et al., 2012). We can therefore assume that these metabolites, at least partly, contribute to demonstrated spasmolytic activity of tested essential oil.

4.2. Spasmolytic activity of essential oil of *C. schoenanthus*

Essential oil from inflorescence of *C. schoenanthus* collected in February 2015 (sample CI-2) exhibited strong and concentration-dependent spasmolytic activity.

Spasmolytic activity against spontanous contractions

The oil (10-130 μg/ml) concentration-dependently inhibited spontaneous contractions of isolated rat ileum. The effect was strong and in concentration of 130 μg/ml comparable (105.23±29.56%) to maximal relaxant effect of atropine obtained in concentration of 6.4 μM in previous series of experiments. The results are presented in Figure 28.

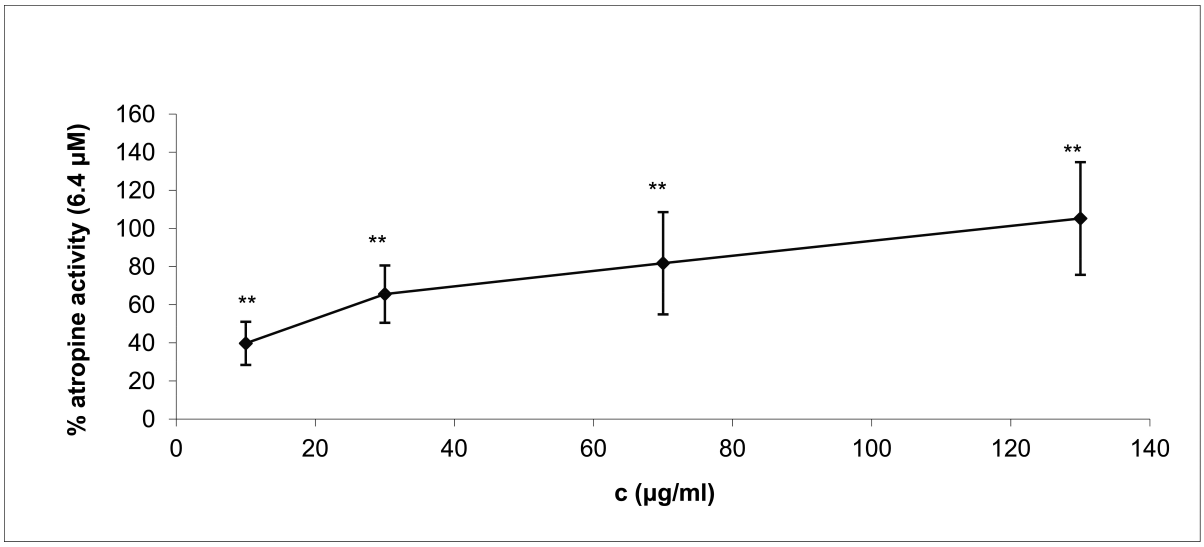


Figure 28. Effect of the essential oil on spontaneous contractions of the isolated rat ileum compared with atropine. Activity is presented as % of maximal spasmolytic effect achieved with atropine at concentration of 6.4 μM (4.44 μg/ml). The concentrations presented on abscissa scale are the final cumulative concentrations and each point represents mean ± S.E.M. of five or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (**$p < 0.01$).

Spasmolytic activity against contractions induced with Ach

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

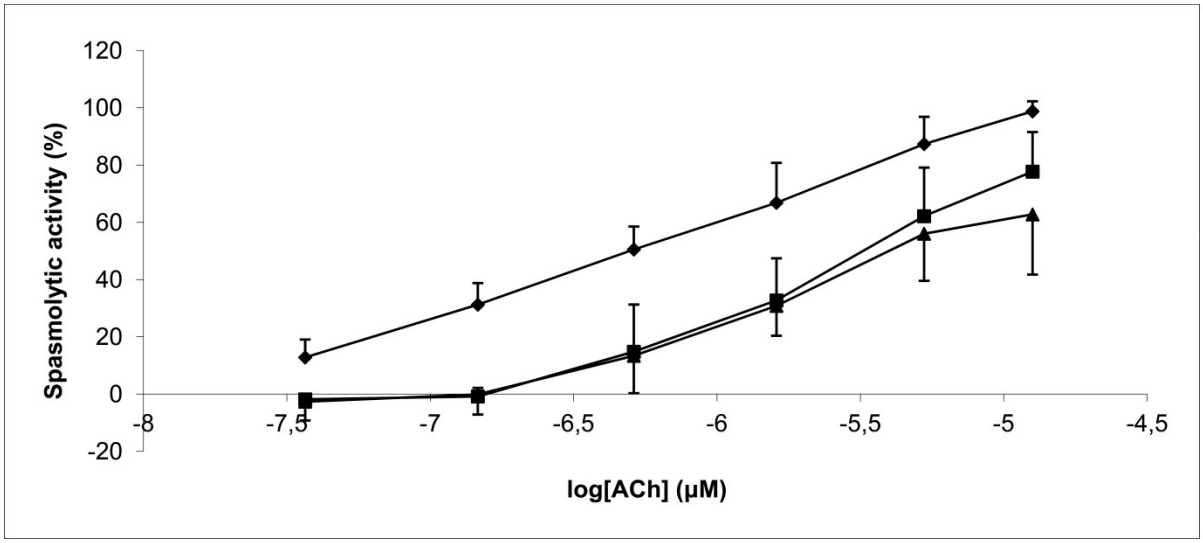


Figure 29. Concentration-response curves of ACh in the absence (rhombus) and presence of 60 µg/ml (square) and 90 µg/ml (triangle) of the essential oil in isolated rat ileum. The values presented on ordinate scale represent responses expressed as % of the maximum response to ACh. Each point represents mean + or - S.E.M. of seven or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*$p < 0.05$, **$p < 0.01$)

In concentration of 90 μg/ml oil exhibited similar activity on lower concentrations of ACh (0.01-0.44 μg/ml), but the effect on contractions induced with higher concentrations was stronger and significant with inhibition of maximal effect of ACh to 62.76±21.00%. In concentration of 120 μg/ml oil exhibited the strongest relaxant activity and completely abolished contractile effect of ACh (data not shown). The results are shown in Figure 29. Atropine, used as a reference drug in this model, exhibited stronger activity and in concentration of of 0.14 μM completely inhibited spasmogenic effect of ACh.

Spasmolytic activity against tonic contractions induced with KCl

The oil demonstrated the strongest activity against tonic contractions induced with KCl (80 mM). In concentration of 30 μg/ml oil inhibited contractile effect of KCl to 19.67±20.26%. The results are presented in Figure 30.

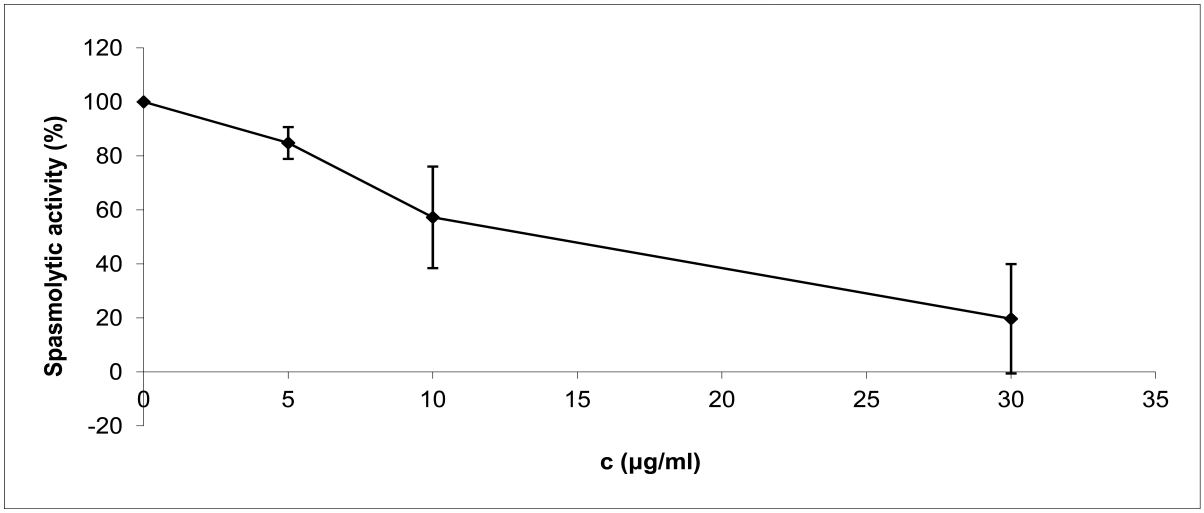


Figure 30. Concentration-related inhibitory effects of the essential oil on contractions induced with KCl (80 mM) in isolated rat ileum. Each point represents mean ± S.E.M. of four or more experiments. Stars show statistically significant differences in comparison with control data – vehicle (Na-CMC) treated group (*$p < 0.05$).

Piperitone, the main metabolite of investigated essential oil (58.7%) was previously shown to exhibit spasmolytic activity. Tested in range of 1-100 μg/ml

piperitone concentration-dependently inhibited contractions of isolated rat uterus contracted with KCl (60 mM) with calculated EC_{50}=10.73±1.27 μg/ml (Ponce-Monter et al., 2008). It was previously shown that limonene, minor component of this oil, exhibit spasmolytic activity as well (Cardoso Lima et al., 2012). 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 (Morita et al., 1996). Additionally, essential oil of *Perovskia abrotanoides* Kar. exhibited relaxant effect on spontaneous and KCl (80 mM) induced contraction of isolated rabbit jejunum. The main metabolite of this oil was δ-3-carene (Shah et al., 2013). Therefore, δ-2-carene present in *C. schoenanthus* essential oil (9.7%) might contribute to demonstrated activity of the oil. It could be postulated that strong spasmolytic activity of *C. schoenanthus* inflorescence essential oil demonstrated in our experiments could be, at least partly, explained with previously corroborated activity of its constituents with piperitone as the main contributor.

V Conclusions

- In this PhD thesis two species of genus *Cymbopogon*, *C. nervatus* and *C. schoenanthus* from Sudan, were analyzed in terms of content and detailed chemical analysis of essential oils obtained from different plant parts. In addition, essential oils from both species were investigated for antimicrobial, antioxidant and spasmolytic activities.
- The yield of oils of *C. nervatus*, collected from Western and Eastern Sudan in May 2014 (summer) and February 2015 (winter), calculated on a dry weight basis, was higher in inflorescence (0.6%-2.1% *v/w*) than in stems (0.1%-0.2% *v/w*) and also was higher in plant material collected during the winter (February 2015).
- All the oils of *C. nervatus* were similar regarding qualitative pattern with some quantitative differences and composed mainly of oxygenated monoterpenes (81.1-94.6%), with the complete absence of acyclic and bicyclic monoterpenes.
- The major constituents in *C. nervatus* were *p*-menthadienols (64.0-80.3%): *trans*-*p*-mentha-1(7),8-dien-2-ol (19.5-32.6%), *cis*-*p*-mentha-1(7),8-dien-2-ol (18.9-23.3%) and *trans*-*p*-mentha-2,8-dien-1-ol (10.6-21.0%).
- The results of the chemical analysis of samples from different localities and from different collecting periods, as well as comparison with literature data suggest that essential oil composition of *C. nervatus* is rather stable, i.e that plant part, origin and time of collection do not affect much on the composition of the essential oil *C. nervatus*.
- 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.
- The oils from stems and inflorescence, as well as the oils originated from different periods of plant material collection, were similar in qualitative composition with some quantitative differences. All investigated essential oils were characterised by high content of oxygenated monoterpenes (50.8-75.5%).
- The most 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 antimicrobial activity of the essential oils of *C. nervatus* and *C. schoenathus* was tested using broth microdilution method against six standard strains of bacteria (*S.*

aureus, S. epidermidis, *E. faecalis, E. coli*, *K. pneumonia*, *P. aeruginosa)* and two strains of fungi *C. albicans*.

- The susceptibility of tested microorganisms to the essential oils of *C. nervatus* and *C. schoenanthus* was similar. The essential oils were active against the strains of *S. aureus*, *S. epidermidis* and *C. albicans* and without inhibition of growth of Gram (-) bacteria in tested concentrations, but the overall antimicrobial activity of *C. nervatus* and *C. schoenathus* oils, tested in this study, was weak.
- The antioxidative properties of inflorescence essential oils of *C. nervatus* and *C. schoenanthus* were estimated by Ferric Reducing Antioxidant Power (FRAP) assay for determination of total antioxidant capacity and by DPPH test for determination of free radical scavenging ability.
- The total antioxidant capacity of essential oils of both species was weaker than the capacity of rutin and ascorbic acid used as the referent antioxidants.
- In the DPPH radical scavenging assay, essential oil from inflorescence of *C. nervatus* showed moderate activity, while essential of C. schoenanthus exhibited weak activity.
- The inflorescence essential oil of *C. nervatus* and *C. schoenanthus* were tested for spasmolytic activity using three different experimental models: against spontaneous contractions, and contractions induced with ACh and KCl. The oils of both species exhibited strong, significant and concentration-dependent spasmolytic activity in all experimental models.

The results of this dissertation showed that inflorescence of both *Cymbopogon* species, which were analyzed, reperesent a rich source of essential oils. The qualitative compositions of those oils are rather stable. Significant spasmolytic activity of *C. nervatus* and *C. schoenanthus* inflorescence essential oils from Sudan was evidenced in this study, emphasizing its beneficial properties, probabely, especially for gastrointestinal complaints.

Obtained data may represent a good basis for further investigations of this essential oil for its medicinal purposes as well as for application in food industry. In addition, high essential oil content and its rather stable composition makes *C. schoenanthus* from Sudan valuable as a source of commercially important monoterpene piperitone.

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VII Supplement

Biography

Eihab Omer Ahmed Mohamed Yousif was born on 06. 07. 1969. in Al kamline dist- Almiaque town in Sudan. He graduated at the Faculty of Pharmacy, MGR Medical University, India in 1998/1999 school year. He worked in the Ministry of Health of Sudan from 2001 to 2002, in the Directorate of Pharmacy and in the private pharmacy. In the period from 2002 to 2011, he was employed as teaching assistant at the Department of Pharmacognosy, Omdurman Islamic University - Faculty of Pharmacy. During this period he completed master level study program (2005 - 2008) at the University of Khartoum - Faculty of Pharmacy, Department of Pharmacognosy, where he defended his master's thesis entitled "Phytochemical and Antimicrobial studies on two medicinal plants (*Cucumis figareis* & *Coccina grandis*)".

During the autumn of 2011, he became a scholar of the Republic of Serbia under the project "World in Serbia". From school year 2012/2013 he enrolled in the PhD study program Pharmaceutical sciences – modul Pharmacognosy at the University of Belgrade - Faculty of Pharmacy.

Authorship Statement

Signed **Eihab Omar Ahmed Mohamed Yousif**

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Belgrade, **18.11.2016** _____

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

Упоредна анализа хемијског састава, антимикробне, антиоксидантне и спазмолитичке активности етарског уља *Cymbopogon nervatus* (Hochst.) Chiov. и *Cymbopogon schoenanthus* (L.) Spreng (Poaceae) из Судана

која је моје ауторско дело.

Дисертацију са свим прилозима предао/ла сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

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(Молимо да заокружите само једну од шест понуђених лиценци.
Кратак опис лиценци је саставни део ове изјаве).

Потпис аутора

У Београду, **18.11.2016**