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Bily Nebié
Laboratory of Chemistry and Renewable Energies, Nazi Boni University, 01 BP 1091 Bobo 01, Burkina Faso

Constantin M Dabiré
[1] Laboratory of Chemistry and Renewable Energies, Nazi Boni University, 01 BP 1091 Bobo 01
[2] Laboratory of Applied Organic Chemistry and Physics, Joseph Ki-Zerbo University, 03 BP 7021 Ouaga 03, Burkina Faso

Schemaeza Bonzi
Natural Systems, Agrosystems and Environmental Engineering, Nazi BONI University, 01 BP 1091 Bobo 01, Burkina Faso

Rémy K Bationo
[1]Laboratory of Applied Organic Chemistry and Physics, Joseph Ki-Zerbo University, 03 BP 7021 Ouaga 03, Burkina Faso
[2] National Center for Research in Science and Technology, Institute for Research in Applied Science and Technology, 03 BP 7047 Ouaga 03, Burkina Faso

Siaka Sosso
Laboratory of Chemistry and Renewable Energies, Nazi Boni University, 01 BP 1091 Bobo 01, Burkina Faso

Roger Ch Nebié
National Center for Research in Science and Technology, Institute for Research in Applied Science and Technology, 03 BP 7047 Ouaga 03, Burkina Faso

Irénée Somda
Natural Systems, Agrosystems and Environmental Engineering, Nazi BONI University, 01 BP 1091 Bobo 01, Burkina Faso

Eloi Palé
Laboratory of Applied Organic Chemistry and Physics, Joseph Ki-Zerbo University, 03 BP 7021 Ouaga 03, Burkina Faso

Pierre Duez
Department of Therapeutic Chemistry and Pharmacognosy, Faculty of Medicine and Pharmacy, University of Mons, 7000 Mons, Belgium

Corresponding Author:
Constantin M Dabiré
[1] Laboratory of Chemistry and Renewable Energies, Nazi Boni University, 01 BP 1091 Bobo 01
[2] Laboratory of Applied Organic Chemistry and Physics, Joseph Ki-Zerbo University, 03 BP 7021 Ouaga 03, Burkina Faso

Chemical composition and antifungal activity of the essential oil obtained by co-distillation of *Cymbopogon citratus* and *Eucalyptus camaldulensis* from Burkina Faso

Bily Nebié, Constantin M Dabiré, Schemaeza Bonzi, Rémy K Bationo, Siaka Sosso, Roger Ch Nebié, Irénée Somda, Eloi Palé and Pierre Duez

Abstract
The objective of this study was to determine the chemical composition of essential oil obtained by co-distillation of *Cymbopogon citratus* and *Eucalyptus camaldulensis* and to compare its antifungal activity with that of the mixture obtained by combining the pure essential oils of the two plants. Essential oils were obtained by hydrodistillation of the dry leaves of *C. citratus* and *E. camaldulensis*, separately, then of the mixture of these leaves in the mass ration 50/50. Essential oils were then analyzed by GC/MS and their antifungal activity on *Phoma sorghina*, *Fusarium moniliforme* and *Macrophomina phaseolina* was evaluated by direct contact method on PDA medium successively at doses of 0.6; 0.2 and 0.05%.
Essential oil obtained by co-distillation mainly contains 1,8-cineole (38.52%) and citral (25.77%). Essential oil of *C. citratus* is dominated by citral (74.32%) and that of *E. camaldulensis* by 1,8-cineole (44.17%). All the essential oils, except that of *E. camaldulensis*, inhibited 100% the mycelial growth of the three fungi at the dose of 0.2%. At this dose, they were more effective than the synthetic fungicide used as control. Essential oil obtained by co-distillation was the most effective on *Phoma sorghina* and *Fusarium moniliforme*.
These results show that co-distillation improved the antifungal efficacy of *C. citratus* and *E. camaldulensis* essences and suggest the use of this combination as a natural fungicide.

Keywords: *C. citratus, E. camaldulensis*, co-distillation, essential oil, chemical composition, antifungal activity

Introduction
Burkina Faso is a Sahelian country where the agricultural sector plays a very important socio-economic role. Among the agricultural products, cereals are the main vegetable productions and constitute the staple food of the majority of the population [1]. However, the post-harvest conservation of these cereals remains a major concern [2]. Indeed, poor harvesting practices and the use of inappropriate storage equipment make the grain vulnerable to fungal infestation including *Phoma sorghina*, *Macrophomina phaseolina* and *Fusarium moniliforme* among many others are the cause of crop quality loss and damping off in Burkina Faso [3]. The severity of attacks on grains by fungi most often forces people to use synthetic pesticides to protect foodstuffs in storage. Yet, continued use of these synthetic chemicals in high doses increases the risk of toxic residues in food products [4] and leads to the development of fungi resistance [5]. Faced with the increasingly persistent toxic effects of synthetic pesticides on humans and the environment, the use of biopesticides is increasingly encouraged as an alternative method, in particular extracts of aromatic plants such as essential oils [6]. This is the case of essential oils of *Cymbopogon citratus* and *Eucalyptus camaldulensis*, two aromatic plants with potential biopesticides grown in Burkina Faso. Indeed, essential oils of these two plants respectively rich in citral and 1, 8-cineole would be antifungal [7, 8] and insecticides [9, 10]. Unfortunately, the use of essential oils as a biopesticide would be limited by many constraints: low extraction yield, high energy consumption linked to their extraction, fairly high production costs, etc. It is therefore necessary to find formulations allowing the use of small quantities of essential oils but sufficiently effective [11]. Thus, several studies showed that the combinations of essential oils allow, in some cases, to increase their effectiveness and therefore allow the use of small quantities [11-13]. In addition, farmers use traditional recipes based on combinations of aromatic plants to effectively protect foodstuffs in storage [14]. On the other hand, very few scientific studies have been devoted to this traditional practice. Thus, the present work aims to determine the chemical composition of essential oil obtained by co-distillation of *Cymbopogon*

citratus and *Eucalyptus camaldulensis* and to compare its antifungal activity to that of the mixture obtained by combining the pure essential oils of the two plants.

Material and methods

Material

Plant material: Plant material consists of dry leaves of *Cymbopogon citratus* (DC.) Stapf and *Eucalyptus camaldulensis* Dehnh. The samples were collected in August 2019 at the site of Nazi-Boni University, Burkina Faso (11°12'N; 4°24'W) and then identified under N° 961 and 963 respectively for *C. citratus* and *H. suaveolens* and deposited in the herbarium of Nazi-Boni University.

Biological material: Biological material included three fungal species namely *Fusarium moniliforme* Sheld, *Phoma sorghina* (Sacc.) Boerema Dorenbosch and Van Kest. and *Macrophomina phaseolina* (Tassi). They were respectively isolated from sorghum (*Sorghum bicolor* L.), cowpea (*Vigna unguiculata* L.) and corn (*Zea mays* L.), purchased in the markets of Bobo-Dioulasso city. The fungi were identified at the Laboratory of Natural Systems, Agrosystems and Environmental Engineering (SyNAIE) of NAZI Boni University according to the method described by Mathur and Kongsdal (2003) [15]. They were then regularly maintained by subculturing on PDA medium until use.

Methods

Extraction of essential oils: Essential oils were extracted by hydrodistillation with a Clevenger-type apparatus from the dry leaves of *C. citratus* and *E. camaldulensis*, separately, then from their mixture in the mass proportions 50/50. Essential oils obtained were dehydrated with anhydrous sodium sulphate and then stored at 4 °C until use. The essential oil extraction yield (R) was determined according to the following formula:

$$R = \frac{\mathrm{Mh}}{Mv} x100,$$

Where Mh is the mass of essential oil obtained and Mv that of the dry plant material used [16].

Analysis of the chemical composition of essential oils: Essential oils were analyzed by an Agilent 8860 type gas chromatograph fitted with a 60 meters DBWAX capillary column; this chromatograph was coupled to an Agilent 5977B type mass spectrometer provided with a quadripolar analyzer. The experimental conditions were described in Table 1.

Table 1: Experimental conditions for GC/MS analysis

Agilent 8860 GC	**Capillary column**	**Type DB Wax (PEG)**
	Internal diameter	0.25 mm
	film thickness	0.25 µm
	Carrier gas	Helium
	Debit	1.0 ml/min
	Injector temperature	250 °C
	Oven programming	2 °C/min from 60 °C to 250 °C
		2 mins at 60 °C
		10 mins at 250 °C
	Sample concentration	20 µL/mL heptane
	Quantity injected	1µL
	Injection mode	Split 1:10
MS 5977B	Interface temperature	250 °C
	Ionization by IE	70 eV

The identification of the different constituents of essential oils was carried both, by comparing the mass spectra of the different constituents with those existing in a NIST database [17] and their retention index (RI) with those of the literature [18].

Antifungal tests: Four essential oils and a synthetic fongicide (Red Caiman) were tested using the direct contact method as described by Adjou *et al*. (2013) with slight modifications [19]. In 9 cm diameter Petri dishes, each containing 25 ml of sterilized culture medium (Potato Dextrose Agar), decreasing quantities of essential oils were added aseptically so as to obtain doses of 0.6; 0.2 and 0.05%. After solidification of the medium, a 5 mm mycelial disk of the fungal strain to be tested was placed in the center of each Petri dish. The synthetic fungicide (Permethrin 25 g/kg + Thiram 250 g/kg) was used as a positive control. It tested at the recommended dose of 0.25%. The negative control consisted of culture medium devoid of essential oils. The inoculated cultures were then incubated at 22±2 °C. for 12 hours of near UV light alternated with 12 h of darkness. Each test is repeated four times. The measurement of mycelial growth was made on the 7th day after incubation for *F. moniliforme* and *P. sorghina* and on the 4th day for *M. phaseolina*. The percentages of mycelial growth inhibition were calculated according to the following formula:

$$\mathrm{I}\,(\%) = \left(1 - \frac{\mathrm{D}}{\mathrm{Dt}}\right) \mathrm{x}\ 100$$

Where D is the average diameter of the mycelial growth in the culture medium containing the essential oil and Dt the average diameter of the mycelial growth in the culture medium without essential oil [20]. The tests were repeated four times.

Statistical analysis: All the Data were analyzed using Microsoft Excel spreadsheet and reported as mean ± standard deviation. ANOVA analysis of variance was performed using IBM SPSS 25.0 software. The comparison of the means was carried out at the 5% threshold by the Student-Newman-Keuls test.

Results and Discussion

Extraction yield of essential oils

Extraction yields of essential oils are recorded in TABLE 2.

Table 2: Extraction yields of essential oils

Plant	Essential oil yield (%)
C. citratus	1.055 ± 0.078
E. camaldulensis	0.970 ± 0.020
C. citratus/E. camaldulensis	0.995 ± 0.007

All essential oils have an extraction yield substantially close to around 1%. The extraction yield of *C. citratus* essential oil is higher than that of the same plant from Southern Benin, which was 0.71% [21]. However, Bassole *et al*. (2011) obtained an essential oil yield of 1.25% from the same plant collected in June 2009 in central Burkina Faso [22]. The extraction yield of *E. camaldulensis* essential oil is comparable to that of the same plant from the North-West of Algeria which was 0.99% [23]. In contrast, the species from Northern Morocco had a higher essential oil yield (1.40%) [7]. The differences in yields observed could be explained, on the one hand, by the difference in the place and/or the collection period [24] and on the other hand, by the influence of the state (fresh or dry) of the material used [25].

Chemical composition of essential oils

Results of chromatographic analyzes of essential oils are presented in TABLE 3. The structures of the main compounds of each essential oil are represented in FIGURES 1 ; 2 and 3. In the essential oil of *C. citratus*, 14 compounds representing 96.16% of the total mixture were identified. This essence mainly contains neral (32.83%), geranial (41.49%) and β-myrcene (13.66%). These compounds have also been identified as major constituents in essential oil of the same plant from central Burkina Faso [22, 24, 26].

28 compounds representing 97% have been identified in the essential oil of *E. camaldulensis* which mainly contains 1, 8-cineole (44.17%), α-pinene (14.70%), o-cymene (14.11%) and α-phellandrene (5.91%). The chemotype of this essence differs from that generally described in literature, by the presence of *o*-cymene (14.11%) instead of *p*-cymene which was most often reported [7, 27]. This difference in chemical composition could be justified by the fact that the composition of an essential oil depends on genetic factors, the age, the season and/or the environment of the plant and the reactions over the plant [24].

As for the essential oil obtained by co-distillation of the two plants, 17 compounds representing 98.88% were identified. Neral (32.83%), geranial (41.49%) and myrcene (13.66%) present only in *C. citratus* essential oil were identified in that obtained by co-distillation at relatively low levels (14.28, 11.49 and 7.09% respectively). Similarly, 1,8-cineole (41.17%), α-pinene (14.70%) and α-phellandrene (5.91%), present only in the essential oil of *E. camaldulensis* were identified in that obtained by co-distillation also at low levels (38.52 ; 8.12 and 2.56% respectively). Conversely, limonene (3.85%) present only in the essential oil of *E. camaldulensis* was identified at a fairly high content (7.42%). We also note the absence of o-cymene in the essence of the mixture and which, however, was present in that of *E. camaldulensis* at a content of 14.11%. These observed variations would be due on the one hand, to a dilution effect linked to the mixtures of plants and on the other hand, to chemical reactions which would be occured during the co-distillation. The essential oil obtained by co-distillation of these two plants contains a molecular diversity coming not only from the constituents initially present in the pure essences but also from the formation of new constituents which were initially absent there. Similar observations had already been reported by Hay (2015) [13] on the co-distillation of Thyme and Rosemary, without giving any further explanation [13]. Studies deserve to be continued in an attempt to elucidate this phenomenon worthy of interest.

Table 3: Chemical composition of the essential oils of *C. citratus*, *E. camaldulensis* and that obtained by the co-distillation of the two plants

RI	Compounds	Content (%)		
		***C. citratus* (CC)**	***E. camaldulensis* (EC)**	**CC/EC**
921.5	3-methylbutanal	-	1.61	2.27
1025.4	*α*-pinene	-	14.70	8.12
1068.5	Camphene	-	0.13	-
1115.6	*β*-pinene	-	0.14	-
1164.7	*β*-myrcene	13.66	0.26	7.09
1170.3	*α*-phellandrene	-	5.91	2.56
1205.9	Limonene	-	3.85	7.42
1220.2	1,8-cineole	-	44.17	38.52
1236.1	cis-β-ocimene	0.36	-	-
1250.2	*γ*-terpinene	-	3.83	1.95
1253.1	Trans-β-ocimene	0.25	-	-
1274.1	*p*-cymene	-	-	0.92
1275.5	*o*-cymene	-	14.11	-
1287.9	Terpinolene	-	0.30	0.41
1299.2	Isopentyl isovalerate	-	0.32	-
1340.3	6-methylhept-5-en-2-one	1.14	-	0.32
1481.4	Lemonellal	0.17	-	-
1550.9	Linalool	1.14	0.15	0.60
1573.0	Pinocarvone	-	0.17	-
1588.4	Endo-fenchol	-	0.20	-
1601.3	2-undecanone	0.39	-	-
1607.2	Terpinen-4-ol	-	1.41	0.50
1611.2	Aromandendrene	-	0.21	-
1661.2	Trans-pinocarveol	-	0.96	-
1683.0	Carvotanacetone	-	0.24	-

1688.0	Neral	32.83	-	11.49
1691.5	Cis-carveol	-	0.25	-
1691.5	Trans-carveol	-	-	0.22
1698.5	Viridiflorene	-	0.23	-
1701.9	*α*-terpineol	-	1.84	1.09
1707.2	Endo-borneol	-	0.33	-
1731.1	Piperitone	-	0.41	-
1737.1	Bicyclogermacrene	-	0.73	-
1738.2	Geranial	41.49	-	14.28
1759.7	Geranyl acetate	0.49	-	-
1771.3	Lemonellol	0.31	-	-
1802.7	Trans-p-mentha-1(7),8-dien-2-ol	-	0.17	-
1805.4	Nerol	0.21	-	-
1811.8	2-tridecanone	0.23	-	-
1811.9	cis-sabinol	-	0.23	-
1853.1	Geraniol	3.49	-	1.12
1893.1	Cis-p-mentha-1(7),8-dien-2-ol	-	0.14	-
Oxygenated monoterpenes		80.78%	50.99%	68.14%
Hydrocarbon monoterpenes		14.27%	43.23%	28.47%
Hydrocarbon sesquiterpenes		-	1.17%	-
Other compounds		1.11%	1.61%	2.27%
Total		96.16%	97%	98.88%

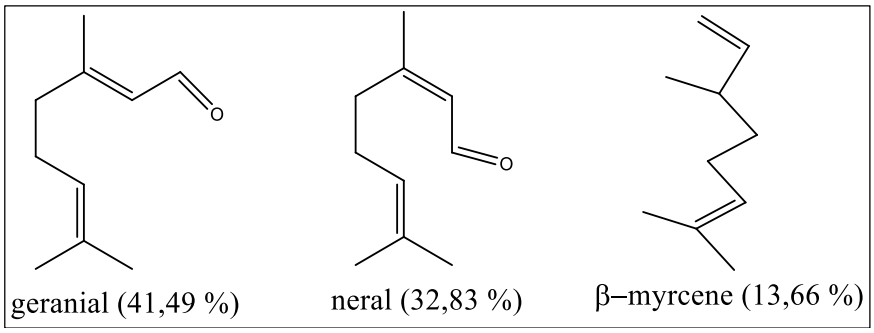

Fig 1: Structures of the main constituents of *C. citratus* essential oil

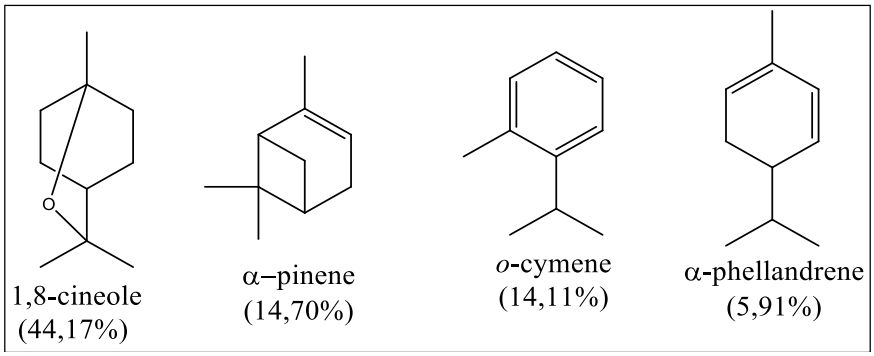

Fig 2: Structures of the main constituents of *E. camaldulensis* essential oil

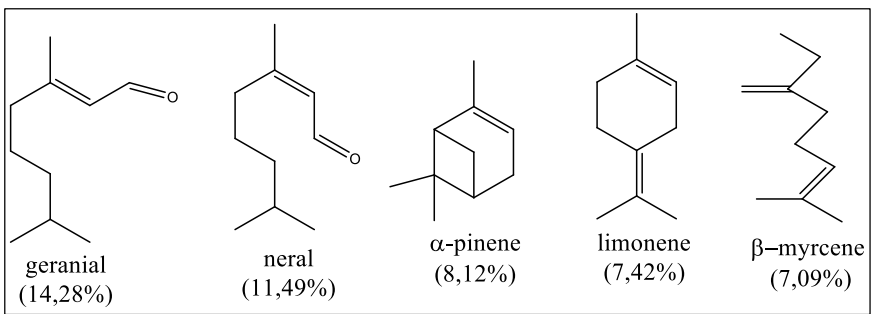

Fig 3: Structures of the main constituents of essential oil obtained by co-distillation of *C. citratus* and *E. camaldulensis*

Antifungal activity of essential oils

Results of antifungal tests showed that all the essential oils had an inhibitory action on the mycelial growth of *Fusarium moniliforme*, *Phoma sorghina* and *Macrophomina phaseolina*. However, this inhibitory action varies according to the essential oils used, the considered fungal species and doses tested (Table 4).

Table 4: Percentage of inhibition of essential oils on the three fungal strains according to the applied doses

Sample	Dosage (%)	Inhibition percentage (%)		
		P. sorghina	*M. phaseolina*	*F. moniliforme*
CC/EC	0.6	100 a	100 a	100 a
CC/EC*	0.6	100 a	100 a	100 a
C. citratus	0.6	100 a	100 a	100 a
E. camaldulensis	0.6	56.87±8.16c	56.04±12.11b	63.61±4.4b
CC/EC	0.2	100 a	100 a	100 a
CC/EC*	0.2	100 a	100 a	100 a
C. citratus	0.2	100 a	100 a	100 a
E. camaldulensis	0.2	38.49 ±10.98d	0th	43.30±2.56c
CC/EC	0.05	44.55±1.74d	22.36±5.40d	41.86±9.83c
CC/EC*	0.05	4.83±1.97th	33.54±4.22c	33.11±3.67d
C. citratus	0.05	40.90±2.4d	39.52±6.62c	27.20±4.96d
Fungicide control	0.25	74.66±11.63b	100 a	62.80±5.17b
ddl		11	11	11
F-value		160	292,419	131.058
P-value		0.000	0.000	0.000

CC/EC: Essential oil obtained by co-distillation of the two plants; CC/EC*: Essential oil obtained by combining pure essences of the two plants: 50/50 (v/v); Standard fungicide (Red Caiman P). The percentages of inhibition assigned the same letter in the same column are not significantly different at the threshold of 5% according to the Student-Newman-Keuls test.

All essential oils, except that of *E. camaldulensis* totally inhibited the mycelial growth of the three fungal species from the dose of 0.2%. At this dose, these essences were more effective than the synthetic fungicide which was tested at a dose of 0.25%. At the lowest dose (0.05%), essential oil obtained by co-distillation presented the highest inhibition rates on *P. sorghina* and *F. moniliforme*. It presented a highly significant difference compared to other essential oils on *F. moniliforme* (Table 4). On the other hand, on *M. phaseolina*, it is the essential oil of *C. citratus* and that obtained by combining pure essences which presented the highest inhibition rates but with a non-significant difference (TABLE 4).

The difference in effectiveness between essential oils could be related to their chemical composition. Indeed, although all essential oils tested are mainly monoterpene (known for their interesting antifungal properties), the different molecules they contain can act differently given the different chemical functions they carry. Thus, the more pronounced antifungal activity of the essential oil of *C. citratus* compared to that of *E. camaldulensis* could be due to the presence of citral. This analysis corroborates that of Tchoumbougnang *et al*. (2009), who reported that monoterpene aldehydes are more active against microbial agents than terpene oxides [28]. In addition, the difference in genetic heritage of fungal species tested would also justify the variation in the effectiveness of essential oils from one fungal species to another. Moreover, the higher antifungal activity on *P. sorghina* and *F. moniliforme* of the essential oil obtained by co-distillation compared to that obtained by combining pure essences would also be linked to their chemical composition. Indeed, the limonene present only in the essence of *E. camaldulensis* at a low content (3.85%) was identified in the essence obtained by co-distillation at a fairly high content (7.42%). Yet, this molecule is known to produce synergistic antimicrobial activity when combined with 1, 8-cineole [29] or citral [26]. Thus, the efficiency of the essence obtained by co-distillation would be linked to the different synergistic interactions between these molecules. Besides, Togola *et al*. (2014) also reported that the mixture of essential oils of *C. citratus* and *E. camaldulensis* causes a synergistic insecticidal effect against *S. oryzae* and *S. cerealella* [30].

Conclusion

This study showed that the co-distillation of *C. citratus* and *E. camaldulensis* provides an essential oil with a chemical composition that is distinguished by the presence (or absence) of certain compounds initially absent (or present) in essential oils of these two plants taken separately. In addition, the essential oil obtained by co-distillation of these two plants has a higher antifungal activity on *P. sorghina* and *F. moniliforme* than pure essential oils and that obtained by combining pure essences. This essence could therefore be a good antifungal candidate to fight against *P. sorghina* and *F. moniliforme*, which until now have remained difficult to control with Red Caiman P, a synthetic fungicide.

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