Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
21(1): 63-69, Jan./Feb. 2011

Antiacetylcholinesterase and antioxidant activity of essential oils from six medicinal plants from Burkina Faso

Martin Kiendrebeogo,*[*,1] Ahmed Y. Coulibaly,[1] Roger C. H. Nebie,[2] Boukaré Zeba,[1] Charles E. Lamien,[1,3] Aline Lamien-Meda,[1] Odile G. Nacoulma[1]

[1]*Laboratoire de Biochimie & Chimie Appliquées, UFR/SVT, Université de Ouagadougou, Burkina Faso,*
[2]*Département Substances Naturelles, Institut de Recherches en Sciences Appliquées et Technologies, Burkina Faso,*
[3]*FAO/IAEA Agriculture and Biotechnology Laboratory, Animal Production Unit, International Atomic Energy Agency, Austria.*

Article

Received 6 Dec 2009
Accepted 7 Jul 2010
Available online 24 Jan 2011

Abstract: In this investigation, we evaluated essential oils from six medicinal plants from Burkina Faso for their antiacetylcholinesterase and antioxidant abilities. The chemotype of most active were also determined. The best antiacetylcholinesterase activities were recorded for the essential oils of *Eucalyptus camaldulensis* (IC50 18.98 µg/mL) and *Ocimum canum* (IC50 36.16 µg/mL). Their chemotype have been related to the 1,8-cineole one. Both essential oils demonstrated a linear mixed non competitive inhibition. The essential oil of *Ocimum basilicum* which belong to the linalool-eugenol chemotype exhibited the best radical scavenging activity (IC50 3.82 µg/mL) and reducing power (531.75 mg AAE/g). In comparison with gallic and ascorbic acids, *O. basilicum* essential oil evidenced interesting antioxidant activities. The antiacetylcholinesterase and antioxidant activities of essential oils were discussed in regard with their chemical composition.

Keywords:
acetylcholinesterase
inhibition mechanism
antioxidant activity
Eucalyptus camaldulensis
Ocimum canum
Ocimum basilicum

ISSN 0102-695X
doi: 10.1590/S0102-695X2011005000008

Introduction

Acetylcholinesterase (AChE, EC. 3.1.1.7) is a key enzyme catalysing the hydrolysis of acetylcholine (ACh) in the nervous system of animals and insects. ACh deficiency in the cerebral cortex of humans is one of the major futures seen in sufferers of Alzheimer's disease (Bierer et al., 1995). Recent studies have pointed out that ß-amyloid peptides found in Alzheimer's disease brain can induce inflammatory process with subsequent liberation of radical oxygen species (Vina et al., 2004; Stuchbury & Munch, 2005) acting as secondary messenger in inflammation. A promising approache for treating Alzheimer's disease is to boost the level of ACh in the brain using AChE inhibitors (Enz et al., 1993). Antioxidants may contribute to this chemotherapy by attenuating inflammation pathways (Gibson & Huang, 2005) through their ability to scavenge free radicals.

Recently, interest has increased in natural substance that could be supplied as food components or specific pharmaceutics for human well being. Among those, essential oils from aromatic and medicinal plants has been known to exhibit antioxidant and antiacetylcholinesterase properties (Perry et al., 2003; Politeo et al., 2007; Sacchetti et al., 2005; Savelev et al., 2003). *Cymbopogon citratus, Cymbopogon giganteus, Eucalyptus camaldulensis, Lippia multiflora, Ocimum canum* and *Ocimum basilicum* are very common in Burkina Faso. Their essential oils are traditionally used in aromatherapy and as insects repellent or insecticide (Nacoulma, 1996).

This paper deals with the antiacetylcholinesterase and antioxidant activity of those six essential oils. The chemotype and inhibitory mechanisms on eel AChE of the most promising essential oils were reported.

Materials and Methods

General procedures

Acetylthiocholine iodide (ATCI), 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB), acetylcholinesterase (AChE) type VI-S from electric eel, tris [hydroxymethane], bovine serum albumin (BSA), magnesium chloride hexahydrate, galanthamine hydrobromide and gallic acid were supplied from Sigma (USA). Trichloroacetic acid and 2,2-dipheny-

l-1-picrilhydrazyl (DPPH) were provided from Fluka Chemika (Switzerland). Potassium hexacyanoferrate [($K_3Fe(CN)_6$] and ascorbic acid were purchased from Labosi (France). Bioassays were performed on a double beam spectrophotometer (Uvikon 923, Bio-Tek Kontron instruments). Composition of essential oils were achieved on a Hewlett-Packard 5890 gas chromatograph coupled with a Hewlett-Packard 5972 mass selective detector.

Plant materials and essential oils extraction

Fresh leaves of *Cymbopogon citratus* DC, *Cymbopogon giganteus* Chiov, *Eucalyptus camaldulensis* Dehnhardt, *Lippia multiflora* Moldenke, *Ocimum canum* Sims and *Ocimum basilicum* L. were collected at Gampela (25 km, east of Ouagadougou; Burkina Faso). Collection and taxonomic identification were carried out by Dr Amadé Ouedraogo, botanist from the Laboratoire de Biologie et d'Ecologie Vegetales (University of Ouagadougou, Burkina Faso).

Fresh leaves were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulphate and stored in dark glass bottle at 4 °C until use.

Antiacetylcholinesterase activity

The inhibitory effect of essential oils on acetylcholinesterase activity was evaluated using and adaptation of the spectrophotometric method of Ellman et al. (1961). Briefly, 100 µL of essential oil (0.1 % in 50 mM Tris-HCl, pH 8 buffer, 10% methanol) was mixed with 100 µL of AChE (0.22 U/mL in 50 mM Tris-HCl, pH 8 buffer, 0.1% BSA) and 200 µL of buffer (50 mM Tris-HCl, pH 8, 0.1% BSA). Mixture was incubated for 5 min at 30 °C in a 1 mL cuvette. Subsequently, 500 µL of DTNB (3 mM in Tris-HCl, pH 8 buffer, 0.1 M NaCl, 0.02 M $MgCl_2$) and 100 µL of ATCI (4 mM in water) were added. A blank was also prepared by replacing AChE with 100 µL of buffer (50 mM Tris-HCl, pH 8 buffer, 0.1% BSA). The reaction was monitored for 5 min at 412 nm and initial velocity (V_0) recorded. Buffer (0.1 % in 50 mM Tris-HCl, pH 8, 10% methanol) was used as negative control. Antiacetylcholinesterase activity (I %) was calculated as following:

$$I\,(\%) = (1 - V_{0\ Sample}/V_{0\ Blank}) \times 100$$

$V_{0\ Sample}$ and $V_{0\ Blank}$ represent the initial velocities of samples and blank. IC50 values were obtained though Log-Probit plotting. Galanthamine HBr was used as positive control.

Enzyme kinetic measurements were carried out to elucidate the inhibitory mechanisms. Enzyme assay experiments were performed in the presence of different sets of concentrations of essential oils (0-0.125% v/v) and substrate (ATCI: 0.1-0.4 mM). The nature of inhibitions was determined by analysis of kinetic parameters (K_m, V_{max}, Ki, K'i) calculated from Lineweaver-Burk plots, Dixon plots (Dixon, 1953) and their secondary replots (Cornish-Bowden, 1974).

Free radical (DPPH) scavenging activity

DPPH radical scavenging activity was measured as described by Velasquez et al. (2003). Briefly, 1.5 mL of a freshly prepared DPPH solution (20 mg/mL in methanol) was added to 0.75 mL of essential oil (0.1% v/v in methanol). After shaking, the mixture was incubated for 15 min in darkness at room temperature and then absorbance was measured at 517 nm against a blank (mixture without essential oil). Inhibition percentage of free DPPH radicals (I %) was calculated following the formula:

$$I\,(\%) = (1 - A_{Sample}/A_{Blank}) \times 100$$

A_{blank} and A_{sample} are the absorbance of the blank and sample reactions. Samples exhibiting more than 50% inhibition were considered for IC50 determination. Essential oil concentration scavenging 50% of DPPH radicals (IC50) was calculated from the plotting of inhibition percentage versus sample concentration. Ascorbic acid and gallic acid were used as positive controls.

Ferric-reducing antioxidant power assay (FRAP)

The Fe(III) to Fe(II) reducing power was evaluated as described by Hinneburg et al. (2006). Briefly, 1 mL of each essential oil sample (0.1% v/v in methanol) was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium hexacyanoferrate (1% in water) solution. After 30 min incubation at 50 °C, 2.5 mL of trichloroacetic acid (10% in water) was added, and the mixture centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of water and 0.5 mL of aqueous FeCl3 (0.1 %), then absorbance was read at 700 nm. Ascorbic acid was used to generate the calibration curve. The reducing power was expressed as mg ascorbic acid equivalent g-1 of pure essential oil (mg AAE/g). Gallic acid was used as positive control.

Gas chromatography-Mass spectrometry (GC-MS)

Separation of samples (0.1 µL) were achieved on

a SPB-1 (*E. camaldulensis, O. basilicum, L. multiflora*, *C. giganteus*) or DB-1 (*O. canum, C. citratus*) capillary columns (length: 30 m; inner diameter: 0.25 mm; film thickness: 0.25 µm) using helium as carrier gas at a flow rate of 1 mL/min. The oven temperature was 40 °C to 240 °C at 2 °C/min and held isothermal for 40 min. The GC injector and transfer line were set at 210 °C and 280 °C respectively. The mass spectrometer parameters for EI (electron impact) mode were ion temperature source 220 °C, electron energy 70 eV and detector temperature 280 °C. Individual constituents were identified by their Kovat's indices referring to the compounds known from the literature data (Adams, 1995) and by comparing their mass spectra with those stored in the Wiley mass spectral database.

Statistical analysis

Assays were performed in triplicate and data presented as mean±standard deviation. Statistical significance of each test (n=3) was evaluated with Turkey test using GraphPad Prism® software at *p* value <0.01 considered as being significant. Correlations between antioxidant data were analysed through Person's correlation coefficient (Thaipong et al., 2006).

Results and Discussion

Acetylcholinesterase enzyme inhibitory activity

As summarized in Table 1, all the essential oils tested exhibited moderate (25-50%) to strong (>50%) inhibitory activity at the concentration of 0.01% (v/v). The best inhibitory activities were recorded for the essential oils of *Eucalyptus camaldulensis* (83%) and *Ocimum canum* (72%). Their antiacetylcholinesterase activities were compared to that of galanthamine HBr through their IC50 values. *E. camaldulensis* (IC50 18.98 µg/mL) is twice more active than *O. canum* (IC50 36.16 µg/mL), both being less potent than our reference galanthamine HBr (IC50 0.19 µg/mL).

The *in vitro* anticholinesterase activity of *E. camaldulensis* and *O. canum* essential oil are very interesting compared to those of *Salvia lavandulaefolia* and *Rosmarinus officinali*s which demonstrated some valuable therapeutic effects with less strong *in vitro* bioactivity. Indeed, the essential oil of *S. lavandulaefolia*, suggested to be relevant in the treatment of dementia of the Alzheimer's type (Perry et al., 2000) exhibited an IC50 of 50 µg/mL (Savelev et al., 2003) while that of *R. officinalis* with an IC50 value of 70 µg/mL (Mata et al., 2007) enhanced the performance and overall quality of memory in healthy adults (Moss et al., 2003). The essential oil of *S. lavandulaefolia* demonstrated also significant effects on cognition (Perry et al., 2003).

Inhibition mechanism

Kinetic parameters (K_m, V_{max}, Ki, K'i) recorded for *E. camaldulensis* and *O. canum* were given in Table 2. Both essential oils decrease the maximum velocity of catalysis ($V_{max\ app}<V_{max}$) as well as the affinity of substrate for the enzyme ($K_{m\ app}>K_m$). Ki and K'i values recorded were different (Ki ≠ K'i).

According to Cornish-Bowden (1974), *E. camaldulensis* and *O. canum* essential oils inhibit the enzyme acetylcholinesterase following a linear mixed non competitive mechanism.

Antioxidant activity

Free radicals-scavenging capacity

The DPPH test intends to measure the ability of essential oils to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) in solution by donation of hydrogen atom or electron. Essential oils exhibited weak to strong free DPPH• scavenging activity (weak: <25%; moderate: 25-50%; strong: >50%) at the tested concentration of 0.1% (v/v). The best free radicals scavenging activity was obtained with the essential oil of *Ocimum basilicum* which scavenged 96% of DPPH•. A moderate activity (39% inhibition) was recorded with *Lippia multiflora* essential oil while the other essential oils gave weak inhibitions. As shown in Table 1, the essential oil of *O. basilicum* (IC50 3.82 µg/mL) was six fold less active than our reference antioxidant standard gallic acid (IC50 0.61 µg/mL) while it exhibit statistically the same activity than ascorbic acid.

Reducing power

The FRAP assay was used to estimate the reducing power of essential oils resulting from their electron-donating capacity. The highest reducing capacity was recorded for the essential oil of *Ocimum basilicum* (531.75 mg AAE/g) followed by those of *Cymbopogon giganteus* (75.74 mg AAE/g) and *Lippia multiflora* (65.49 mg AAE/g). The reducing power of *O. basilicum* essential oil (531.75 mg AAE/g) was slightly more important than that of gallic acid (445.40 mg AAE/g) as summarized in Table 1.

A very significant correlation (r=0.96, $p<0.01$) was recorded between DPPH and FRAP assays, indicating that essential oils we tested had comparable activities in the two antioxidant methods. This is not always the case when testing the antioxidant activities of plant extracts. A significant correlation was found

between DPPH and FRAP assays with methanol extract of guava fruit while its dichloromethane extract did not show any correlation (Thaipong et al., 2006) nor the hydrodistilled extracts of some herbs and spices (Hinneburg et al., 2006). The antioxidant activities evaluation of eleven essential oils did not mention any concordance using DPPH, photochemiluminescence and ß-carotène tests (Sacchetti et al., 2005).

Taking in account the DPPH and FRAP models to evaluate the antioxidant capacity of the screened essential oils, *Ocimum basilicum* seems to be a promising source of natural antioxidant.

Chemical composition of most active essential oils

Essential oils of *E. camaldulensis* and *O. canum* exhibited the best antiacetylcholinesterase activity while O. basilicum was the most antioxidant. The hydrodistillation yield from fresh leaves was 2%, 1.5% and 0.2% respectively. Chemical composition of essential oils is summarized in Table 3. The most abundant components in *E. camaldulensis* essential oil were 1,8-cineole (33.9%), α-pinene (12.5%), *p*-cymene (12.3%) and limonene (11.5%). Smaller amount of viridiflorol, spatulenol, bicyclogermacrene, α-terpineol, terpin-1-en-4-ol and α-phellandrene were also detected. Composition pattern recorded for the essential oils of *O. canum* was 1,8-cineole (59.9%), camphor (8.1%), ß-pinene (5.4%), α-terpineol (4.6%) and α-pinene (4.5%) while that of *O. basilicum* contain mainly linalool (48.7%), eugenol (27.5%), trans-α-bergamotene (5.4%) and δ-cadinol (3.4%). Major components encountered in essential oils of *O. canum* and *O. basilicum* are remarkably variable in occurrence and concentration. Several chemotypes have been reported (Chalchat et al., 1999; Ekundayo et al., 1989; Grayer et al., 1996; Keita et al., 2000; Martins et al., 1999; Yayi et al., 2001). Fewer chemotypes have been reported for *Eucalyptus camaldulensis* essential oil (Dagne et al., 2000; Samate et al., 1998).

Since medicinal or pesticidal activity of essential oils depends obviously on their chemical profile, reports on their biological properties have little value if their chemotype have not been determined (Grayer et al., 1996). Hence, depending on their major constituents as stated by Grayer's chemotype classification system (Grayer et al., 1996), the essential oil of *O. basilicum* could be related to the linalool-eugenol chemotype and those of *O. canum* and *E. camaldulensis* to the 1,8-cineole chemotype.

Table 1. Antiacetylcholinesterase and antioxidant activities of essential oils.

Essential oils	Antiacetylcholinesterase activity		Radical DPPH scavenging activity		Reducing power
	Inhibition (%)	IC50 (µg/ml)	Inhibition (%)	IC50 (µg/ml)	(mg AAE/g)
Cymbopogon citratus	30.98±0.97 [a]	n.d	7.48±0.25 [a]	n.d	47.57±2.56 [a]
Cymbopogon giganteus	36.20±2.20 [a]	n.d	18.76±0.13 [b]	n.d	75.74±2.14 [b]
Eucalyptus camaldulensis	83.41±1.33 [b]	18.98±0.74 [a]	3.68±0.25 [a]	n.d	6.47±1.34 [c]
Ocimum canum	72.13±0.49 [c]	36.16±1.48 [b]	5.20±0.51 [a]	n.d	9.35±0.93 [c]
Ocimun basilicum	32.43±2.49 [a]	n.d	95.70±0.20 [c]	3.82±0.98 [a]	531.75±10.31 [d]
Lippia multiflora	43.08±2.72 [d]	n.d	39.29±1.65 [d]	n.d	65.49±1.37 [b]
Galanthamine HBr (10 µg/ml)	98.28±1.20 [e]	0.19±0.02 [c]	n.d	n.d	n.d
Ascorbic acid (3 µg/ml)	n.d	n.d	86.16±0,32 [c]	1.80±0,43 [a]	n.d
Gallic acid (1.5 µg/ml)	n.d	n.d	91,40±0,37 [c]	0.61±0.01 [b]	445.40±1.70 [e]

Values were expressed as mean±SD of three replicates; n.d.: Not determined. Antiacetylcholinesterase activity was evaluated at the final concentration of 0.01% (v/v) essential oil. Antioxidant activities were evaluated at the final concentration of 0.1% (v/v) essential oil. IC50 expressed the concentration of essential oil (µg/ml) inhibiting 50% of acetylcholinesterase activity or scavenging 50% of free DPPH. Reducing power was given as mg Ascorbic Acid Equivalent/g of essential oil (mg AAE/g). Data from the same column, marked with the same letter doesn't shown statistical differences by Turkey test (p<0.01)

Table 2. Acetylcholinesterase inhibition parameters.

Essential oils	Km, Vm	Ki (µg/mL)	K'i (µg/mL)
Eucalyptus camaldulensis	$K_{m\ app}>K_m$ $V_{m\ app}<V_m$	8,52±0,90 a	39,56±2,60 b
Ocimum canum	$K_{m\ ap}p>K_m$ $V_{m\ app}<V_m$	7,71±0,06 a	48,46±0,66 b

Values were expressed as mean±SD of three replicates; Michaelis constant (K_m), apparent Michaelis constant ($K_{m\ app}$), maximum velocity (V_{max}), apparent maximum velocity ($V_{max\ app}$). Dissociation constants of the complex [Enzyme]-Inhibitor (Ki) and [Substrate-Enzyme]-Inhibitor (K'i). Data from the same line, marked with the same letter doesn't shown statistical differences by Turkey test (p<0.01)

Bioactivity related to chemical composition

Major compounds identified in essential oils (Table 3) were 1,8-cineole (33.9%), α-pinene (12.5%), *p*-cymene (12.3%), limonene (11.5%) for *E. camaldulensis*; 1,8-cineole (59.9%), camphor (8.1%) for *O. canum* and linalool (48.7%), eugenol (27.5%) for *O. basilicum*. Except eugenol, all these compounds have been previously found to inhibit individually AChE; 1,8-cineole being the most potent followed by α-pinene, camphor and linalool (Savelev et al., 2003; Miyazawa et al., 1997). Mixtures of 1,8-cineole/α-pinene demonstrated a minor synergistic impact while 1,8-cineole/camphor combinations antagonize the antiacetylcholinesterase activity (Savelev et al., 2003). Individual inhibitory activity of 1,8 cineole and α-pinene as well as the antagonism impact of 1,8-cineole/camphor mixture may justify the anticholinesterase potency of *E. camaldulensis* essential oil compared to *O. canum.*

The antioxidant capacity of *O. basilicum* essential oil could be mainly related to the occurrence of eugenol as assessed by Politeo et al. (2007).

Conclusion

The results of this work point out that all the essential oils tested possess anticholinesterase activity. Meanwhile, the most potent inhibitors were *E. camaldulensis* followed by *O. canum* essential oils. Both essential oils inhibit AChE though a linear mixed non competitive mechanism. On another hand,

Table 3. Composition of most active essential oils.

Compounds (KI)	Composition (%)		
	E. camaldulensis	*O. canum*	*O. basilicum*
Monoterpene hydrocarbons	41.6 [c]	15.5 [c]	2.4 [c]
α-pinene (934[a], 933[b])	12.5	4.5	-
camphene (949[a])	n.i	1.1	n.i
ß-pinene (973[a], 969[b])	-	5.8	-
myrcene (990[a], 986[b])	-	1.4	-
α-phellandrene (993[b])	3.8	n.i	n.i
p-cymene (1010[b])	12.3	n.i	n.i
limonene (1026[a], 1022[b])	11.5	-	-
trans-ß-ocimene (1048[b])	n.i	n.i	1.2
Oxygenated monoterpenes	41.8 [c]	78.1 [c]	82.5 [c]
1,8-cineole (1026[a], 1018[b])	33.9	59.9	1.6
linalool (1098[a], 1103[b])	-	1.2	48.7
camphor (1124[a])	n.i	8.1	1.2
terpin-1-en-4-ol (1172[a], 1162[b])	1.7	1.7	-
α-terpineol (1182[a], 1172[b])	2.6	4.6	-
bornyl acetate (1276[a], 1271[b])	n.i	1.4	1.1
eugenol (1337[b])	n.i	n.i	27.5
Sesquiterpenes hydrocarbons	2.9 [c]	5.2 [c]	9.6 [c]
ß-caryophyllene (1421[a], 1398[b])	-	1.7	n.i
trans-α-bergamotene (1433[a], 1427[b])	n.i	1	5.4
α-farnesene (1483[a])	n.i	2	n.i
bicyclogermacrene (1481[b])	1.9	n.i	-
γ-cadinene (1500[b])	n.i	n.i	1.1
Oxygenated sesquiterpenes	7 [c]	Not determined[c]	4.3 [c]
spatulenol (1549[b])	2.5	n.i	-
viridiflorol (1558[b])	1.8	n.i	n.i
globulol (1563[b])	1	n.i	n.i
δ -cadinol (1622[b])	n.i	n.i	3.4

Compounds representing less than 1% in all essential oils were not listed; (-) compounds identified at a concentration less than 1%; n.i. Non identified compounds. [a,b]Kovat's indices (KI) obtained with respect on DB-1 and SPB-1 columns; [c]Total percentage of each class of monoterpene and sesquiterpene identified.

E. camaldulensis, O. canum and *Cympobogon citratus* essential oils show very weak antioxidant activities while the essential oil of *O. basilicum* exhibited promising antioxidant abilities. Through GC-MS analyses, we related *E. camaldulensis* and *O. canum* to the 1,8-cineole chemotype and *O. basilicum* to the linalool-eugenol one.

Acknowledments

This research was financially supported by the International Foundation for Science (Sweden) through the project F3979-1 granted to the main author. We are also grateful to the International Atomic Energy Agency (Vienna) for providing the basic equipments of the laboratory through the Technical Cooperation Project BKF 5002.

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***Correspondence**

Martin Kiendrebeogo
Laboratoire de Biochimie & Chimie Appl iquées, UFR/SVT, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso
martin.k@univ-ouaga.bf
Tel. +226 70 60 85 90
Fax: +226 50 30 72 42