Antioxidant potentiality of three herbal teas consumed in Bandundu rural areas of Congo

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Abstract

The aim of this study was to evaluate and compare the radical scavenging and particulary the cellular antioxidant activities of *Lantana montevidensis*, *Lippia multiflora,* and *Ocimum gratissimum* leaves often consumed as herbal teas in a rural area of Bandundu (Democratic Republic of Congo). This area is severely affected by konzo, which is related to oxidative damage. Consequently, dietary supplements with proven antioxidant potentialities could be of real interest to promote in this area. Phytochemical screening by TLC and HPLC of aqueous and organic extracts revealed the presence of verbascoside as a major phenolic compound. Verbascoside in *L. montevidensis* and *O. gratissimum* is reported here for the first time. All extracts displayed high ABTS and DPPH radical-scavenging activities at the concentration range of 1–40 µg mL^{-1} according to order: *L. multiflora* > *O. gratissimum* > *L. montevidensis*. *L. multiflora* showed the best cellular antioxidant activity using DCFH-DA on HL-60 monocytes assay at 1–20 µg mL^{-1}. These herbal teas may be used as nutraceuticals for their potent antioxidant activity.

Keywords: Congo D.R; Kahemba; konzo; *Lantana montevidensis*; *Lippia multiflora*; *Ocimum gratissimum*; oxidative stress

1. Introduction

A recent survey of traditional food resources in Kahemba city showed that leaves of *Lantana montevidensis, Lippia multiflora,* and *Ocimum gratissimum* are consumed as herbal teas in the form of infusions. Kahemba, a rural area of Bandundu in the Democratic Republic of Congo, has a specific significance due to recurring outbreaks of a specific disease called konzo which is a distinct neurological entity with selective upper motor neurone damage. This disease is induced by chronic cassava cyanogenic poisoning. Recent studies suggest that disease development may be mediated through oxidative damage (Bumoko et al. 2015).

Lantana montevidensis Spreng (Verbenaceae) is native from Brazil and has been used in folk medicine as carminative and antiseptic. Previous studies showed the presence of phenolic compounds and triterpenes as its major chemical constituents. Extracts of this plants exhibited high antiproliferative, antibacterial, anti-inflammatory, antipyretic and antioxidant activities (Makboul et al. 2013; Sousa et al. 2015). Sousa et al (2015) reported that ethanolic extract exhibited high scavenging activity of DPPH radical related to its phenolic acids and flavonoids content.

Lippia multiflora Moldenke (Verbenaceae) is an African plant, also used in folk medicine with antihypertensive, antifungal, antioxidant and antiviral activities. Extracts of *L. multiflora* are known to have an excellent antioxidant activity related to their abundance of phenylpropanoids such

as verbascoside (Arthur et al. 2011). *Ocimum gratissimum* Linn (Labiateae) is widely distributed in the tropical area and is used for medicinal and culinary purposes. It showed antimicrobial, anti-inflammatory, antioxidant properties. Extracts of this plants exhibited high antioxidant activity mainly dependent on some phenolic compounds such as caffeic, ferulic, rosmarinic acids…(Hakkim 2008; Chiu et al. 2013).

To our knowledge, few investigations have been performed on the antioxidant capacities of traditional food plants from Kahemba. The present work aimed to investigate and to compare the radical-scavenging and antioxidant activities of the leave extracts from the three herbal teas collected and consumed in Kahemba, using ABTS and DPPH assays and a cell-based assay.

2. Results and discussion

2.1. Phenolic compounds content

TLC fingerprints revealed that verbascoside (or acteoside) (Figure S1) is one of the major phenolic acids in these herbal teas. Its presence in the three species was confirmed by comparison of HPLC retention time and UV-spectrum with verbascoside used as standard (Figure S2- S4). Verbascoside and isoverbascoside were first isolated in 1963 from *Verbascum sinuatum* L (Scarpati, Delle Monache 1963). *L. multiflora* leaves are already recognised as a source of verbascoside and its isomers, beside many biological properties(Arthur et al. 2011). Nevertheless, if previous phytochemical studies on *L. montevidensis* (Sousa et al. 2015) and *O. gratissimum* (Chiu et al. 2013) reported that their leaves contain a diversity of flavonoids and phenolic acids such as caffeic acid, chlorogenic acid, luteolin, quercetin, and rutin, the presence of verbascoside, and furthermore as one of the major phenolic compound, is described here, to the best of our knowledge, for the first time. Eventuellement, voici une autre suggestion de phrase: "… and rutin, but, to the best of our knowledge, this is the first report showing the presence of verbascoside in these two plant species.

2.2. Radical scavenging and Cellular antioxidant activities

All extracts had significant scavenging effects with antiradical activities connected to their ability to scavenge ABTS and DPPH radicals according to their IC_{50} (Table S1), that ranged from 7.56 to 29.1 µg mL^{-1}. IC_{50} values for organic and aqueous extracts showed that *L. multiflora* is the most active followed by *O. gratissimum* and *L. montevidensis*. A good scavenging activity of DPPH radical was reported in *L. multiflora* infusion (Arthur et al. 2011) and in the essential oil from leaves of *O. gratissimum* and *L. montevidensis* (Chiu et al. 2013; Sousa et al. 2015). Verbascoside was thought to be the major molecule responsible for the antioxidant capacity in *L. multiflora* leaves (Arthur et al. 2011) and could have a similar role for *L. montevidensis and O. gratissimum*.

DCFH-DA is useful to indirectly measure the effect of intracellular antioxidant activities in scavenging the reactive oxygen species (ROS)(Girard-Lalancette, Pichette 2009). Figure S5 shows that the addition of extracts at concentrations ranging from 1 to 20 µg mL^{-1} resulted in a dose-dependent decrease of the ROS-induced fluorescence. All extracts at 1 µg mL^{-1} (except for *O. gratissimum*) induced a significant inhibition ($p < 0.0001$) of the intracellular ROS production by HL-60 cells compared to DMSO taken as control. At 5 µg mL^{-1}, *L. multiflora* exerted already more than 50 % inhibitory effect while *L. montevidensis* and *O. gratissimum* achieved this only from 20 µg mL^{-1}.

Altogether, the three tea extracts showed good antioxidant and radical-scavenging activities but *L. multiflora* in both cases presents the most efficient effects. The aqueous extracts used in local medicine showed similar antioxidant capacity or slightly lower compared to organic extracts. Interestingly, verbascoside, mostly present in *L. multiflora,* exhibited a higher cellular antioxidant activity but a lower radical-scavenging capacity compared to gallic acid taken as positive control. López-Alarcón and Denicola (2013) showed that a good antioxidant is not just a good radical scavenger. Verbascoside is well known for its numerous biological activities including anti-oxidative, anti-apoptosis and anti-inflammatory effects. The in vivo effects of verbascoside could also be assigned to its metabolites such as caffeic acid and ferulic acid (Alipieva et al. 2014). However, the inhibition of ROS production is not probably due to verbascoside exclusively since the extracts also contain other phenolic compounds that may have synergistic effects on this inhibition.

Concerning konzo, improved processing methods to remove cyanogens from cassava prior to human consumption remain the main preventive strategy. Nevertheless, enhancement of human cyanide detoxification capabilities, perhaps through dietary supplementation may be critical to the prevention of konzo (Bumoko et al. 2015). It was shown that polyphenols have the potential to confer benefit in diverse neurodegenerative disorders associated with oxidative damage (Vauzour et al 2014). By their relevant antioxidant potentiality, these herbal teas could provide protection against oxidative damage under different disease conditions including konzo but in the future, it will be interesting to determine more specifically if the herbal tea consumption will contribute to prevent konzo.

3. Conclusion

Verbascoside is found to be the major phenolic compound of *L. montevidensis, L. multiflora, and O. gratissimum* and this is described for the first time for *L. montevidensis* and *O. gratissimum*. All the extracts exhibited an evident antioxidant activity in the selected *in vitro* antioxidant assays; particularly this is also the first report showing the potential inhibitory effect of

L. montevidensis and O. gratissimum on intracellular ROS production. The promotion of these plants as teas could contribute to diversifying diets and to increase antioxidant consumption in Kahemba's population, which consumes a diet largely dependent on cassava with a low intake of fruits and vegetables. However, *in vitro* findings, such as the antioxidant activities we have measured, are of uncertain relevance to the *in vivo* situation in healthy humans. Further studies are needed, especially on neuronal cells and in vivo to demonstrate the benefit of these extracts.

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Supplementary material

Experimental details, figures, and tables relating to this article are available online

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