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Anti-inflammatory activity of *Crateva adansonii* DC on keratinocytes infected by *Staphylococcus aureus*: From traditional practice to scientific approach using HPTLC-densitometry

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ABSTRACT

Ethnopharmacoligical relevance: Leaves of *Crateva adansonii DC* (Capparidaceae), a small bush found in Togo, are widely used in traditional medicine to cure infectious abscesses. Traditional healers of Lomé harvest only budding leaves early in the morning, in specific area in order to prepare their drugs.
Aim of the study: The main goal was to validate the ancestral picking practices, and to assess the activity of *C. adansonii* medicine towards infectious abscesses.
Materials and methods: A phytochemical screening of various *C. adansonii* leaf samples was performed using an original HPTLC-densitometry protocol and major flavonoids were identified and quantified. *C. adansonii* samples were collected in different neighborhoods of Lomé, at different harvesting-times and at different ages. Radical scavenging capacity, using DPPH assay, was used to quickly screen all extracts. Extracts were tested for anti-*Staphylococcus aureus* activity and anti-inflammatory effect on human primary keratinocytes infected by *S. aureus*. IL6, IL8 and TNFα expression and production were assessed by RT-PCR and ELISA assays.
Results: Using antioxidant activity as selection criteria, optimal extracts were obtained with budding leaves, collected at 5:00 am in Djidjolé neighborhood. This extract showed the strongest anti-inflammatory effect on *S. aureus*-infected keratinocytes by reducing IL6, IL8 and TNFα expression and production. None of the extracts inhibited the growth of *S. aureus*.
Conclusions: Those results validate the traditional practices and the potential of *C. adansonii* as anti-inflammatory drug. Our findings suggest that traditional healers should add to *C. adansonii* leaves an antibacterial plant of Togo Pharmacopeia, in order to improve abscess healing.

1. Introduction

Crateva adansonii DC also known as *Crateva guineensis* Schumach & Thonn, belongs to the family Capparidaceae. This species is confined to Africa but bears very close affinity to the Asian *Crateva religiosa* G. Forst with which it has been equated by some authorities (Igoli et al., 2014). This moderately sized deciduous tree is found throughout the tropics especially along the river banks. The leaves are

Abbreviations: AUC, area under the curve; BPE, Bovine pituitary extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl free radical; CH_2Cl_2, methylene chloride; DNI, direct normal irradiation; EDTA, ethylene diamine tetraacetic acid; EGF, epidermal growth factor; EtOAc, ethyl acetate; HPTLC, High Performance Thin Layer Chromatography; ICH, International Conference of Harmonization; IL6, Interleukine 6; IL8, Interleukine 8; K-SFM, Keratinocyte-Serum Free Medium; LOD, limit of detection; LOQ, limit of quantification; MAPK, mitogen activated protein kinase; MeOH, methanol; MOI, multiplicity of infection; MTT, 3-(4,5-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; *n*-BuOH, *n*-butanol; NF-κB, nuclear factor-kappa B; PBS, phosphate buffer saline; PVGIS, PhotoVoltaic Geographical Information System; r^2, correlation coefficient; RDSC, Radical DPPH Scavenging Capacity; rpm, round per minutes; RSD, Relative standard deviation; RT-PCR, real time polymerase chain reaction; SEM, standard error of the mean; TE, trolox equivalent; TLC, Thin Layer Chromatography; TNFα, Tumor Necrosis Factor α; v/v, volume/volume; XTT, 3′-{1-[(phenylamino)carbonyl]-3,4-tetrazolium}-*bis*(4-methoxy-6-nitro)benzene sulfonic acid hydrate; w/w, weigh/weigh

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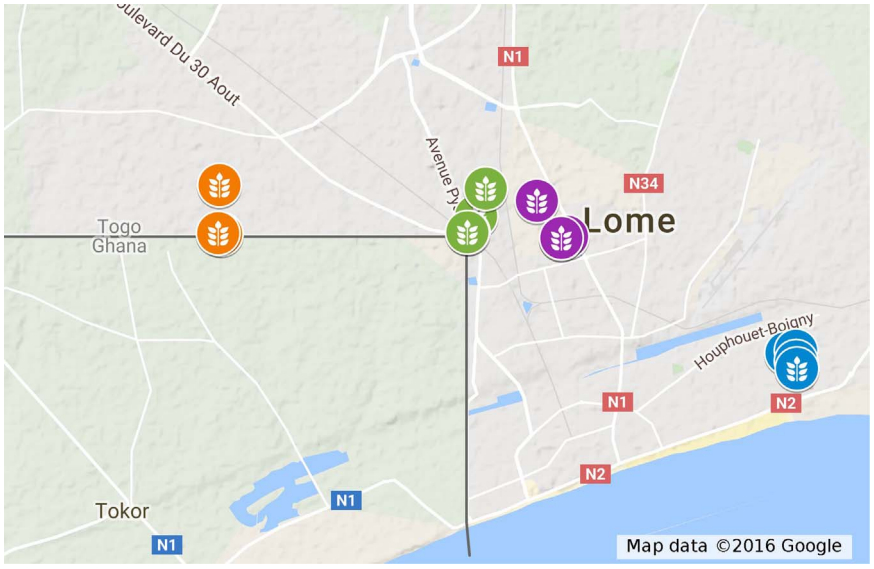

Fig. 1. Localization of the different collection points of leaves of *C. adansonii* in Lomé: Adidogomé in orange, University of Lomé in purple, Bé in blue and Djidjolé in green. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

trifoliate and ovate to oblong in shape. The flowers are white or creamy and occur at the terminal corymbs when the tree is completely without leaves. The edible fruits which are 3.5–5 mm large are spherical in shape. The barks are gray, smooth, and sometimes horizontally wrinkled (Akanji et al., 2013; Nkeiruka and Samuel, 2015).

Traditionally, barks, leaves, flowers, and root barks are extensively used in folk medicine for the cure of many disease conditions. In Senegal, the roots are used in the treatment of syphilis, jaundice, and yellow fever (Igoli et al., 2014). In India and Nigeria, the bark is useful in skin diseases and snakebites (Gupta et al., 2006; Akanji et al., 2013) while flowers are astringent (Maruthupandian and Mohan, 2010). The powdered bark is useful in kidney, bladder and urinary tract disorders, including urinary tract infections with burning micturition or urolithiasis. Concerning the validation of ethnomedicinal potential of *Crateva adansonii* DC for urinary tract disorder, an anti-urolithiasis activity of bark petroleum ether extract was observed in rats (Gupta et al., 2006). The bark or leaves decoction is widely used as a stomachic and tonic, while leaves also figure in treatments of intermittent fevers and inflammation, *e.g.* rheumatic pains, swellings and asthma (Borikini and Omotayo, 2012; Igoli et al., 2012; Gitte et al., 2012; Gupta et al., 2006).

The *in vitro* antimicrobial and antifungal activities of *Crateva adansonii* DC extracts of leaves have been previously reported (Sahoo et al., 2008; Agboke et al., 2011), while crude hexane and ethyl acetate extracts or isolated phytoconstituents of leaves presented moderate anti-trypanosomal activity *in vitro* (Igoli et al., 2012, 2014). Leaves extracts also inhibited *in vitro* xanthine oxidase activity that might be helpful in preventing or slowing the progress of gout (Abdullahi et al., 2012).

Phytochemically, the leaves contain flavonoids, tannins, saponins, cardiac glycosides, terpenes and alkaloids (Abdullahi et al., 2012; Borikini and Omotayo, 2012). Lately, aurantiamide acetate, pyropheophorbide A, ethyl pyropheophorbide A and purpurin-18 ethyl ester were also isolated from leaves of *C. adansonii* DC (Igoli et al., 2014).

As a part of our ongoing research on traditional herbs, we turned out our attention to the ethnomedicinal use of *C. adansonii* DC in Togo. Indeed, the Togolese Pharmacopoeia relates the use of this plant mainly for the treatment of abscesses, wounds, skin diseases, as dressing paste on the lesional parts. Leaves of *C adansonii* are crushed just before use to form a plaster. The plaster is then put directly on the abscess. The exposure to the plaster is usually brief, *i.e.* less than 5 min, due to an important prickly activity. Picking leaves of *C. adansonii* DC is an art from *Egbétôs*, *i.e.* traditional healers in Mina language. According to their empirical knowledge, herbalists harvest only budding leaves very early in the morning. However, no scientific evidence supports either this specific harvest practice or the medicinal use of this plant in management of skin disorders.

Wound healing is a natural body reaction leading to the restoration of structural and functional integrity of injured tissues. Inflammation, proliferation and remodeling are three overlapping and continuous wound healing phases. The healing properties of phytomedicines have been widely used by traditional healers in many countries (Süntar et al., 2012; Reddy et al., 2013). The medicinal value of these plants lies in their various health-sustaining constituents, which act on different intracellular signaling pathways *e.g.* NF-κB, MAPK (Nicolaus et al., 2017; Bui et al., 2014). These constituents include various members of chemical families like alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Balekar et al., 2012; Arct and Pytkowska, 2008).

The main goal of the present study was to validate traditional harvesting practices and to investigate the biological effects of *C. adansonii* DC leaves. Thus, we studied the *in vitro* antioxidant and anti-inflammatory activities of *C. adansonii* DC leaves extracts using DPPH scavenging assay and human primary keratinocyte cultures infected by *Staphylococcus aureus*.

2. Materials and methods

2.1. Chemicals

Quercitrin and isoquercitrin standards were purchased from Extrasynthèse (Genay, France). 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH$^{\bullet}$) was purchased from Sigma Aldrich (St Louis, MO, USA). Trolox standard reagent was purchased from Acros Organics (Geel, Belgium). Acetic acid, formic acid, petroleum ether, methylene chloride (CH_2Cl_2), ethyl acetate (EtOAc), methanol (MeOH) were purchased from Carlo Erba (Val de Reuil, France). Water was purified by a Milli-Q-system (Millipore Corporation, Bedford, MA, USA).

2.2. Plants

2.2.1. Samples collection

The leaves of *C. adansonii* were collected in September 2013, in different areas of Lomé, the capital of Togo: Adidogomé, Bé, Campus of the University of Lomé, and Djidjolé (Fig. 1). GPS coordinates of