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Research article

Phytochemical study of medicinal plants used against buruli ulcer by Ntandu people in Kongo Central, DRC

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Abstract: In Africa, ancestors are known to possess phytotherapy knowledge. This knowledge is transmitted from one generation to another through oral tradition. Based on experience, this knowledge is unaware of the chemical composition of the plants used. The study is to justify its scientific basis in the treatment of Buruli ulcer. Ethnobotanical data are collected from older men, traditional healers, herbalists, practitioners and patients who have suffered from Buruli ulcer. The species mentioned in the recipes were screened for the detection of major chemical groups. *Aloe tenuifolia*, *Annona senegalensis*, *Brillantaisia owariensis*, *Vernonia amygdalina* and *Strychnos icaja* are involved in the management of Buruli ulcer. Chemical screening has revealed the presence, to varying degrees, of the following secondary metabolites: tannins, alkaloids, saponosides, free quinones, anthocyanins, bound quinones, terpenoids, polyphenols, steroids, coumarins and reducing sugars. The presence of these metabolites provides a scientific basis for Ntandu endogenous knowledge. These findings give credence to the ethnomedical use of in the treatment of Buruli ulcer in Ntandu people.

Keywords: Endogenous knowledge - Ntandu people - *Mycobacterium ulcerans* - Medicinal plants - Phytochemistry.

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INTRODUCTION

In order to restore disturbed health, the population uses medicinal plants. Thus, important data are collected in the Democratic Republic of the Congo (DRC) (Ngbolua *et al.* 2013, Ngbolua *et al.* 2016). In Africa, ancestors are reputed to possess phytotherapeutic knowledge. They transmit it from one generation to another through the oral tradition. Qualified as empirical, this knowledge is not based on any theory and is unaware of the chemical composition of drugs used for health care (Nacoulma 1996).

Nowadays, the medicinal virtues of plants are, more and more recognized. Indeed, various authors have reported the presence of secondary metabolites that may act actively against certain pathogens, or even proven their effectiveness in the laboratory against certain diseases (Kikakedimau *et al.* 2012, 2013, Mbaya *et al.* 2014, Wanzala *et al.* 2017).

Buruli ulcer was recorded for the first time in 1897 at Buruli village in Uganda. The first sightings were published in Australia in 1937 as Bairnsdale ulcer and then in the former Belgian Congo in 1942 (Aubry 2017). Buruli ulcer caused by *Mycobacterium ulcerans*, a pathology that causes large and deep skin lesions causing serious functional sequelae in 25% of cases. Although it is the third most common mycobacteriosis in the world, after tuberculosis and leprosy, Buruli ulcer remains a shadow disease due to the localization of endemic foci in remote rural areas. There has been a dramatic increase in cases worldwide since the 1980s. (WHO 2002, 2003, Ouattara *et al*. 2002, Mougin 2009, Abgeguen *et al*. 2010).

To date, 36 countries, mostly in tropical hot and humid climates, have reported Buruli ulcer in Africa, South Asia and the Western Pacific. Although other outbreaks exist in Australia, French Guinea, Peru, Papua New Guinea and Japan, Africa appears to be the most affected region (WHO 2012). This massive increase in the number of cases and countries affected by this scourge over the last 30 years led the World Health Organization

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(WHO) to launch a specific control program in 1998 which was intensified in 2004 (Josse *et al.* 2004, WHO 2004, 2008, 2009, Quentin *et al.* 2014).

In the DRC, Van Oye & Baillon (1950) claimed to find the first case of Buruli ulcer but, the disease existed in the Kongo Central province well before the 1935s. Since then, many cases were more and more registered and Songololo territory in Kongo Central Province remains the main focus (Meleney & Johnson 1950).

The antiquity of this mycobacteriosis in this province raises the following questions:

• Is there any endogenous knowledge against Buruli ulcer in Ntandu people?

• If so, is its effectiveness based on any foundation?

The plausible hypotheses can be summarized as follows: The Ntandu people, has for many years, faced Buruli ulcer, otherwise known as "mbasu", by developing endogenous knowledge. For its anchoring in the local culture, herbal medicine must has proven its effectiveness.

The study aims to promote Congolese biodiversity and promote the green economy through the census and popularization of plant species likely to treat Buruli ulcer. It consists in verifying the scientific value of information collected from traditional healers, herbalists and other former Buruli ulcer patients.

MATERIAL AND METHODS

Material (plant species) selection

Inquiry questionnaires were sent to traditional healers, herbalists, and former Buruli ulcer patients to collect data from the field. Traditional healers cited five species: *Aloe tenuifolia* Lam., *Annona senegalensis* subsp. *oulotricha* Le Thomas, *Brillantaisia owariensis* P. Beauv, *Vernonia amygdalina* Delile and *Strychnos icaja* Baill. Three species were collected in Kasangulu from the Sisters of St. Mary of Kisantu in Kasangulu and the other two in Kimpese in the territory of Songololo.

Method of preparation

The aerial parts (leaves and stems) and the underground part (root) were dried at 40°C in an oven, after drying, the samples were milled at the Thomas brand mill and sieved in order to collect the powder.

Preparation of the aqueous and organic phases

Five grams of the powder of each species were introduced into an Erlenmeyer flask containing 200 mL of distilled water and then refluxed for 15 minutes. The mixture was cooled for 24 minutes and placed in a separating funnel in which five mL of dichloromethane (CH_2Cl_2) were added thereto. After manual stirring for 3 to 5 minutes, the gas escapes from the separating drum until exhaustion.

Phytochemical Screening

The roots decoctions were separately prepared for each species. These analyzes were used to identify secondary metabolites: alkaloids, saponosides, tannins, anthocyanins, quinones, polyphenols, reducing sugars, terpenoid, steroids and coumarins. The different chemical groups are characterized according to the techniques described in Wagner (1983), Békro *et al.* (2007) and N'Guessan *et al.* (2009).

RESULTS

Ethnobotanical data

Ethnobotanical data on families, plant species and parts or organs used in Buruli ulcer herbal medicine are presented in table 1.

Table 1. Plant species used against buruli ulcer by Ntandu people.

Family	Species	Part of the plants used
Asparagaceae	*Aloe tenuifolia* Lam	Leaves
Annonaceae	*Annona senegalensis subsp. oulotricha* Le Thomas	Leaves
Acanthaceae	*Brillantaisia patula* P. Beauv.	Leaves
Asteraceae	*Vernonia amygdalina* Delile	Leaves
Loganiaceae	*Strychnos icaja* Baill	Stem

The analysis of this table reveals that 5 plant species belonging to 5 families are used in the phytotherapy of Buruli ulcer among the Ntandu of the Lukaya District in Kongo Central. It appears that the leaves predominate in the recipes compared to the stems.

Phytochemical Screening

The phytochemical screening findings of the studied plants are given below (Table 2).

Table 2. Different major groups of compounds found after screening.

Extracts	Secondary metabolites	Plant species								
		Aloe tenuifolia		*Brillantaisia owariensis*		*Annona senegalensis subsp. oulotricha*		*Vernonia amygdalina*		*Strychnos icaja*
		Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots	Stem
Aqueous	Alkaloids	+	+	+	+	-	-	+	+	+
	Saponosides	-	-	-	-	+	-	+	+	+
	Tannins	+	+	+	-	+	+	+	+	+
	Anthocyanins	+	+	+	+	-	+	+	+	-
	Free quinones	+	+	-	-	-	+	-	-	-
	Polyphenol	+	+	+	+	+	+	+	+	+
	Reducing sugars	+	+	+	+	+	+	+	+	+
Organic	Quinone related	+	+	-	-	+	+	+	-	+
	Terpenoids	+	+	-	-	-	+	+	-	+
	Steroid	+	+	+	+	+	+	+	+	-
	Coumarins	-	+	+	+	+	+	+	+	+

Note: +, Presence of the active ingredient; -, Absence of the active ingredient.

In view of the results obtained after the phytochemical screening, it emerges that:

- Alkaloids were found in the aqueous extracts of the plant species studied with the exception of *Annona senegalensis* subsp. *oulotricha* Le Thomas.
- Saponosides are absent in aqueous extracts of *Aloe tenuifolia* Lam and *Brillantaisia owariensis* P. Beauv.
- The tannins are present in the aqueous extracts of the plant species studied with the exception of the roots of *Brillantaisia owariensis* P.Beauv.
- Anthocyanins were detected in the aqueous extracts of the plant species studied with the exception of leaves of *Annona senegalensis* subsp. *oulotricha* Le Thomas and stalks of *Strychnos icaja* Baill.
- Free quinones are found in the aqueous extracts of some species while those bound are identified in organic extracts;
- Polyphenols and reducing sugars are ubiquitous in the aqueous extracts of the species studied.
- Steroids and coumarins are identified in organic extracts of different species. They are respectively absent in the *Strychnos icaja* Baill stem and *Aloe tenuifolia* Lam leaves.

DISCUSSION

In 2004, the WHO issued a recommendation to use antibiotic therapy in the treatment of Buruli ulcer, while surgical interventions were the basis of treatment. An 8-week antibiotic therapy reduces the size of the lesions and reduces the extent of surgery, if necessary (Lagarrigue *et al.* 2000, Kanga *et al.* 2003, WHO 2004).

In practice, the medical prescription includes rifampicin (10 mg kg^{-1}) once a day orally and streptomycin (15 mg kg^{-1}) once a day by intramuscular injection for four weeks. The evaluation takes place after four weeks. In case of regression or stabilization, treatment with antibiotic continues for eight weeks. In case of extension, the surgery is associated with the anatomo-pathological examination. Antibiotic therapy is not very effective on bone lesions (Darie 2003, Aubry 2017).

Herbal medicine could be an alternative to this treatment. Indeed, Bi *et al.* (2016) demonstrated the anti-ulcer effects of herbaceous ingredients on animals while Choi *et al.* (2015) shows the use of *Brillantaisia madagascariensis* T. Anderson ex Lindau in Tanzania for its anti-proliferation effects.

Some listed species are already tested by various authors. Ibrahim *et al.* (2007) provided information on the toxic and anti-inflammatory activities of *Vernonia amygdalina* while Ezeonu *et al.* (2016) reported the immunomodulatory properties of the same species.

Alembert *et al.* (2014) isolated from the roots of *Strychnos icaja* an asymmetric alkaloid called "Strycnobaillonine". Further, Philippe *et al.* (2003) isolated from the roots five alkaloids: three monomers, protostrychnine and genostrychnine, pseudostrychnine already found in the leaves of the plant, strychnogucin C, a new bisindolic alkaloid and strychnohexamine, the first indolomonoterpenic trimeric alkaloid of natural origin.

The latter showed antiplasmodial activity against the *Plasmodium falciparum* FCA strain close to 1 µm. The use of *Strychnos icaja* in the treatment of Buruli ulcer may be justified by the presence of alkaloids which have proved themselves in other conditions.

CONCLUSION

The aim of the study was to promote Congolese biodiversity and promote the green economy through the identification and dissemination of plant species likely to treat Buruli ulcer. This valorization passes explanations based on recognized and proven scientific evidence.

According to ethnobotanical surveys, the Buruli anti-ulcer floret of Ntandu endogenous knowledge is composed of 5 species. These are: *Aloe tenuifolia* Lam.; *Brillantaisia owariensis* P. Beauv.; *Annona senegalensis* subsp. *oulotricha* Le Thomas; *Vernonia amygdalina* Delile; *Strychnos icaja* Baill

The phytochemical analysis of the aqueous and organic leaves and root extracts revealed that these species secrete secondary metabolites recognized as being effective against bacterial germs.

These results confirm the hypotheses that the Ntandu people, confronted with Buruli ulcer problems, in other words "mbasu" for many years, have developed endogenous knowledge. The plants used secrete secondary metabolites capable of acting actively against *Mycobacterium ulcerans*.

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