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Original Article

Microscopic and UV/Vis spectrophotometric characterization of *Cissampelos pareira* of Brazil and Africa

Niara Moura Porto[a], Yuri Lima de Barros[b], Ionaldo J.L. Diniz Basílio[b], Maria de Fátima Agra[a,*]

[a] *Laboratório de Taxonomia e Farmacobotânica, Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*
[b] *Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, Universidade Federal da Paraíba, João Pessoa, PB, Brazil*

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ABSTRACT

Cissampelos pareira L., belonging to Menispermaceae family, has worldwide distribution, occurring in tropical and subtropical regions of the Americas, Africa and Asia. It is the most popular species of *Cissampelos*, known for its medicinal uses of leaves and roots. The study aims to find distinctive leaf anatomical characters, and also demonstrate the importance of spectral data to identify *C. pareira* samples, in order to contribute to its taxonomy and quality control of its drugs. Anatomical leaf analyses were performed by optical and scanning electron microscopy. The spectral profile was obtained from methanolic extracts of *C. pareira* samples from Brazil and Africa, with application of UV–vis spectrophotometry data, which were analyzed by principal component analysis (PCA). Some anatomical characters such as leaf epidermal cells walls, stomata, trichomes, mesophyll, features of midrib and petiole, and the spectral profile within the wavelength ranging between 770 and 240 nm (eight bands) differs between Brazilian and African samples. The results represent an additional support to the taxonomy of *C. pareira*, and the quality control of their leaf drugs, mainly in relation to misidentified samples.

Introduction

Cissampelos pareira L., belonging to Menispermaceae family, has worldwide distribution, occurring in tropical and subtropical regions of the Americas, Africa and Asia (Ortiz, 2001). In Brazil, it is encountered in different types of vegetation, from Caatinga, Atlantic Forest and Amazon forest (Braga, 2015). According to Schmelzer and Gurib-Fakim (2008), in Africa this species occurs in subtropical forest, savannah, deciduous shrubs, often persisting in cleared land and plantations, also in secondary vegetation and near rock outcrops.

It is the most popular species of *Cissampelos* not only for its wide distribution, but mainly because its leaves and roots are widely used as medicinal. According to Napralert (2013), *C. pareira* has more than eighty folk names. In Brazil, it is known as "parreira", "abuta", and "parreira-brava" (Lewis and Elvin-Lewis, 1977; Rury, 1983); in Africa, it is called in folk medicine as "chegonde" and "karigi-munana" (Hedberg et al., 1983; Rukunga et al., 2009); and in India, it is known as "ambastha", "patha" and "laghupatha" (Vaidya, 1988).

In many ethnobotanical reports, the leaves of *C. pareira* are recognized as a natural medicine for various purposes. The leaf juice is used as antiseptic, anthelmintic, insecticidal and parasiticidal, and against dermatitis (Singh and Ali, 1992), asthmas (Singh and Maheshwari, 1994), genitourinary disorders (Sanchez Medina et al., 2001), diarrhea, dysenteries and gastrointestinal disorders (Kumar et al., 2006; Kamble et al., 2008), antifertility (Ganguly et al., 2007; Priya et al., 2012), and antidiabetic (Yadav et al., 2013). The topical use of leaves is indicated to treat hemorrhages from cuts, burns and wounds (Ramasubramaniaraja and Babu, 2010; Shukla et al., 2012), and also to treat abscesses (Abbasi et al., 2010; Haque et al., 2011). In addition, in India, the leaves are also used as cattle feed to increase milk production, and also in some food systems as thickeners, gelling agents, texture modifiers and stabilizers (Vardhanabhuti and Ikeda, 2006; Priya et al., 2012), *inter alia.*

The leaves of *C. pareira* have been reported to be a rich source of isoquinoline and bisbenzylisoquinoline alkaloids (Shukla et al., 2012), such as berberine (Kupchan et al., 1960a), curine (Chowdhury, 1972), hayatine (Sharma, 1987) and magnoflorine (Ahmad et al., 1992). In addition, have also been isolated essential oil (Kupchan et al., 1960b), flavonoids (Ramirez et al., 2003; Amresh et al., 2007a), polysaccharides (Vardhanabhuti and Ikeda, 2006), and pectin (Singthong et al., 2004; Arkarapanthu et al., 2005) have also been isolated.

* Corresponding author.
E-mail: agramf@ltf.ufpb.br (M. de Fátima Agra).

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Biological and pharmacological activities of leaves and aerial parts (leaves and branches) of *C. pareira* were demonstrated in several studies. The cissampeloflavone, isolated from leaves, showed activity against *Trypanosoma cruzi* and *T. brucei rhodesiense* (Ramirez et al., 2003). The plant extract exhibited antifungal activity against *Aspergillus niger* and *Saccharomyces cerevisiae* (Kumar et al., 2006). The ethanol extract of the aerial parts showed anti-inflammatory and analgesic activities (Amresh et al., 2007b). The contraceptive and cytotoxic effects were demonstrated by Priya et al. (2012) and Ganguly et al. (2007), respectively. The anti-diabetic activity was confirmed by Jannu et al. (2011) and Yadav et al. (2013). In addition, a preliminary study carried out by Thakur and Rana (2013) confirmed the anxiolytic effect of *C. pareira* leaves.

According to Rhodes (1975) and Hoot et al. (2009), *C. pareira* has problems in its interspecific delimitation with imprecise limits, mainly caused by its wide distribution and great plasticity of their vegetative forms. On the other hand, the leaf anatomical studies have shown to be an additionall support to the plant taxonomy, as already done in *Solanum* (Nurit-Silva et al., 2007; Nurit-Silva and Agra, 2011; Sampaio et al., 2014), and also to the Menispermaceae family, including *Cissampelos* by De Wet et al. (2002), Porto et al. (2008, 2011, 2012), for example.

The spectroscopic chemical techniques have emerged and contributed as an additional tool to contribute to plant taxonomy, and also as a support to the quality control of herbal drugs, allowing information to be obtained without the need for previous isolation of chemical constituents, as demonstrated before for *Baccharis* (Lonni et al., 2005) and *Solanum* (Basílio et al., 2012).

Although the leaves of *C. pareira* are commonly used in traditional medicine, and there is evidence of many activities of their compounds, a literature survey showed a lack of studies of the leaf comparative anatomy, as well as spectroscopic analysis of UV–visible of the leaf extracts. In this way, this study aimed to find leaf anatomical characters, distinctive to *C. pareira*, on samples of plants from Brazil and Africa, revealing the importance of anatomical studies combined with spectral data, would be useful to the quality control of its drugs, as well as to the taxonomy of *C. pareira*.

Materials and methods

Plant material

Botanical expeditions and field observations were carried out by N.M. Porto, in areas of Atlantic Forest and Rain Forest, for sample collection of Menispermaceae, including leaves of *Cissampelos pareira* L. in the following Brazilian States: Alagoas, Pará Maranhão, Paraíba, Pernambuco and Sergipe (Table 1). For each individual, an average of three leaf samples were taken from the second to the fifth nodes of the leaf blades and the proximal, median and distal portions, and petiole were fixed in FAA (50%) for 24 h (Johansen, 1940), and preserved in ethanol 70 GL. The other part of fertile material was pressed and dried for herbaria, according to Bridson and Forman (1999). The voucher specimens were deposited at the Herbarium Prof. Lauro Pires Xavier (JPB), of the Universidade Federal da Paraíba.

In addition, leaf samples from herbarium specimens identified as *C. pareira* were also analyzed from the following herbaria, acronyms by Thiers (2015): Herbarium of Centro de Pesquisas do Cacau (CEPEC), Herbarium Prof. Jayme Coelho de Morais (EAN), Herbarium of Embrapa Amazônia Oriental (IAN), Herbário Prof. Lauro Pires Xavier (JPB), Herbário Museu Paraense Emílio Goeldi (MG), Herbarium Jardim Botânico do Rio de Janeiro (RB),

Table 1
Selected voucher specimens of *Cissampelos pareira* and species of outgroup.

Species	Specimen code	Country, State and Municipality	Voucher specimen	Herbarium
Anomospermum chloranthum	AC	Brazil, Pará, Santarém	M Silva 2619	MG
Anomospermum steyermarkii	AS	Brazil, Roraima, Uaicá	GT Prance s/n	MG
Cissampelos andromorpha	CA1	Brazil, Paraíba, Conde	NM Porto 30	RB
	CA2	Brazil, Pará, Belém	NM Porto 45	JPB
	CA3	Brazil, Pernambuco, Catende	NM Porto 07	JPB
	CA4	Brazil, Espírito Santo, Santa Teresa	W Pizziolo 329	RB
Cissampelos pareira	CP1	Brazil, Rondônia, Porto Velho	JA Silva 39	IAN
	CP2	Brazil, Pará, Monte Alegre	RL Fróes 30443	IAN
	CP3	Brazil, Distrito Federal, Brasília	HS Irwin s/n	IAN
	CP4	Brazil, Santa Catarina, Ipumirim	AL Gasper 2020	RB
	CP5	Brazil, Goiás, Pirenópolis	HS Irwin s/n	RB
	CP6	Brazil, Mato Grosso do Sul, Corumbá	A C. Cervi 3276	RB
	CP7	Africa, Ethiopia, Ghion	JW Ash 655	MO
	CP8	Africa, Tanzania, Tanga district	H Faulkner 5631	MO
	CP9	Africa, Uganda, Kyadondo	PK Rwaburindore 205	MO
	CPa	Brazil, Bahia, Filadélfia	AM Giulietti 1886	CEPEC
	CPb	Brazil, Mato Grosso do Sul, Corumbá	A Pott 3158	RB
	CPc	Brazil, Bahia, Coribe	MM Lopes 1374	CEPEC
	CPd	Brazil, Paraíba, Maturéia	MF Agra 5061	JPB
	CPe	Brazil, Santa Catarina, Capão Alto	M Verdi 1156	RB
Cissampelos sympodialis	CS1	Brazil, Paraíba, João Pessoa	MF Agra 7133	JPB
	CS2	Brazil, Ceará, Fortaleza	Celismar s/n	JPB
	CS3	Brazil, Bahia, Juazeiro	Zehntren 211	RB
Cissampelos tropaeolifolia	CT1	Brazil, Sergipe, Capela	NM Porto 19	JPB
	CT2	Brazil, Alagoas, Coruripe	NM Porto 47	JPB
	CT3	Brazil, Maranhão, Ribeirãozinho	NM Porto 48	JPB
	CT4	Brazil, Pará, Conceição do Araguaia	T Plowman 8755	IAN
Hyperbaena domingensis	HD	Brazil, Pernambuco, Sirinhaém	M Oliveira 1553	UFP
Orthomene hirsuta	OH	Brazil, Amazonas, São Gabriel	GA Black 48-2473	IAN
Orthomene schomburgkii	OS	Brazil, Pernambuco, Igarassu	BS Amorim 1668	UFP
Sciadotenia brachypoda	SB	Brazil, Amazonas, São Paulo Olivença	NT Silva 4146	IAN