Phytochemical prospection of the floral extract of *Ouratea spectabilis* (Mart.) Engl. (Ochnaceae)

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Abstract

Ouratea spectabilis is a tree species belonging to the genus *Ouratea* and the family Ochnaceae. This study aimed to report the first report on the phytochemical constitution of the floral extract of *O. spectabilis*. Flowers were collected from individuals of *O. spectabilis* in a Cerrado area in Rio Verde, Goiás, Brasil in 2022. The extract was produced from 150g of floral organ. The extract yield was determined by the difference in mass, visual analysis, crystallinity and homogeneity were performed by the organoleptic method, pH and electrical conductivity were determined using a pHmeter and conductivitymeter both digital. The qualitative phytochemical profile was performed for several groups of phytomolecules of the special metabolism of this plant using specific reagents. The floral extract yield was 11.81%, several phytochemical groups were observed, such as phenolics, saponins, flavonoids, steroids, triterpenoids, organic acids, reducing sugars, aromatic compounds, alkaloids and amino acids. The floral extract of *Ouratea spectabilis* showed high potential for future work on chemical quantification and assays with different biological models.

Keywords: *Ouratea* genus, Phenolics, Alkaloids, Antioxidant activity, Hemolytic activity

Resumo

Ouratea spectabilis é uma espécie arbórea pertencente ao gênero *Ouratea* e a família Ochnaceae. Este estudo teve por objetivo, reportar o primeiro relato sobre a constituição fitoquímica do extrato floral de *O. spectabilis*. Flores foram coletadas em indivíduos de *O. spectabilis* em área de Cerrado sentido restrito em Rio Verde, Goiás, Brasil em 2022. O extrato foi produzido a partir de 150 g de órgão floral. O rendimento de extrato foi determinado pela diferença de massa, a análise visual, cristalinidade e homogeneidade foram realizadas pelo método organoléptico, o pH e a condutividade elétrica foram determinados em pHmetro e condutivímetro ambos digitais. O perfil fitoquímico qualitativo foi realizado para diversos grupos de fitomoléculas do metabolismo especial desse vegetal utilizando reagentes específicos. O rendimento de extrato floral foi de 11,81%, foram observados diversos grupos fitoquímicos como, fenólicos, saponinas, flavonoides, esteroides, triterpenoides, ácidos orgânicos, açúcares redutores, composto aromático, alcaloides e aminoácidos. O extrato floral de *Ouratea spectabilis* demonstrou alto potencial para futuros trabalhos de quantificação química e ensaios com diferentes modelos biológicos.

Palavras-chave: Gênero *Ouratea*, Fenólicos, Alcaloides, Atividade antioxidante, Atividade hemolítica

Resumen

Ouratea spectabilis es una especie arbórea perteneciente al género *Ouratea* y a la familia Ochnaceae. Este estudio tuvo como objetivo informar el primer informe sobre la constitución fitoquímica del extracto floral de *O. spectabilis*. Las flores se recolectaron de individuos de *O. spectabilis* en un área de Cerrado en Rio Verde, Goiás, Brasil en 2022. El extracto se produjo a partir de 150 g de órgano floral. El rendimiento del extracto se determinó por diferencia de masa, el análisis visual, la cristalinidad y la homogeneidad se realizaron por el método organoléptico, el pH y la conductividad eléctrica se determinaron mediante un pHmetro y conductímetro, ambos digitales. Se realizó el perfil fitoquímico cualitativo para varios grupos de fitomoléculas del metabolismo especial de esta planta utilizando reactivos específicos. El rendimiento de extracto floral fue de 11,81%, se observaron varios grupos fitoquímicos como fenoles, saponinas, flavonoides, esteroides, triterpenoides, ácidos orgánicos, azúcares reductores, compuestos aromáticos, alcaloides y aminoácidos. El extracto floral de *Ouratea spectabilis* mostró un alto potencial para trabajos futuros sobre cuantificación química y ensayos con diferentes modelos biológicos.

Palabras clave: Género *Ouratea*, Fenólicos, Alcaloides, Actividad antioxidante, Actividad hemolític

1. Introduction

The genus *Ouratea* is circumscribed in the botanical family Ochnaceae (27 genera), with ~600 species tropicalis distributed in South America and the African continent. The *Ouratea* species are evergreen trees, shrubs, and shrublets (Carvalho et al., 2000; Chacon, 2011 Fidelis et al., 2014). In Brasil, this genus is present in the various phytophysiognomies of the Cerrado domain (Rocha et al., 2020).

Several species of *Ouratea* are used in folk medicine to treat gastric discomfort, dysentery and diarrhea (Mbing et al., 2006); as an astringent and tonic (Estevam et al., 2005), as well as for the treatment of inflammation-related diseases such as rheumatism, sprains, and arthritic disorders (Carbonari et al., 2006). The genus *Ouratea* has scientifically described flavonoids and biflavonoids that exhibit antiviral, antimicrobial, and cytotoxic activities, in addition to inhibitory activity of the topoisomerase enzyme (Suzart et al., 2007).

Among the great genetic diversity of this flora, *Ouratea spectabilis* (Mart. ex Engl.) Engl. (Figure 1) is described in areas of Cerrado *sensu stricto* (Mecina et al., 2014). *Ouratea spectabilis* is popularly known as "folhas de serra (pt) or sawblade leaves (in)", morphologically, it is an arboreal plant, presenting extrafloral, deciduous, heliophytic nectary and indifferent to soil conditions (Montesinos & Oliveira, 2014; Alves -Silva & Del-Claro, 2016). In folk medicine *O. spectabilis* is used to treat gastric and rheumatic disorders (Paulo et al., 1986). Studies revealed the presence of 6,6"-di-genkwanin, 7,7"-di-methoxyflavone (3,3")-linked biflavone *O*-methyl ethers, named ouratein A (**1**), B (**2**), C (**3**), and D (**4**) where they exhibit important activities in several models and biological assays (Felício et al., 1995; Rocha et al., 2020). The effect of the strata show strong antiviral inhibition on Herpes virus type 1 (HSV-1) as described by Brandão et al. (2011) and with important anti-inflammatory properties by Rocha et al. (2020).

Especially *O. spectabilis* presents other important biological activities from leaf extracts and their fractions, with an allelopathic effect on lettuce seeds and cytotoxic effect on the mitotic index in the study by Mecina et al. (2014). Molecules isolated from the leaf extract of *O. spectabilis* showed an inhibitory effect on aldose reductase (AR) from two phytocompounds. The extract from the trunk of *O. spectabilis* demonstrated in a study, activity of inhibiting TNF release by lipopolysaccharide (LPS)-stimulated THP-1 cells, indicating its potential anti-inflammatory activity (Campana et al., 2015).

In this sense, studies on the floral organ of *Ouratea* are still scarce, with only two phytochemical studies for *O. hexasperma* by Suzart et al. (2012) and *O. lancifolia* by Menzes Filho et al. (2021), both evaluating in particular the strata of this organ. This study aimed to evaluate the qualitative phytochemical composition of the floral hydroethanolic extract of *Ouratea spectabilis* collected in a Cerrado area, restricted sense, in the Goiás State, Brasil.

Figure 1: Individual of *Ouratea spectabilis* in flowering period. Photo by Menezes Filho, A. C. P., 2022.

2. Materials and Methods

2.1 Plant material and preparation of extract

O. spectabilis flower were collected from specimens at Universidade de Rio Verde, Rio Verde, Goiás State, Brasil, (17°47'14.0'' S and 50°58'01.8''W), June 5, 2022. The plant species O. spectabilis was identified by a dichotomous key for the genus *Ouratea* by the first author of this study. A voucher specimen was deposited in the Herbarium of the Instituto Federal Goiano, Rio Verde, Goiás State, Brasil. (HRV: 13831). For preparation the extract, 1 kg of flowers were collected from 6 individuals of *O. spectabilis*. The flowers were weighed 150g and processed in a domestic processor with a 70% (*v/v*) hydroethanolic extractor solution. The hydroethanolic extract was obtained by mechanical agitation for 72 h at 25 °C. The extract was filtered and rotary evaporated at 68 °C to remove the ethanol, then, the sample were frozen and lyophilized to obtain the dry floral extract.

2.2 Physicochemical prospection

Visual color, transparency, opacity, crystallinity and aroma were performed by organoleptic testing. The yield determined in percentage described by Alves et al. (2011), after lyophilization process. Red% = [(g dry extract x 100)/g vegetable drug]. The pH of the extract of *O. spectabilis* was determined using a pH meter.

2.2 Phytochemical assay

The phytochemical tests to detect the presence of heterosides, saponins (foamy and hemolytic), tannins (blue (hydrolyzable or gallic) and green (condensed or catechic) coloration), reducing sugars, no-reducing sugars, resins, amino acids, purines, aromatic and aliphatic compounds, polysaccharides, carboxylic acids, oxylates, flavonoids, steroids, triterpenes, coumarines, quinones, organic acids, alkaloids and depsides & depsidones were performed following the method described by Matos (1997), Madike et al. (2017), Mehdi et al. (2019) and Menezes Filho et al. (2022). These tests were based on the visual observations of color modification or precipitate formation after the addition of specific reagents (-) negative (+) present.

2.3 Statistical analysis

Results are the mean of three replicates followed by ± standard deviation (SD). The statistical program used was Microsoft Excel (2010).

3. Results and Discussion

The yield of the floral extract was 11.81 ± 0.31, visual color (dark red), aromatic, homogeneous, clear and crystalline, and pH = 5.96 ± 0.43. The compounds denominated as special metabolites are slightly produced by the plants and have a relevant role in their adaptation to the environment and protection, presenting a wide number of biological properties. The floral hydroethanolic extract of *O. spectabilis* exhibited the presence of several phytochemical groups such as heterosides, tannins, reducing sugars no-reducing sugars, amino acids, aromatic compounds, carboxylic acids, flavonoids, steroids, triterpenes, coumarins, organic acids, alkaloids and depsides & depsidones (Table 1).

Several phytochemical groups are described for the Ochnaceae family, including lignans, di- and triterpenoids, steroids, monosaccharides, depsides and triacylglycerides (Velandia et al., 2002; Fidelis et al., 2014). Suzart et al. (2012) found for the floral extract of *O. hexasperma* the flavonoids, swertisin, swertiajaponin, rutin, vitexin and orientin; terpnoids 3-β-*O*-acyl-olean-12-en-28-oic acid; steroids sitosterol, stigmasterol and 3-*O*-β-glycopyranosylsitosterol. Results like those of this study were described by Menezes Filho et al. (2021), evaluating by screening the main phytochemical groups of the floral extract of *O. lancifolia*, describe the presence of cardiac glycosides, foamy saponins, alkaloids, organic acids, reducing sugars, non-reducing sugars, hemolytic saponins, phenols, tannins (condensed or catechic), flavonoids, depsides & depsidones, derivatives of benzoquinones, anthraquinones, flavonols, flavanones, flavanols and double olefins.

Table 1. Results of preliminary phytochemical screening of 70% hydroethanolic extract from flowers of *Ouratea spectabilis*.

Compounds	**Results**
Heterosides	+
Foamy sponins	+
Hemolytic saponins	+
Tannins	Green
Reducing sugars	+
No-reducing sugars	+
Resins	-
Amino acids	+
Purines	-
Aromatic & aliphatic compounds	Red
Polysaccharides	-
Carboxylic acids	+
Oxylates	-
Flavonoids	+
Steroids	+
Triterpenes	+
Coumarins	+
Quinones	-
Organic acids	+
Alkaloids	+
Depsides & depsidones	+
Proteins	-
Phlobatannins	-
Cardiac glycosides	-
Double olefins	-

Note: (-) Negative test. (+) Positive test. Green: condensed or catechic. Red: Aromatic compounds. Source: Authors, 2022.

Studies evaluating the compounds isolated or in synergism in *Ouratea*, have demonstrated important biological activities as antiviral from the leaf extracts of *O. lucens* on Herpes virus 1 (HSV-1) and (HSV-2), poliovirus, vesicular stomatitis virus (VSV), and parainfluenza-3 virus (Bagla et al., 2012) and (Roming et al., 1992); *O. castaneifolia*, *O. semiserrata* and *O. spectabilis* on viruses (HSV-1), Vaccinia virus (VACV) and Murine Encephalomyocarditis virus (EMCV) (Brandão et al., 2011). Molecules such as agathisflavone, 7”-methyl-agathisflavone and lupeol in *O. parviflora* showed efficacy in inhibiting HSV-1 and HSV-2 in the study by Araújo et al. (2011).

In assays on Sarcoma 180 and Walker 256 cancer cell lines, flavanol dimer and proanthocyanidin A and B isolated from the root extract in *Ouratea* sp. demonstrated antitumor activity (Oliveira et al., 1972). Grynberg et al. (1994) also obtained potential antitumor activity against Murine tumors using the isolated molecules 7”-*O*-methylagathisflavone and amentaflavone from *Ouratea* sp. Daniel et al. (2007) evaluated the molecules 7,7”-dimethyllanaraflavone, agathisflavone and 7”-methylagathisflavone isolated from the leaf extract of *O. hexasperma* where they observed important inhibition on HT-29 colon adenocarcinoma, NGI-H460 non-small cell lung carcinoma, MCF- 7 breast cancer cell, OVGAR-3 ovarian adenocarcinoma cells and RXF-393 renal cell carcinoma.

Anti-inflammatory effects are described for the extract of *O. parviflora* leaves, in addition to being an antioxidant in the reduction of DPPH free radical, hydroxyl radical, superoxide anion and lipid peroxidation (Carbonari et al., 2006). The stem and leaves extracts of *O. semiserrata* showed exceptional inhibition on 5-lipoxygenase with antiasthma effect in the study by Braga et al. (2000). Other diverse activities are described for *Ouratea*, such as hepatoprotective (*O. parviflora*) Carbonari et al. (2006), enzyme inhibition (*O. spectabilis*) Felício et al. (1995), vasodilator (*O. semiserrata*, *O. spectabilis* and *O. castaneifolia*) by Côrtes et al. (2002) and Valadares et al. (2003), antimalarial (*O. nitida*) Estevam et al. (2005), antimicrobial (*O. sulcata*, *O. multiflora*) Pegnyemb et al. (2005) and Carbonezi et al. (2007).

4. Conclusions

This was the first report evaluating the phytochemical composition of the floral extract of *Ouratea spectabilis*. The preliminary phytochemical prospection showed for the extract, a great diversity of groups of phytochemical compounds, especially flavonoids, phenolics, steroids, tannins and reducing sugars.

Future studies should be carried out quantifying these phytocompounds in the floral extract of *O. spectabilis* and evaluating possible positive effects on different biological models, such as antioxidant, anti-inflammatory, photoprotective, antibacterial and antifungal.

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