***Original Research Article***

**Byrsanthus brownii Guill., Chemical profile and potential antioxidant**

**Abstract**

*Byrsanthus brownii* Guill (Salicaceae) is a plant used in traditional Congolese medicine to treat malarial fevers, amoebic dysentery, high blood pressure, physical and sexual asthenia. This work aimed to determine the chemical profiles and then evaluate the antioxidant activity of extracts from different parts of *Byrsanthus brownii* Guill. The extracts were obtained by maceration in methanol. The contents of total polyphenols and total flavonoids were determined respectively by the Folin-ciocalteu reagent and by aluminum trichloride and sodium nitrite. The chemical profiles were determined by LC-MS/MS. The antiradical activity was evaluated by trapping the free radical DPPH. The total polyphenol contents obtained are 288.87 ± 0.14 mgEAG/g.Ms (root barks), 271.12 ± 0.12 mgEAG/g.Ms (trunk barks) and 174.77 ± 0.17 mgEAG/g.Ms (leaves). The total flavonoid contents are 104.09 ± 0.03 mgEQ/gMs (trunk barks), 85.50 ± 0.01 mgEQ/gMs (root barks) and 34.20 ± 0.01 mgEQ/gMs (leaves). The antioxidant activity is better in trunk barks (IC50= 141.53 μg/mL) and in root barks (IC50 = 154.06 μg/mL).

**Keywords**: *Byrsanthus brownii* Guill., polyphenols, flavonoids, LC-MS/MS, antioxidant activity.

**1-Introduction**

*Byrsanthus brownii* Guill., also called *Byrsanthus epigynus* Mast. or Anetia *Byrsanthus* Steud. is a species of the Salicaceae family. Previously classified in the Flacourtiaceae family (Pellegrin, 1921), *B. brownii* is a shrub or shrub of the periodically flooded forests of the Congo River and its tributaries (Miabangana et al., 2018). Its foliage is gray green, its flowers are green and yellow-white, thick, its wood is pink. The genus *Byrsanthus*, which has only one species, seems to have a limited phytogeographic distribution. Its distribution is from West Africa (Guinea, Senegal and Sierra Leone) to Central Africa (Gabon, Central African Republic, Congo, Democratic Republic of Congo and the Cabinda enclave). In Congo, its distribution seems to be poorly circumscribed. This species is found all along the Congo River, particularly on M'Bamou Island (northern district of Brazzaville) and Kombé (southern district of Brazzaville). *B. brownii* is used in traditional Congolese medicine to treat several pathologies such as amoebic dysentery, high blood pressure, physical and sexual asthenia (Miabangana et al., 2018). It is therefore likely that *Byrsanthus brownii* extracts contain bioactive molecules with interesting pharmacological properties such as phenolic compounds. To our knowledge, *Byrsanthus brownii* extracts have never been the subject of either phytochemical or pharmacological studies. This work therefore constitutes a first investigation in this direction. The objective of this study is therefore to determine the chemical composition and then evaluate the antioxidant activity of the extracts of this species.

**2- Material and methods**

**2-1- Plant material**

The plant material consisted of leaves, trunk bark and roots of *Byrsanthus brownii* collected in May in Kombé (south of Brazzaville). The plant was authenticated by botanists from the national herbarium of the National Institute for Research in Exact and Natural Sciences under the references. The different organs (figure 1) were dried for two weeks at room temperature, protected from sunlight and light. The plant material was finely ground using a Philips type electric grinder. The powder of each organ was stored in plastic bags before extraction.



Figure 1: *Byrsanthus brownii* in its natural environment

**2-2- Obtaining the extracts**

50g of the plant powder of the leaves, trunk barks and roots previously delipidated with hexane was macerated with 500mL of methanol under stirring for 24 hours. The mixture was then filtered using filter paper. The operation was repeated several times until the plant material was exhausted. The filtrates were mixed and then evaporated to dryness, using a Büchi brand rotary evaporator. The extraction yield was calculated by the following formula:

$Rdt=\frac{Mext.}{Mv}$x100

Mext: mass of the extract; Mv: mass of the plant material

**2-3-Determination of total polyphenols**

The content of total polyphenols was determined by the method described by Singleton et al., (1999); Waterhouse et al., (2002) with minor modifications. 0.1g of dry extract of each organ (leaves, trunk bark, root bark) was dissolved in 100 mL of distilled water and then in a test tube, 0.3mL of the solution was mixed with 1.5mL of Folin-Ciocalteu reagent diluted 1/10. After 5 minutes, 1.2 mL of aqueous sodium carbonate solution (Na2CO3) was added. The mixture was then incubated in the dark for 2 hours at room temperature. The absorbance of the mixture was read with a spectrophotometer at 760 nm, against a blank. In parallel, a range of standard solutions (gallic acid) was prepared. The results are expressed in milligram equivalent of gallic acid per gram of dry matter (mg EAG/g.Ms).

**2-4-Determination of total flavonoids**

The content of total flavonoids in the extracts was determined by the method described by Kouamé et al., (2021) with some modifications. 0.1g of plant extract was dissolved in 100 mL of distilled water. In a test tube, 1mL of the solution was taken and then successively added to 4 mL of distilled water and 0.3 mL of a sodium nitrite solution (NaNO2 5%). At t = 5 minutes, 0.3 mL of an aluminum chloride solution AlCl3 (10%) was added. At t = 6 min, 2 mL of the sodium hydroxide solution (NaOH, 1N) were added. The mixture was incubated in the dark for 30 minutes at room temperature. Absorbance was measured using a UV-visible spectrophotometer at 510 nm against a blank. The contents were calculated from a standard solution of quercetin. The results are expressed in milligram equivalent of quercetin per gram of dry matter (mg EQ/g.Ms).

**2-5- Determination of chemical profiles**

The chemical profiles of the extracts of different parts of Byrsanthus brownii were determined by HPLC-MS on a UHPLC Ultimate 3000 RSLC system (Thermo Fisher Scientific Inc., Waltham, MA, USA) coupled with a rapid quaternary separation pump (Ultimate autosampler) and a DAD detector. The compounds were eluted on an Uptisphere Strategy C18 column (250 4.6 mm, 5 m, Interchim, Montluçon, France). The mobile phase consisted of a mixture of water acidified with 0.1% (v/v) formic acid (phase A) and acetonitrile also acidified with 0.1% (v/v) formic acid (phase B). The phase A gradient was programmed as follows: 100% (0 min), 80% (10 min), 73% (35 min), 0% (40–50 min), and 100% (51–60 min). The flow rate was 0.8 mL/min and the injection volume was 5 µL. The UHPLC system was connected to an Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Ionization was obtained by electrospray in positive (ESI+) or negative (ESI-) mode with molar masses between 80 and 1200 g/mol. The source parameters were as follows: spray voltage at 3 kV; capillary temperature at 320 °C; auxiliary gas temperature at 400 °C; envelope gas (nitrogen), sweep sheath and auxiliary gases at a flow rate of 50, 10 and 2 arbitrary units respectively and a collision cell between 10 and 50 eV. The spectrum analysis data were obtained with a resolution of 70,000 while those of the MS/MS were obtained with a resolution of 17500. The data were processed with the Freestyle software in negative mode.

**2-5- Evaluation of the antiradical activity**

The antiradical activity was evaluated by the method described by Brand-Williams et al., (1995) with some modifications. 3 mL of a methanolic solution of 2,2-diphenyl-1-picrylhydrazine (18µM) was mixed with 3 mL of a range of methanolic solutions (200µg/mL; 600µg/mL and 1000µg/mL) of each sample. The mixture was homogenized and then incubated in the dark at room temperature for 30 minutes. The absorbance was read at 517 nm against a blank. Ascorbic acid was used as a reference. The tests were performed in triplicate.

**2-6- Statistical treatment**

The tests were performed in triplicate. The results are expressed as mean ± standard deviation.

**3- Results and discussion**

**3-1- Extraction yields**

 The extraction yields obtained are 6.42%; 6.58% and 6.64%, respectively for the extract of leaves, root barks and trunk barks. The yields are almost the same in all organs (Table 1).

Table 1. Yield of methanolic of extraction of each organ of plant

|  |  |  |  |
| --- | --- | --- | --- |
| Organes | Leave |  Bark |  Root |
| Masse of plant (g) | 50 | 50 | 50 |
| Masse of extract (g) | 3.21 | 3.32 | 3.29 |
| Yield (%) | 6.42 | 6.58 | 6.64 |

**3-2- Total polyphenol contents**

The total polyphenol contents were determined from a gallic acid calibration curve with equation Y = 5.8591X + 0.079 and regression coefficient R² = 0.9971 (figure 2).

**Figure 2.** Calibration curve of gallic acid

The total polyphenol contents were 174.77 ± 0.17 mg EAG/g.Ms, 271.12 ± 0.169 mg EAG/g.Ms and 288.87 ± 0.14 mg EAG/g.Ms, respectively for the leaf, trunk bark and root bark extracts. The root bark contained slightly more total polyphenols than the trunk bark (Figure 3). The leaves had the lowest total polyphenol content.

Figure 3.Total polyphenol contents of extracts of *Byrsanthus brownii* GUILL.

**3-3- Total flavonoid content**

The total flavonoid contents were determined using a calibration curve obtained with quercetin with equation Y=1.345x +0.002 and regression coefficient R2=0.998 (figure 4).

Figure 4.Quercetin calibration curve

The total flavonoid contents were 34.20 ± 0.01 mgEQ/g.Ms, 85.50 ± 0.01 mgEQ/g.Ms and 66.91 ± 0.03 mgEQ/g.Ms, respectively for leaf, stem bark and root bark extracts (Figure 5). The stem bark is rich in total flavonoids compared to other parts of the plant.

Figure 5. Total flavonoid contents of leaves, trunk barks (E-T) and root barks (E-R)

**3-4- Chemical profile**

LC/MS study of extracts from different organs of *Byrsanthus brownii* allowed the identification of compounds such as citric acid, tachioside, scutellarin, dihydroammiol glucoside, apigenin-o-glucuronide, lanceoloside A isomer, kaempferol, kaempferide and acacetin in the leaves. Some compounds identified in the leaves are sometimes found in the extracts of the trunk bark and roots (Figure 6, Table 2). The identified compounds belong to the flavonoid family, some of which have already been identified in other species. Indeed, tachioside was identified in the inner part of the bark of *Betula platyphylla* (Liimatainen, 2013); Kaempferide was identified in Australian *lemongrass* extract and *Phrynium capitatum* (Fathoni et al., 2022; Ali et al., 2022); lanceoloside A isomer in *Juglans regia* extracts (Ventura et al., 2023); apigenin-o-glucuronide in *Salvia ceratophylla* extracts (Sengul et al., 2021); stecullarin in *Scutellariae barbatae* extracts (Hon-Yeung et al., 2009); acacetin in *Codiaeum variegatum* extracts (Rashwan et al., 2024); dihydroammiol glucoside was identified in *Ammi visnaga* L extracts (El Karkouri et al., 2020; Khalil et al., 2020).



Leave



Bark of the trunk



Root bark

Figure 6. Negative ion LC/MS chromatograms of methanolic extracts of *Byrsanthus brownii* Guill.

Table 2. Compounds identified in leaves, trunk barks and root barks of *Byrsanthus brownii*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **N°** | **Rt (min)** | **Compound** | **Formula** | **M-Hexp (m/z)** | **MS/MS Fragment** | **Extracts** | **Reference** |
| **Leave** | **Sterm bark** | **Sterm root** |
| 1 | 4.1 | NI | C6H10O6 | 177.0399 | 162/176/78 | + | - | - |   |
| 2 | 7.08 | Citric acid | C6H8O7 | 191.0192 |  | **+** | **-** | **-** | Standard |
| 3 | 12.42 | Tachioside | C13H18O8 | 301.923 | 85/259 | **+** | **+** | **+** | Liimatainen, 2013 |
| 4 | 19.5 | Scutellarin | C21H18O12 | 461.0720 | 461/285/136/219/424/86 | **+** | **-** | **-** |  Hon-Yeung et al., 2009 |
| 5 | 21.81 | dihydroammiol glucoside | C20H24O11 | 439.1240 | 303/439/133/89 | **+** | **+** | **+** | El Karkouri et al., 2020 ; Khalil et al., 2020 |
| 6 | 24.87 | Apigenin-O-glucuronide | C21H18O11 | 445.0771 | 269/113/85/175/445 | **+** | **-** | **-** | Sengul et al., 2021 |
| 7 | 28.52 | Lanceoloside A isomer | C20H22O9 | 405.1186 | 121/139/283/405 | **+** | **+** | **+** | Ventura et al., 2023 |
| 8 | 39.45 | Kaempferol | C15H10O6 | 285.0399 | 285/286/257 | **+** | **-** | **-** |  Standard |
| 9 | 39,76 | NI | C27H30O12 | 545.1659 | 145/347/414/263 | **+** | **+** | **+** |   |
| 10 | 40.28 | NI | C27H28O12 | 543.1503 | 274/168/199/175 | **+** | **+** | **+** |  |
| 11 | 40.64 | Kaempferide | C16H12O6 | 299.0556 | 283/151 | **+** | **-** | **-** | Fathoni et al., 2022 ; Ali et al., 2022 |
| 12 | 42.05 | Acacetin | C16H12O5 | 283.0606 | 283/176/118/96 | **+** | **-** | **-** |  Rashwan et al., 2024 |

     

Figure 7. Structures of compounds identified in *Byrsanthus brownii* extracts

**3-5- Antiradical activity**

The antiradical activity of the different parts (leaves, trunk bark and root bark) of Byrsanthus brownii was evaluated by the free and stable radical DPPH trapping method. The results show that only the extracts of the trunk bark and roots have an inhibitory effect on the free radical DPPH. The concentrations that inhibit 50% of the free radicals obtained are 141.54 and 154.06 µg/mL, respectively for the trunk bark (E-T) and the root bark (E-R) as shown in Figure 8. These concentrations are close to that of ascorbic acid used as a reference (IC50=103.87 µg/mL). This antiradical activity can be correlated with the contents of total polyphenols and total flavonoids. Indeed, extracts containing high total polyphenol and total flavonoid contents have shown interesting activity, this is the case for trunk bark and roots. However, the extract of trunk bark is more active compared to other organs of the plant. Moreover, several authors report that phenolic compounds, particularly flavonoids, are excellent natural antioxidants, due to their antioxidant power and their ability to trap free radicals (Dias et al., 2021). These compounds have antioxidant, anti-inflammatory, anticancer, and antimicrobial properties (Liimatainen, 2013; Hon-Yeung et al., 2009; El Karkouri et al., 2020; Sengul et al., 2021; Ventura et al., 2023; Fathoni et al., 2022; Rashwan et al., 2024).

Figure 8. Antioxidant activity of the extracts in *Byrsanthus brownii*

**Conclusion**

he objective of this study was to determine the chemical profiles and then evaluate the antioxidant activity of the leaves, trunk barks and root barks of Byrsanthus brownii Guill. The extracts were obtained by maceration in methanol. The extraction yields are almost the same in all organs. The total polyphenol content is higher in the root barks than in the other parts of the plant, i.e. 288.86 ± 0.14 mgEAG/gMs. While the trunk barks are richer in total flavonoids, i.e. 85.502 ± 0.01 mgEQ/g.Ms. Both parts of the plant, namely the trunk barks and the root barks, significantly inhibited the DPPH free radical with IC50s of 141.54 and 154.06 µg/mL, respectively. Byrsanthus brownii extracts can be a source of natural antioxidants that can be used in several sectors, including the food industry, the pharmaceutical industry and cosmetology.

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