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PHYTOCHEMICAL STUDY AND EVALUATION OF THE IN VITRO ANTIOXIDANT ACTIVITY OF THE MEDICINAL PLANT BRUGMANSIA CANDIDA PERS. USED IN THE TREATMENT OF MADNESS IN THE SANKURU PROVINCE (DR CONGO)

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Abstract

The species Brugmansia candida Pers. was introduced in the Sankuru province of the Democratic Republic of Congo (DRC) by Catholic or Protestant missionaries as an ornamental plant. They were mainly interested in its decorative appearance and beautiful multi-colored flowers, which give off a pleasant scent in the evening and at night. B. candida is known for its psychotropic and hallucinogenic properties. In the Sankuru province, it is known as a strong drug that is easier to obtain than local drugs. The objective of this work was therefore to highlight the phytochemical compounds of this plant and to evaluate their antioxidant activity. This phytochemical analysis was carried out following the method described by Harbone (1998). The polyphenolic compounds were assayed by the spectrometric method and the antioxidant activity was determined using the DPPH and ABTS trapping tests. These analyses showed that aqueous

extracts of B. candida leaves contain polyphenols, anthocyanins, tannins, leucoanthocyanidins, alkaloids, bound quinones, and saponins, but no flavonoids. On the other hand, in organic extracts of the leaves of this species, steroids, terpenoids, and free quinones were detected. As for the antioxidant activity, the ABTS radical showed interesting IC50 values, namely 38.95 ± 0.15 µg/mL for aqueous extracts and 21.15 ± 0.08 µg/mL for organic extracts. In conclusion, B. candida leaves are a source of active compounds and have a significant antiradical profile. It can therefore be suggested that this species be integrated into alternative medicine for the management of certain psychosomatic diseases. However, its excessive use should be discouraged by government authorities in the same way as tobacco.
Keywords: *Phytochemistry, antioxidant, psychotropic, Brugmansia candida, DR Congo*

1. Introduction

The species *Brugmansia candida* was introduced to the Sankuru province in the Democratic Republic of Congo (DRC) by Catholic or Protestant missionaries who were probably interested in its appearance since it is a very beautiful decorative plant with magnificent flowers of various colors, which give off a lovely scent in the evening and at night. Initially, this species was introduced and cultivated as an ornamental plant. It has subsequently adapted to local environmental conditions and currently grows spontaneously and naturally throughout the Sankuru province in DR Congo.

Best known for its psychotropic and hallucinogenic properties, *B. candida* is considered and widely used in the Sankuru province by local people as medicinal as a strong drug, that is easier to obtain than local drugs. Its resemblance to tobacco and its violent and unpleasant effects have earned it the nickname “Ofokafoka”. Indeed, the word “Ofokafoka” comes from “Foka”, which means tobacco in Tetela (the language spoken in the Sankuru province), and “Ofokafoka” means something that looks like tobacco (something that acts like tobacco). In certain circumstances, Ofokafoka means stronger, producing more effects than local tobacco. In addition to its psychotropic properties, the B. candida plant has many medicinal virtues. In Sankuru province, all parts of the plant are used in traditional medicine: leaves, flowers, seeds, stems and roots.

The genus *Brugmansia* comprises 7 species, many cultivars, as well as spontaneous or artificial hybrids, in a variety of colors and shapes. Depending on their size at maturity, small and medium-sized species such as *B. versicolore* and *B. sanguinea* are distinguished, and large species such as *B. candida,* which produces magnificent decorative flowers. B. candida Pers. is a vigorous shrub 2 to 5 m high, belonging to the Kingdom Plantae, the Embranchment Spermatophytes, the Sous Embranchment Angiospermae, the class Dicotyledones, the order Solanales, the family Solanaceae, and the genus *Brugmensia* (Stevens (2012). It is a very decorative tropical plant whose flowers are in the form of huge colorful and fragrant trumpets (Bongbeme, 2015). It is also called Angel’s Trumpet, White Brugmensia, Jimson Weed, Angel’s Trumpet, etc.

The species *B. candida* has simple, alternate, dark, or variegated yellow leaves, semi-evergreen or deciduous, depending on the climate. Many varieties have been developed for ornamental gardens. In temperate climates, flowering occurs from June to October. It can be early in greenhouses or winter gardens. The fruits are not prickly and keep for a long time (several decades). Its large flowers (up to 30 cm) are hanging trumpets of various colors, directed towards the ground at an angle of about 40°. In the evening and at night, they give off a strong and pleasant scent, particularly attractive to nocturnal insects. This scent has earned them the nickname “angel’s trumpet” (Ibrahim et al., (2017).

Plants of the species *B. candida* have psychotropic and hallucinogenic properties. Several authors report that *Brugmansia* hallucinogens cause violent and unpleasant effects. They are widely used in several Latin American communities: during initiation or shamanic rituals, for seduction, rape, even murder, to correct unruly children, to hypnotize people who are robbed without realizing it or remembering it when they wake up, mixed with corn beer or tobacco, they were administered to wives and slaves as a drug before being buried with their lords

(Bongbeme, 2015). Many nicknames attributed to the plant testify to its toxicity: "devil's herb", "sorcerer's herb", "magician's herb", "rapist's herb", "thorny apple", etc. The plant *B. candida* has many therapeutic properties. In Sankuru province, the leaves, flowers, seeds, roots, and stems of B. candida are widely used in various ways to treat a wide range of skin, stomach, and muscle diseases, as an anti-inflammatory to treat pain, rheumatism, etc., and sometimes as a poison (Niedz et al., 2012).

According to Alvarez (2008) and Gonzalo et al. (2015) all parts of the plant are toxic The seeds, for example, contain high concentrations of poisons and ingestion can cause muscle paralysis, confusion, dry mouth, diarrhea, hallucinations, and death. Although very attractive for their flowers, all varieties of B. candida are toxic if ingested (Athony et al., 2009). They mainly contain two alkaloids: hyoscyamine and scopolamine, which are responsible for an atropinoid syndrome if ingested after prolonged contact with the sap, stems, leaves, flowers, or fruits. It is advisable to wash your hands after prolonged contact with the plant and to keep it out of the reach of children. In the past, drugs in general and strong drugs, in particular, were the preserve of adults, especially the elderly, as a stimulant (Odavia, 2015). Today, it is increasingly young people who are attracted to drugs. This is explained by the current context: youth unemployment and the loss of purchasing power of the population, leading to an increase in crime and the number of people developing mental disorders. This explains the craze among young people for the leaves of *B. candida*, which are a powerful and easy-to-obtain medicine, as the plant grows in all habitats in the DRC.

The fact that *B. candida* is commonly used in Sankuru Province for medicinal purposes prompted us to undertake this study. The main objective pursued was to determine the chemical compounds present in the leaves of this species through phytochemical analyses and to evaluate their antioxidant activity. To detect the phytochemical compounds present in this plant, the Harbone (1998) method was used. The polyphenolic compounds were evaluated by the spectrometric method and the antioxidant activity using the DPPH and ABTS trapping tests.

2. Material and methods

2.1. Material

The plant material used in this study consists of *Brugmansia candida* leaves that was collected in the experimental botanical garden of the Department of Biology of the Faculty of Science and Technology of the University of Kinshasa and dried at Laboratory room temperature. Qualitative phytochemical analyses were carried out at the Laboratory of Phytochemistry of Natural Substances and Medicinal Chemistry of the Faculty of Sciences of the University of Kinshasa. On the other hand, quantitative phytochemical and biological analyses were carried out at the Laboratory of Analysis and Research on Food and Nutrition (LARAN) of the Department of Biology of the Faculty of Sciences and Technology of the University of Kinshasa.

2.2. Methods

2.2.1 Preparation and packaging of samples

After harvesting, the leaves of *B. candida* were sent to the laboratory and then dried in an oven (Fisher Scientific model 665 fc), at a temperature of 40 °C for three days. After drying, the samples were ground using an electric grinder (IKA MF10 basic) and sieved to obtain a fine and homogeneous powder. The powders obtained were stored in glass containers in the laboratory until the time of analysis.

2.2.2 Phytochemical analysis

1. Chemical screening (qualitative analysis

The use of this test provides a general idea of the secondary metabolites, with potential biological or therapeutic properties, present in the leaves of B. candida. The characterization reactions were carried out in tubes (solution tests) (Harbone 1998). Indeed, this is a qualitative analysis based on coloring and/or precipitation reactions.

2. Preparation of aqueous and organic extracts

The **aqueous and organic extracts** were prepared as follows: 10 g of the powder of the species leaves was placed in 100 ml of distilled water and boiled at 100 °C for 15 minutes in a water bath. The decoc-

tion obtained was filtered hot through cotton and collected in an Erlenmeyer flask. For the organic extracts, we weighed 10 g of the powder and added 100 ml of ethyl acetate for at least 1 hour, filtered the solution on filter paper using a funnel, and collected the filtrate in an Erlenmeyer flask.

3. Aqueous phase test

a. Polyphenol detection

To detect polyphenols, 1 mL of aqueous extract of B. candida leaves was placed in a test tube. Then a few drops of Burton's reagent ($FeCl_3$) 2% and K3Fe(CN)6 1% (1:1/v/v) were added using a Pasteur pipette. This reagent can be used to highlight the presence of polyphenols when the solution becomes intense blue (sometimes accompanied by a precipitate). In case of a positive test, we systematically looked for the following different polyphenolic compounds: flavonoids, quinones, anthocyanins, tannins, leucoanthocyanidins.

b. Flavonoid detection

Shinoda's reagent was used to detect flavonoids. This reagent consists of a mixture of various reagents, namely: 95% ethyl alcohol, concentrated HCl, distilled water (1:1:1 v/v/v), and Mg or Zn shavings. For this purpose, 1 ml of aqueous extract was taken with a Pasteur pipette and placed in a test tube. Then, Shinoda's reagent, a few Mg shavings, and a few drops of stirred isoamyl alcohol were added and this mixture was left to stand. The formation of a thin film of orange (flavones), cherry red (flavonols), or purplish coloring indicates the presence of flavonoids.

c. Search for Anthocyanins

To highlight anthocyanins, hydrochloric acid (HCl 20%) concentrated at 20% was used. For this purpose, 1 mL of aqueous extract was placed in a test tube, to which a few drops of HCl 20% were added. This mixture was then heated in a water bath. A purplish coloration of the anthocyanin chloride develops and can crystallize.

d. Leucoanthocyanidin detection

The Shinoda's reagent was used to detect leucoanthocyanidins. One mL of aqueous extract was placed in a test tube. A few drops of Shinoda reagent, and isoamyl alcohol were then added and the whole mixture was then heated in a water bath. The presence of a red or purplish color in the supernatant layer indicates a positive test.

e. Detection of tannins

Tannins are water-soluble phenolic compounds that, in addition to the classic reactions of phenols, have the properties of precipitating proteins and alkaloids. To detect them, we used Stiansny's reagent $FeCl_3$ 2% (formaldehyde 30% + concentrated HCl, 2:1), CH3COONa. First, 1 mL of aqueous extract is placed in a test tube to which a few drops of FeCl3 1% are added. The appearance of a green color with or without precipitate generally indicates the presence of tannins. In case of a positive test, the catechic tannins and gallic tannins can be differentiated as follows: 1 mL of the aqueous extract is placed in a test tube to which a few drops of Stiansny's reagent are added. The mixture obtained is then heated in a water bath at 90 °C for 30 minutes. The appearance of a brown precipitate indicates the presence of catechic tannins. In this case, it is necessary to filter this mixture and then saturate the filtrate with CH_3COONa crystals. Finally, 1 mL of 2% $FeCl_3$ must be added. When the blackish color appears, this indicates the presence of gallic tannins.

f. Search for alkaloids

The search for alkaloids was carried out using Dragendorff reagents ($Bi(NO_3)_3$ 0.85 g; glacial CH3COOH 10 ml; KI 8 g and distilled water 80 ml) and 0.1 N HCl. We proceeded as follows: 1 ml of slightly acidified aqueous extract with a few drops of 0.1 N HCl, added a few drops of Dragendorff reagent. A red-orange precipitate forms and indicates the presence of alkaloids.

g. Search for saponins

Saponins are genuine steroidal or terpenic glycosides characterized by their surfactant properties. Their dissolution in water forms a foaming solution. To detect them, we placed 1mL of aqueous extract in a test tube to which we added a few drops of distilled water which was then shaken vigorously. The formation of a foam of at least 1 cm in height for 15 minutes indicates the presence of saponins, which corresponds to a positive test.

h. Search for bound quinones

This was done using the Borntrager reagent (NaOH 10% or NH4OH 10%). For this purpose, 1mL of the aqueous extract was placed in a test tube, to which the Borntrager's reagent was added and the mixture was

shaken vigorously. The color ranging from orange to bright red characterized that the test is positive, which indicates the presence of bounds quinones.

4. Tests on the organic phase

a. Detection of steroids and triterpenoids

To detect steroids and triterpenoids, 1 mL of organic extract was added to the Liebermann-Buchardat reagent. A purple coloration of this mixture indicates the presence of triterpenoids and steroids. Separately, triterpenoids form a purple complex while steroids develop a green coloration.

b. Detection of free quinones

Free quinones are highlighted by treating the organic extract with Borntrager reagent. The appearance of a coloration ranging from orange to bright red indicates the presence of free quinones.

2.2.3 Polyphenolic compound assays (quantitative analyses)

Polyphenolic compounds were detected through cold extraction. For this purpose, 40 mg of B. candida leaf powder was weighed and placed in glass flasks. Then, 40 mL of distilled water was added. Afterward, the flasks were then placed in the ultrasonic bath for 45 minutes. The macerates obtained were filtered using Whatman paper n°1. The filtrates were used for the analysis of the determination of the content of polyphenolic compounds (total polyphenols, flavonoids, and tannins).

a. Total polyphenols determination

The determination of the total polyphenol content was carried out using the Folin-Ciocalteu reagent (Mbadiko et al., 2019; Mbemba et al., 2023; Nyamangombe et al., 2023). For this purpose, we prepared a reaction mixture comprising: 0.2 mL of extract, 2 mL of distilled water, and 0.2 mL of Folin reagent. We added 0.4 mL of sodium carbonate (20%) after 3 minutes of incubation. The mixture obtained was shaken well and then incubated for one hour at laboratory temperature and protected from light. We also carried out the same operation for the blank, except that instead of the extract, we added 0.2 mL of 80% methanol. The absorbances were read on a spectrophotometer at 725 nm. Each assay was repeated in triplicate. A standard concentration range with gallic acid (0.016 mg/ml to 0.125 mg/ml) was prepared to calculate the concentration of total polyphenols contained in the analyzed sample. The amount of total polyphenols obtained is expressed in mg of gallic acid equivalent (GAE)/g of dry matter using the following equation from the calibration line.

$$Y = 7.2852x + 0.0581$$

Where y = the absorbance of the extract, and x = gallic acid equivalent (mg/g).

b. Determination of Total flavonoids

Aluminum trichloride $AlCl_3$ forms a yellow complex with flavonoids that absorbs at 415 nm (Heimler et al., 2006; Mbadiko et al., 2019). For this purpose, we prepared a reaction mixture composed of 1 mL of the extract to be analyzed and 1 mL of 2% $AlCl_3$ (dissolved in methanol). The mixture obtained was shaken well, then incubated at laboratory temperature, and protected from light for one hour. The same operation was carried out for the blank, except that instead of the extract, 1 mL of methanol was added. We also prepared a standard range with quercetin (0.03125 to 0.125 µg/ml) to calculate the flavonoid concentration contained in the analyzed sample. The absorbances were measured using a spectrophotometer at 415 nm. Note that the mixtures were prepared in triplicate for each analysis and the average value was retained. Finally, the flavonoid content was expressed as mg quercetin equivalent (EQ)/g dry matter using the calibration line equation:

$$y = 22.382x - 0.15$$

Where: y = the absorbance of the extract, and x = the equivalent of quercetin (mg/g).

c. Tannin determination

The principle of this determination is based on the fixation of the aldehyde group of vanillin on carbon 6 of the A cycle of catechin to form a red chromophore complex that absorbs at 510 nm (Anthony S. J., Zuchowski W., Setzer, W. N., 2009). The reaction mixture containing 0.5 mL of organic extract, 1.5 mL of a vanillin solution (4% in methanol), and 1.5 mL of concentrated hydrochloric acid was prepared and all this was well shaken. After 30 minutes of incubation at laboratory temperature, and protected from light, we measured the absorbances with a spectrophotometer at 510 nm. The mixtures were prepared in triplicate for each analysis and the average value was retained. The tannin concentration was deduced from a calibration range established

with catechin (0 to 1 mg/mL). The total tannin content of the extracts is expressed in mg catechin equivalent per gram of dry matter using the calibration line equation:

$$y = 34.358x - 0.0132$$
$$R_2 = 0.9994,$$

Where: x = the absorbance, and y = the equivalent of catechin (mg/g).

2.2.4. Evaluation of biological activity

a. Preparation of total dry extracts

The evaluation of the biological activity was done as follows: 10 g of the powder of the analyzed sample was macerated in 100 mL of distilled water for 24 hours. The macerate was filtered using Wathman paper No. 1 and the filtrate was placed in a Petri dish which was then placed in an oven (Memmer) at 40 °C for 24 hours. The total dry methanolic extracts were prepared as follows: 10 g of the powder of the analyzed sample was macerated in 100 mL of an 80% methanol solution for 24 hours. The macerate was then filtered using Wathman paper No. 1 and the filtrate was placed in a Petri dish. This was placed in an oven (Memmer) at 40 °C for 24 hours.

b. Evaluation of antioxidant activity

1. DPPH (2,2 DiPhenyl-1- Picrylhydrazyl) radical test

The method reported by (Heimler et al., 2006; Mayele et al., 2024) was used to evaluate the antioxidant power by the DPPH test. The chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is a purplish free radical that absorbs at the wavelength of 517 nm. The reduction of the free radical DPPH to DPPH-H (2,2-diphenyl-1-picrylhydrazine) produced when single electron pairs cause representative decolorization reflects the ability of an extract to scavenge free radicals. This discoloration of the radical measured by the spectrophotometer at 517 nm is proportional to the concentration of antioxidants (Mbadiko et al., 2019; Mbemba et al., 2023). To prepare the DPPH radical, 3.2 mg of DPPH was dissolved in 100 mL of 80% methanol. The solution thus obtained was kept away from light for at least one hour. After incubation, the absorbance of the DPPH solution was adjusted to 0.7 ± 0.05 using 80% methanol.

To prepare the different concentrations of the analyzed extracts, 40 mg of total dry extracts were weighed and diluted in 4 mL of distilled water. From this mother solution (10 mg/mL), we made successive dilutions to have a concentration range from 10 to 6 mg/mL Then, 20 µL of each concentration level of extract to be analyzed was taken and placed in test tubes to put the sample in contact with the DPPH. We then added 1980 µL of the DPPH · radical analysis solution. The same operation was carried out for the control solution (DPPH · radical solution), except that instead of the extract, we added 20 µL of 80% methanol. The different solutions prepared were incubated away from light for 30 minutes.

We used quercetin, gallic acid, and catechin as reference antioxidants (positive control). They were prepared under the same conditions as the analyzed extracts. The reaction solutions were prepared in triplicate. The absorbance reading and the determination of the DPPH · inhibition power were done via the absorbances at 517 nm using a spectrophotometer (Jenway 7615). In addition, the percentage of inhibition of the radical by the sample was determined using the following formula:

$$\%\ inhibition = \frac{1 - A_x}{A_c} \times 100$$

Where: A_x = absorbance of the DPPH radical in the presence of the extract, and A_c =: absorbance of the DPPH radical (control solution or blank).

Finally, the IC50 values of the different samples were determined using the Grap Pad Prism 10.4 software.

c. ABTS (2,2'-azino-bis-3ethylbenzthiazoline-6-sulfonic acid) test

By reacting with potassium or sodium persulfate, ABTS (2,2'-azino-bis-3ethylbenzthiazoline-6-sulfonic acid) forms the cationic radical ABTS · + of blue to green color. The addition of an extract containing antioxidant compounds reduces this radical and causes a discoloration which is measured by spectrophotometer at 734 nm. The discoloration of the ABTS · + radical is proportional to the antioxidant concentration (Mbadiko et al., 2019).

The preparation of ABTS · + was carried out by diluting 32.49 mg of the ABTS radical in 1500 µL of distilled water (solution A). Then, 8 mg of potassium persulfate ($K_2S_2O_8$) was weighed and dissolved in 1142 µL of distilled water (solution B). The two solutions were then mixed in equal volume

(1:1). The resulting mixture was stored away from light for 12 to 16 hours to obtain the ABTS · + stock solution which was then diluted with methanol to obtain an analysis solution with an absorbance of 0.800 to 1.000.

The different concentrations of the analyzed extracts were prepared from a stock solution of 10 mg/mL of the analyzed basic extracts. Subsequently, a range of concentrations ranging from 10 to 6 mg/mL was prepared by performing successive dilutions. To bring the sample into contact with ABTS ·+, 20 µL of each concentration level of extracts to be analyzed were taken and placed in the test tubes. We then added 1980 µL of the ABTS · radical analysis solution. The same operation was carried out for the control solution (ABTS ·+ solution), except that instead of the extract, 20 µL of 80% methanol was added. The different solutions obtained were finally incubated away from light for 30 minutes. Gallic acid was used as a reference antioxidant (positive control). The different solutions concerned were prepared under the same conditions as the extracts analyzed. Note that each mixture was prepared in three repetitions.

The absorbance reading and the determination of the ABTS · + inhibitory power were carried out at 734 nm using a Jenway 7615 spectrophotometer, for the blank (methanol), the control solution, and the solutions of the samples analyzed. The determination of the ABTS ·+ radical inhibitory power of the analyzed extracts was calculated according to the following formula:

$$\%\ inhibition = \frac{1 - A_x}{A_c} \times 100$$

Where: Ax = the absorbance of the ABTS ·+ radical in the presence of the extract.

Ac = the absorbance of the ABTS ·+ radical (control solution or blank)

The IC50 values of the samples studied were determined using the Graph Pad Prism 10.4 software.

III. Results

3.1. Phytochemical analyses

3.1.1 Data from Phytochemical screening in solution

Phytochemical screening was carried out following the precipitation coloring reactions for the search for secondary metabolites and the results are recorded in Table 1 below. This table shows the results of the qualitative analysis of aqueous and organic extracts of B. candida leaves.

Table 1. *Results of chemical screening of B. candida leaf extracts*

Substances sought	Reagents used	Results
	Aqueous phase	
Total Polyphenols	Burton	+
Flavonoïds	Shinoda	–
Anthocyanins	Hydrochloric Concentrated acid 20%	+
Tannins	$FeCl_3$ 2%.	+
Leucoanthocyanins	Shinoda	+
Alkaloids	Dragendorff	+
Saponins	Agitation	+
Related Quinones	Borntrager	+
	Organic Phase	
Steroids	Liebermann	+
Triterpenes	Liebermann	+
Free quinones	Borntrager	+

Legend: +: Presence of the substance sought; -: Absence of the substance sought

It appears that aqueous extracts of B. candida leaves contain polyphenols, anthocyanins, tannins, leucoanthocyanidins, alkaloids, bound quinones, and saponins, but

they do not contain flavonoids. On the other hand, organic extracts of leaves of this same plant species contain steroids, terpenoids, and free quinones

This table presents the results of the qualitative analysis of aqueous and organic extracts of *B. candida* leaves. It can be seen that aqueous extracts of B. candida leaves contain polyphenols, anthocyanins, tannins, leucoanthocyanidins, alkaloids, bound quinones, and saponins. On the other hand, they do not contain flavonoids. On the other hand, organic extracts of leaves of this same plant species contain steroids, terpenoids, and free quinones.

3.1.2 Determination of total polyphenol, flavonoid, and tannin contents.

Table 2 below presents the results of the analysis concerning the contents of polyphenols, flavonoids, and total tannins.

Table 2. *Total polyphenol, flavonoid, and tannin contents (mean, n = 4)*

Extracts	Total Polyphenols (mg EAG/g MS)	Total Flavonoids (mg EQ/g)	Tannins (mg EC/g)
B. candida	163.8 ± 6.5	46.1 ± 0.2	17.7 ± 0.7

Legend: GAE/g DM: Equivalent of gallic acid per gram of dry matter; – EQ/g DM: Equivalent of quercetin per gram of dry matter; – EC/g DM: Equivalent to catechin per gram of dry matter

The results in Table 2 show that among these chemical compounds, total polyphenols are the highest, followed by flavonoids and tannins, the lowest. Such high content of polyphenolic compounds in B. candida leaf extracts makes them responsible for antioxidant activity.

3.2. Evaluation of antioxidant activity in vitro

Table 3 below summarizes the results of the antioxidant properties from the different leaf extracts of B. candida. It appears from this Table that the different leaf extracts of B. candida have low antioxidant properties since the IC50 values are greater than 10 µg/mL This indicates a low antioxidant activity of different extracts analyzed.

Table 3. *Screening of antioxidant activity by the ABTS and DPPH test, expressed in terms of IC50 and µg/mL (mean ± SD, n=4)*

	IC_{50} (µg/mL)			
Plants used	DPH		ABTS	
	Aqueous extract	Organic extract	Aqueous extract	Organic extract
B. candida	2491.25 ± 1.06	Nd	38.95 ± 0.15	38.95 ± 0.15
Gallic Acid	0.42±0.10		22.32±3.4	

Legend: Nd: not determined

IV. Discussion and conclusion

Phytochemical screening revealed on the one hand that the aqueous extracts of *B. candida* leaves contain polyphenols, anthocyanins, tannins, leucoanthocyanidins, alkaloids, bound quinones, and saponins and on the other hand do not contain flavonoids. In addition, the organic extracts revealed the presence of steroids, terpenoids, and free quinones in the leaves of *B. candida* Pers. These results are consistent with those of Bongbeme (2015) on aqueous and organic extracts of B. candida leaves. Gonzalo (2015) and Ibrahim et al. (2017), also brought to light the presence of alkaloids in *B. candida* leaf extracts. This confirms the toxicity of *B. candida* leaf extracts. Moreover, quantitative analysis of polyphenolic compounds

showed that B. candida leaf extracts have a high content of total polyphenols (163.8 ± + 6.5 mg EAG/g DM), followed by a medium content of flavonoids (46.1 ± 0.2 mg EQ/g) and a low content of tannins (17.7 ± 0.7 mg EC/g).

Furthermore, the results of antioxidant activity revealed that the different extracts of *B. candida* leaves analyzed in this work have a low antioxidant power for the DPPH test (aqueous extracts) (2491.25 ± 1.06 µg/mL), since the IC50 values are greater than 10 µg/m, far from that of quercetin used as a control. According to Mbemba et al. (2023), a low IC50 value corresponds to a higher antioxidant activity of the extract, while a high IC50 value means that the antioxidant activity is high, which is not the case for the values obtained in this study. As for the ABTS test, the organic extracts showed a high antioxidant power compared to the aqueous extracts. The values obtained in this case (38.95 ± 0.15 µg/m) are significantly lower compared to the control. The difference between the two tests lies in their reaction mechanism. Indeed, the ABTS radical reacts at the same time with hydrophilic and lipophilic compounds while DPPH only reacts with hydrophilic compounds (Floegel et al., 2011).

In conclusion, this work further shows that aqueous extracts of *Brugmansia candida* leaves contain polyphenols, anthocyanins, tannins, leucoanthocyanidins, alkaloids, bound quinones, and saponins, but no flavonoids. On the other hand, organic extracts of B. candida leaves contain free steroids, terpenoids, and quinones.

Indeed, Petricevich et al. (2020), Algradi et al. (2021), and Pundira et al. (2022) pointed out that the species of the family Solanaceae in general, and those of the *Brugmansia* genus in particular, are very rich in phytochemical compounds. These are particularly alkaloids, steroids, polyphenols, terpenes, tannins, etc. All these chemical compounds are of obvious interest for the different pharmacological and therapeutic activities. Among these different compounds, tropane alkaloids predominate. The latter are among those that present toxic effects. Alvarez (2008) also highlights the fact that all parts of B. candida are toxic. Therefore, their use for therapeutic purposes requires great caution because of the potential risks.

In conclusion, this study showed that *B. candida* is rich in chemical compounds like most other species of the Solanaceae family. Among these compounds, total polyphenols appear to be dominant. These are followed by flavonoids and tannins whose contents appear to be the lowest. As for the screening of antioxidant activity, the analysis of different extracts of Brugmansia candida leaves by the ABTS test showed a higher antioxidant power compared to DPPH. This study has therefore made it possible to highlight the phytochemical compounds, biological activities, and toxicity of *B. candida*. Such results demonstrate the therapeutic potential of this species. This species can therefore play a major role in pharmacological and therapeutic activities. However, its use requires great caution due to their toxicity.

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Declaration of available data

The sharing of available data does not apply to this article, as no new data was created as part of this study. However, we are willing to answer any question regarding this article.

Author contributions

Project conception, lead author, and funding (on own funds): N. L. G. Writing – original project execution: N. L. G.; M. M. B., MAI., D. A. Writing – revision: N. L. G., MMD., M. M. B., M. A. I., D. A. Editing – English translation: M. M. D. All authors have read and accepted the final version of the manuscript.

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Conflicts of interest

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Declaration of available data

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