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Phytochemical study and dosage of polyphenols and flavonoids of the methanolic extract of the leaves and stem bark of *Gardenia ternifolia*

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Abstract

Gardenia ternifolia is a medicinal plant used in Senegal and in several African countries for the treatment of many pathologies. According to the results of the bibliography, the four parts of the plant, mainly used for medicinal purposes, are recommended. Therefore, the chemical studies made on the extracts of the leaves, roots, seeds and fruits show that they are rich sources of secondary metabolites. The phytochemical study carried out on the hexane, ethyl acetate, methanolic and aqueous extracts of the leaves and stem bark of the *Gardenia ternifolia* plant reveals the presence of polyphenols, flavonoids, alkaloids, gallic and catechin tannins, sterols, polyterpenes, coumarins, mucilages, catechols and Leucoanthocyanins in the two different organs of this plant. The successive extracts obtained with hexane, ethyl acetate, methanol and water gave yields varying respectively from 0.14 to 3.94% for the four leaves extracts and 0.08 to 1.62% for the four stem bark extracts. The determination of the total polyphenols of the methanolic extracts respectively gave variable contents between 1.57 ± 0.05 and 7.00 ± 0.044 µg EAG/mg for the leaves and the bark of the stems. Compared to flavonoids, the levels vary respectively between 1.57 ± 0.05 and 15.45 ± 0.066 µg EQ/mg for stem bark and leaves. In general, the methanolic extract and the aqueous extract are the richest in secondary metabolites, polyphenols and flavonoids. This suggests that these families of compounds are responsible for the biological activity of the plant in the treatment of certain types of diseases. However, in-depth tests on the antioxidant, antidiabetic and antimicrobial activities are necessary in order to identify the full therapeutic potential of this plant.

Keywords: *Gardenia ternifolia*; Phytochemical; Polyphenols; Flavonoids

1. Introduction

Since antiquity, the concern of man has been the satisfaction of his food needs. He thus developed an intimate relationship with the environment around him. To heal himself, he learned at his expense to discern the plant and animal resources necessary for his survival. For this he was inspired by the mores of animals, his experience and sometimes even his imagination.

In recent years, many studies have been devoted scientifically to medicinal plants, certain therapeutic virtues have been confirmed and the active ingredients responsible have been isolated.

In Senegal, as everywhere in Africa, people do not hesitate to use medicinal plants to treat themselves because it is a country known for its biodiversity which has a particularly rich and varied flora. Medicinal plants are very little explored from a chemical point of view and yet they constitute a significant source of natural bioactive substances. This work is part of the logic of valorization of the Senegalese flora. To this end, we carried out the phytochemical study of the leaves

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and stem bark of *Gardenia ternifolia*, belonging to the Rubiacea family, used in the pharmacopoeia for its many biological properties. Its leaves are used for the treatment of cancer, sickle cell, anemia, diarrhea and liver necrosis, but also act as voltage regulators. They have antimalarial, antibacterial, antimicrobial and antioxidant properties **[1]**. For the stem bark, unlike the leaves already studied, they have not been the subject of chemical studies according to our bibliographical research.

These scientific results justify the choice made on this plant, in order to determine the nature of the bioactive molecules it contains, the content of phenolic compounds and flavonoids in the methanolic extract of the leaves and stems.

2. Material and methods

2.1. Plant material

Stem bark Leaves

Figure 1 Stem bark and leaves of *Gardenia ternifolia*

The choice of *Gardenia ternifolia* is justified by its use in traditional Senegalese medicine for the management of numerous pathologies, the results of which are palpable. The material consists of leaves and stem bark (Figure 1). They were harvested in December 2021 in Taïba Ndiaye (west of Senegal) and then identified at the Fundamental Institute of Black Africa (IFAN) in the African Lebrun and Stock plant database. After harvest, the plant material is dried in the dark and then pulverized using an electric grinder. The powder obtained, put in condition, is used for the various chemical experiments.

2.2. Extraction

The extraction method used is maceration which is a form of solid-liquid extraction at room temperature. Indeed, in this extraction, we used 20 g of material powder in 100 mL of hexane. A maceration is made for 15 min under a magnetic stirrer at room temperature. After filtration on filter paper, the same solvent was renewed to obtain two (02) hexane fractions, which are combined to obtain the hexane extract. The same process was repeated successively, first with ethyl acetate, then with methanol and finally with distilled water. In each case, filtrates 1 and 2 are combined and then concentrated using a rotary evaporator.

2.3. Phytochemical screening

Phytochemical screening is a qualitative analysis based on precipitation and/or coloring reactions. It makes it possible to define the presence or absence of secondary metabolites which may be found in a sample. In this work, the screening concerns the search for: alkaloids, polyphenols, tannins, flavonoids, saponins, sterols and polyterpenes, leucoanthocyanins, catechols, mucilages. We tested the presence of these different chemical groups by referring to the techniques described in the work of Diallo and Békro. **[2], [3].** Polyphenols and tannins were identified by the $FeCl_3$ test and Stiasny's reagent; flavonoids, leucoanthocyanins and catechols by reaction with cyanidin; saponins by the foam test; sterols and polyterpenes by the Liebermann-Burchard test; mucilages by the absolute ethanol test and alkaloids by Mayer's tests **[3], [4].**

2.4. Content of total phenolic compounds

The content of total phenolic compounds was determined with the Folin-Ciocalteu reagent **[5]**. Indeed, 40 µL of each extract are taken and supplemented to 200 µL with distilled water. A volume of 150 µL of Folin-Ciocalteu reagent, 600

µL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water are additionally added. After 30 minutes of incubation in the dark, the absorbance is read at 760 nm from a UV/Visible spectrometer of the Perkin-Elmer Lamda 365 type. The measurement was compared with a standard curve of gallic acid prepared from a 0.1 mg/mL stock solution of gallic acid.

2.5. Flavonoid content

The total flavonoid content was calculated by the method described by Dirar et al. **[6]**. This method consists of adding 2.5 mL of a 2% ethanolic $AlCl_3$ solution to 500 µL of each extract. The mixtures are incubated for 1 hour at room temperature and the absorbance is read at 425 nm. The flavonoid content is expressed in terms of Quercetin equivalent (EqQ) by reference to the calibration curve plotted with a concentration range obtained from a 0.1 mg/mL quercetin stock solution.

3. Results and discussion

3.1. Mining rate

The results of the extraction using different solvents are presented in the table below:

Table 1 Extraction rates of leaves and stem bark in different solvents

Plant	Parts	Solvents	Mass of samples (g)	Mass obtained (g)	Mining rate (%)
Gardenia ternifolia	Leaves	Hexane	5	0,007	0,14
		Ethyl acetate		0,015	0,3
		Methanol		0,195	3,9
		Aqueous		0,197	3,94
	Stem bark	Hexane	5	0,004	0,08
		Ethyl acetate		0,01	0,2
		Methanol		0,075	1,5
		Aqueous		0,081	1,62

The analysis of Table 1 shows that the extraction rates increase with the polarity of the solvents. Indeed, the best extraction rates are obtained with water and methanol which are the most polar solvents. It can also be noted that these two solvents (methanol and water), of similar polarity, give almost the same extraction rates. The lowest extraction rate is obtained with hexane, the less polar solvent. These values obtained suggest the richness of the plant in polar secondary metabolites, but poor in lipid compounds.

Moreover, by making a comparison between extracts of the same solvent, we find that the extraction rates obtained with the leaves are higher than those obtained with the stems.

3.2. Phytochemical screening

The organic and aqueous extracts of the leaves and stems of the *Gardenia ternifolia* plant were subjected to phytochemical screening, the results of which are presented in the table below:

The results of the phytochemical screening showed:

- The presence of polyphenols, flavonoids, alkaloids, sterol, polyterpenes, mucilage, gallic tannins and catechic tannins in both parts of the plant;
- A strong presence of polyphenols in both parts of the part;
- A strong presence of alkaloids in the stems and a weak presence in the leaves;
- A strong presence of mucilage in the leaves and weak presence in the stems
- The absence of saponins in both parts of the plant
- The absence of coumarins in the leaves and their presence in the stems.

Table 2 Phytochemical screening results

	Leaves				**Stem bark**			
	Hex	**AcOEt**	**Met**	**Aq**	**Hex**	**AcOEt**	**Met**	**Aq**
Polyphenols	-	+	++	++	-	+	++	++
Flavonoids	-	+	+	-	-	-	+	-
Alkaloids	-	-	+	-	++	+	+	-
Sterols-polyterpenes	++	+	+	-	++	+	+	-
Leucoanthocyanins and catechols	-	-	+	-	-	+	-	-
Coumarins	-	-	-	-	-	-	-	+
Saponines	-				-			
Mucilage	++				+			
Gallic tannins	-	-	++	-	-	-	+	-
Catechic tannins	-	-	++	++	-	-	+	++

Legend : Hex = hexane; Met = methanol; Aq = aqueous; AcOEt = ethyl acetate; + : positive; ++ : very positive; - : Negative

The phytochemical screening carried out showed the presence of the main groups of chemical compounds that were tested, with the exception of saponins. However, they are distributed very differently in the two parts of the plant and according to the nature of the extraction solvent. These results also revealed a more marked presence of secondary metabolites in the methanolic and aqueous extracts. This suggests the importance of the polarity of solvents for the extraction of polar compounds.

It is further noted that the methanolic extracts are richer in secondary metabolites than the aqueous extracts. This result could be justified by the sequential extraction method used. Indeed, methanol has a polarity close to water, therefore it would have extracted practically all the polar compounds contained in the sample.

In summary, the results of the phytochemical screening showed the richness and variability of the secondary metabolites contained in the *Gardenia-ternifolia* plant.

3.3. Content of total polyphenolic compounds and flavonoids

The polyphenol contents of methanolic extracts of stem and leaf bark are determined from the gallic acid calibration curve shown in Figure 2 below, plotted using gallic acid solution as the standard solution.

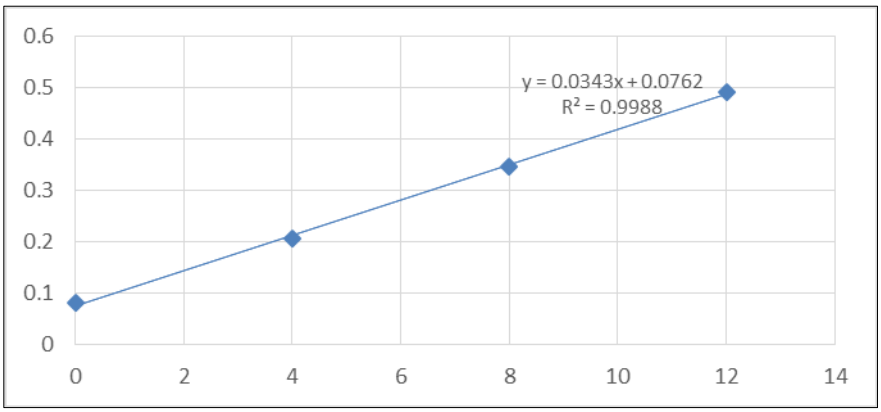

Figure 2 Gallic Acid Calibration Curve

The flavonoid contents of the methanolic extracts of the stem and leaf barks are determined from the quercetin calibration curve shown in Figure 3, plotted using the quercetin solution as the standard solution.

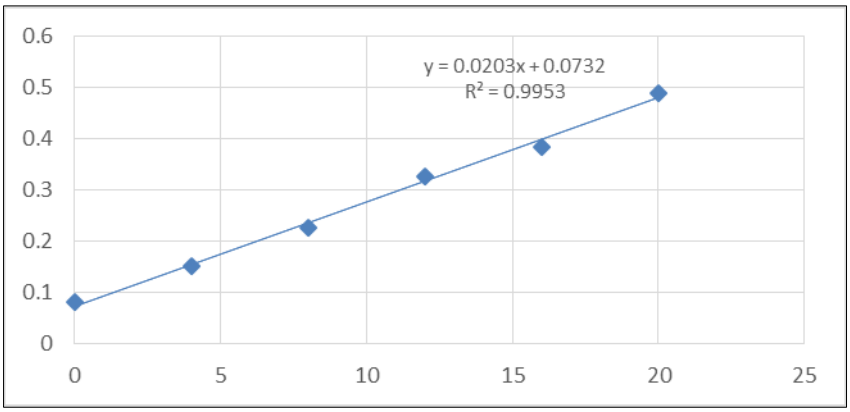

Figure 3 Quercetin calibration curve

The results obtained are illustrated in figures 4 and 5 in the form of a histogram

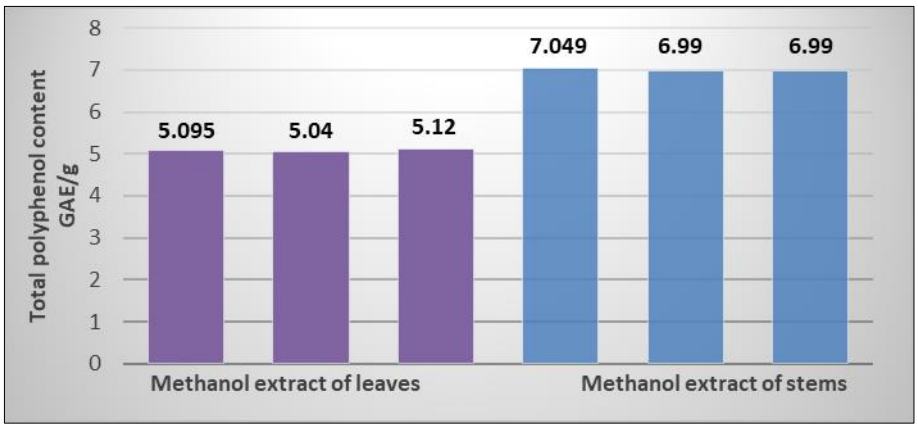

Figure 4 Polyphenol content of methanolic extracts from the leaves and stems of the *Gardenia ternifolia* plant

The dosage of phenolic compounds was carried out according to the Folin-Ciocalteu method. From the results, it appears that the stem barks show a higher polyphenol content with a maximum average value of 7,000 ± 0.044 µg gallic acid equivalent (µgEAG/g) of extract (Figure 4). Furthermore, the leaves of *Gardenia ternifolia* contain a lower level of polyphenols than the stems. Since flavonoids are considered to be the most widely distributed group of phenolic compounds, we assessed the flavonoid content of the two organs of *Gardenia ternifolia* (leaves and stem bark).

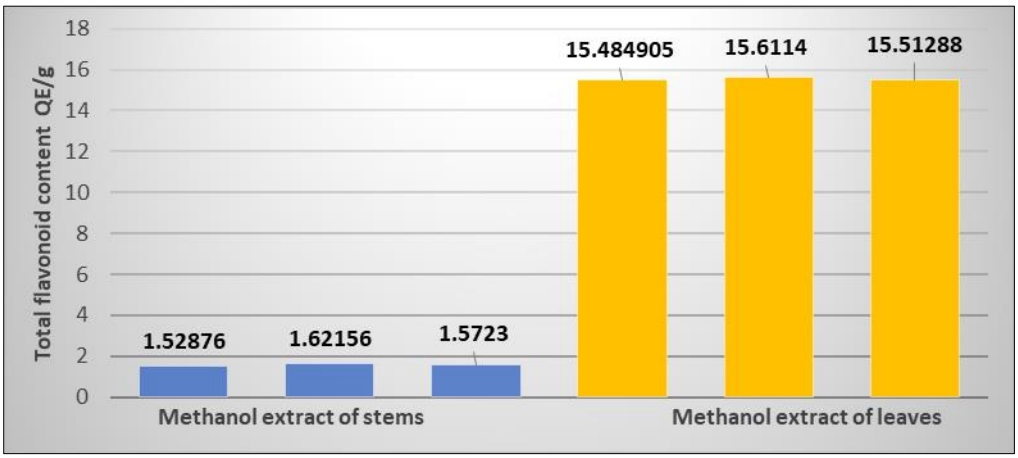

Figure 5 Flavonoid content of methanolic extracts from the leaves and stems of the *Gardenia ternifolia* plant

As shown in Figure 5, we note that the methanolic extracts of the two plant organs reveal high flavonoid contents with a maximum value of 15.454 ± 0.066 µg Quercetin equivalent (µg EQ/g) for the methanolic extract of the leaves. It is noted that the leaves are richer in flavonoids, compared to the stem barks. Since flavonoids are considered to be the most widely distributed group of phenolic compounds, this suggests that flavonoids are not the predominant phenolic compounds in the stem barks of *Gardenia ternifolia*. Thus, we can postulate that flavonoids are not the main phenolic compounds of *Gardenia ternifolia*.

4. Conclusion

In this work, we have tried to establish a correlation between the use in traditional medicine of *Gardenia ternifolia* and the nature of the compounds that this plant contains. The qualitative analysis through the phytochemical screening showed the richness in secondary metabolites of the two parts of this plant which can be at the origin of their uses as medicine. The stem bark is the richest part in polyphenols with a content of 7.00 ± 0.044 µg/mg of gallic acid equivalent. Compared to flavonoids, the leaves constitute the richest part with a content of 15.454 ± 0.066 µg/mg quercetin equivalent.

The comparison of these two results makes it possible to say that the molecules responsible for the biological activity of the two organs of *Gardenia ternifolia* are not mainly phenolic compounds and that the bark of the stem seems to be the part best indicated in the use of this plant.

Further study to determine the antioxidant, antimicrobial and antidiabetic activities in the leaves and stem bark of this plant may provide insight into the nature of its active ingredients. And finally, a study of isolation and identification of the molecules responsible for these biological activities could be expected.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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