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Phytochemical Study, in vitro Antioxidant Activities and in vitro Determination of Free Glucose Concentration when Brought into Contact with Extracts of *Tetracera rosiflora* (*Dilleniaceae*) Leaves

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Abstract

Tetracera rosiflora Gilg (Dilleniaceae) is a medicinal plant traditionally used in the Congolese pharmacopoeia to treat various diseases, including diabetes. This study aimed to determine the phytochemical composition, evaluate the in vitro antioxidant potential, and assess the glucose-lowering capacity of *T. rosiflora* leaf extracts. Phytochemical screening revealed the presence of coumarins, iridoids, anthocyanins, polyphenols, flavonoids, and tannins. Quantitative assays indicated total polyphenols (42.12 ± 1.14 mg GAE/g), flavonoids (0.090 ± 0.002 mg QE/g), and anthocyanins (0.30 ± 0.07 mg CE/g). The quantification of mineral elements by x-ray fluorescence revealed 29 elements with hight conecntration of Ca, K, S and Mg. The organic extracts exhibited strong antioxidant activity by scavenging ABTS and DPPH radicals, with higher efficacy in the ABTS assay (IC_{50}: 5.48 ± 3.23 µg/mL for ethanol extract; 25.65 ± 8.67 µg/mL for dichloromethane extract) compared to DPPH (IC_{50}: 10.81 ± 0.86 µg/mL and 96.16 ± 16.73 µg/mL, respectively). Furthermore, in vitro glucose-binding assays demonstrated that organic extracts markedly reduced free glucose concentrations, confirming their potential antidiabetic activity. These findings support the traditional use of *T. rosiflora* and highlight its promise as a source of natural antioxidant and hypoglycemic agents.

Keywords: *Tetracera rosiflora*; antioxidant activity; glucose-lowering effect; phytochemicals; traditional medicine.

INTRODUCTION

Humanity has long faced diseases that threaten survival, quality of life, and public health systems. Among these, diabetes mellitus and oxidative stress remain major contributors to global morbidity and mortality. Over time, advances in scientific disciplines—particularly pharmacology—have significantly improved our understanding of these conditions and enhanced strategies for their management. Nevertheless, their growing prevalence and associated complications continue to pose serious challenges worldwide. Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion, insulin action, or both. It is associated with severe long-term complications affecting both microvascular and macrovascular systems, including neuropathy, nephropathy, retinopathy, and cardiovascular diseases. Globally, the burden of diabetes continues to rise, making it one of the leading causes of death. In the Democratic Republic of Congo (DRC), the disease affects hundreds of thousands of individuals, with type 2 diabetes accounting for approximately 80% of cases, while type 1 diabetes also impacts a significant number of children. Beyond its prevalence, the severity of diabetes lies in its chronic nature and its strong association with debilitating complications. Closely linked to diabetes is oxidative stress, a condition resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defense systems. This imbalance leads to damage of vital biomolecules such as lipids, proteins, and DNA, thereby contributing to the development and progression of various metabolic disorders, including diabetes. The relationship between oxidative stress and diabetes is bidirectional: oxidative stress can contribute

to the onset of diabetes, while diabetes itself can exacerbate oxidative damage. Furthermore, oxidative stress plays a critical role in the development of diabetic complications, as patients often exhibit weakened antioxidant defenses, making them more susceptible to cellular damage. Given this interconnection, effective diabetes management should go beyond glycemic control and include strategies to combat oxidative stress. Although conventional hypoglycemic drugs are widely used and effective in regulating blood glucose levels, they do not offer a definitive cure and are often associated with side effects and high treatment costs. These limitations have driven the search for safer, more accessible, and cost-effective therapeutic alternatives. In this context, medicinal plants represent a promising source of bioactive compounds with both antidiabetic and antioxidant properties. Numerous studies have demonstrated the therapeutic potential of plant-derived compounds, particularly those belonging to phytochemical groups such as flavonoids and tannins. These compounds are known for their ability to reduce blood glucose levels and neutralize free radicals, thereby helping to prevent or mitigate oxidative damage. In the Democratic Republic of Congo, traditional medicine plays a central role in healthcare delivery, especially in rural areas where access to modern medical services may be limited. The country's rich biodiversity, encompassing forests, savannahs, and mountainous regions, supports an immense variety of plant species, many of which are used in traditional healing practices. Despite this wealth of botanical resources, systematic documentation and scientific validation of antidiabetic plants remain incomplete. This study contributes to the valorization of Congolese medicinal flora by focusing on Tetracera rosiflora Gilg, a plant belonging to the Dilleniaceae family. This family comprises numerous species known for their ethnopharmacological applications in treating a wide range of ailments, including inflammatory conditions, gastrointestinal disorders, infections, and metabolic diseases such as diabetes. T. rosiflora is traditionally used for various therapeutic purposes, yet its scientific evaluation remains limited. The main objectives of this study are threefold: to analyze the phytochemical composition of T. rosiflora leaves, to evaluate their in vitro antioxidant activity, and to assess their glucose-lowering potential through interaction assays with free glucose. By exploring these properties, the research aims to provide scientific evidence supporting the traditional use of this plant and to identify potential natural compounds that could contribute to the development of new, safer antidiabetic therapies. Overall, this work highlights the importance of integrating traditional knowledge with modern scientific approaches in the search for effective and sustainable solutions to global health challenges such as diabetes and oxidative stress.

MATERIALS AND METHODOLOGY

Plant Material

In this study, *Tetracera rosiflora Gild*, a plant selected based on ethnobotanical reports from the Lukala population in Kongo Central Province, was collected in January 2023 from the commune of Mont-Ngafula (Kimwenza), Kinshasa Province, DRC. The plant was authenticated at the Herbarium of the National Institute for Agronomic Studies and Research (INERA), Faculty of Sciences, University of Kinshasa (UNIKIN). Collected samples were air-dried at ambient temperature (±27°C) for two weeks' laboratory.

Phytochemical Screening

Preliminary phytochemical screening was conducted on aqueous or methanolic extracts obtained by macerating 10 g of T. rosiflora leaf powder in 100 mL of solvent at room temperature for 24 h, followed by filtration. Total polyphenols were detected using Burton's reagent; flavonoids with Shinoda's reagent in the presence of a few magnesium shavings; bound quinones with Borntrager's reagent; anthocyanins with 20% HCl; leucoanthocyanins with Shinoda's reagent and a few drops of isoamyl alcohol; tannins using a mixture of 2% FeCl3 and Stiasny's reagent. Alkaloids were detected in the acidified aqueous extract (0.1N HCl) using Dragendorff's reagent. Steroids and terpenes were identified in the organic extract using Liebermann-Burchard reagent, while free quinones were detected using Borntrager's reagent. (Dinangayi Tshilanda et al., 2019) (Mpiana et al., 2010) (Ngbolua et al., 2022).

- ***Total Polyphenols Quantification***

Total polyphenol content was determined using the Folin-Ciocalteu method (Tshibangu et al., 2016). Results were expressed as mg of gallic acid equivalents (GAE)/g of dry extract based on the calibration equation: $y = 0.0037x + 0.0218$ ($R^2 = 0.9899$), where x represents concentration and y represents absorbance.

- ***Total Flavonoids Quantification***

Total flavonoid content was estimated by UV-visible spectrophotometry at 415 nm using aluminum chloride, which forms a yellow complex with flavonoids (Tshibangu et al., 2016). The content was calculated using the equation: $y = 0.0542x - 0.0367$ ($R^2 = 0.987$), where x is the absorbance and y is the quercetin equivalent (mg/g).

- ***Anthocyanins Quantification***

Anthocyanins were quantified according to Lebreton et al. (1967). The maximum absorbance wavelength was identified from the absorption spectrum of the n-butanol extract between 480 and 600 nm. The anthocyanin content (%) was calculated using: Anthocyanins = $(\gamma A/\varepsilon) \times M \times V \times d / p$, where $\gamma = 6$ (correction factor), A = absorbance at maximum wavelength, ε = molar

extinction coefficient of cyanidol (34,700), M = molar mass of leucocyanidol (306 g/mol), V = volume of n-butanol solution (mL), d = dilution factor, and p = dry weight of plant material (g).

Determination of mineral elements by X-ray fluorescence

X-ray fluorescence (XRF) spectroscopy was employed for the determination of mineral elements. The analysis was carried out by irradiating the sample with primary X-rays generated by an energetic excitation source, inducing the emission of characteristic secondary X-rays from the elements present.

For elemental analysis, a portion of the powdered sample was compressed into a pellet using a hydraulic press and subsequently analyzed using an XEPOS X-ray fluorescence spectrometer. This analytical technique enables the simultaneous detection and quantification of multiple mineral elements within the sample.

Evaluation of Antiradical Activity

Decoction: 10 g of powder in 100 mL water, boiled 3–5 min, filtered, concentrated using a rotary evaporator, dried 24 h at 37°C, and stored at +4°C.

Percolation: 10 g powder moistened with methanol/dichloromethane (1:1) for 15 min, placed in a percolator, 20 mL solvent added and renewed until exhaustion (final volume 200 mL), concentrated and dried, stored at +4°C.

Sample preparation: 10 mg dry extract dissolved in 1 mL methanol (organic extracts) or DMSO-water (1:1, aqueous extracts), then diluted to 0.1–0.5 mg/mL.

ABTS assay: ABTS radical prepared by mixing ABTS (20 mM) with potassium persulfate (10 mM) in equal volumes, incubated in the dark 12–16 h, diluted to OD 0.6–0.8, mixed with 20 µL sample and 1980 µL radical solution, incubated 30 min in the dark, OD read at 734 nm. The inhibition rate was calculated using the formula below.

(%) = [(Abs control – Abs sample)/Abs control] × 100. IC50 determined by linear regression

DPPH assay: 3.2 mg DPPH in 100 mL 80% methanol, adjusted to Abs = 0.70 ± 0.05, mixed with 20 µL sample and 1980 µL DPPH solution, incubated 30 min in dark, Abs measured at 517 nm, inhibition calculated as above (Gulcin & Alwasel, 2023). The IC50 values of different samples were determined by linear regression using Origin 8.5 Pro software.

Glucophage Activity / Free Glucose Binding Assay

This assay evaluates the capacity of plant extracts to interact with glucose in vitro. Using a non-stoichiometric approach, the ability of the extracts to bind (complex) free glucose was estimated, thereby indicating their potential "glucophage" activity. The concentrations of free and bound glucose were expressed as the percentage decrease in free glucose or the percentage increase in bound glucose, respectively. The study was performed not only on total aqueous extracts and their binary and ternary mixtures but also on tannin and flavonoid fractions, which were further separated according to their structural classes using diethyl ether, ethyl acetate, and butanol (Demartis et al., 2018)

Although in vitro conditions differ from in vivo physiology, this assay provides insights into one potential mechanism underlying the hypoglycemic effects of plant extracts. For the assay, 0.01 mL of a glucose solution (4 g/L) was mixed with 10 µL of the aqueous extract or each flavonoid and tannin fraction (aglycones, mono-, di-, and triglycosides) at concentrations of 1, 2, and 4 mg/mL. The control consisted of 0.01 mL of distilled water. The mixtures were incubated at 37°C for 15 min, and the amount of unbound glucose was determined using the glucose oxidase method (Sok Yen et al., 2021).

RESULTS AND DISCUSSION

Chemical Screening

Phytochemical screening of *Tetracera rosiflora* leaves revealed that the aqueous extract is rich in polyphenols, including tannins and anthocyanins, while leucoanthocyanins and bound quinones were present in trace amounts. Alkaloids were not detected. The plant also contains appreciable amounts of saponins and flavonoids. In the organic extract, terpenes were abundant, whereas free quinones and steroids were absent. These results are consistent with previous reports on related species, such as *A. alboviolaceum* (Bensaad et al., 2021), which contains anthocyanins, flavonoids, alkaloids, polyphenols, and triterpenes, and *A. angustifolium* (Rakotonirina, 2019), which shows flavonoids, leucoanthocyanins, and triterpenes. Similar secondary metabolites, including tannins, saponins, flavonoids, phenolic acids, iridoids, anthocyanins, anthrones, anthraquinones, and terpenes, have been reported in *Ficus exasperata* and *Lippia multiflora* (Masengo et al., 2023). Variations in metabolite content may reflect differences in plant parts, developmental stage, and environmental conditions such as temperature, sunlight, drought, or salinity, which can influence secondary metabolite biosynthesis (Gbolo et al., 2022; Ngbolua et al., 2021). Overall, *T. rosiflora* from the DRC is rich in total polyphenols and flavonoids, supporting its traditional pharmacological use, as these compounds are associated with multiple biological activities (Masengo et al., 2023; Bougandoura & Bendimerad, 2013; Bukatuka et al., 2016).

Total Polyphenol and Total Flavonoid Content

The total polyphenol content of *Tetracera rosiflora* was 42.12 ± 1.14 mg GAE/g, while the total flavonoid and anthocyanin contents were 0.090 ± 0.002 mg QE/g and 0.30 ± 0.07 mg QE/g, respectively. These results indicate

that *T. rosiflora* is particularly rich in phenolic compounds, notably flavonoids. Variations in polyphenol content when compared with others autors are influenced by several factors, including genetic, geographic, climatic, and physiological conditions, as well as the plant's maturation stage and storage duration. Such environmental and biological factors affect secondary metabolite biosynthesis, which may explain observed differences between species. The high levels of polyphenols and flavonoids in *T. rosiflora* support its traditional medicinal use, as these compounds are known for diverse biological activities (Ngbolua et al., 2022; Bukatuka et al., 2016; Bougandoura & Bendimerad, 2013).

Mineral content

This study revealed the presence of twenty-nine (29) mineral elements in the investigated plant. Among these, six macroelements—potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sulfur (S), and chlorine (Cl)—were identified. Calcium, potassium, sulfur, and magnesium were present at relatively high levels, whereas phosphate and chloride contents were comparatively low. Sodium was not detected due to the absence of its measurement. The predominance of these macroelements highlights the nutritional and physiological relevance of the plant. Calcium and potassium play critical roles in maintaining electrolyte balance, cellular permeability, and cardiovascular function. Calcium, in particular, is essential for bone structure, blood coagulation, and muscle contraction. In the context of sickle cell disease, calcium ions are known to influence red blood cell hydration and rigidity. Intracellular accumulation of Ca^{2+} promotes cellular dehydration, increases intracellular hemoglobin S (HbS) concentration, and accelerates HbS polymerization, leading to membrane stiffening. Calcium, together with potassium, is also involved in the regulation of the Gardos channel, which controls ion transport across erythrocyte membranes (Kitadi et al., 2020; 2021). The calcium content of this plant may therefore contribute to modulating Ca^{2+} transmembrane transport, improving erythrocyte hydration and potentially enhancing anti-sickling effects. Magnesium, another abundant macroelement, plays a crucial role in maintaining osmotic balance in plasma and extracellular fluids and acts as an essential enzymatic cofactor. Previous studies have shown that magnesium can reduce the proportion of abnormal erythrocytes and improve red blood cell hydration in sickle cell disease (Kitadi et al., 2022), further supporting the therapeutic potential of this plant. In addition to macroelements, several trace elements were detected and quantified. Iron (Fe), aluminum (Al), and manganese (Mn) were present at relatively high levels, whereas cobalt (Co) and molybdenum (Mo) were detected at low concentrations. The high iron content is particularly noteworthy, as iron is indispensable for hemoglobin synthesis, and iron deficiency is a well-known cause of anemia (Kitadi et al., 2022). Other trace elements such as zinc (Zn), copper (Cu), and iodine (I) were also identified. Zinc is involved in numerous enzymatic processes related to erythropoiesis, while copper is a component of key enzymatic systems, including cytochrome oxidase, and plays an indirect role in iron metabolism.

Overall, the mineral composition observed in this study is consistent with previous reports on medicinal plants used in the management of sickle cell disease. Comparisons with the work of Kitadi et al. (2021), Oyeleke et al. (2022), and Kasiama (2023) indicate that the present plant exhibits higher concentrations of key macroelements such as calcium, potassium, and magnesium. However, studies by Nzuzi P.N. et al. (2024) reported higher iron contents in *Jatropha podagrica* and *Euphorbia hirta*, with comparable sodium and calcium levels. These comparisons further support the mineral richness of the studied plant and its potential relevance in traditional and complementary therapeutic applications.

Anti-Radical Properties of *Tetracera rosiflora*

The results of the antioxidant activity assessment are summarized in Table 4.

The antioxidant potential of *T. rosiflora* extracts was evaluated using DPPH and ABTS radical scavenging assays. IC_{50} values represent the concentration required to reduce the initial radical concentration by 50%, with lower IC_{50} values indicating stronger antioxidant activity. All extracts demonstrated significant radical scavenging capacity, with ABTS assay results generally showing greater activity than DPPH. This difference may be attributed to the distinct reaction mechanisms of the assays, as DPPH predominantly reacts with hydrophilic compounds, whereas ABTS can interact with both hydrophilic and lipophilic compounds (Birindwa et al., 2019 Masengo et al., 2022). The observed antioxidant activity correlates with the high total phenolic and flavonoid content of *T. rosiflora*, suggesting that these compounds are major contributors to its radical scavenging capacity. Although the extracts exhibited lower activity than gallic acid, they displayed substantial antioxidant potential compared to other plant species. Notably, aqueous extracts (decoctions) showed similar activity to methanolic extracts, supporting the traditional use of *T. rosiflora* leaves in medicinal decoctions. Our results are consistent with previous studies reporting appreciable antioxidant activity in plant extracts, including *Ledermanniella schlechteri*, *Lippia multiflora*, (Aganga & Mosase, 2001) *Satureja calamintha ssp. Nepeta Marrubium deserti* and *Origanum compactum* (Bouyahya et al., 2017). These studies demonstrate that phenolic-rich plant extracts generally exhibit significant free radical scavenging activity, though the magnitude depends on extraction method, solvent polarity, and chemical composition. Overall, the high radical

scavenging capacity of *T. rosiflora* extracts highlights their potential to mitigate oxidative stress and supports further investigation into the specific bioactive compounds responsible for these effects.

Free glucose concentration

This investigation aimed to evaluate the *in vitro* ability of *T. rosiflora* extracts to reduce free glucose levels using UV–Visible spectrophotometry at 505 nm, according to the glucose oxidase enzymatic method (Bahati et al., 2017). The results demonstrated a clear dose-dependent response, indicating that increasing extract concentration led to a significant exponential reduction in free glucose content. Variation of free glucose during the in vitro glycosylation reaction in the presence of different total aqueous and organic extracts of *Tetracera rosiflora is presented in* figure 1. The figure illustrates the capacity of the extracts to reduce free glucose concentration, reflecting their potential glucophage activity.

This figure illustrates the reduction of free glucose following the addition of aqueous and organic extracts of *Tetracera rosiflora*. The total organic extract exhibited significantly higher glucose-lowering activity than the aqueous extract, in comparison with the metformin control. The half-maximal inhibitory concentration (IC_{50}) value further confirmed that the organic extract was more potent than the aqueous one. This higher activity may be attributed to the greater solubility and concentration of bioactive secondary metabolites such as flavonoids, tannins, and phenolic compounds in organic solvents. These phytochemicals are known for their antioxidant, antiglycation, and hypoglycemic properties, which could synergistically contribute to the reduction of glucose levels in solution. Our results are in agreement with those of Lahouel et al. (2016), who reported strong glucose-lowering potential in flavonoid extracts of *Rumex repens*. Similar findings were described by (Bahati et al., 2017) in *Lippia multiflora* and *Vernonia* extracts, as well as by Rouamba et al. (2025), who demonstrated significant antiglycation activity in tannin- and flavonoid-rich extracts of *Raphia gentiliana*, *Lippia multiflora*, and *Sesamum indicum*. Collectively, these findings suggest that *T. rosiflora*, particularly its organic extract, exhibits promising *in vitro* hypoglycemic potential. The observed activity may be linked to the synergistic action of phenolic and flavonoid constituents that interfere with glucose oxidation and glycation pathways. Further phytochemical and mechanistic studies are warranted to isolate and characterize the active compounds responsible for these effects and to assess their potential for therapeutic application in diabetes management.

CONCLUSION

This study investigated the chemical composition and *in vitro* biological activities of *Tetracera rosiflora*, with a particular focus on its antioxidant and glucose-lowering potential. Phytochemical screening revealed that the plant is rich in coumarins, iridoids, anthocyanins, total polyphenols, flavonoids, and tannins, indicating a diverse array of bioactive secondary metabolites. Quantitative assays showed a total polyphenol content of 42.12 ± 1.14 mg EFA/g, a total flavonoid content of 0.090 ± 0.002 mg EQ/g, and an anthocyanin content of 0.30 ± 0.07 mg EQ/g, confirming the plant's strong phenolic character. The leaves of this plant are rich in mineral elements, with a total of twenty-nine (29) identified. Calcium, potassium, sulfur, and magnesium are the most abundant, whereas phosphate and chloride are present in relatively low amounts. Overall, the plant exhibits a favorable mineral profile, which may contribute to its notable biological properties. The *in vitro* antioxidant assays demonstrated significant radical-scavenging activity, particularly against the ABTS radical, where the ethanolic extract (IC_{50} = 5.48 ± 3.23 µg/mL) exhibited higher efficiency than the dichloromethane extract (IC_{50} = 25.65 ± 8.67 µg/mL). In comparison, the DPPH assay showed relatively lower but still notable activity (DCM: 96.16 ± 16.73 µg/mL; ETOH: 10.81 ± 0.86 µg/mL), suggesting that the antioxidant potential is both solvent-dependent and concentration-dependent. Furthermore, the glucose-lowering tests revealed that the organic extracts exhibited greater hypoglycemic potential than the aqueous ones, as evidenced by lower IC_{50} values in glucose inhibition assays. This observation supports the idea that nonpolar or moderately polar solvents enhance the extraction of active constituents responsible for the modulation of glucose metabolism. Overall, the findings highlight the pharmacological potential of *T. rosiflora* as a promising source of natural antioxidants and hypoglycemic agents. Its rich polyphenolic and flavonoid profiles may contribute synergistically to its biological activities.

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Table 1. secondary metabolites in *Tetracera rosiflora*.

Métabolites secondaires	***Tetracera rosiflora***
1.Polyphenols	
✔ Flavonoids	+
✔ Anthocyanins	+
✔ Leucoanthocyanins	+
✔ Tanins	+
✔ Quinons	+
2.Alcaloids	-
3.Saponines	+
4.Triterpenoids	+
5.Steroids	-
6. Quinons	-

Table 2. Polyphenols and flavonoids content in *Tetracera rosiflora* leaves.

	Teneur en polyphénols totaux (EAG)	Teneur en flavonoïdes (EQ)	Teneurs en anthocyanes (EC)
Tetracera rosiflora	42.12 ± 114	0,090 ± 0,002	0,30 ± 0,07

Table 3. The results of the mineral element assays

ELEMENTS	***T.rosiflora***
	T1(ppm)
Na	ND
Mg	15,9
Al	134,2
Si	2,1
P	38,9
S	7,4
Cl	195,2
K	8,5
Ca	4,1
Ti	2690,4
V	ND
Cr	30929,3
Mn	659,1
Fe	148,5
Ni	152024,1
Cu	14564,3
Zn	4373,3
Se	994269,3
Br	1619,0
Rb	6232,3
Sr	1449,7
Mo	340905,9
Ag	ND
Cd	2806265,3
I	1199013,1
Cs	14896574,0
Ba	10711,1
Pb	424181,0
Th	ND

Table 4. The screening of antioxidant activity using the ABTS and DPPH assays, expressed as IC_{50} values (µg/mL) and reported as mean ± SD (n = 6).

Echantillon	ABTS (µg / mL)	DPPH (µg / mL)
	T. rosiflora	*T. rosiflora*
DCM	25.65 ± 8.67	96 .16± 16.73
ETOH	5.48 ± 3.23	10.81 ± 0.86
Acide gallique	0.71 ± 0.08	1.07 ± 0.10

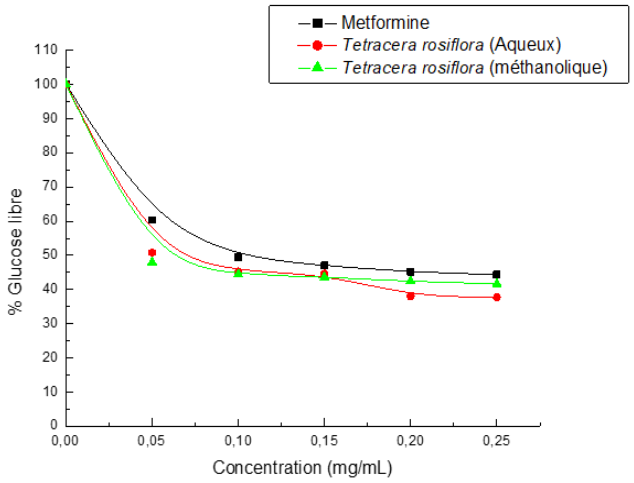

Figure 1. Free glucose levels in the aqueous and methanolic extracts of *Tetracera rosiflora.*

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