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Microscopic Features, Chromatographic Fingerprints and Antioxidant Property of *Tetracera rosiflora* Gilg

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Abstract: *Tetracera rosiflora* Gilg is a medicinal plant used in the Congolese pharmacopeia for the treatment of various diseases. The aim of this work was to achieve the micrographic analysis of the powder of leaves of *T. rosiflora* from Pumbu City (Kinshasa) and Lukala areas (Kongo Central), to determine their chemical composition and to evaluate the antioxidant activities of their different extracts. Extracts from leaves of this species were characterized for their chemical fingerprint by Thin Layer Chromatography and their in vitro antioxidant activities were evaluated using ABTS, DPPH assays. Microscopically leaves can be characterised by non-glandular trichomes, sinuous anticlinal epidermal cells, and parenchymal cells with cristal of calcium oxalate, polycytic stomata and spiraled vessels. Thin Layer Chromatography showed that leaves of *Tetracera rosiflora* contain varied phytochemicals such as anthocyanins, flavonoids, coumarins, iridoids, phenolic acid, tanins and the terpenes. All tested extracts (methanolic and dichloromethane) have exhibited high antiradical activities. The obtained results showed that the interesting bioactivities of *T. rosiflora* correlated with the chemical composition, which depends on several factors including climatic conditions. *T. rosiflora* is a phytogenetic resource of the Congolese pharmacopoeia with promising therapeutic potentials for the management of various pathologies associated with oxidative stress.
Keywords: *Tetracera rosiflora*, micrographic analysis, antioxidant activities, phytochemicals, polyphenols.

INTRODUCTION

Tetracera rosiflora Gilg is a medicinal plant used in the Congolese pharmacopeia for the treatment of various diseases. Species of Tetracera genus have been used in folk medicine for the treatment of various diseases and infections in the world. In traditional Indian medicine, some species have been used as febrifuge, diuretic agent, antidiabetic agent and against dysentery, hepatitis and blennorrhagia [1-5]. *Tetracera indica* Merr for exemple, is used for healing fever, flu, sinus symptoms, skin rashes, itching, piles, mouth ulcer, diarrhoea, insects bites and diabetes [6]. The root and stem of *Tetracera scandens* are used to treat hepatitis, swelling and gout in Vietnam [7].

Presently, scientists have become more fascinated towards ethnomedicinal plants around the world and a numerous biological studies are currently undergoing in regard to discover safe and effective drugs from natural sources [6].

To our knowledge, only few investigations have been performed on the phytochemical composition and biological properties of of *T. rosiflora*.

The aim of this work is to achieve the micrographic analysis of the powder of leaves of *T. rosiflora* from Pumbu City (Kinshasa) and Lukala areas (Kongo Central) in Democratic Republic of the Congo (DRC), to determine their chemical composition, to evaluate the antioxydant activities of their different extracts.

Extracts from leaves of this species are characterized for their chemical fingerprint by Thin Layer Chromatography and their *in-vitro* antioxidant activities are evaluated using ABTS and DPPH assays.

MATERIALS AND METHODS

Plant Materials

The leaves of *Tetracera rosiflora* were

collected from the area of Lukala, Kongo Central and the Pumbu City, Kinshasa in DRC. They were dried at room temperature. The identities of the plants were confirmed by Mr. Landu of the National Institute for Research in Agronomics (INERA) of the University of Kinshasa (Democratic Republic of Congo). Voucher specimens (ML/2015) were deposited at the Herbarium of University of Kinshasa. The powdered leaves were stored in the dark at room temperature and used for solvent extraction.

Chemicals and Reagents

All solvents used were of analytical and HPLC grade and purchased from Merck (Hohenbrunn, Germany) and VWR Chemical Prolabo (Leuven, Belgium). 2,2'-Azino-bis-(3-ethylbenzothiazoline- 6-sulfonic acid) diammonium salt (ABTS), 2-Aminoethyldiphenylborat, Folin-Ciocalteu's phenol reagent and sodium persulfate were purchased from Sigma (Bornem, Belgium). 1,1-diphenyl-2-picrylhydrazyl (DPPH) Gallic acid (purity: 97%) was purchased from Sigma-Aldrich (Bornem, Belgium).

Preparation of extracts

Methanolic and dichlorométhane extracts were prepared by percolation with methanol and dichloromethane respectively from 10 g of leaves powder to obtain 200 mL of percolate. Evaporation of the solvent was performed under reduced pressure (at 40 °C). The extracts were then weighed and kept in dark hermetic flasks at 4 °C.

Microscopic analysis

Powder observations were made using lactic acid reagent (Steimetz reagent) [8]. Observations were made with a Hund WETZLAR microscope and pictures were taken with Digital Camera CANON IXUS 165.

Phytochemical analysis

The phytochemical screening was performed following the standard techniques [8-10]. Analytical TLC of 10 µL of solution for 10 mg/mL of methanolic and dichloromethane extracts were carried out on normal phase Silica gel 60 F_{254} plates (Merck), using different eluents [11].

Cell-free assays

Evaluation of radical scavenging activity ABTS radical scavenging capacity

ABTS assay was based on the method described previously [12].

DPPH radical scavenging capacity

DPPH assay was performed according to the method described previously by Floegel *et al*., [13] with slight modifications. A solution of 0.004% of DPPH in 80% (v/v) methanol was prepared one hour before use. The absorbance of the solution was adjusted to 0.75 ± 0.03 at 517 nm using fresh 80% (v/v) methanol. Then 0.02 mL of standard or sample were mixed with 1.98 mL of DPPH solution and incubated for 30 min in the dark. The decrease of absorbance was monitored at 517 nm with a Spectrophotometer Perkin Elmer Lambda 5.

The antiradical capacity analysis was performed on methanolic dry extracts. Gallic acid was used as positive control and ABTS•+ and DPPH scavenging activities of extracts were expressed as IC_{50} values. Each sample was measured in triplicate.

Statistical Analysis

Results were expressed as mean values ± standard deviation (SD). IC_{50} were calculated with GraphPad Prism 6.0 under application of the function "log (inhibitor) vs. normalized response-variable slope" after converting the concentrations into their decimal logarithm. One-way analysis (ANOVA) and Student's paired t-test were used to compare scavenging capacities determined by ABTS and DPPH assays and the level of statistical significance was set at $p < 0.05$, for two-sided testing.

RESULTS AND DISCUSSION

Botanical microscopic characters

Powders of the leaves of the two sample of *Tetracera rosiflora* treated with Steimetz reagent showed the following specific botanical microscopic characters : no glandular and unicellular trichromes, polycytic stomates, polygonal epidermal cells, spiral vessels, parenchyma cells (Figure-1).

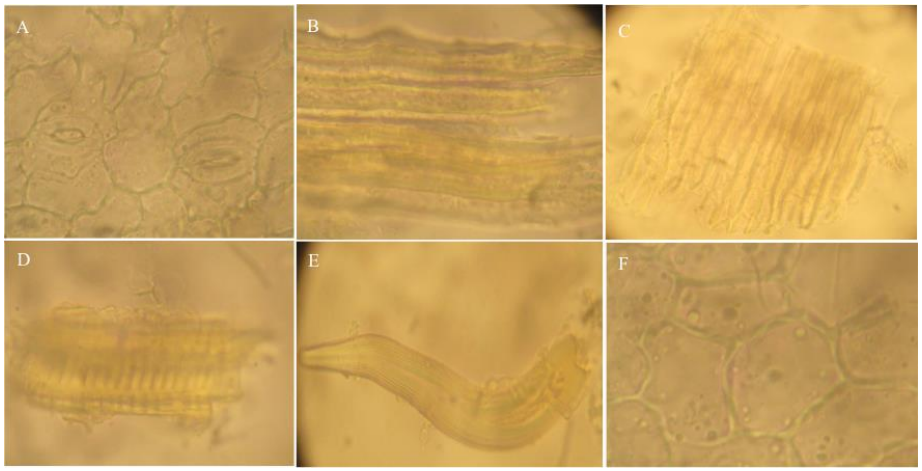

Fig-1: Microscopic characters of leaves powder of *Tetracera rosiflora*: Polycytic stomates (A), Fibers (B), Allonged Parenchyma cells(C), Spiral vassels (D) Smooth non glandular trichoma (E) and Epidermic cells (F) at 10X.

Microscopy is useful for the identification and authentification of botanicals and for detecting their adulteration. Gurav and Gurav [14] reported that microscopic analysis is one of the cheapest methods to correctly identify the drugs and the raw materials from herbal medicines; The knowledge of microscopic details of plants in crude and powder form is vital for the evaluation of medicinal plants in every way.

Chemical composition

Results of phytochemical screening are given in Table-1.

Table-1: Phytochemical screening of the plant samples

Secondary metabolites	*T. rosiflora* from Lukala	*T. rosiflora* from Pumbu City
Alkaloids	-	-
Anthocyanins	+	+
Cardiac glycosids	-	-
Anthraquinones	+	+
Flavonoids	+	+
Quinones	+	+
Tanins	+	+
Steroids	-	-
Terpenoids	+	+

Phytochemical Analysis revealed the presence of anthocyanidins, anthraquinones, flavonoids, iridoids, phenolic acids and terpenoids in the two items of *T. rosiflora*.

Figure-2 gives TLC chromatograms of methanolic and dichloromethane extracts from *T. Rosiflora* leaves.

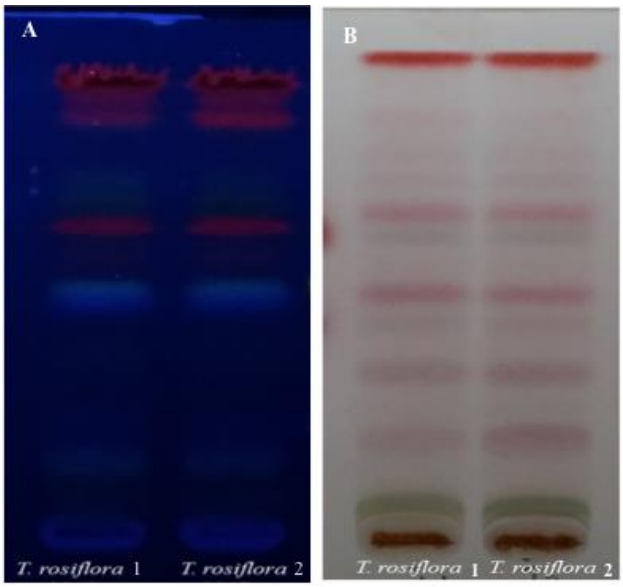

Fig-2: TLC chromatogram of methanolic extract from *T. Rosiflora* (1: from Pumbu City and 2 from Lukala), developed with ethyl acetate/formic acid/methanol/water (20:0.5:2.5:2; v/v/v/v) and visualized at 365 nm with Natural Products-PEG reagent (A); TLC chromatogram of dichloromethane developped with toluen/ ethyl acetate (9:1) and visualized at visible with anisaldehyde sulfuric (B).

Results obtained with TLC analysis are in accordance with those of the standard techniques. As shown in the figure-2, *T. rosiflora* contains phenolic acid as major phenolic compounds which detected as blue fluorescent spots (A) and terpenes (B). The fingerprints of the two items of *Tetracera rosiflora* are identical for all secondary metabolites.

There are few reports on the phytochemical screening of *Tetracera rosiflora*. However, previous studies on others species of *Tetracera*, reported the presence of alkaloids, anthocyanins, flavonoids, terpenes such secondary metabolites [1, 3, 5-7]. For Polyphenols, apigenin, kaempferol and their glycosides were found to be typical flavonoids of the genus *Tetracera* [5]. Alhassan *et al*., [15] have identified the new sulphated flavone with a strong inhibitory activity against alpha-glucosidase in the leaves of *Tetracera indica*.

Polyphenol contents

Results from the quantitative determination of anthocyanins, flavonoids and total phenolic content are summarized in Table-2.

Table-2: Total anthocyanins, flavonoids, tannins and total phenolic contents of selected vegetables

Secondary metabolites	*T. rosiflora* from Lukala	*T. rosiflora* from Pumbu City
Anthocyanins (mg CE/g DW)	0.30 ± 0.07	0.47 ± 0.14
Flavonoids (mg QE/g DW)	0.090 ± 0.002	0.070 ± 0.001
Total phenolic content (mg GAE/g DW)	42.12 ± 1.14	28.13 ± 1.18

Anthocyanin content was determined as catechin equivalents in milligrams per gram of dry weight (mg CE/g DW), while total polyphenol contents were calculated as gallic acid equivalents in milligrams per gram of dry weight (mg GAE/g DW). Flavonoid content was determined as quercetin equivalents in milligrams per gram of dry weight (QE/g DW). The anthocyanin and total phenolic contents varied significantly ($P < 0.05$) between the studied items.

Antioxidant activity

Antioxidant activity of *T. rosiflora*, determined by two biochemical *in vitro* methods is presented in Table-3 and is expressed as IC_{50} values.

Table-3: IC_{50} values (µg / mL) and GAE of methanolic extracts of selected traditional vegetables on ABTS and DPPH assays (Mean ± SD, n = 6)

Samples	ABTS (µg / mL)		DPPH (µg / mL)	
	T. rosiflora from Lukala	*T. rosiflora* from Pumbu City	*T. rosiflora* from Lukala	*T. rosiflora* from Pumbu City
Dichloromethane extract	25.65 ± 8.67	5.66 ± 0.52	96 .16± 16.73	103.28± 25.60
Methanolic extract	5.48 ± 3.23	10.51 ± 0.86	10.81 ± 0.86	9.59 ± 0.57
Gallic acid	0.71 ± 0.08		1.07 ± 0.10	

IC_{50} is the amount of antioxidant necessary to decrease the initial concentration of radical by 50%. Lower IC_{50} value indicates a higher antioxidant activity. All the extracts had significant scavenging effects with antiradical activities connected to their ability to scavenge free radicals according to their IC_{50}. The antioxidant activity of extracts varied significantly ($P < 0.05$) between the studied items in ABTS assay; while in DPPH assay, the difference is not significative. The inhibition effects of extracts from Lukala item was more high than those of Pumbu City item for the methanolic extracts and for dichloromethne extracts the Pumbu City was the more active. The difference can be explained by qualitative and quantitative chemical composition that depends with ecological conditions [16, 17].

Our results obtained from ABTS and DPPH assays show that all the extracts possessed high antioxidant properties. Our results were correlated between the two biochemical methods used and the differences in the capacity of extracts to scavenger the radicals were observed between the two methods. This difference could be attributed to reactional mechanisms. In fact, DPPH react only with the hydrophilic compounds in the contrary of ABTS [13]. The highest radical-scavenging capacity of extracts from Lukala item is correlated with their higher total phenolic content. By their relevant antioxidant potentiality of his extracts, *T. rosiflora* could provide protection against oxidative damage under different disease conditions but in the future, it will be interesting to characterize the molecules responsible of bioactivities.

CONCLUSION

The microscopic analysis of powders from leaves of *Tetracera rosiflora* allowed the identification of specific botanical microscopic characters. Phytochemical analysis indicated that phenolic compounds, terpenes are major secondary metabolites of leaves from this species. All extracts exhibited good antioxidant activity with Lukala item as the most actives. However, these antioxidant *in vitro* activities should be complemented in the future by further studies by evaluating the cellular antioxidant and the *in vivo* antioxidant activities. This could lead to the valorization of this plant, which could be promoted as medicine with high antioxidant capacity.

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REFERENCES

1. Adekunle, A. A., Duru, C., Odufuwa, O. M., & Jäger, A. K. (2003). Antifungal activity and phytochemical screening of the crude extracts of Khaya ivorensis Juss (Meliaceae) and Tetracera potatoria L.(Dilleniaceae). *South African journal of botany*, *69*(4), 568-571.
2. Kukongviriyapan, V., Janyacharoen, T., Kukongviriyapan, U., Laupattarakasaem, P., Kanokmedhakul, S., & Chantaranothai, P. (2003). Hepatoprotective and antioxidant activities of Tetracera loureiri. *Phytotherapy Research*, *17*(7), 717-721.
3. de Lima, C. C., Lemos, R. P. L., & Conserva, L. M. (2013). Chemical Constituents, Larvicidal Effects and Radical Scavenging Activity of Tetracera breyniana Schltdl.(Dilleniaceae).
4. Umar, A., Ahmed, Q. U., Muhammad, B. Y., Dogarai, B. B. S., & Soad, S. Z. B. M. (2010). Anti-hyperglycemic activity of the leaves of Tetracera scandens Linn. Merr.(Dilleniaceae) in alloxan induced diabetic rats. *Journal of Ethnopharmacology*, *131*(1), 140-145.
5. Lima, C. C., Lemos, R. P., & Conserva, L. M. (2014). Dilleniaceae family: an overview of its ethnomedicinal uses, biological and phytochemical profile. *Journal of Pharmacognosy and Phytochemistry*, *3*(2).
6. Hasan, M. M., Ahmed, Q. U., Soad, S. Z. M., Latip, J., Taher, M., Syafiq, T. M. F., ... & Zakaria, Z. A. (2017). Flavonoids from Tetracera indica Merr. induce adipogenesis and exert glucose uptake activities in 3T3-L1 adipocyte cells. *BMC complementary and alternative medicine*, *17*(1), 431.
7. Muliyah, E., Sulistijorini, S., Sulistyaningsih, Y. C., & Rafi, M. (2018). Tetracera scandens as a Medicinal Plant: Secretory Structures, Histochemistry, and Antibacterial Activity. *Journal of Tropical Life Science*, *8*(1).
8. Bahati, L. M., Kapepula, P. M., Kabamba, N. N., Moni, B., Kafuti, G. M., Mungitshi, P. M., ... & Mutunda, C. M. (2017). Microscopic Features, Chromatographic Fingerprints and Antioxidant Property of Some Unconventional Green Leafy

Vegetables Consumed in Bandundu, DR Congo. *Pharmacognosy Communications*, *7*(4), 158-163.

9. Mpiana, P. T., & Tshibangu, S. T. D. (2016). Les alicaments et la drépanocytose: une mini-revue. *Comptes Rendus Chimie*, *19*(7), 884-889.
10. Tshishimbi, J. M., Mudogo, V., & Mpiana, P. T. (2017). Bioactive compounds from eight plant species traditionally used in Madagascar as medicines: A mini-review.
11. Staudinger, H. W. (2013). Chromatographic Fingerprint Analysis of Herbal Medicines, *Springer International Publishing*.
12. Kabuyi, M. K., Kapepula, P. M., Kabengele, J. K., Moni, B., Makengo, G., Mungitshi, P. M., ... & Kabamba, N. N. (2017). Selenium content and antioxidant potential of some edible wild mushrooms from Bandundu Area, DR Congo. *Natural Resources*, *8*(02), 103.
13. Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of food composition and analysis*, *24*(7), 1043-1048.
14. Gurav, S. S., & Gurav, N. S. (Eds.). (2014). *Indian herbal drug microscopy* (220). New York: Springer.
15. Alhassan, A. M., Ahmed, Q. U., Latip, J., & Shah, S. A. A. (2018). A new sulphated flavone and other phytoconstituents from the leaves of Tetracera indica Merr. and their alpha-glucosidase inhibitory activity. *Natural product research*, 1-8.
16. Liu, W., Liu, J., Yin, D., & Zhao, X. (2015). Influence of ecological factors on the production of active substances in the anti-cancer plant Sinopodophyllum hexandrum (Royle) TS Ying. *PLoS one*, *10*(4), e0122981.
17. Mohamadi, N., & Rajaei, P. (2016). Effects of Different Ecological Condition on the Quality and Quantity of Essential Oils of Artemisia persica Boiss. Populations from Kerman, Iran. *Journal of Essential Oil Bearing Plants*, *19*(1), 200-207.