

Phytochemical profile and antibacterial photodynamic activity of *Centella asiatica* L. (Apiaceae), *Citrus grandis* L. (Rutaceae) and *Zanthoxylum gillettii* De Wild. (Rutaceae) leaves extracts

ABSTRACT

Aims: The objective of this study was to determine the phytochemical profile of *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* leaves, in solution and the thin layer chromatography chemical screening and to evaluate the antibacterial photodynamic activity of the species extracts.

Materials and Methods: The search of the secondary metabolites in solution and by thin layer chromatography, the alkaloids and polyphenols extraction were carried out using standard protocols.

The antibacterial photodynamic activity was performed using microdilution method in solution and solid culture media.

Results: *Centella asiatica* and *Citrus grandis* showed the same phytochemical profile, with the presence of alkaloids, terpenoids, steroids and polyphenols such as: flavonoids, free quinones, anthocyanins, leuco-anthocyanins and tannins. *Zanthoxylum gillettii* extract showed similar profile with the absence of anthocyanins and leucoanthocyanins. The polyphenolic extracts of both *Centella asiatica* and *Citrus grandis* leaves were active against *Staphylococcus aureus* and *Escherichia coli* after one and two hours of solar exposition, respectively. Whereas for *Zanthoxylum gillettii* extract, one hour was sufficient to completely destroy both bacterial strains.

Conclusion: polyphenolic extracts from *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* leaves showed a photodynamic effect on *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Centella asiatica*, *Citrus grandis*, *Zanthoxylum gillettii*, photodynamic, antibacterial activity.

1. INTRODUCTION

Water is essential for life, it constitutes almost 70% of the human body weight. However, the access to drinking water is still a problem in some countries, especially in developing countries. It is noted that nearly 2.1 billion people, or 30% of the world's population, still do not have access to domestic drinking water supply services [1].

In fact, the poor quality of sanitation facilities and contaminated water cause the death of nearly 36.000 children under the age of five around the world. This as a result of water-borne diseases such as diarrhea, cholera, dysentery, hepatitis A and typhoid fever. In Democratic Republic of the Congo (DRC), in 2018, 31.387 cases of cholera were recorded with a case fatality rate of 3.32% [2].

Insufficient sanitation and drinking water distribution infrastructures is a worrying problem in DRC, where nearly 76% of urban and rural sources of drinking water supply present microbial and viral contaminations. This including pathogenic microorganisms of fecal origin [3], especially in rural areas where only 19% of the population has access to a basic level of water service [4].

The DRC is committed, through the National Healthy Schools and Village Program, to achieving the Sustainable Development Goals (SDGs) by 2030, and more specifically goal six, which aims to guarantee access for all to water and sanitation and to ensure sustainable management of water resources. The National Strategic Development Plan 2017-2022 (NSDP) aims to "ensure equitable

access for the entire population to drinking water, at an affordable cost, as well as to adequate sanitation and hygiene services” and plans to achieve an overall drinking water supply rate of at least 70% [4].

Although the country has more than 50% of the water reserves of the African continent, it is sad to notice, three years after the start of the NSDP, that the number of Congolese with easy access to drinking water, until the year 2020, was only around 30% [5].

Methods such as disinfection by chlorination, ozonation, UV, etc. are used to alleviate the problems associated with the supply and distribution of drinking water, however these methods can present some risks to human health and are not easily accessible [6].

Due to the problems presented by the above methods, the use of easily accessible water disinfection methods, even in rural areas, such as solar disinfection is advised. However, its effectiveness depends on the variations in climatic conditions and the required time for its implementation [6]. The use of singlet oxygen generated by the combined action of a photosensitizer and light, capable of causing the death of microbial cells, is therefore essential [7, 8].

This work aims to evaluate the antibacterial photodynamic activity of *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* leaves. So, a phytochemical profile evaluation was necessary, in order to know the different secondary metabolites groups present in these plants.

2. MATERIAL AND METHODS

2.1 Plant material

The leaves of *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* were collected on the site of the University of Kinshasa, in the Garden of Kimbaseke (Kinshasa) and in Mayombe forest (Kongo-central), respectively, in DRC. These plant species were identified at the herbarium of the National Institute of Agronomic Studies and Research (INERA), housed at the Faculty of Sciences of the University of Kinshasa. The powder of the air and shade-dried leaves of each species was obtained by grinding, using a Sinbo CBD-2001 type grinder.

2.2 Bacterial strains

Two bacterial strains references *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used.

2.2 Phytochemical screening, in solution and by Thin Layer Chromatography

The search of the secondary metabolites was carried out in aqueous and organic solutions, following a well known protocol [9].

The Thin Layer Chromatography (TLC) was performed to confirm the presence of the secondary metabolites following the standard protocol described by Wagner [10].

2.3 Extraction of alkaloids

The plant material (15g) previously delipidated using petroleum ether was macerated in 30 mL of 10% acetic acid in ethanol, for 48 hours. The pH of the obtained filtrate was adjusted to 9 by adding ammonia.

The extraction of the alkaloids was carried out using liquid-liquid extraction with 50 mL (x3) of chloroform.

2.4 Polyphenols Extraction

The extraction of the polyphenols was carried out following the protocol used by Guendouzen & Haddouche in 2016 [11].

2.5 Photosensitization test

By one hand, each extract (0.025g) was dissolved in 1 mL of distilled water (solution A) and by the other hand, 350 mL of distilled water contained in each of two Erlenmeyer were respectively polluted with *Escherichia coli* and *Staphylococcus aureus*. Then, each part of polluted water was distributed

into seven beakers at the rate of 50 mL each, to which were added 200 μ L of each solution A (2 extracts per plant). The mixture contained in each beaker was distributed in 10 tubes (5 mL each) of which 5 were placed under the sun and 5 others in the dark, in order to compare their antimicrobial activity with the time evolution (one hour apart for five hours). Polluted water without plant extract was used as a negative control.

Furthermore, the inoculation of the Salt Agar culture medium was carried out to search for *S. aureus* in each tube, during 48 hours of incubation, while for *E. coli*, a part of the content of each tube was inoculated on the Mac conkey solid medium and incubated for 48 hours.

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening in solution

The chemical screening result in solution in table 1

Table 1. Chemical screening in solution

Metabolites	Plants		
	<i>C. asiatica</i>	<i>C. grandis</i>	<i>Z. gillettii</i>
Total polyphenols	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Bound quinones	+	+	+
Free quinones	+	+	+
Anthocyanins	+	+	-
Leuco-anthocyanins	+	+	-
Tanins	+	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Terpens	+	+	+

Legend :

+ : presence

- : absence

This table reveals a similar phytochemical profile for *C. asiatica* and *C. grandis*, with the presence of polyphenols (flavonoids, free quinones, anthocyanins, leuco-anthocyanins, and tannins), alkaloids, terpenoids and steroids and the absence of saponins. While for *Z. gillettii*, a similar profile to that of the two aforementioned plants is observed. The sole difference was the absence of leuco-anthocyanins and anthocyanins. The result of the phytochemical screening of *C. asiatica* leaves corroborates those of the studies carried out by Arumugam, in India and by Noverita, in Indonesia [12, 13].

For *C. grandis*, the profile is different from that of the same species collected in India where the tannins and steroids were absent [14]. This could be due to the ecology difference.

3.2 Phytochemical screening by TLC



The result of the extracts by TLC chemical screening of *C. asiatica*, *C. grandis*, *Z. gillettii* given in figures 1



leaves

are to 4.

Figure 1: Chromatoplate for alkaloids

Stationary phase: Silicagel 60F₂₅₄
Mobile phase: Toluene / Ethyl acetate /
diethylamide (35: 10: 5)
Developer: NaNO₂ 5%

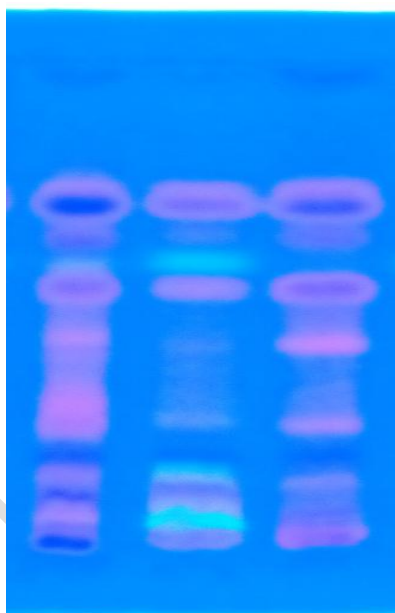


Figure 3: Chromatoplate for coumarins

Stationary phase: Silicagel 60F₂₅₄
Mobile phase: Toluene / Ethyl acetate (31:
2.3 saturated with 10% acetic acid).
Developer: 10% KOH in ethanol and UV
lamp at 366 nm.

Figure 1 indicates the presence of terpenes, anthracene derivatives, coumarins and alkaloids. The alkaloid molecules of the same type were observed for *C. grandis* and *Z. gillettii* by highly visible yellow spots with the R_f value of 0.65 and brown spots at R_f of 0.57.

Figure 2: Chromatoplate for flavonoids

Stationary phase: Silicagel 60F₂₅₄
Mobile phase: ethyl acetate / formic acid/
glacial acetic acid / Water (50: 6.5: 6.5:
13.5).
Developer: Neu reagent.

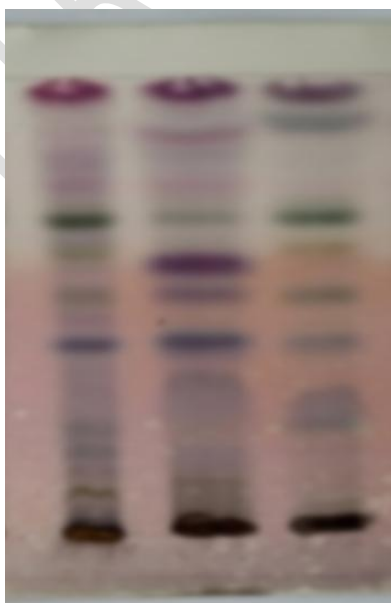


Figure 4: Chromatoplate for terpenoids

Stationary phase: Silicagel 60F₂₅₄
Mobile phase: Toluene / Ethyl acetate (93: 7).
Developer: Sulfuric anisaldehyde.

Flavonoids were identified on the chromatoplate of Figure 2 by the yellow and brown spots that were noted in all the three samples. In addition, the blue and green colored spots with the Rf of 0.70 and 0.17, respectively, were also reported in the three plants. This could correspond to the same type of flavonoids molecules, but in different quantities. For the brown spots visible at Rf of 0.6, their presence was detected only in *Z. gillettii* and *C. asiatica*.

The coumarins in Figure 3 showed blue, violet and brown colors spots after spraying with 10% of ethanolic KOH and observation at 366 nm. A similar profile was observed for samples from all the three plants with blue colored spots showing the presence of coumarins at Rf of 0.70, 0.52 and 0.20 and fluorescent spots of brown color with Rf values of 0.22 and 0.15.

The presence of the triterpenoids was noticed by the maroon colored spots in the figure of chromatogram 4, after visualization with sulfuric anisaldehyde and heating within some minutes. From this chromatoplate, a similar profile is noticed in the three plants with the spots at values of Rf 0.67, 0.50 and 0.42.

The presence of the spots of the same colors and at the same Rf indicates the presence of the same type of molecules, in the different samples.

4. Alkaloids and polyphenols extraction

The table 2 gives extraction yield values for total alkaloids and polyphenols in the three plants.

Table 2: Percentage extraction yields

Extracts	Yields (%)		
	<i>C. asiatica</i>	<i>Z. gillettii</i>	<i>C. grandis</i>
Total alkaloids	10.46	16.23	14.68
Total polyphenols	11.72	15.64	13.71

From this table it can be seen higher values yields of alkaloids and polyphenols in *Z. gillettii*, while the lowest values are found in *C. asiatica*. These yields are close to what is reported in the literature [15, 16].

5. Antibacterial photodynamic activity of total alkaloids and polyphenolic extracts

5. 1 Antibacterial photodynamic activity of total alkaloids extract

The bacterial presence in water polluted with *S. aureus* and *E. coli* was observed after their contact with alkaloid extracts of *C. asiatica*, *C. grandis* and *Z. gillettii*. Indeed, no bactericidal and / or photodynamic action has been observed after exposure to sunlight and darkness. This corroborates, by one hand, the results of Taba & Luwenga (1999) on the leaves of *Cassia alata*, *Cassia occidentalis*, *Carica papaya*, *Phyllanthus niruri*, who noted the absence of a photodynamic effect of alkaloid extracts against *E. coli* and by the other hand, a bactericidal effect of the same extracts had been observed in the shade. Sunda *et al.* (2008) noted a bactericidal effect exhibited by alkaloid extracts in the dark and in the presence of light. This was not the case in the present study [17, 18].

In addition, several studies have reported the antibacterial activity of *C. asiatica*, *C. grandis*, and *Z. gillettii* on many bacterial strains including *S. aureus*, *E. coli* [12, 19]. Thus, this anti-bacterial activity of the extracts of the leaves of these three plants in previous studies would be due to the presence of other metabolites other than alkaloids [20-22].

5. 2 Antibacterial photodynamic activity of total polyphenolic extract

The result of the evaluation of the photosensitizing effect observed during the contact between polyphenolic extracts and polluted water in the presence of light showed that the polyphenolic extracts of these three plants exhibit a bactericidal effect before and after exposure to solar radiation. In the shade, the extracts of *C. asiatica* and *C. grandis* exhibit a bactericidal effect against *E. coli* after five hours of contact with polluted water, while for *Z. gillettii*, the absence of bacteria was noticed after four hours.

For the polyphenolic extract of *Z. gillettii*, a bactericidal effect was observed after only three hours of contact with *S. aureus* in the shade and the same effect was manifested for *C. asiatica* and *C. grandis* from four hours of shade exposure. This may be due to the difference of polyphenol concentrations in the three plants.

The effect of light is evidenced by the exposure of the extracts of *C. asiatica*, *C. grandis* and *Z. gillettii*, thus causing a significant reduction in the exposure time. The absence of bacteria is observed for the polyphenolic extracts of these three plants after one hour of solar exposure for *S. aureus*.

However, for *E. coli*, total destruction is observed after two hours of exposure for the leaves of *C. asiatica* and *C. grandis* and only one hour for *Z. gillettii* leaves. The presence of bacteria is observed for the negative control under the sun as well as in the dark.

It therefore seems obvious that an interaction between light and the molecules of the extracts leading to the production of reactive oxygen species causing the death of bacteria has occurred [24].

A difference in time for complete destruction of *E. coli* by *C. asiatica*, *C. grandis* and *Z. gillettii* extracts, compared to the time required for complete inhibition of *S. aureus* may be explained by the complexity of the membrane of negative gram bacteria which may be difficult to penetrate [25, 26].

This photosensitizing activity of the polyphenolic extracts of these three plants is due to the presence of quinones, coumarins and flavonoids, in these samples. In fact, these three metabolites often have the ability to produce singlet oxygen in the presence of an excitation source [18, 27, and 28]. The table 3 below shows the treatment of water polluted with *S. aureus* and *E. coli* by extracts of polyphenols from three plants.

Table 3: Water polluted treatment with *S. aureus* and *E. coli* by polyphenol extracts of the three plants.

Species	<i>S. aureus</i>										<i>E. coli</i>									
	Darkness					Sun light					Darkness					Sun light				
	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h	1h	2h	3h	4h	5h
<i>C. asiatica</i>	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-
<i>C. grandis</i>	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-
<i>Z. gillettii</i>	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Negative control					+					+					+					+

Legend :

+ : Presence

- : Absence

Conclusion

The phytochemical screening of *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* leaves revealed a similar profile for *Centella asiatica*, *Citrus grandis* with the presence of polyphenols (flavonoids, free quinones, anthocyanins, leuco-anthocyanins, tannins), alkaloids, terpenoids and steroids, and the absence of saponins. For *Zanthoxylum gillettii*, a similar profile was observed but with the absence of leuco-anthocyanins and anthocyanins.

The evaluation of the photosensitizing activity allowed the demonstration of the polyphenolic extracts antibacterial photodynamic activity of the *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gillettii* leaves. Indeed, the polyphenolic extracts of the *Centella asiatica* and *Citrus grandis* leaves completely inhibited the presence of *S. aureus* and *E. coli* after one hour and two hours, respectively. For *Zanthoxylum gillettii* extracts, one hour was sufficient to completely destroy both bacterial strains.

A bactericidal effect was observed for the polyphenolic extracts of these three plants. This effect was observed after four hours of contact for *Centella asiatica* and *Citrus grandis* on *S. aureus*, while for *E. coli*, it took five hours of contact. For *Zanthoxylum gillettii*, complete inhibition occurred after three and four hours for *S. aureus* and *E. coli*, respectively.

The antibacterial photodynamic effect reduced the time required for the complete destruction of bacteria. Therefore, the leaves of all three plants can be used to destroy positive and negative gram bacteria from water.

The determination of the specific chemical groups responsible for this activity is undergoing.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCES

1. WHO/UNICEF JMP (2021), Progress on household drinking water, sanitation and hygiene 2000-2020: Five years into the SDGs
2. UNICEF, République Démocratique du Congo, 2017-2018, Multiple Indicator Principaux résultats Cluster Survey. French
3. Programme des Nations Unies pour l'Environnement (PNUE). Problématique de l'eau en République Démocratique du Congo, Rapport technique 2011.
4. Programme National École et Village Assainis (PNEVA). ATLAS 2018. Accès à l'eau potable, à l'hygiène et à l'assainissement des communautés rurales et périurbaine en République Démocratique du Congo, 2018.
5. Radio Okapi, Ministre congolais des Ressources hydrauliques, Available on : <https://www.radiookapi.net/2020/03/23/actualite/societe/rdc-seuls-30-de-congolais-ont-acces-facile-leau-potable-officiel> accessed 22/3/ 2020
6. Cowie BE, Porley V and Robertson N. Solar Disinfection (SODIS) Provides a Much Underexploited Opportunity for Researchers in Photocatalytic Water Treatment (PWT). ACS Catal. 2020; 10 : 11779–11782
7. Salman HE, Rajab ZN, Khalil BI, To develop dental prostheses and improve the addition of polymers (Atridox, Fort Collins) in dental procedures. Eurasia J Biosci 2020; 14 : 629-643
8. Zhen X, Chudal L, Pandey NK, Phan J, Ran X, Amador, Huang EX, Johnson O, Ran Y, Chen W, Hamblin MR, Huang L, Une puissante combinaison de nanoparticules de cuivre-cystéamine avec de l'iode de potassium pour la destruction bactérienne. Science et génie des matériaux 2020; 110(C) : 154-185
9. Tshilanda DD, Inkoto C, Mpongu K, Mata S, Mutwale P, Tshibangu DST, Bongo G, Ngbolua KN,, Mpiana PT. Microscopic Studies, Phytochemical and Biological Screenings of Ocimum canum, International Journal of Pharmacy and Chemistry 2019; 5(5): 61-67
10. Wagner H, Bladt S, Zgainski EM. Plant drug analysis: A thin layer chromatography, Atlas 1996. 1
11. Guendouzen R et Haddouche L. Extraction des métabolites secondaires (composés phénoliques et huiles essentielles) et évaluation de l'activité antioxydante chez l'espèce Juniperus oxycedrus, Mémoire de Master, Université A/Mira de Bejaia, 2016 .French
12. Arumugam T, yyanar M, Pillai YJK, Sekar T. Criblage phytochimique et activité antibactérienne des feuilles et des callosités extraits de *Centella asiatica*. bangladesh J. Pharmacol 2011; 6 : 55-60
13. Noverita SV, Luthfi AM and Justin A. Morphology of leaves and content of secondary metabolites asiaticoside in some accession of pegagan (*Centella asiatica* L. Urban) in North Sumatera; Proceedings of The 2nd Annual International Conference Syiah Kuala University 2012; 1(2):
14. Jabamalaiaraj A, Dorairaj S, Yadav SA, Bathrachalam C. détection de groupe fonctionnel et activité antimicrobienne de feuille extraits de *Citrus grandis* (L.) Contre pathogènes cliniques sélectionnés. Indo American Journal of Pharmaceutical Research 2015; 5(5) : 2231-6876
15. Farhana NI and Masrina MN. Comparative studies on different extraction methods of *Centella asiatica* and bioactive compounds effects on antimicrobial activities. Antibiotics 2021 ;10(457) :
16. Wafula RG. phytochemical investigation of *zanthoxylum gillettii* (rutaceae) for antiplasmodial biomolecules, University of Nairobi 2014

17. Taba KM et Luwenga E, L'effet de la photosensibilisation des extraits de plantes dans la désinfection de l'eau. Med. Fac. Landbouwkundige, Univ. Gent 1999; 64(1) :177-181. French
18. Sunda M, Rosillon F, Taba KM., Contribution à l'étude de la désinfection de l'eau par photosensibilisation avec les extraits de plantes. European Journal of Water Quality 2008; 2(39) : 199-209
19. Siti M. Antimicrobial activity of *Centella asiatica* and *Gigantochloa apus*. J Basic Clin Physiol Pharmacol 2021; 32(4) : 755-759.
20. Wardatul HI, Tamri, Lamek M., Marpongahtun. Preparation and characterization of bacterial cellulose supplemented centella asiatica l. Urban extract to improve mechanical properties. Rasayan Journal of Chemistry 2021; 14(01) : 09-15
21. Sholeh S, Mansour A, Reza SG, Nasrin A. Evaluation of Antibacterial Activities of Citrus limon, Citrus reticulata, and Citrus grandis Against Pathogenic Bacteria. Int J Enteric Pathog. 2016; 4(4) :
22. Nguyen XN, Pham MQ and Nguyen TH, Alkaloids and Their Pharmacology Effects from Zanthoxylum Genus. Bioactive Compounds in Nutraceutical and Functional Food for Good Human Health 2020
23. Arfao A, Ewoti OVN, Onana MF, Djimeli C, Tchakonté S, Kobbe N, Nola MD, combined effect of eucalyptus microcorys aqueous extract and light on pathogenic escherichia coli survival in aquatic microcosm. Journal of Microbiology, Biotechnology and Food Sciences 2020; 9(5) :1003-1008
24. Maisch T, Photoantimicrobials-An update. Translational Biophotonics 2020, 2:e 201900033
25. Shah FA, Mahmood Q, Iqbal A et Mackie RI, Inhibition de la production de biométhane sous antibiotiques dans la litière de volaille. Journal arabe des géosciences 2020; 13(614) : 358-389
26. Narayan MR, Review : Dye sensitizer solar cells based on natural photosensitizers, Renewable and sustainable energy reviews, 2012, 16 : 208-215
27. Ryberg EC, Knight J et Kim J-H., Farm-to-Tap Water Treatment : Naturally-Sourced Photosensitizers for Enhanced Solar Disinfection of Drinking Water, ACS ES&T Engineering 2020 : <https://dx.doi.org/10.1021/acsestengg.0c00067>