International Journal of Pathogen Research

9(2): 1-8, 2022; Article no.IJPR.84456
ISSN: 2582-3876

Phytochemical Profile and Antibacterial Photodynamic Activity of *Centella asiatica* L. (Apiaceae), *Citrus grandis* L. (Rutaceae) and *Zanthoxylum gilletii* De Wild. (Rutaceae) Leaves Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPR/2022/v9i230219

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/84456

Original Research Article

Received 10 December 2021
Accepted 12 February 2022
Published 15 February 2022

ABSTRACT

Aims: The objective of this study was to determine the phytochemical profile of *Centella asiatica, Citrus grandis* and *Zanthoxylum gilletii* 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.

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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 gilletii* 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 gilletii* extract, one hour was sufficient to completely destroy both bacterial strains.
Conclusion: Polyphenolic extracts from *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gilletii* leaves showed a photodynamic effect on *Escherichia coli* and *Staphylococcus aureus*.

Keywords: Centella asiatica; Citrus grandis; Zanthoxylum gilletii; secondary metabolites; 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 [2]. 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 [3]. 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].

The approach at the individual or family level is to boil water or make use of chlorinated products [6].

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 [7].

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 adviced. 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 gilletii l*eaves. So, a phytochemical profile evaluation was necessary, in order to know the different secondary metabolites groups present in these plants [9].

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Centella asiatica*, *Citrus grandis* and *Zanthoxylum gilletii* 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 and samples deposited 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.

2.3 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 the dried and powdered plant material (10 g) was repeatedly extracted by cold percolation with 95% ethanol and water (100 mL × 2) for 48 hours. Chemical screening was performed to investigate the presence of alkaloids, saponins, total polyphenols, flavonoids, tannins, anthocyanis, leuco-anthocyanins, quinones, terpenes and steroids according to standard protocol [10].

The Thin Layer Chromatography (TLC) was performed to confirm the presence of the secondary metabolites following the standard protocol based on the observation of spots of various colours to identify different secondary metabolites [11].

2.4 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.

2.5 Polyphenols Extraction

The extraction of the polyphenols was carried out following the protocol used by Guendouzen & Haddouche in 2016. It consists of dissolving 0.8 g of the vegetable grind (foliage and cones) in 32 mL of 96% ethanol. The mixture is stirred for two hours at room temperature followed by centrifugation for 10 minutes to 6000 rpm. The supernatant is recovered in test tubes and then kept coo [11].

2.6 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 µL 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 Manitol 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 MacConkey 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.

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. In *Z.gilletii*, a similar profile to that of the two aforementioned plants is observed. The sole difference was the absence of leuco-anthocyanins and anthocyanins (Table 1). 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].

Table 1. Chemical screening in solution

Metabolites	Plants		
	C. asiatica	***C. grandis***	***Z. gilletii***
Total polyphenols	+	+	+
Saponins	-	-	-
Flavonoids	+	+	+
Bound quinones	+	+	+
Free quinones	+	+	+
Anthocyanins	+	+	-
Leuco-anthocyanins	+	+	-
Tannins	+	+	+
Alkaloids	+	+	+
Steroids	+	+	+
Terpens	+	+	+

Legend: +: presence; -: absence

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 leaves extracts by TLC chemical screening of *C. asiatica*, *C. grandis, Z. gelletii* are given in Figs. 1 to 4.

Fig. 1. Chromatoplate for alkaloids
Stationary phase: Silicagel 60F$_{254}$; Mobile phase: Toluene / Ethyl acetate / diethylamide (35: 10: 5); Developer: NaNO$_2$ 5%

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

Flavonoids were identified on the chromatoplate of Fig. 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. gilletii* and *C. asiatica*.

Fig. 2. Chromatoplate for flavonoids
Stationary phase: Silicagel 60F$_{254}$; Mobile phase: ethyl acetate / formic acid/ glacial acetic acid / Water (50: 6.5: 6.5: 13.5); Developer: Neu reagent.

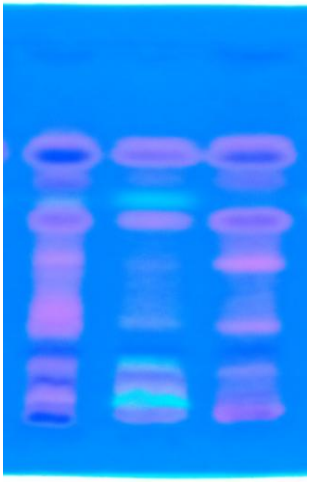

Fig. 3. Chromatoplate for coumarins
Stationary phase: Silicagel 60F$_{254}$; Mobile phase: Toluene / Ethyl acetate (31: 2.3 saturated with 10% acetic acid). Developer: 10% KOH in ethanol and UV lamp at 366 nm

The coumarins in Fig. 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 anysaldehyde 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.

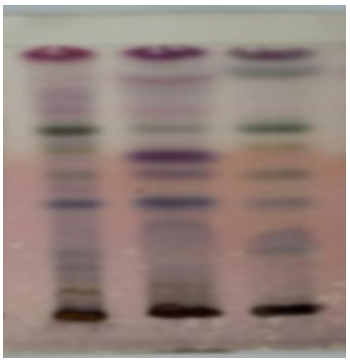

Fig. 4. Chromatoplate for terpenoids
Stationary phase: Silicagel 60F$_{254}$; Mobile phase: Toluene / Ethyl acetate (93: 7); Developer: Sulfuric anysaldehyde.

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.

From this table it can be seen higher values yields of alkaloids and polyphenols in *Z. gilletii*, while the lowest values are found in *C. asiatica* (Table 2). These yields are close to what is reported in the literature [15,16].

Table 2. Percentage extraction yields

Extracts	Yields (%)		
	C. asiatica	***Z. gilletii***	***C. grandis***
Total alkaloids	10.46	16.23	14.68
Total polyphenols	11.72	15.64	13.71

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. gilletii*. 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].

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

Species	*S. aureus*										*E. coli*									
	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
C. asiatica	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-
C. grandis	+	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-	-	-
Z. gilletii	+	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-
Negative control					+					+					+					+

Legend: +: Presence; -: Absence

In addition, several studies have reported the antibacterial activity of *C. asiatica, C. grandis*, and *Z. gelletii* 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. gilletii*, the absence of bacteria was noticed after four hours (Table 3).

For the polyphenolic extract of *Z. gilletii*, 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. gilletii*, 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. gilletii* leaves. The presence of bacteria is observed for the negative control under the sun as well as in the dark (Table 3).

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 [23,24].

A difference in time for complete destruction of *E. coli* by *C. asiatica, C. grandis* and *Z. gilletii* extracts, compared to the time required for complete inhibition of *S. aureus* may be explained by the complexity of the membran 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 [18,27,28]. The Table 3 bellow shows the treatment of water polluted with *S. aureus* and *E. coli* by extracts of polyphenols from three plants.

6. CONCLUSION

The phytochemical screening of *Centella asiatica, Citrus grandis* and *Zanthoxylum gilletii* 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 gilletii*, 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 gilletii* 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 gilletii* 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 gilletii*, 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.

7. RECOMMENDATION

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

DISCLAIMER

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.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://dx.doi.org/10.1021/acsestengg.0c00067

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/84456