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**Original Research Article**

# Phytochemical Screening and *in Vitro* Evaluation of Antioxydant and Antibacterial Properties of *Acmella caulirhiza* (Asteraceae)

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## Abstract

The present study was aimed to evaluate the antibacterial and antioxidant properties of *Acmella caulirhiza*. The plant was harvested, identified, dried and pulverized. Five extractions were carried out: one by decoction and four by maceration with distilled water, ethanol/water mixture (70/30, *v/v)*, methanol, and 95° ethanol as solvents. Phytochemical screening of *A. caulirhiza* extracts was performed according to the Sofowora method. The determination of the minimum inhibitory concentrations by the microdilution method in a liquid medium, allowed the evaluation of the antibacterial activity on *Pseudomonas aeruginosa* NR 48982, *Staphylococcus aureus* ATCC 43300, methicillin-resistant Staphylococcus *aureus* ATCC 33591, *Klebsiella pneumonia* NR 41897. Antioxidant activity was performed by DPPH radical scavenging and $Fe^{3+}$ reduction tests. All *A. caulirhiza* extracts contained phenolic compounds (flavonoids, tannins, coumarins), mucilages, and resins. However, alkaloids, saponosides, cardiac glycosides, and anthocyanins were absent. They showed antibacterial activity with minimum inhibitory concentration values ranging from 8 to 32 mg/mL). The antioxidant activity of the DPPH extracts had concentrations (IC50) ranging from 0.098 ± 0.011 to 3.9040 ± 0.319 mg/mL. The $Fe^{3+}$ ion reducing activities of the extracts ranged from 53.120 ± 0.443 to 22.267 ± 1.006 µg/mL. The extracts of *Acmella caulirhiza* contain secondary metabolites that can justify the antibacterial and antioxidant activities. The use of this plant in traditional medicine in the management of infectious pathologies could be justified.

**Keywords:** *Acmella caulirhiza*; Antioxidant activity, antibacterial activity; Phytochemical screening.



## 1. INTRODUCTION

Bacterial infections continue to be the leading cause of morbidity and mortality worldwide despite the discovery of many antibiotics [1]. The use of antibiotics has significantly reduced the morbidity of bacterial infections [2]. However, bacterial resistance is increasingly being observed, leading to treatment failures [3, 4]. According to WHO, *Pseudomonas aeruginosa*, *Klebsiella* sp, methicillin-resistant *Staphylococcus aureus* (MRSA) are bacteria with a high multidrug resistance potential [5]. Oxidative stress is a risk factor for morbidity and mortality in most infectious diseases [6]. Therefore, the exploration of natural resources with antibacterial and antioxidant potential appears to be a promising alternative that can be used to treat infectious diseases. Indeed, 80% of the African population uses medicinal plants for their primary health care [7, 8]. *Acmella caulirhiza* is a plant that belongs to the Asteraceae family used in traditional medicine to treat oral diseases, urinary tract infections, diaper rash, and tonsillitis by the people of the Njidara village in the Noun Division, in Cameroon. Numerous studies have been carried out on the antipyretic, anti-inflammatory and analgesic properties of *A. caulirhiza* [9, 10]. The aim of this study was to evaluate the antibacterial and antioxidant activities of *A. caulirhiza* extracts used traditionally to treat infectious diseases.

## 2. MATERIALS AND METHODS

### 2.1. Study area

The study took place in the Laboratory of Pharmacognosy and Pharmaceutical Chemistry of the Faculty of Medicine and Biomedical Sciences (FMSB) and the Laboratory of Phytobiochemistry and Study of Medicinal Plants of the Faculty of Science of the University of Yaoundé I. The study was authorized by the Institutional Research Ethics Committee of the FMSB (N°175/UYI/FMSB/VDRC/DAASR/CSD).

### 2.2 Plant material

The entire plant of *Acmella caulirhiza* was collected in the village of Njidara located in the Noun Division (West Cameroon Region). *Acmella caulirhiza* is referenced at the National Herbarium of Cameroon in

Yaoundé in comparison with specimen N° 66203 HNC of the Herbarium Collection N° 137.

### 2.3 Biological material

Four (4) bacterial strains were used including *Pseudomonas aeruginosa* NR 48982, *Staphylococcus aureus* ATCC 43300, Multi-resistant *Staphylococcus aureus* ATCC 33591, *Klebsiella pneumonia* NR 41897 available in the Laboratory of Phytobiochemistry and Study of Medicinal Plants of the Faculty of Sciences, the University of Yaoundé I.

### 2.4. Plant extraction

The plant extract was air-dried for 14 days and then pulverized. Sixty milligrams of powder were immersed in 600 mL of solvent. On the one hand, four extractions were carried out by maceration for 48 hours with regular stirring using distilled water, an ethanol/water mixture (70/30, *v/v*), methanol, and 95° ethanol as solvent. Another extraction by decoction using distilled water as solvent was also carried out for 30 minutes at 100°C. The solutions were successively filtered using hydrophilic cotton and Whatman paper N°1. The filtrates obtained were dried in an oven at 45°C.

### 2.5. Phytochemical screening

The phytochemical screening was performed according to Sofowora *et al.*, [11].

### 2.6. Preparation of the 1% stock extract solution

Half a gram of each dried extract was dissolved in 25 mL of distilled water. The mixture was homogenized and then made up with 50 mL of distilled water.

### 2.7. Antibacterial activity

#### 2.7.1. Preparation of bacterial inocula

The bacterial inocula were prepared according to the recommendations of the Clinical and Laboratory Standard Institute (CLSI) [12].

#### 2.7.2. Preparation of stock solutions of extracts and reference antibacterial

In particular, the methanolic and ethanolic extracts were dissolved in dimethylsulphoxide 1%. The concentration of the stock solutions was prepared at 100 mg/mL.

#### 2.7.3. Determination of the Minimum Inhibitory Concentration of extracts

It was carried out according to liquid micro-dilution as described by CLSI [12].

### 2.8. Assessment of antioxidant activity

#### 2.8.1. DPPH- radical scavenging test

It was carried out according to the method described by Komsok *et al.*, [13].

#### 2.8.2. $Fe^{3+}$ ion reducing activity or FRAP (Ferric Ion Reducing Antioxidant Power)

It was determined according to Path Canada's modified protocol [14].

### 2.9. Statistical analysis

Data were entered and analysed using Word and Excel 2016 (Microsoft Office 2016, USA). Describe statistics involved the presentation of data as the percentage in tables and graphs and as mean ± standard errors on the mean (SEM) for the variables analysed.

## 3. RESULTS

### 3.1 Phytochemical screening

The aqueous extracts contained phlobotannins and quinones. Oxalates, saponosides and steroids were identified only in the ethanolic extracts (Table 1). On the other hand, alkaloids, anthocyanins, betacyans, and cardiac glycosides were absent in the different extracts of *A. caulirhiza* studied.

**Table 1: Major groups of secondary metabolites present in A. caulirhiza**

| Secondary metabolites | Test/reagents | EAD | EM | EAM | EHE | EE |
|---|---|---|---|---|---|---|
| Alkaloids | Wagner and Valse mayer | - | - | - | - | - |
| Polyphenols | $FeCl_3$ 3%. | + | + | + | + | + |
| Flavonoids | $H_2SO_4$ | + | + | + | + | + |
| Tannins | $CuSO_4 + NH_3$ | + | + | + | + | + |
| Mucilage | Absolute ethanol | + | + | + | + | + |
| Saponosides | Foam | - | - | - | - | + |
| Steroids | $H_2SO_4 + CH_3COOH$ | - | - | - | - | + |
| Resins | $CH_3COOH + H_2SO_4$ | + | + | + | + | + |
| Phlobotannins | HCl 1% | + | - | + | - | - |
| Anthocyanins | $H_2SO_4 + NH_3$ | - | - | - | - | - |
| Betacyans | NaOH 2N | - | - | - | - | - |
| Coumarins | $FeCl_3$ 10% | + | + | + | + | + |
| Quinones | Concentrated HCl | + | - | + | - | - |
| Oxalates | $CH_3COOH$ glacial | - | - | - | + | + |
| Cardiac glycosides | $HCl + FeCl_3$ 3% + $H_2SO_4$ | - | - | - | - | - |

*+ Present; - Absent;* 1*: Aqueous extract by maceration;* EHE*: Hydro-ethanolic extract;* EAD*: Aqueous extract by decoction;* EM*: Methanolic extract;* EE*: Ethanolic extract*

### 3.2. Antibacterial activity

All *A. caulirhiza* extracts inhibited the growth of all bacteria strains tested. The different MICs observed varied between 8, and 32 mg/mL (Table 2). Compared to ciprofloxacin, these extracts remain weakly active.

**Table 2: Minimum inhibitory concentrations obtained with the different extracts**

| Extracts | MIC (mg/mL) | | | |
|---|---|---|---|---|
| | ***P. aeruginosa*** | ***S. aureus*** | **Multi-resistant *S. aureus*** | ***K. pneumonia*** |
| Aqueous extract (maceration) | 32 | >32 | 32 | 32 |
| Hydroethanol extract | >32 | 32 | 8 | 16 |
| Aqueous extract (decoction) | >32 | >32 | >32 | >32 |
| Methanolic extract | >32 | 16 | >32 | 16 |
| Ethanolic extract. | 16 | 8 | 8 | 16 |
| Ciprofloxacin | 0,031 | 0,015 | 0,031 | 0,015 |

### 3.3. Antioxidant activity

#### 3.3.1. DPPH● radical scavenging test

The ethanolic extract showed the best free radical scavenging power compared to the other extracts (Table 3).

**Table 3: DPPH● radical scavenging concentrations by the different extracts**

| Extracts | IC50 (mg/ml) | EC50 (mg/ml) | Anti-radical power |
|---|---|---|---|
| Aqueous extract by maceration | 2,2670 ± 0,080c | 113,100 ± 2,722c | 0,898 ± 0,0219a |
| Hydroethanol extract | 1,2052 ± 0,071b | 60,2617 ± 3,588b | 1,663 ± 0,1024a |
| Aqueous extract by decoction | 3,9040 ± 0,319d | 195,200 ± 15,982d | 0,514 ± 0,040a |
| Methanolic extract | 1,0200 ± 0,041b | 51,000 ± 2,075b | 1,963 ± 0,080a |
| Ethanolic extract | 0,098 ± 0,011a | 4,933 ± 0,575a | 20,457 ± 2,413b |
| Vitamin C | 0,001 ± 0,0001a | 0,059 ± 0,000a | 1672,300 ± 11,186c |

The ethanolic extract showed the best free radical scavenging power compared to the other extracts.

#### 3.3.2. Reducing activity of $Fe^{3+}$ ions/FRAP (Ferric Ion Reducing Antioxidant Power)

None of the tested extracts showed significant $Fe^{3+}$ ion reducing activity compared to vitamin C (Table 4).

**Table 4: Fe3+ inhibitory concentration 50 (IC50) by extracts**

| Extracts | EAM | EM | EE | EAD | EHE | Vitamin C |
|---|---|---|---|---|---|---|
| IC50 (ug/mL) | 53,12 ± 0,443d | 23,333 ± 3,403b | 22,267 ± 1,006b | 42,271 ± 2,805c | 25,305 ± 1,802b | 0,038 ± 0,001a |
| EAM: *Aqueous extract by maceration*, EHE*: Hydro-ethanolic extract*, EAD: *Aqueous extract by decoction*, EM*: Methanolic extract*, EE*: Ethanolic extract.* | | | | | | |

## 4. DISCUSSION AND CONCLUSION

The present study was aimed to evaluate the *in vitro* antibacterial, and antioxidant properties of *A. caulirhiza*. The phytochemical screening revealed that all plant extracts contained phenolic compounds (flavonoids, tannins, coumarins), as well as mucilages and resins. The aqueous extracts contained phlobotannins, and quinones. On the other hand, only the hydroethanolic, and ethanolic plant extracts contained oxalates. Furthermore, none of the five extracts contained alkaloids, anthocyanins, betacyans, and cardiac glycosides. In addition, only the ethanolic extract contained steroids and saponosides. These results are different from those obtained by Shefali *et al.,* [15] and Matchuenkam *et al.,* [16] who found that the methanolic, and ethanolic extracts of *A. caulirhiza* leaves contained alkaloids, phenolic compounds, and glycosides. Azame *et al.,* [17] also showed the presence of polyphenols, steroids, alkaloids, terpenoids, and reducing sugars in the aqueous extract of *A. caulirhiza* leaves, and the absence of saponins, and phlobotannins. The place of harvesting, the time of harvesting, environmental factors, the level of maturity of the plant, as well as the nature of the solvent used might have influenced the phytochemical composition we found in our study as previously reported by other studies [18, 19]. However, the findings of the present study corroborate with the results of the study done by Jespher *et al.,* [20]; this similarity particularly concerns the presence of polyphenols and mucilages and the absence of no alkaloids, saponosides and steroids in the aqueous extract of *A. caulirhiza*, respectively.

The different plant extracts of *A. caulirhiza* inhibited the growth of different bacterial strains tested with MIC values between 8, and 32 mg/mL. The aqueous extract obtained by maceration was found to be active against *Pseudomonas aeruginosa*, methicillin-

resistant *Staphylococcus aureus* (MRSA) and *Klebsiella pneumonia*. The hydroethanolic extract was found to be active against *Staphylococcus aureus*, MRSA and *K. pneumonia*. The antibacterial activity of the hydro-ethanolic extracts of our plant against the WHO priority organisms such as MRSA, *Klebsiella pneumoniea* and *Ps, aeruginosa* is of high public health relevance (21). On the other hand, the aqueous extract from the decoction was not active on any of the bacterial strains. The methanolic extract was only active on *Staphylococcus aureus* and *Klebsiella pneumonia*. The ethanolic extract inhibited the growth of all bacterial strains. These results are similar to those obtained by Shefali *et al.* [15] who found that the macerated aqueous extract of *A. caulirhiza* was active on *P. aeruginosa*, and inactive on *K. pneumonia* while the methanolic extract was active on *K. pneumonia*. They are also similar to those obtained by Matchuenkam *et al.* [16] who showed that the ethanolic extract of *A. caulirhiza* was inactive on *K. pneumoniae*, *S. aureus* and *Staphylococcus* spp. The antibacterial activity of *A. caulirhiza* shown in the present study is probably related to the presence of phenolic compounds such as flavonoids, coumarins and tannins in the five plant extracts. These compounds are known for their numerous therapeutic properties [22, 23]. Flavonoids are thought to have antibacterial and antiviral properties [22]. They act by inhibiting bacterial DNA gyrase, and also by sequestering the substrates necessary for microbial growth [24]. Concerning Tannins, there is evidence of their antibacterial, antifungal, and anti-diarrhoeal properties [22]. Indeed, they also have a toxic activity against bacteria, yeasts and dermatophytes. Moreover, their effects might be due to their ability to complex transport proteins [23]. Furthermore, saponosides, and steroids, found only in the ethanolic extract of *A. caulirhiza*, could be responsible for the marked antibacterial activity of this extract towards different bacterial strains.

Evaluation of the antioxidant activity of the whole plant of *A. caulirhiza* showed that the aqueous, hydro-ethanolic, methanolic, and ethanolic extracts scavenged the DPPH● radical. However, these plant extracts do not have a significant antioxidant activity compared to that of the vitamin C with the highest antiradical power. The ethanolic extract showed the best anti-free radical activity. The results obtained with the different plant extracts tested are different from those obtained by Orapin *et al.*, [25] following a methanolic extraction of *A. caulirhiza* leaves. They are also different from those of Sana *et al.*, [26] with the same extraction. The differences observed can be attributed to the nature of the extraction solvent, the parts of the plant studied which are critical factors that can influence the composition and the content of secondary metabolites in an extract [27].

In conclusion, this study found that several secondary metabolites are present in the different plant extracts. In addition to their antioxidant activity, these plant extracts are capable of inhibiting the growth of the WHO priority bacterial strains *in vitro*. In perspective, further research should be considered to translate this knowledge from the present study into potential therapeutic applications.

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## REFERENCES

1. Madrid, L., Seale, A. C., Kohli-Lynch, M., Edmond, K. M., Lawn, J. E., Heath, P. T., ... & Schrag, S. (2017). Infant group B streptococcal disease incidence and serotypes worldwide: systematic review and meta-analyses. *Clinical infectious diseases*, *65*(suppl_2), S160-S172.
2. Schwarz, S., Loeffler, A., & Kadlec, K. (2017). Bacterial resistance to antimicrobial agents and its impact on veterinary and human medicine. *Advances in Veterinary Dermatology*, *8*, 95-110.
3. Diallo, O., Baron, S., Jimeno, M. T., Abat, C., Dubourg, G., Chaudet, H., ... & Rolain, J. M. (2019). Mortalité des bactériémies causées par des bactéries difficiles à traiter («Difficult to Treat Resistance» DTR). *Médecine et Maladies Infectieuses*, *49*(4), S48.
4. Duval, M., & Cossart, P. (2019). A new mechanism of antibiotic resistance: ribosome recycling. *Medecine sciences: M/S*, *35*(8-9), 613-615.
5. World Health Organization. (2017). WHO releases list of bacteria against which new antibiotics are urgently needed. 2017 [Accessed 25 February 2021]. Available from: https://www.who.int/fr/news
6. Ivanov, A. V., Bartosch, B., & Isaguliants, M. G. (2017). Oxidative stress in infection and consequent disease. *Hind Pub Corp*, 2017, 3p. https://doi.org/10.1155/2017/3496043
7. Titanji, V. P., Zofou, D., & Ngemenya, M. N. (2008). The antimalarial potential of medicinal plants used for the treatment of malaria in Cameroonian folk medicine. *African journal of traditional, complementary, and alternative medicines*, *5*(3), 302-321.
8. Organisation Mondiale de la Santé (OMS). (2013). Stratégie pour la médecine traditionnelle 2014-2023. Genève; 72p.
9. Chakraborty, A., Devi, B. R. K., Sanjebam, R., Khumbong, S., & Thokchom, I. S. (2010). Preliminary studies on local anesthetic and antipyretic activities of Spilanthes acmella Murr. in

experimental animal models. *Indian Journal of Pharmacology*, *42*(5), 277-279.
10. Prachayasittikul, V., Prachayasittikul, S., Ruchirawat, S., & Prachayasittikul, V. (2013). High therapeutic potential of Spilanthes acmella: a review. *EXCLI journal*, *12*, 291-312.
11. Sofowora, A. (1993). Medicinal plants and traditional medicine in Africa. John Wiley and sons LTD, 97-145.
12. Clinical and Laboratory Standards Institute (CLSI). (2012). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Ninth Edition. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Pennsylvania, USA, 32(2), 88p.
13. Komsok, T. J., Chelea, M., Kouamouo, J., Ipowo, T. V R, Fekam, F., & Ngoupayo, J. (2019). Phytochemical, cytotoxic, anti-hemolytic and antioxidant activities of leaf and bark of extracts of *Azadirachta indica* (Meliaceae). *Pharm Chem Journ*, 6(3), 99-111.
14. Path Canada. (1994). Report on research findings: field test methods for the determination of iron in fortified foods. 15 P.
15. Arora, S., Vijay, S., & Kumar, D. (2011). Phytochemical and antimicrobial studies on the leaves of Spilanthes acmella. *J. Chem. Pharm. Res*, *3*(5), 145-150.
16. Raissa, M. G. S., René, F. K. P., Fokam, M., Aude, M., Michel, Y. D. A., Aristide, M. M., ... & Roger, K. J. (2020). Extracts from acmella caulirhiza potentiate the activity of ciprofloxacin against multidrugresistant bacteria expressing extended-spectrum beta-lactamases. *World Journ Pharm Resch*, 9(3), 187-202.
17. Azame T. L., Tembe F., Njinkio B. N., Ngoupayo J., & Fokunang C. N. (2020). Phytochemical Screening, Healing Activity and Acute Toxicity of *Musanga Cecropioides* Root Sap and Aqueous Extract of the Whole Plant *Acmella Caulirhiza* on Wistar Rats. *Health Scie and Dis*, 21(1), 1-10.
18. Badiaga, M. (2011). Ethnobotanical, phytochemical study and biological activities of *Nauclea latifolia* Smith, an African medicinal plant collected in Mali [Thesis] Doctorate in organic chemistry. Bamako: Université Blaise Pascal, 184 p.
19. Dougnon, V. T., Klotoé, J. R., Sènou, M., Roko, G. O., Dougnon, G., Fabiyi, K., ... & Baba-Moussa, L. (2017). Chemical composition, cytotoxicity and antibacterial activity of selected extracts of Euphorbia hirta, Citrus aurantifolia and Heterotis rotundifolia on Enteropathogenic Bacteria. *EC Microbiology*, *12*(4), 180-195.
20. Onyango, J. N., Onyancha, J. M., Onyuka, J. O., Ochora, J. M., Getonto, P. O., & Maina, C. W. (2017). Phytochemical Studies of Acmella Caulirhiza and Spermacoce Princeae used by postpartum mothers in Nyamira County, Kenya. *International Journal of Scientific and Research Publications*, 7(8), 591-599.
21. Sabbagh, P., Riahi, S. M., Gamble, H. R., & Rostami, A. (2019). The global and regional prevalence, burden, and risk factors for methicillin-resistant Staphylococcus aureus colonization in HIV-infected people: a systematic review and meta-analysis. *American journal of infection control*, *47*(3), 323-333.
22. Benguerba, A. (2008). Phytochemical and butanol phase study of *Inula crithmoides* L. [Master's thesis]. Constantine: Université Mentouri Constantine, 110p.
23. Abudunia, A. M. (2018). Phytochemical Study, Biological and Pharmacological Screening of Flowers of *Calendula arvensis* [Thesis] Doctorate in Drug Sciences. Rabat: University of Mohamed V, 199p.
24. Elkolli, M. (2016). Structure and Activity of Natural Substances: Principles and Applications. Université Ferhat Abbas de Sétif.
25. Wongsawatkul, O., Prachayasittikul, S., Isarankura-Na-Ayudhya, C., Satayavivad, J., Ruchirawat, S., & Prachayasittikul, V. (2008). Vasorelaxant and antioxidant activities of Spilanthes acmella Murr. *International Journal of Molecular Sciences*, *9*(12), 2724-2744.
26. Sana, H., Rani, A. S., & Sulakshana, G. (2014). Determination of antioxidant potential in Spilanthes acmella using DPPH assay. *Int. J. Curr. Microbiol. Appl. Sci*, *3*(7), 219-223.
27. Fonmboh, D. J., Abah, E. R., Fokunang, T. E., Herve, B., Teke, G. N., Rose, N. M., ... & Ntungwen, F. C. (2020). An Overview of Methods of Extraction, Isolation and Characterization of Natural Medicinal Plant Products in Improved Traditional Medicine Research. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, 9(2), 31-57.