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Full Length Research Paper

Phytochemical screening, anti-radical and antibacterial activities of leaf extract from *Combretum glutinosum* Perr. Ex DC., a plant used in treatment of urinary infections in Benin

Emmanuel O.D. Bakrou[1]*, Alphonse Sako Avocefohoun[1,2], Papin Sourou Montcho[1], Alassane Abdou-Karim Youssao[1] and Virgile Ahyi[2]

[1]Laboratory for Study and Research in Applied Chemistry, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01BP 2009 Cotonou, Benin.
[2]Inter-Regional Génie Industriel et Biotechnologies (IRGIB), Africa University, Rue 1886, Behind the Air France Building, Route de l'Aéroport, Cotonou, Littoral, Benin.

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Infectious diseases represent a major global health challenge, with bacterial infections accounting for approximately 70% of mortality cases. This study aimed to evaluate the medicinal potential of *Combretum glutinosum* Perr. Ex DC. by identifying its secondary metabolites and assessing their antioxidant and antibacterial activities. Secondary metabolites in the leaves of *C. glutinosum* were identified using specific staining and precipitation reactions. The antibacterial activity of the extracts was evaluated using the macrodilution method. The leaves of *C. glutinosum* exhibited a diverse range of secondary metabolites, with a total flavonoid content of 16.78 mg EQ/g extract, total phenolic content of 93.291 mg EAG/g extract, and total tannin content of 0.583 mg EL/g extract. The hydroethanolic extract demonstrated notable bactericidal activity against multiple strains, including *Staphylococcus aureus*, *Streptococcus* species, *Klebsiella pneumoniae*, and *Escherichia coli*. The DPPH radical scavenging assay revealed a dose-dependent increase in antioxidant activity, with the lowest half-maximal inhibitory concentration (IC_{50}) observed at 3.5 mg/mL for the methanolic extract of *C. glutinosum*. These findings support the traditional use of *C. glutinosum* in treating urinary infections and highlight the plant's potential as a source of antibacterial and antioxidant agents.

Key words: *Combretum glutinosum*, secondary metabolites, bactericidal and antioxidant activities.

INTRODUCTION

Plants from the Combretaceae family are well known for their therapeutic applications, attributed to a diverse array of secondary metabolites. This family comprises approximately twenty genera and around six hundred species. The genus *Combretum* is the largest within this family, encompassing 370 species distributed across tropical and

*Corresponding author. E-mail: orouemmanuelb@gmail.com.

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subtropical regions of Asia, Africa, and Brazil (Bekele and Lemma, 2021). Traditionally, *Combretum* species have been used in herbal medicine throughout African and Asian countries to treat various health conditions, demonstrating activities such as abortifacient, antibacterial, anticancer, antifungal, antimicrobial, antischistosomal, antitussive, antiviral, and molluscicidal effects (Bisoli et al., 2020). *Combretum glutinosum*, a member of the *Combretum* genus, is indigenous to regions extending from Senegal to Cameroon and Sudan. This species is widespread, often abundant, and typically grows gregariously. In Niger, *C. glutinosum* is found predominantly on plateaus throughout the country. It is a tree or shrub that can reach up to 12 meters in height and 60 centimeters in diameter, often exhibiting twisted or branched growth at the base (Malgras, 1992). In Senegal, *C. glutinosum* is the most commonly prescribed species by traditional therapists for various ailments ailments (Breman and Kessler, 1995)

The plant contains several bioactive compounds, including combreglutinine, a tannin with promising medicinal properties, particularly for the treatment of hepatitis B. Traditionally, the leaves are used for their diuretic, cholagogue, purifying, and febrifuge properties, typically prepared as a decoction or infusion at a ratio of five leaves per liter of water (Enda Tiers Monde, 2003). Locally, *C. glutinosum* Perr. Ex DC. is known by various names: Doso or Mangbevide (Fongbé), Bekpagorou, Batoko (Bariba), Okan (Yoruba), and Dooki or Ndodi (Peulh) (Burkill, 1985). The therapeutic applications of *C. glutinosum* are broad, including treatments for malaria, hypertension, anemia, bilious fever, gonorrhea, syphilis, wounds, coughs, jaundice, pneumonia, and spasms (Haidara et al., 2023; Malgras, 1992). In Benin, nearly every hamlet or small village hosts traditional healers or therapists (Konkon et al., 2017). Historically, communities have relied on botanical remedies before the widespread availability of synthetic drugs. Although synthetic alternatives now dominate the market, they often present limitations such as high costs, inaccessibility for rural populations, and the growing problem of antimicrobial resistance (Ladekan-Yayi et al., 2021). There is thus an urgent need to explore alternative treatments using readily available local resources to address public health challenges, particularly in developing countries (Mangambu et al., 2014).

C. glutinosum has been documented in the Beninese flora for its medicinal applications in treating various microbial diseases (Adjanohoun et al., 1991). This study aims to evaluate the efficacy of hydroethanolic leaf extracts of *C. glutinosum* against multiple resistant microbial strains, using the extraction methods described by Feknous et al. (2014).

Specifically, the present study evaluates the phytochemical composition, antioxidant properties, and antibacterial activities of hydroethanolic extracts of *C. glutinosum* leaves. Given its extensive use in traditional medicine in Benin, especially for treating urinary tract infections, it is critical to conduct phytochemical and antimicrobial analyses of this extract.

MATERIALS AND METHODS

Plant

The plant material consisted of leaves of *C. glutinosum* harvested in northern Benin (Alibori Department).

Microbial strains

The study tested sixteen microbial strains, including bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *E. coli* ATCC 25922, *Streptococcus* D, *Klebsiella pneumoniae*) and a fungal strain (*Candida albicans* MHMR). The bacterial strains were clinical isolates obtained from urine, semen, and vaginal swabs. All strains were sourced from the collection of the Laboratory of Biology and Molecular Typing in Microbiology, Department of Biochemistry and Cellular Biology, Faculty of Sciences and Techniques, University of Abomey-Calavi, Benin.

Collection of plant

After the plant sample was identified at the National Herbarium of Benin, located at the University of Abomey-Calavi, it was cataloged as YH 755/HNB. The leaves of *C. glutinosum* were dried in the laboratory at 20 ± 2°C until the plant material stabilized, after which they were ground into a fine powder.

Preparation of hydroethanolic extract

The hydroethanolic extract was obtained by macerating the powdered leaves in a water-ethanol mixture (30:70). The resulting macerate was filtered through Whatman paper, and the filtrate was then concentrated using a rotary evaporator. The concentrate was further dried in a drying oven at 40ºC until completely evaporated (Ouardi, 2019).

Preliminary phytochemical screening

Secondary metabolites of *C. glutinosum* were identified using specific coloration and precipitation reactions for each class of metabolite (Onoh, 2021; Alain et al., 2022). Table 1 shows the methods for identifying secondary metabolites from *C. glutinosum* leaves.

Dosage of phenolic compounds

Total phenols

The total phenolic content was determined using the Folin–Ciocalteu reagent (Koudoro et al., 2021).

Total flavonoids

Total flavonoids were quantified using the aluminum chloride ($AlCl_3$) method (Koudoro et al., 2021).

Condensed tannins

Condensed tannins were measured using the Butanol-HCl method.

Table 1. Methods for identifying secondary metabolites from *C. glutinosum* leaves.

Secondary metabolites	Chemical test
Alkaloids	Mayer's test and Dragendroff's test
Anthocyanes	HCl and NH_3 test
Anthraquinones	Borntrager's test
Coumarins	UV 365 nm
Flavonoids	Shibita's reaction test
Tannins	Ferric chloride and sodium acetate test
Saponins	Frothing test
Leucoanthocyanins	Bate-Smith and Metcalf
Mucilages	flaky test
Cyanogenic derivatives	picric acid test
Reducing compound	Fehling's test
Sterols and terpenes	Liebermann-Burchard's test)

The reaction mixture consisted of 0.5 mL of extract, 3 mL of butanol-HCl (95:5), and 0.1 mL of ferric solution (2% ferric ammonium sulfate diluted in 2N HCl). Samples were incubated in a boiling water bath for 60 minutes. Absorbance was measured at 550 nm, and results were expressed as leucoanthocyanidin equivalents, according to the following formula:

$$T\ (mgEL/gEx) = (A \times 78.26 \times FD)$$

where A: absorbance measured at 550 nm; FD: dilution factor. The dilution factor is equal to 1 if the extract is prepared at 200 mg in 10 mL of solvent and the measured absorbance is less than 0.6 (Dohou et al., 2003).

Anti-radical activity

The anti-radical activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. This method is based on the ability of the extract to trap free radicals in a DPPH solution, which is observed as the disappearance of the purple color of DPPH. The samples were left in the dark for one hour, and absorbances were measured at 517 nm (Alain et al., 2022). The percentage of radical scavenging was calculated using the following formula:

$$P = (Ab\text{-}Ae)\ /Ab$$

where P: trapping percentage; Ab: absorbance of blank, Ae: Absorbance of sample.

Antimicrobial activity

Sensitivity test

The sensitivity of the various extracts was assessed using the method described by Chabi et al. (2014) on Mueller-Hinton (MH) solid medium. A preculture from the previous day (1 colony in 1 mL of Mueller-Hinton broth) was diluted to achieve a turbidity of 0.5 on the McFarland scale (~10^8 CFU/mL) and further adjusted to 10^6 CFU/mL using sterile distilled water. A volume of 1,000 µL of this bacterial suspension was used to flood Petri dishes containing Mueller-Hinton agar (Bio-Rad, France). Using a perforator, sterile paper discs of 6 mm diameter were prepared and aseptically placed on the inoculated agar surface. Each disc was then impregnated with 30 µL of the test extract under aseptic conditions. Experiments for each extract were performed in duplicate, and a negative control was included using solvent instead of extract. Plates were left at room temperature for 15 to 30 min to allow diffusion before incubation at 37ºC for 24 and 48 h. After incubation, inhibition zones were measured using a graduated ruler.

Determination of minimum inhibitory and bactericidal concentrations (MIC/MBC)

Minimum Inhibitory and Bactericidal or Fungicidal Concentrations (MIC/MBC) were determined using the microdilution method, with iodonitrotetrazolium employed as an indicator of microbial viability (Singleton et al., 1999).

Statistical analysis

Microsoft Excel was used to calculate means, standard deviations, and to generate illustrative graphs. Comparisons of the anthelmintic effects according to dose, time, and plant were performed using the Student-Newman-Keuls (SNK) procedure in the *agricolae* package of R software, followed by GraphPad software for two-way ANOVA with multiple comparisons to calculate the IC_{50} of larval migration. Differences were considered statistically significant at the 5% threshold.

RESULTS AND DISCUSSION

Secondary leaf metabolites of *C. glutinosum*

Leaves of *C. glutinosum* were found to contain catechic tannins, flavonoids, anthraquinones, coumarins, reducing compounds, sterols, triterpenes, and saponosides. However, cyanogenic derivatives, gallic tannins, and alkaloids were absent. These findings regarding leaf metabolites of *C. glutinosum* are consistent with the work of Ousmane et al. (2017) on species from Senegal, but differ from the findings of Yahaya et al. (2012) and Edeoga et al. (2005), who reported the presence of alkaloids. Table 2 shows the methods for the

Table 2. Methods for the identification of secondary metabolites of *C. glutinosum.*

Secondary metabolites		*C. glutinosum*
Alkaloids		Absent
Tannins	catechisms	Present
	gallic	Absent
Flavonoids		Present
Anthocyanins		Absent
Leuco-anthocyanes		Absent
Saponosides		Present
Reducing compounds		Present
Mucilages		Absent
Cyanogenic derivatives		Absent
Coumarines		Present
Sterols and triterpenes		Present
Anthraquinones		Present

Table 3. Phenolic compounds content in the *C. glutinosum* leaves extract.

Test	Quantity
Total phenol content (mg EAG/gEx)	93.291
Total flavonoid content (mg EQ/gEx)	16.78
Condensed tannin content (mg EL/g Ex)	0.583

identification of secondary metabolites of *C. glutinosum.* Table 3 shows the phenolic compounds content in the *C. glutinosum* leaves extract.

Phenolic compounds contained in *C. glutinosum*

DPPH

The curve showing the percentage of DPPH radical scavenging as a function of the concentration of the hydroethanolic extract of *C. glutinosum* leaves is presented in Figure 1. A progressive increase in the scavenging rate was observed with increasing concentrations of the extract. From the percentage scavenging curve, the concentration of hydroethanolic extract required to scavenge 50% of DPPH radicals (IC_{50}) was determined to be 3.5 mg/mL. In comparison, for *C. glutinosum* species from Senegal, Sene et al. (2018) reported an IC_{50} of 0.65 mg/mL. Similarly, Sore et al. (2012) demonstrated significant antiradical activity in leaves of *C. glutinosum* collected in Senegal. Tahiri et al. (2022) reported an IC_{50} of 0.055 mg/mL for the ethanolic extract of *C. glutinosum* leaves from the Ivory Coast.

Diameter of inhibition (DI) of hydroethanolic extract of C. glutinosum on microbial strains selected

Table 4 presents the inhibition diameters of *C. glutinosum* hydroethanolic extract, Ciprofloxacin, and Amoxicillin against the 16 microbial strains tested. The hydro-ethanolic extract of *C. glutinosum* inhibited 56.25% of the strains, with inhibition zone diameters ranging from 10 mm to 22 mm. The following strains were insensitive to the hydroethanolic extract: *K. pneumoniae* (vaginal sample), *S. aureus* (vaginal sample), *S. aureus* (semen), *S. aureus* (urine), *E. coli* (vaginal sample), *C. albicans* (MHMR), and *Streptococcus* D (semen). In comparison, the synthetic antibiotics Ciprofloxacin and Amoxicillin inhibited 75% and 25% of the tested strains, respectively. The inhibition zones for Ciprofloxacin ranged from 13.5 mm to 41 mm, while those for Amoxicillin ranged from 13 mm to 19 mm. These results are consistent with the findings of Yahaya et al. (2012), who reported that *C. glutinosum* extract inhibited multiple microbial strains, with variation in inhibition zone diameters among different strains.

MIC and MBC of hydroethanolic extract of *C. glutinosum* on selected microbial strains

Table 5 presents the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the hydroethanolic extract of *C. glutinosum* leaves and the synthetic antibiotics Amoxicillin and Ciprofloxacin. The MICs of the hydroethanolic extract ranged from 0.39

Table 4. Inhibition diameters of *Combretum glutinosum* extract on selected microbial strains.

Microbial strains (Origin)		Extract [DI (mm)]	Ciprofloxacin [DI (mm)]	Amoxicillin [DI (mm)]
	PV	-	41.5±0.5	19.5±0.5
K. pneumoniae	SP	10.0±0.0	38.0±0.0	16.0±1.0
	UR	10.5±0.5	36.0±0.5	-
	PV	-	18.5±0.5	-
S. aureus	SP	-	18.5±1.5	-
	UR	-	38.0±0.0	-
	PV	-	41.5±3.5	13.0±0.0
E. coli	SP	22.0±0.0	13.5±0.5	-
	UR	17.0±0.0	14.5±0.5	-
	PV	14.0±0.0	-	-
Streptococcus D	SP	-	40.0±0.0	13.5±1.5
	UR	15.0±0.0	40.0±0.0	-
C. albicans	MHMR	-	-	-
E. coli	ATCC 25922	19.5±0.5	-	-
S. aureus	ATCC 29213	13.0±0.0	38.0±0.0	-
S. pneumoniae	ATCC 49619	20.5±1.5	-	-

PV: Vaginal sample; SP: sperm; UR: urine; *S. aureus*: *Staphylococcus aureus*; *S.pneumoniae*: *Streptococcus pneumoniae*; *E. coli: Escherichia coli*; DI: Inhibition diameter; -: absence of inhibition; *K. pneumoniae*: *Klebsiella pneumoniae*; *C. albicans: Candida albicans.*

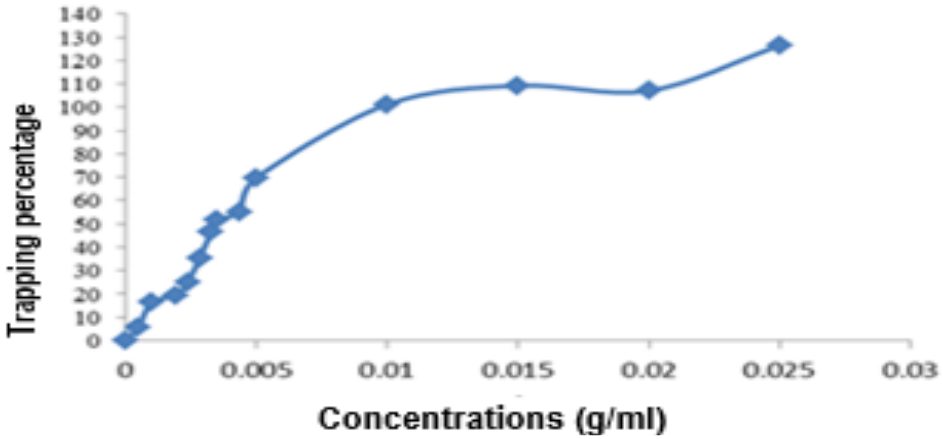

Figure 1. DPPH free-radical scavenging activity in different concentrations of hydroethanolic extract of *C. glutinosum* leaves.

to 1.56 mg/mL, while MBCs varied between 1.56 and 10 mg/mL. The following strains were insensitive to the extract: *K. pneumoniae* (vaginal sample), *S. aureus* (vaginal sample, semen, urine), *E. coli* (vaginal sample), and *C. albicans* (MHMR). For Ciprofloxacin, MICs ranged from 0.125 to 5 mg/mL, and MBCs ranged from 0.31 to 20 mg/mL. Amoxicillin inhibited only *K. pneumoniae* (vaginal swab), *E. coli* (vaginal swab), *Streptococcus* D (semen), and *E. coli* (ATCC 25922) at an MIC of 10 mg/mL, while it exhibited bactericidal activity against *K. pneumoniae* (semen) at 20 mg/mL. Overall, the hydroethanolic extract of *C. glutinosum* leaves demonstrated a broader spectrum of antimicrobial activity than the two synthetic antibiotics used as reference standards. These findings are consistent with those of Ousmane et al. (2017), who reported that hydroethanolic extracts of *C. glutinosum* leaves exhibited bactericidal activity against *S. aureus*, *Enterococcus faecalis* and

Table 5. Minimum Inhibitory Concentration (MIC) and Bactericidal Concentration (MBC) of hydroethanolic extract of *C. glutinosum.*

Strains/Origin		*C. glutinosum*		Amoxicillin		Ciprofloxacin	
		MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
	P V	-	-	10		0.625	2.5
K. pneumoniae	S P	0.39	1.56	5	20	0.625	2.5
	U R	0.39	10	-	-	1.25	5
	P V	-	-	-	-	0.156	0.625
S. aureus	S P	-	-	-	-	5	20
	U R	-	-	-	-	2.5	10
	P V	-	-	10	-	1.25	5
E. coli	S P	0.39	1.56	-	-	0.156	0.31
	U R	0.78	3.125	-	-	0.156	0.625
	P V	0.78	3.125	-	-	-	-
Streptococcus D	S P	-	-	10	-	1.25	5
	U R	1.56	6.25	-	-	2.5	10
C. albicans	MHMR	-	-	-	-	-	-
E. coli	ATCC 25922	0.39	1.56	10	-	2.5	10
S. aureus	ATCC 29619	0.78	3.125	-	-	-	-
S. pneumoniae	ATCC 49619	1.56	6.25	-	-	0.625	2.5

PV: Vaginal sample; SP: sperm; UR: urine; *S. aureus: Staphylococcus aureus; E. coli: Escherichia coli;* MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; -: absence of inhibition; *K. pneumoniae: Klebsiella pneumoniae; C. albicans: Candida albicans.*

Pseudomonas aeruginosa.

Conclusion

Infectious diseases have intensified the exploration of medicinal plants as viable treatments for various pathologies. This study focuses on the leaves of *C. glutinosum* Perr. Ex DC., which are traditionally used in Benin for the treatment of urinary tract infections. By assessing the phytochemical composition and evaluating the biological activity of the hydroethanolic extract, we identified a rich array of secondary metabolites, including tannins, flavonoids, coumarins, anthraquinones, sterols, and triterpenes, which may contribute to the observed antimicrobial effects. These findings substantiate the traditional use of *C. glutinosum* and highlight its potential as a source of effective herbal remedies. The promising results of this study open avenues for future research. Bio-guided fractionation techniques could facilitate the isolation and characterization of the specific compounds responsible for antimicrobial activity, which is crucial for developing novel therapeutic agents. Furthermore, a comprehensive investigation into the mechanisms of action of these metabolites could lead to innovative formulations tailored to current clinical challenges. Integrating *C. glutinosum* into preventive and complementary medicine strategies may also provide sustainable solutions to the growing problem of antibiotic resistance. This work not only has the potential to enrich traditional pharmacopoeias but also promotes the valorization of local plant resources while advancing research in ethnopharmacology.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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