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Research Article

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Healing and antibacterial activity of the powder and shell extracts of *Achatina fulica* Bowdich (Achatinidae) detected *in vitro* and *in ovo*

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Abstract

The study consisted in evaluating *the in vitro* healing and antibacterial activity of the powder and shell extracts of *Achatina fulica* (Achatinidae).

A chemical screening of the extracts and powder was carried out after extraction by successive maceration of 48 hours of the powder with different solvents. Healing activity was assessed based on the angiogenic process of fertilized nine-day-old *Gallus gallus* eggs. Antibacterial activity was determined by the liquid macro-dilution method on two pathological specimens (*Staphylococcus aureus* and *Streptococcus agalactiae*) and three reference strains (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700602). The chemical screening of the extracts and shell powder revealed the presence of secondary metabolites, in particular: flavonoids, alkaloids, anthocyans, coumarins, saponosides, catechic tannins, triterpenes, anthocyans, sugars and proteins. Angiogenic activity was observed resulting in a significant increase in the number of chorioallantoic membrane vessels in batches treated with shell powder and aqueous extract at a dose of 80 µg compared to the batch treated with NaCl. The highest antibacterial activity was observed with a Minimum Inhibitory Concentration (MIC) of 0.78 mg/mL on *Escherichia coli* powder and on *Klebsiella pneumoniae* with hydroethanolic extract. Minimum Bactericidal Concentrations (MBCs) ranged from 25 to 50 mg/mL. The CMB/CMI ratios show that the powder and

its extracts are bacteriostatic with a higher activity of the powder against *Klebsiella pneumoniae*, for a CMB/CMI ratio of 8.

The powder and aqueous extract of *Achatina fulica* shells would have healing and antibacterial activity. These angiogenic and antibacterial effects would be beneficial in the healing of infectious wounds and would justify the traditional use of this snail in wound management.

Keywords: *Achatina fulica*, healing, antibacterial, MIC, MBC, CMB/CMI ratios.

Introduction

A wound is defined as a destruction of the integrity of the skin, mucous membranes, and organs [1]. Wounds are a common reason for admission to emergency hospital facilities [2]. Chronic wounds are increasing in prevalence with the advent of chronic diseases and comorbidities (obesity, smoking, atherosclerosis) worldwide, particularly in Africa [3]. According to statistics from the European Wound Management Association in 2010, 1% to 2% of people worldwide suffer from a chronic wound during their lifetime. In developed countries, the incidence of chronic wounds ranges from 2% to 6%, while in developing countries, the incidence has increased significantly from 12% to 41% [4].
An ulcer wound is an incision that develops on the skin as a result of an injury, poor blood circulation, or pressure that generally has a low tendency to heal [5]. Hospitalization and mortality from ulcer wounds have increased in recent years and infectious complications (mainly related to *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus*) account for more than 60,000 deaths per year in Australia [6]. In Cameroon, there is a 21.8% prevalence of ulceration risk in diabetes, the main one being the diabetic foot with a prevalence of 11.8% [7].

Healing is a complex and dynamic mechanism that involves physiological inflammation, extracellular membrane production, and angiogenesis. Treatments, often limited by their potential adverse effects, unavailability, and high costs in low-income countries, lead populations to use traditional medicine, which is often effective and less expensive [8]. The work of Magne and*al.*[9] has demonstrated the healing activity of the leaves of *Kalanchoe crenata*[9]. In 2012, the angiogenic properties of *Ziziphus xylopyrus* promoting wound healing were demonstrated in a study conducted by Jena *et al*. [10].

Achatina fulica (Achatinidae) snail from East Africa [11] is attracting increasing interest. Its flesh is used on the one hand as a nutrient and on the other hand, preventing anaemia, cardiovascular diseases, diabetes, and hypertension [12], in cosmetology and traditional medicine, its drool is endowed with regenerative, repairing, antibacterial and healing activity [13,14,15]. Its shell is traditionally used as a bleaching agent in tooth brushing, the treatment of infections such as gonorrhea, and can be mixed with other ingredients to make concoctions [16]. Scientific data on the healing action of *Achatina fulica* shells are rare. Regarding its traditional use [12], our investigation focused on the antibacterial and healing properties of *Achatina fulica* powder and shell extracts.

Materials and Methods

The shells of *Achatina fulica* were collected from live snails purchased in Yaoundé, the capital of the Central Cameroon region, at the Mfoundi market. The identification was made at the Institute of Agricultural Research and Development (IRAD) in Yaoundé. The eggs of *Gallus gallus*, fertilized for 9 days, were purchased from NAAPCAM SARL in Nkolfoulou, Yaoundé.

The bacterial strains involved in ulcer wound infections have been, for some, isolated from pathological products (*Staphylococcus aureus* and *Streptococcus agalactiae*) and for others, obtained from the reference strain bank of the microbiology laboratory of the Bangangté mountain clinics (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700602).

1. The acquisition of the powder:

The modified protocol used was the one described by *Kouadio et al*. [17]. *Achatina fulica* was separated from her shell with a sharp hook. The shells were cleaned thoroughly with distilledwater, dried for two days at room temperature, and then weighed. They were dried in an oven for two weeks at 37°C, then weighed and sprayed. The powder was sieved with a 160 µm sieve.

2. Protocol for the extraction of *Achatina fulica* shell powder:

The shell powder was macerated for 48 hours in distilled water, ethanol/water mixture (70/30 v/v), or dichloromethane. The powder was put into glass vials with 2 L of each solvent. The mixture was stirred manually with a glass rod several times a day. The mixture was filtered using Whatman N˚3 paper. Maceration was repeated three times. The filtrates of each solvent were concentrated by a rotary evaporator at 40˚C and the pressure varies according to the solvent (850 mbar for dichloromethane and 175 mbar for hydroethanolic). The extracts obtained were placed in an oven at 45° C to remove solvent residues. The various extracts were weighed using a precision balance. The yield expressed as a percentage is calculated according to the formula of *Falleh et al.* [18]. Ratio between the mass of dry residue obtained after extraction (M') and the mass of the animal material used (M) (RE% = (M' /M) x100).

3. Qualitative chemical screening:

It was carried out according to the methods described by *Bruneton* [19].

4. Evaluation of the healing activity:

Made from a solution of sildenafil, powder solution and various snail shell extracts.

Sildenafil Solution: One tablet of sildenafil (Rigid® 50 mg) was sprayed in a mortar and the resulting powder was dissolved in 3 mL of 0.9% NaCl to obtain a concentration of 16.67 mg/mL: Volumes of this stock solution (2.8 µL and 28 µL) were each completed to 10 mL with 0.9% NaCl solution, giving concentrations of 4.47µg/mL and 47.45 µg/mL respectively.

Solution of the powder and various extracts: A mother solution of the powder or various extracts was previously prepared by dissolving 240 mg of the powder or extracts in 3 mL of 0.9% NaCl to obtain a concentration of 80 mg/mL. Solutions to be used were obtained by diluting 20 µL of stock solution with 0.9% NaCl in sufficient quantity for 20, 40, and 80 mL to obtain solutions at concentrations of 20 µg/mL, 40 µg/mL and 80 µg/mL respectively, to be applied to sterile discs.

The procedure used was based on the formation of new blood vessels from preexisting vessels (angiogenesis) on the chorioallantoic membrane (CAM) of fertilized hen eggs aged 9 days, which is the one described by *Basanta* and*al.* [16]. On the 9th day of incubation, it consisted in creating a small window in the inner tube of the fertilized eggs and applying a sterile methylcellulose disc to the junction of two large vessels. Each disc had a diameter of 6 mm. The fertilized eggs thus prepared were distributed in a batch of 5 eggs comprising (a control group that have received NaCl (0.9%), two positive controls treated with sildenafil at 4.74 and 47.45 µg/mL respectively, three test groups for the powder or each shell extract that have each received the powder or extract at concentrations of 20, 40 and 80 µg/mL respectively).

The discs in each batch were soaked with 10 µLof sildenafil solvent, diluted powder, or extracts of *Achatina fulica* powder and powder at different concentrations. After 72 hours, the new vessels formed were counted. The area of the membrane where the disc was applied was filmed to determine the capillary density.

5. Evaluation of antibacterial activity:

In vitro evaluation of antibacterial activity was based on the ability of the powder and Achatina fulica extracts to inhibit the growth of certain bacteria by disc diffusion in Muller Hinton agar medium. For this purpose, two pathological specimens: Staphylococcus aureus and Streptococcus agalactiae, three reference strains Pseudomonas aeruginosa (ATCC 27853), Escherichia coli (ATCC 25922) and Klebsiella pneumoniae ATCC (700602) were used.

Muller Hinton and Sabouraud dextrose agar culture media were prepared according to the manufacturer's recommendations. They were cooled to 45ºC in a water bath, and in identified sterile petri dishes, 15 mL of agar media were respectively dispensed.

Shell powder and extracts of*achatina fulica* antibacterial activity: the powder and extracts were used at a concentration of 50mg/mL. Dilutions of Reason 2 of the stock solution were then made to obtain the other concentrations. The bacterial strains used were stored in the laboratory and replanted 24 hours before each activity test and incubated at 37ºC to obtain a young culture and isolated colonies. Near the flame of a Bunsen burner, colonies with 18 to 24 hours of life were sampled using a platinum loop for each bacterial strain. They were introduced into 3 ml of sterile physiological water turbidity similar to that of point 0.5 on the McFarland scale.

The powder and extracts were declared sterile, if no colony was visible on the agar boxes.

Sterility test:

Twenty milligrams of the powder and extracts were added to the tubes containing 2 mL of sterile distilled water. The mixture was homogenizedand allow to stand for 5 minutes. The mixtures were inoculated on single-compartmented Petri dishes containing Muller Hinton agar and Sabouraud agar and incubated at 37°C for three days with a check every 24 hours.

The powder and extracts were declared sterile if no colony was visible on the agar boxes.

Antibacterial activity test:

The powder and extracts were declared sterile if no colony was visible on the agar boxes. It was carried out by the modified solid diffusion method developed by *Konanet al.* [20]. Using a sterile swab, the bacterial inoculum was spread on the agar surface. On sterile discs (diameter 6 mm) were placed 15 µg/mL of each of the powder solutions and extracts at concentrations of 50 mg/mL, 25 mg/mL and 12.5 mg/mLThe discs were placed on the surface of the inoculated agar (7 discs/box, with about 30 mm between the discs and 15 mm from the edge of the box). The plates were placed in the incubator at 37°C aerobically for 24 hours. The corresponding inhibition diameter was measured in millimetres (including disc diameter). The reading and interpretation were conducted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CA-SFM-2013) [21].

Determination of the MICs of *Achatina fulica* shell extracts and powder:

The protocol used was the one described by Konan *et al.*, [20]. Each tube in the dilution range including control tubes contained 1 mL of Mueller Hinton broth. In the first tube of the dilution range, 1 mL of 50 mg/mL stock solution was introduced. Dilutions of reason 2 were made in Mueller Hinton broth (MHB) to obtain a concentration range between 25 mg/mL and 0.048 mg/mL of each solution (aqueous, hydroethanol

and dichloromethane extracts or *Achatina fulica* powder). A bacterial inoculum (15 µl) was added to each tube. The entire set was incubated at 37°C for 24 hours. Turbidity was assessed visually.

The MIC of the test solution was deduced from the first tube in the range within which growth did not occur (no turbidity, no deposition of bacterial products). Tubes containing only 2 mL of MHB were used as negative growth χontrol, while tubes containing 2 mL of MHB and 15 µL of inoculum were used as positive growthχontrol and other containers containing 1 mL of MHB + 1 mL of extract solution were used as growth inhibition χontrol. Each extract was tested in triplicate. The smallest concentration at which no visible growth was observed was considered as the minimum inhibitory concentration.

Determination of minimum bactericidal concentrations (MBCs):

This parameter was determined by subculturing in solid media and the preparations collected from the tubes were used to determine the MICs. In each of the tubes in the dilution range within which visible growth was not observed and in the control tubes used to determine the IJC, samples were taken and streaked on Mueller Hinton Agar (MHA). The seeded cans were incubated for 18 to 24 hours at 37°C.

The lowest concentration at which no visible growth was observed was considered as the minimum bactericidal concentration.

CMB/CMI ratio: This R ratio (CMB/CMI) is used to confirm the bacteriostatic or bactericidal nature of a substance [22]. When this ratio is greater than or equal to 4, the substance is considered bacteriostatic; if it is equal to 1, then the substance is called "absolutely bactericide".

Statistical analysis

The results were expressed as an average ± standard error of average (ESM) or as percentages. The different means were compared using the One-way ANOVA test followed by the Dunnett multiple comparison post-test. The differences were considered significant at $p < 0.05$.

Results

1. Phytochemical study

1. Phytochemical study

1.1. Moisture content and physical characteristics of the *Achatina fulica* shell

The moisture content and physical characteristics of the shell and shell powder are recorded in Table I.

Table I : Moisture content and physical characteristics of the *Achatina fulica* shell

	Weight (g)	Physical characteristics:appearance
Freshshells	1525	Brown with white lines and white tip
Shells after drying in the oven	1455	Brown with white lines and white tip
Shell powder	1280	Light brown, fine particles 160 µm, metal smell

After drying for two weeks in a 37°C oven of 1525 g of fresh shells of *Achatina fulica* at 37°C, 1455 g of shells were obtained, giving a water content of 4.59%,and 1280 g of fine powder.

1.2. Yield and physical aspects of the extracts

The extraction yields and physical appearance of aqueous, hydroethanolic, and dichloromethane extracts have been summarized in Table II.

Table II: Dry extract weight, extraction efficiency, and physical characteristics of extracts

Extraction solvents	Distilled water (Aqueousextract)	Ethanol 70°C (Hydroethanolicextract)	Dichloromethane (Organicextract)
Quantity of dry powder	400	400	400
Quantity of dry extract (g)	2,50	1,71	7,07
Yield (%)	0,62	0,43	1,76
Physical characteristics : (color/consistency)	Light green/teapowder	Brown / pasty and sticky	Light brown/powder forming words

The extraction yields were 0.62%, 0.43%, and 1.76% for aqueous, hydroethanolic, and dichloromethane extracts, respectively. The highest yield was with dichloromethane.

II.1.3. Qualitative phytochemical screening of *Achatina fulica* powder and shell extracts

Phytochemical screening carried out on the various extracts and shell powder revealed several groups of chemical compounds as shown in Table III.

Tableau III :Phytochemical screening of *Achatina fulica* shell extracts and powder

Essais	Specimen			
	Aqueousextract	**Hydroethanolicextract -**	**Dichloromethane extract -**	**powder**
Phenols	-	-	-	-
Flavonoides	+++	+++	+++	+++
Alcaloides	-	-	+++	+++
Anthocyanes	+-	+++	+++	-
Coumarines	-	-	+	+
Saponosides	-	-	++	+
Tanins catéchiques	+++	+++	-	+++
Triterpenes	+++	+++	+++	+++
Anthraquinones	+	-	++	++
Sugar	-	+++	+++	-
Proteines	+	-	+++	+++

Legends:
-: absence; ++++: significant presence; +: low presence; ++: medium presence

It shows that 82% of the compounds sought are present in the dichloromethane extract compared to 73% in the powder, 55% in the aqueous extract, and 45% in the hydroethanol extract. 18% of the compounds were found in abundance in the powder and its extracts (flavonoids and triterpenes). Phenols were not found in the shell of *Achatina fulica*.

2. Healing activity *in ovo* (angiogenesis) of the powder and aqueous powder extracts

Macroscopically observed results using a magnifying glass revealed the formation of new vessels for each treatment. A significant increase in the number of chorioallantoic membrane vessels was observed in batches treated with aqueous extract and shell powder at a concentration of 80 µg/mL compared to the batch treated with NaCl (normal control). The number of vessels decreased from 2.0 ± 0.6 in the normal control batch to 5.0 ± 0.3 and 4.0 ± 0.6, respeχtively, in the batch treated with *Achatina fulica* aqueous extract or shell powder at a concentration of 80 µg/mL, an increase of 150% and 100% respectively. There was also a significant increase ($p<0,05$) in the number of vessels of 100% in the batch treated with sildenafil at a concentration of 47.45 µg/mL compared to the normal control. (Figure 1).

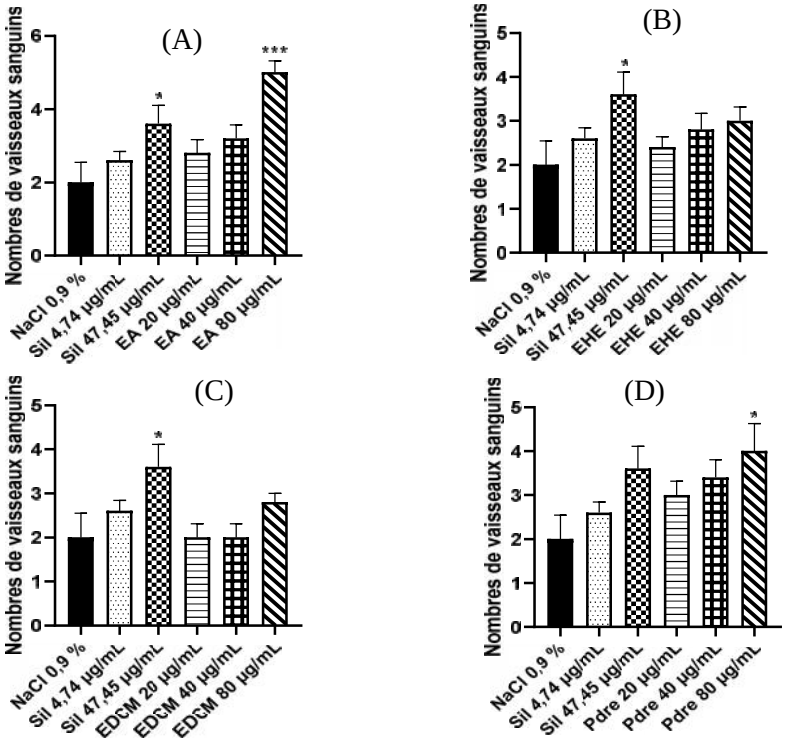

Figure 1 : Effect of extracts (aqueous (A), hydroethanolic (B), dichloromethane (C)) and shell powder (D) of *Achatina fulica* on the angiogenic process of the chorioallantoic membrane of the fertilized chicken egg.
Legends:

*Each bar represents the average of each group ± ESM; n = 5. ***p < 0.001 ; * p < 0.05 : significant difference in comparison with the normal batch. NaCl= Sodium chloride 0.9%; sil = Sildenafil; EA = Aqueous extract; EHE = Hydroethanol extract; EDCM = Dichloromethane extract; Pdre = Powder; Sil 4.74 µg/mL = Positive control 1; Sil 47.45 µg/mL = Positive control 2; EA, EHE, EDCM-Pdre = test batches treated with different doses of aqueous extract, hydroethanol, dichloromethane and Achatina fulica shell powder.*

It should be noted that hydroethanolic (Figure 2.B) and dichloromethane (Figure 2.C) extracts at this concentration of 80 µg/mL caused an insignificant increase in the number of vessels by 50% and 40% respectively compared to the normal control.

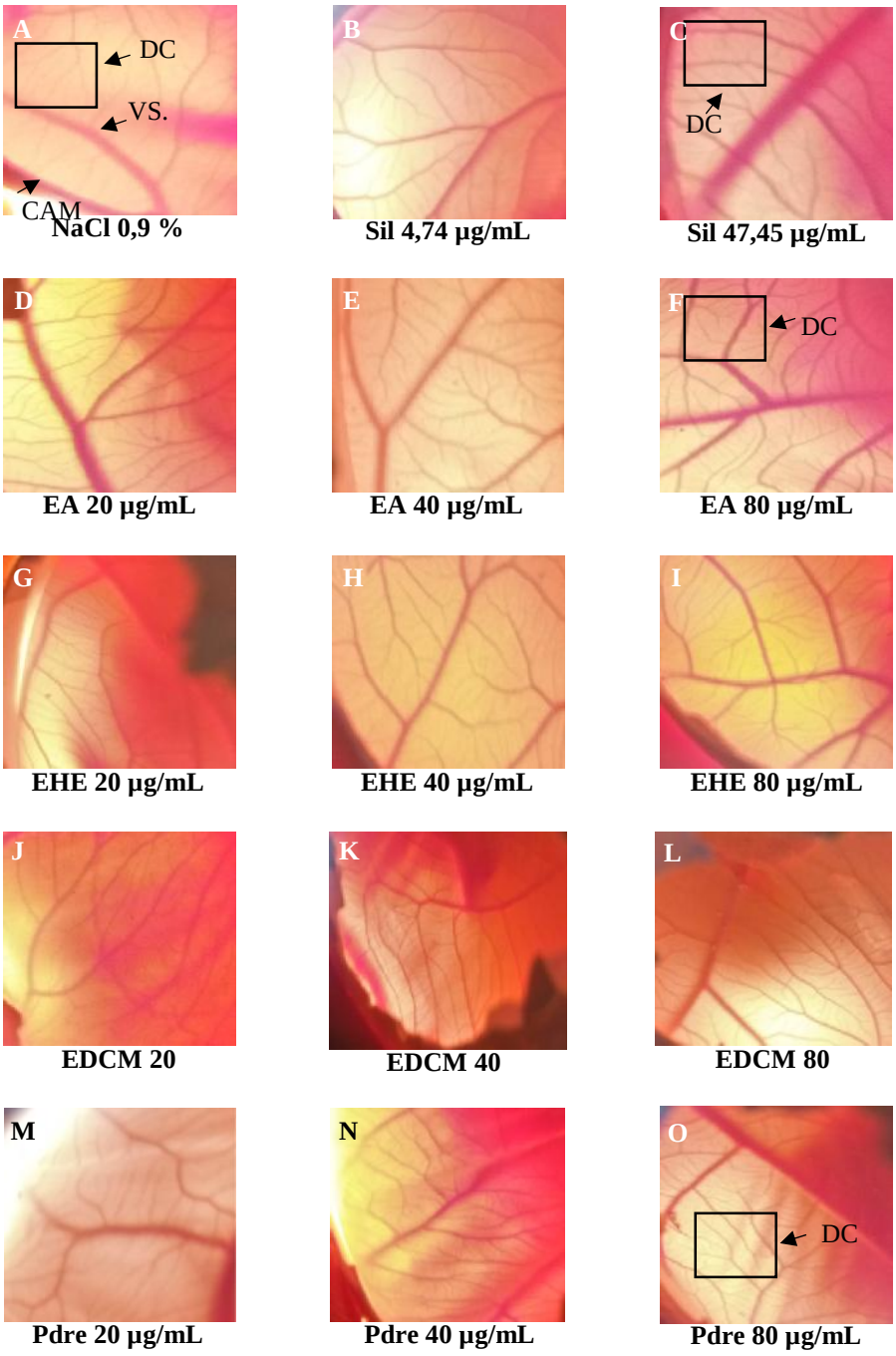

Figure 2 : Photograph of CAM blood vessels 72 hours after the various treatments (1 × 24 MP).
Each microphotograph represents the chorioallantoic membrane (CAM). VS.C = Central Vessels; DC = Capillary; the black circle represents where the disc was placed; NaCl= Sodium chloride 0.9%; if = Sildenafil; EA = Aqueous extract; EHE = Hydroethanolic extract; EDCM = Dichloromethane Extract; Pdre = Powder; NaCl = Normal Control; Sil = Positive Control; EA + EHE + EDCM + Pdre = Test Groups treated with different doses of Achatina fulica shell extracts and powder

The digital images show a newly formed vascular network at the CAM level in batches treated with different concentrations of *Achatina fulica* shell extracts or powder.

The CAM photograph shows one of the central vessels (VS.C) and a low capillary density (DC) in the normal control batch (Figure 2.A). In the positive control group 2 (sildenafil 47.45µg/mL), there was a slight increase in capillary density (Figure 2.C). At the CAM level, in batches treated with extracts and powder of *Achatina fulica* at a concentration of 80 µg/mL, there was a good increase in capillary density (Figure 2, F, O) compared to the normal control (Figure 2.A).

3. Antibacterial activity

3.1. Sterility test of extracts and powder

After three days of incubation of the extracts and powder, no colony was observed on MHA and Sabouraud.

3.2. Sensitivity testing of powder extracts and reference antibiotics on the strains studied

The evaluation of the activity of the extracts and powder against the five bacteria used yielded the results reported in Table IV.

Tableau IV : Activité des extraits et de la poudre sur les souches bactériennes utilisées

Bacteria investigated	Inhibition diameters (mm)													
	EA mg/Ml			**EHE (mg/mL)**			**EDCM (mg/mL)**			**Pdre (mg/mL)**			**CN (µg)**	**CIP(µg)**
	12,5	**25**	**50**	**12,5**	**25**	**50**	**12,5**	**25**	**50**	**12,5**	**25**	**50**	**30**	**5**
Staphylococcus aureus	8 ± 0,2	9 ± 0	11 ± 0,2	8 ± 0	10 ± 0	10 ± 0	6 ± 0	6 ± 0	10 ± 0	10 ± 0	10 ± 0	13 ± 0	17 ± 0	14 ± 0
Streptococcus agalactiae	6 ± 0	9 ± 0,3	12 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	6 ± 0	8 ± 0,5	12 ± 0	30 ± 0	31 ± 0
Pseudomonas aeruginosa	10 ± 0	12 ± 0	13 ± 0	8 ± 0	10 ± 0	10 ± 0	6 ± 0,6	8 ± 0	9 ± 0,2	6 ± 0	8 ± 0	12 ± 0	23 ± 0	33 ± 0
Eschérichia coli	8 ± 0	11 ± 0	12 ± 0,5	8 ± 0	9 ± 0	9 ± 0	8 ± 0	9 ± 0	11 ± 0	8 ±0,2	8 ± 0	11 ± 0	12 ± 0	35 ± 0
Klebsiella pneumoniae	7 ± 0	8 ± 0	9 ± 0	6 ± 0	6 ± 0	8 ± 0	6 ± 0	6 ± 0	6 ± 0	8 ± 0,5	9 ± 0	10 ± 0	27 ± 0	30 ± 0

EA: Aqueous extract; EO: Organic extract; EHE: Hydroethanol extract; Pdre: Powder; CN: Gentamicin; CIP: Ciprofloxacin; 6 mm corresponds to an absence of an inhibition zone

Table IV presents the inhibition diameters of *Achatina fulica* shell powder and its extracts against the different bacteria studied. The powder and its extracts, in contact with the selected bacteria, showed a variation in the inhibition diameters measured around the discs for more than half of the bacteria. Inhibition diameters varied from 6 to 13 mm. The largest diameters were obtained at doses of 50 mg/mL, particularly with aqueous extract and powder. The aqueous extract is more effective (93.33% of values > 6mm) than the powder (86.66%), the hydroethanol extract (66.66%) and the dichloromethane extract (46.66%). Gentamicin and ciprofloxacin have larger inhibition diameters than powder and our extracts except gentamicin on *Escherichia coli* for aqueous extracts at doses of 50 mg/mL. All extracts and powders showed inhibitory activity on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*; only

aqueous extract and powder showed inhibitory activity on *Streptococcus agalactiae*; while aqueous, hydroethanolic extracts and powder showed inhibitory activity on *Klebsiella pneumoniae*.

3.3 Minimum inhibitory concentration and minimum bactericidal concentration

The data recorded during the determination of the MIC and CMB of the extracts and powder were recorded in Table V.

Table V :CMI and CMB of *Achatina fulica* shell extracts and powder

Bacteria investigated	Specimen											
	EA (mg/mL)			**EHE (mg/mL)**			**EDCM (mg/mL)**			**Pdre (mg/mL)**		
	CMI	CMB	R	CMI	CMB	R	CMI	CMB	R	CMI	CMB	R
Staphylococcus Aureus	6,25	-	-	12,5	-	-	6,25	-	-	1,5625	-	-
Streptococcus agalactiae	3,125	50	16	1,5625	-	-	1,5625	-	-	3,125	-	-
Pseudomonas Aeruginosa	3,125	50	16	1,5625	50	32	1,5625	50	32	1,5625	25	16
Escherichia coli	3,125	50	16	6,25	-	-	3,125	50	16	0,78125	-	-
Klebsiella pneumonia	1,565	-	-	0,78125	-	-	6,25	-	-	3,125	25	8

EA: Aqueous extract; EHE: Hydroethanol extract; EDCM: Dichloromethane extract; Pdre: Powder; MIC: Minimal inhibitory concentration; CMB: Minimal bactericidal concentration; R = CMB/CMI ratio; -: No activity.

Table V presents the antibacterial tests carried out to detect the inhibitory and bactericidal activities of *Achatina fulica* shell powder and its extracts. It appears that the lowest MICs were obtained with powder and hydroethanol extract, particularly at 0.78125 mg/mL. The lowest CMB was obtained with powder, especially at 25mg/mL. We found that 100% of the bacteria tested were sensitive to the powder and its extracts. The latter were 100% bacteriostatic on all strains with a lower ratio CMB/CMI equal to 8 obtained with the powder. We found the particularity of *Staphylococcus aureus* which did not reveal any CMB value with all extracts and powder.

Discussion

The extraction of shell powder with different solvents gave a better yield (1.76%) with dichloromethane suggesting that *Achatina fulica* shell powder would be rich in low polar compounds. This contrasts with the yields obtained from aqueous and hydroethanolic extracts.

The results of the chemical screening of *Achatina fulica* shell powder and its extracts showed that flavonoids and triterpenes are mainly present. These results support those of *Sadjadi and al.* [23], who found an abundance of triterpenes in gastropod molluscs. They also agree with those of *Blunt and al.* [24] who illustrated the presence of bioactive compounds such as terpenes, alkaloids, anthocyanins, coumarins, saponosides, catechic tannins, and anthraquinones in molluscs. Proteins were detected in powder, aqueous extracts and dichloromethane. This would confirm the results of *Ejeomoand al.* [25] who reported the presence of protein in the shell of *Achatina fulica.*

The effects of the powder and extracts were evaluated *in ovo* on the formation of new vessels from vessels in the chorioallantoic membrane. *Achatina fulica* shell powder and aqueous extract, unlike hydroethanolic and dichloromethane extracts, showed a significant increase in the capillary density at a concentration of 80 µg/mL. This increase in hair density would indicate an angiogenic activity of the aqueous extract and shell powder of *Achatina fulica* compared to the normal control. These results indicate that both the powder and the shell extract of *Achatinata fulica* contain metabolites capable of inducing the angiogenesis process. In this study, sildenafil at a concentration of 47.45 µg/mL also induced an increase in capillary density as in the work of *Dogancı*and*al*[25]. Previous studies have shown that sildenafil has pro-angiogenic activity and may be able to promote the healing of dog-induced wounds [26] and pressure ulcers in hospitalized patients [6]. Sildenafil acts by mimicking the action of NO which leaves via soluble guanylate cyclase induces the synthesis and release of VEGF. VEGF increases the vascular permeability that is necessary to initiate the angiogenic process. Previous studies have shown the presence of chitosan (a polyglucosamine derived from chitin) in the shell of *Achatina fulica*. The latter has healing and antibacterial properties [27]. Chitosan has the ability to stimulate healing by activating fibroblasts, endothelial cells and inflammatory cells such as macrophages. The latter activated generate the production of growth factors (Transforming Growth Factor TGF-β1, Platelet Derived Growth Faχtor, PDGF) and cytokines that would promote fibroblast proliferation, collagen synthesis; important elements during the granulation phase, the initial stage of tissue reconstruction preceding the re-epithelialization of the damaged area. Chitosan has activity on both Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*, *Escherichia coli*) bacteria. The pharmacological activity of this compound could also justify the antibacterial and healing activities demonstrated in this study [28].

Antibacterial activity has shown that *Achatina fulica* shell powder and its extracts have inhibitory activity on the *in vitro* growth of the bacteria studied. The powder and aqueous extract at a concentration of 50 mg/mL were found to be more effective on all strains than hydroethanolic and dichloromethane extracts with an inhibition diameter of 13 mm respectively with Staphylococcus aureus and *Pseudomonas aeruginosa*. This result could be explained by the fact that the active compounds appear to be soluble in water or remain active in the powder. *Achatina fulica* powder and its extracts are 100% bacteriostatic against all bacteria studied. The results indicated that the MICs range from 0.78 to 6.25 mg/mL while the CMBs range from 25 to 50 mg/mL. *Klebsiella pneumonae* and *Escherichia coli* are sensitive to relatively low MIC values (0.78125 mg/mL) for the powder and hydroethanolic extract respectively. The lowest CMB values were obtained with 25 mg/mL powder for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These results confirm those of *Ulagesan et al.* [29] which revealed the antibacterial properties of proteins extracted from the flesh of *Achatina fulica* at a lower MIC value (100 µg/mL) on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This difference could be justified by the part of the animal material used that suggests that the proteins responsible for antibacterial activity in the flesh are more active than the compounds present in shell powder and its aqueous extract. In addition, the technique of purifying proteins isolated from the flesh could increase the antibacterial activity.

Although MICs appear to be independent of the type of Gram, CMB values are mostly obtained in Gram-negative individuals. These are frequently involved in ulcer wound infections. The proliferation of ulcerous wound germs is induced by skin barrier breakdown, maceration, poor hygiene, and biofilm formation [30]. This activity on Gram negative can be considered against other bacterial types responsible for wound infections. The lowest CMB/CMI ratios were obtained with powder and aqueous extract, particularly at 8 and 16, suggesting that the latter would be more active than hydroethanolic and dichloromethane extracts.

The powder and aqueous extract reveal angiogenic and antibacterial properties that are favourable for the care of ulcerous wounds. Phytochemical caracterisation revealed the presence in the dichloromethane extract of 82% of secondary metabolites, of which 27% and 9%, respectively, were absent in the powder and aqueous extract, it is likely that the activity of the extract is not necessarily proportional to the number of secondary metabolites it contains [31]. Indeed, the optimal efficacy of the extract may not be due to the number of secondary metabolites found, but to the combined or uncombined action (inhibition) of the different compounds present [31]. The tannins present in the powder and aqueous extract would have potentiated the antibacterial and angiogenic action of the other metabolites, while its absence in the organic extract had inhibitory effects. Chemical groups such as tannins, flavonoids, alkaloids, anthraquinones, and terpenes are known to have antibacterial properties. Tannins, for example, are known for their ability to inhibit the growth of many microorganisms, including bacteria. In addition to this property, tannins have astringent and healing properties. Flavonoids have antifungal and antibacterial properties [32].

Conclusion

Chemical screening revealed that the main compounds common to *Achatina fulica* powder and shell extracts are flavonoids and triterpenes. The powder and aqueous shell extract of *Achatina fulica* at a dose of 80 µg/mL demonstrated healing activity resulting in an angiogenic process demonstrated by a significant increase in hair density. The antibacterial activity of the powder and its extracts revealed a bacteriostatic effect. The lowest MIC was in the order of 0.78 mg/mL for powder on Escherichia coli and the lowest CMB/CMI ratio of 8 on *Klesiella pneumoniae*. The best activity was obtained with the powder and its aqueous extract.

Data availability:

No DATA were used to support this study

What is known:

- Angiogenesis, a complex physiological process which is necessary to restore blood flow to the tissue after a wound, is one of the different phases leading to wound healing.
- It has long been known that neovascularization occurs as part of the normal wound regeneration process. Proliferating capillaries deliver oxygen and micronutrients to the growing tissues and remove catabolic waste products. In traditional medicine, the use of certain animals in wound management is common.
- In Brazil, researchers have shown the healing effect of powdered snail shells of *Megalobulimus lopesi* (giant South American snail) after topical administration to second-degree wounds. These results supported the southern Brazilian folkloric use of *M. lopesi* shell powder [8]. In Africa, there is increasing interest in *Achatina fulica* (snail). Its slime is used in wound treatment.

What this study adds:

- This work confirms the angiogenic effect of *Achatina fulica* shell powder and highlights its antibacterial activity
- The shell powder of *Achatina fulica* is beneficial in the healing process of ulcerative wounds.
- The work presents the relationship between the phytochemical composition of the shell powder and its pharmacological activity.

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Competing interest

No conflicts of interest have been reported on this work.

Financial competing interests

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Non-financial competing interests

No non-financial competitors (political, personal, religious, ideological) are declared here.

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