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The pharmacological activities of *Albizia ferruginea* and *Newbouldia laevis* on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Epidermophyton species*

Aude Périne MINYEM NGOMBI-AFUH [2], NNANGA NGA [2] and Joseph NGOUPAYO [1,*]

[1] *Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I, Republic of Cameroon.*

[2] *Department of Galenic Pharmacy and Pharmaceutical Legislation, Faculty of Medicine and Biomedical Sciences, The University of Yaoundé I, Republic of Cameroon.*

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Abstract

Following studies made on the standardization of a traditional recipe made of *Albizia ferruginea* and *Newbouldia laevis*, we carried out studies on pharmacological effects on microorganisms found on dermatosis: two bacteria *Staphylococcus aureus*, *Pseudomonas aeruginosa* and three species of *Epidermophyton*.

Materials and Methods: Following the harvest of the truck pulps of *Albizia ferruginea* and *Newbouldia laevis* at Eloundem, they were dry for a week in an ambient environment, followed by a grinding to a granular powdered form. A powder mass of 250 g was used for the extract, using a Soxhlet from BEHR LABOR-TECHNIK. Therefore, a mixture of ethanol-water in a volume ratio of 70/30 was used as a solvent. The renewal of fungi strain was made by regeneration and viability testing phase. The antifungal antivity was made using Disc diffusion method and the broth dilution method.

Results: MICs were respectively: MIC was 12.5 mg/mL on *Staphyloccocus aureus* and 6.25 mg/mL on *Pseudomonas aeruginosa*; *Trichophyton rubrum*: 250 mg/mL for *Albizia ferruginea* and 250 mg/mL for *Newbouldia laevis; Trichophyton interdigitale*: 125 mg/mL for *Albizia ferruginea* and 125 mg/mL for *Newbouldia laevis; Trichophyton violaceum*: 125 mg/mL for *Albizia ferruginea* and 250 mg/mL for *Newbouldia laevis.*

Conclusions: Our different plant extracts would be a possibility to explore in the study of *in vivo* antibacterial and anti-dermatophytic activities.

Keywords: *Albizia ferruginea*; *Newbouldia laevis*; Traditional medicine Africa; Dermatosis; Antimicrobial; Plant extracts

1. Introduction

Many plants have been used in traditional medicine. Following the studies made by Abondo-Ngono et *al* in 2012, a traditional recipe made of a mixture *Albizia ferruginea* and *Newbouldia laevis* has been acquired [1]. The tradipratician has been using them throughout many generations mixed up together to traditionally treat dermatosis caused by fungi. Preliminary studies showed pharmacognosy and phytochemical standardization of these two plants [2, 3].

Following the studies, we evaluated the pharmacological effects of *Albizia ferruginea* and *Newbouldia laevis* on two bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* and three species of *Epidermophyton.*

* Corresponding author: NGOUPAYO Joseph

2. Material and methods

2.1. Plant material and microorganisms

The plant material used are *Albizia ferruginea and Newbouldia laevis* truck pulps harvested of February 1, 2016, at Eloundem in the vicinity of Yaoundé, Cameroon. Subsequently, the specimens were properly identified and compared to 49871/HNC specimen for *A. ferruginea* and 29469/HNC specimen for *Newbouldia laevis* at the Cameroonian National Herbarium [2, 3].

2.2. Cell media used

- Mueller Hinton agar (Biomerieux®) for the subculture of strains;
- Mueller Hinton Broth (MHB) Liofilchem® for the determination of Minimum Inhibitory Concentration (MIC)
- Culture Medium Sabouraud Chloramphénicol Agar Scharlau®.

2.3. Preparation of plant extracts

The pulps were dry for a week in an ambient environment, followed by a grinding to a granular powdered form. A powder mass of 250 g was used for the maceration. Therefore, a mixture of ethanol-water in a volume ratio of 70/30 was used as a solvent [2, 3].

2.4. Renewal of bacterial strains

The different strains of bacteria were isolated by the streak method on specific medium and incubated at 37 °C for 24 hours in order to obtain young isolated colonies, which were used to make the inocula. Using a platinum loop, a pure colony of microorganism was picked and streaked on Mueller Hinton agar poured into Petri dishes. The plates were incubated at 37 °C for 24h [4,5].

For each bacterial strain, a 0.5 Mc Farland suspension is made in saline. This suspension corresponds to a concentration of approximately 1,000,000 bacteria/mL [4,5].

2.4.1. Preparation of the extract solutions to be tested

The plant extract solutions to be used are prepared at a concentration of 20000 µg/ mL. The dissolving solvent is Mueller Hinton broth [4,5].

2.5. Evaluation of the antibacterial activity of *Albizia ferruginea* and *Newbouldia laevis*

Antibacterial activity was assessed by determining the minimum inhibitory concentrations (MICs). The liquid dilution macromethod is used to determine this parameter. This experiment was performed on the following reference strains reference strains: *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC25923.

2.5.1. Principle

Its determination is based on the evaluation of the susceptibility of a microorganism to an antimicrobial substance. It is the lowest concentration that will inhibit any visible growth of a microorganism after incubation at 37 °C for 18 to 24 hours.

2.5.2. Method

The technique used is the liquid macro-dilution technique 1000 µL of Mueller Hinton broth is introduced from the first to the last tubes (control tubes) of the dilution range. Then 1000 µL of each extract at 200 000µg/mL extract is added to the first tube of the dilution range. From this arrangement, cascade dilutions of reason 2 are performed in Mueller Hinton broth to obtain a concentration range of broth to give a concentration range of 200,000 µg/mL to 195.3 µg/mL of extract [4,5].

Next, 15µL of bacterial inoculum is added to each tube and incubated at 37°C.

After 18 to 24 hours, the turbidity is first assessed visually and then the tubes are centrifuged at a speed of 5000 rpm for 5 minutes. The MIC of the extract is deduced from the first tube in the range in which growth did not occur (absence of turbidity).

The MIC of the tested extract is deduced from the first tube in the range in which growth did not occur (absence of turbidity, absence of deposition of bacterial products) [4,5].

NB: Positive control: broth - microorganism; Negative control 1: simple broth broth; Negative control 2: broth - extract.

2.6. Renewal of the fungal strains [6].

2.6.1. Strain regeneration and viability testing phase

- Prepare the Sabouraud-Chloramphenicol and place the still warm tubes in a slanted position so as to obtain a small slope and a deep pellet.
- Inoculate with a sterile loop and inoculate the pellet in the centre puncture, then inoculate the slope in streaks.
- Incubate in the oven for 24 hours at 25-30°C (72 hours).
- Close tubes tightly with screw caps.
- Store between 25 and 30°C

2.6.2. Reproducing strains: checking the viability of strains

- - Transplant if necessary every week by scraping the culture under the oil layer.
- - Stumps kept pure in this way usually survive for several years.

2.6.3. Preparation of the spore suspension

Spores suspensions were prepared from 10-day-old cultures, incubated at 30 °C on Sabouraud-Chloramphenicol medium. Fungal colonies were immersed with 5mL of API solution and the culture surface was gently scraped with a sterile loop.

2.6.4. Preparation of the extract solutions to be tested

The plant extract solutions to be used are prepared at a concentration of 2000 mg/ mL. The dissolving solvent is Mueller Hinton broth.

2.6.5. Disc diffusion method

Firstly, the antifungal activity will be determined by the disc diffusion method [7]. 100 µl of suspension containing 106 CFU/ml of microbial cells will be spread on petri dishes containing Sabouraud Chloramphenicol [7]. The discs (6 mm in diameter) will be separately impregnated with 15 µl of various extracts and placed on the agar that will have already been inoculated with the selected micro-organism [7]. A disc of appropriate reference antifungal agent will be applied to each petri dish (Griseofulin 250 mg/disc); it will serve as an effective positive control against the three fungal species [7]. Blank discs will be used as a negative control. The plates will be stored at 4 °C for 1 hour. Then, they will be incubated for 48h at 30 °C. Antifungal activity will be assessed by measuring the diameter of the growth inhibition zone in millimetres (including the diameter of the 6 mm disc).

2.6.6. Determination of Minimum Inhibitory Concentration

The antifungal activity of different extracts will be studied using the broth dilution method [7]. The fungal culture used (2 × 108 CFU/mL) will be diluted in peptone water (0.1% w/v) to 104 and 105 CFU/mL. Then 100 µl of each culture will be suspended in Sabouraud Chloramphenicol broth containing different concentrations of each extract ranging from 31.25 to 500 mg/mL. The positive control will be Sabouraud broth inoculated only with the microbial suspension. The uninoculated tube containing the extract will be the negative control. The tubes will be incubated for 72 h at 30 °C. Microbial growth will be indicated by the presence of turbidity on the bottom of the tube. The MIC is defined as the lowest concentration of a given extract capable of interrupting, in a liquid medium and under perfectly defined conditions, any visible growth of a given microbial strain. The first tube, in ascending order, that did not produce a tube bottom will therefore correspond to the MIC [7].

3. Results

3.1. Antibacterial activity on hydro-ethanolic mixture of *Albizia ferruginea* and *Newbouldia laevis*

Table 1 showed Minimal Inhibitory Concentrations of hydro-ethanolic extract of mixture of *Albizia ferruginea* and *Newbouldia laevis*.

Table 1 Minimal Inhibitory Concentrations (MIC) of hydro-ethanolic extract on mixture of *Albizia ferruginea* and *Newbouldia laevis*

	Concentrations (mg/mL)					
Microorganisms	**100 mg/mL**	**50 mg/mL**	**25 mg/mL**	**12,5 mg/mL**	**6,25 mg/mL**	**3,125 mg/mL**
Staphylococcus aureus	-	-	-	σ	+	+
Pseudomonas aeruginosa	-	-	-	-	σ	+

- : Absence of turbidity; + : Presence of turbidity; σ: Value of MIC

Table 2 showed Minimal Inhibitory Concentrations of hydro-ethanolic extract of mixture of ***Albizia ferruginea*** and ***Newbouldia laevis***.

Table 2 Minimal Inhibitory Concentrations (MIC) on aqueous extract of mixed of *Albizia ferruginea* and *Newbouldia laevis*

	Concentrations (mg/mL)					
Microorganisms	**100 mg/mL**	**50 mg/mL**	**25 mg/mL**	**12,5 mg/mL**	**6,25mg/mL**	**3,125mg/mL**
Staphylococcus aureus	-	-	-	σ	+	+
Pseudomonas aeruginosa	-	-	-	σ	+	+

- : Absence of turbidity;+ : Presence of turbidity; σ: Value of MIC

3.2. Antidermatophytic activity on hydro-ethanolic mixture of *Albizia ferruginea* and *Newbouldia laevis*

3.2.1. Diameters of inhibition

Table 3 represents the inhibition diameters observed with *Newbouldia laevis* extract on *Trichophyton rubrum*

Table 3 Inhibitory diameters of *Trichophyton rubrum* (*Newbouldia laevis*)

Trichophyton rubrum	
***Newbouldia laevis* Extract**	**Inhibitory diameters**
Griseofuline 250 mg/mL	2 cm
500 mg/mL	1.4 cm
250 mg/ mL	1.6 cm
125 mg/ mL	1 cm

Table 4 represents the inhibition diameters observed with *Newbouldia laevis* extract on *Trichophyton violaceum*.

Table 4 Inhibitory diameters of *Trichophyton violaceum* (*Newbouldia laevis*)

Trichophyton violaceum	
***Newbouldia laevis* Extract**	**Inhibitory diameters**
Griseofuline 250 mg/mL	2,6 cm
500 mg/mL	2 cm
250 mg/mL 125 mg/mL	1.5 cm 1 cm

Table 5 represents the inhibition diameters observed with *Newbouldia laevis* extract on *Trichophyton interdigitale*.

Table 5 Inhibitory diameters of *Trichophyton interdigitale* (*Newbouldia laevis*)

Trichophyton interdigitale	
***Newbouldia laevis* extract**	**Inhibitory Diameter**
Griseofuline 250 mg/mL	5,3 cm
500 mg/mL	1.5 cm
250 mg/ mL	1.5 cm
125 mg/mL	1 cm

Table 6 showed that *Albizia ferruginea* ethanolic extract showed a similar inhibition diameter of 2 cm at 500 mg/mL than Griseofulin 250 mg/mL.

Table 6 Inhibition diameter of Trichophyton rubrum (*Albizia ferruginea*)

Trichophyton rubrum	
***Albizia ferruginea* Extract**	**Inhibition Diameter**
Griseofulin 250mg/mL	2 cm
500 mg/mL	2 cm
250 mg/mL 125 mg/mL	1.5 cm 1 cm

Table 7 showed that *Albizia ferruginea* ethanolic extract showed a similar inhibition diameter of 2 cm at 250 mg/mL than Griseofulin 250 mg/mL 2.1 cm.

Table 7 Inhibition diameter of *Trichophyton violaceum* (*Albizia ferruginea*)

Trichophyton violaceum	
***Albizia ferruginea* Extract**	**Inhibition Diameter**
Griseofulin 250 mg/mL	2.1 cm
500 mg/mL	2 cm
250 mg/mL	2 cm
125 mg/mL	1,5 cm

Table 8 showed that *Albizia ferruginea* ethanolic extract showed inhibition diameters of 3 cm at 500 mg/mL, 2 cm at 250 mg/mL, 1.5 cm at 125 mg/mL and 4.5 cm at Griseofulin 250 mg/mL.

Table 8 Inhibition diameter of *Trichophyton interdigitale* (*Albizia ferruginea*)

Trichophyton interdigitale	
***Albizia ferruginea* Extract**	**Inhibition Diameter**
Griseofulin 250 mg/mL	4.5 cm
500 mg/mL	3 cm
250 mg/mL	2 cm
125 mg/mL	1.5 cm

The figures below (Figure 2, Figure 3 and Figure 4) represent the studies of the activities of the extracts on *Trichophyton rubrum*, *Trichophyton violaceum* and *Trichophyton interdigitale.*

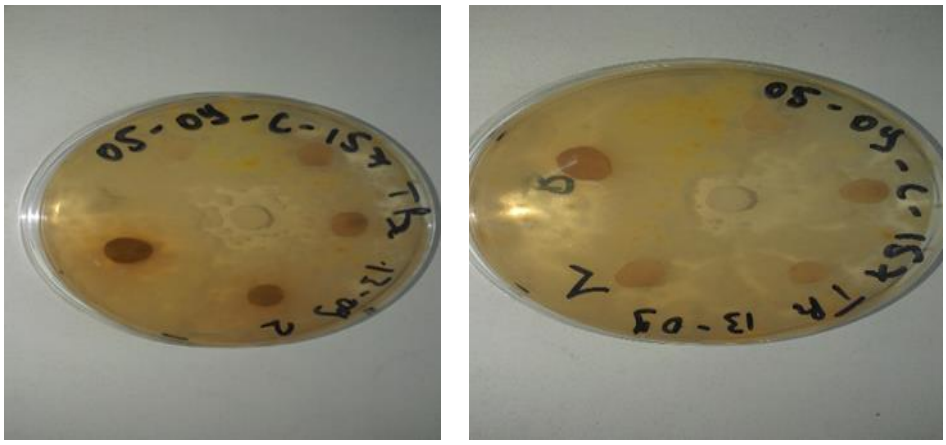

Figure 1 Studies of the activity on *Trichophyton rubrum*

Figure 2 Studies of the activity on *Trichophyton violaceum*

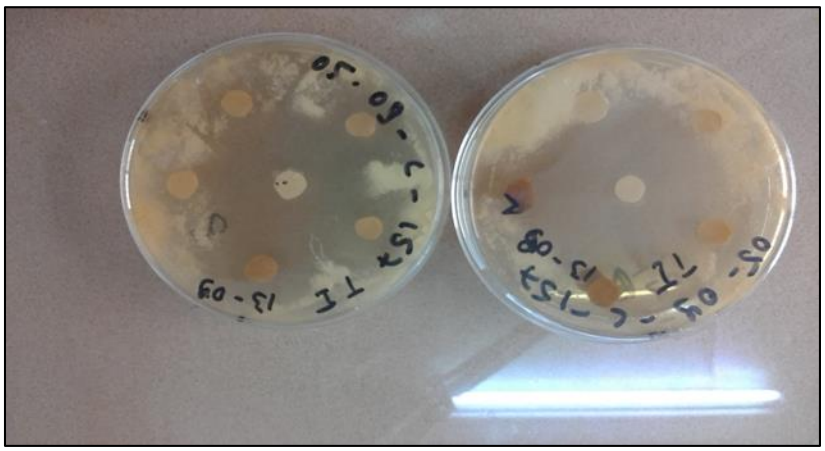

Figure 3 Studies of the activity on *Trichophyton interdigitale*

- Minimal Inhibitory Concentrations of *Albizia ferruginea* Extract on three species of *Trichophyton* genus

Studies made by MINYEM [3] showed that hydro-alcoholic extracts of *Albizia ferruginea* showed an activity on *Trichophyton rubrum, Trichophyton violaceum and Trichophyton interdigitale.* Griseofulin was used as the reference.

Table 9 showed that hydro-alcoholic extracts of *Albizia ferruginea* showed an activity on *Trichophyton rubrum*, *Trichophyton violaceum* and *Trichophyton interdigitale*.

Table 9 Minimal Inhibitory Concentrations of *Albizia ferruginea*

	Concentrations *Albizia ferruginea* (mg/mL)					
Microorganisms	**1000 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62,5 mg/mL**	**31,25 mg/mL**
Trichophytum rubrum	-	-	σ	+	+	+
Trichophyton interdigitale	-	-	-	σ	+	+
Trichophyton violaceum	-	-	-	σ	+	+

- : Absence of turbidity; + : Presence of turbidity; σ: Value of MIC

Table 10 represents the different MICs obtained by testing the activity of the *Newbouldia laevis* extract on the different dermatophytes.

Table 10 Minimal Inhibitory Concentrations of *Newbouldia laevis*

	Concentrations *Newbouldia laevis* (mg/mL)					
Microorganisms	**1000 mg/mL**	**500 mg/mL**	**250 mg/mL**	**125 mg/mL**	**62,5 mg/mL**	**31,25 mg/mL**
Trichophyton rubrum	-	-	σ	+	+	+
Trichophyton interdigitale	-	-	-	σ	+	+
Trichophyton violaceum	-	-	σ	+	+	+

+ : Observed turbidity; - : No turbidity ; σ= MIC Value

4. Discussion

4.1. For *Pseudomonas aeruginosa* and *Staphylococcus aureus*

In order to demonstrate the activity of our extracts, we determined the antimicrobial activity of the aqueous and hydroalcoholic extract mixture.

With the aqueous extract, the MIC is 12.5 mg/mL on *Staphyloccocus aureus* and *Pseudomonas aeruginosa*.

With the hydroalcoholic extract, the MIC was 12.5 mg/mL on *Staphyloccocus aureus* and 6.25 mg/mL on *Pseudomonas aeruginosa*.

According to the classification of Kuete et *al* the activity of plant extracts is considered significant when the MIC < 0.1 mg/mL, moderate when 0.1 mg/mL ≤ MIC ≤ 0.625 mg/L and low when the activity is >0.625 mg/mL. We can therefore say that our extracts have low activity. This could be due to the harvest period [8].

4.2. For *Trichophyton rubrum*

With *Newbouldia laevis* extracts, we obtained at 500 mg/mL an inhibition diameter of 1.4 cm while at 250 mg/mL a diameter of 1.6 cm. These values were lower than the reference (2 cm). Thus, although this extract has activity on *Trichophyton rubrum*, it is slightly lower than the reference.

With *Albizia ferruginea* extracts, we obtained at 500 mg/mL an inhibition diameter of 1.4 cm while at 250 mg/mL a diameter of 2 cm. These values were lower than the reference (2 cm) for the 500 mg/mL concentration while it was identical to the reference for the 250 mg/mL concentration. This difference observed at the 500 mg/mL concentration could be explained by a poor distribution of the extract during the impregnation of the extract on the disc. Thus, although this extract has activity on *Trichophyton rubrum*, it is slightly higher than the reference.

4.3. For *Trichophyton violaceum*

With *Newbouldia laevis* extracts, we obtained at 250 mg/mL a diameter of 1.5 cm. This value was lower than the reference (2.6 cm). The lack of inhibition observed with the 500 mg/mL concentration would be due to a poor distribution of the extract during the impregnation of the extract on the disc. Thus, although this extract has activity on *Trichophyton violaceum*, it would be slightly higher than the reference.

With *Albizia ferruginea* extracts, we obtained at 250 mg/mL a diameter of 2 cm and at 125 mg/mL a diameter of 1.5 cm. This value was lower than the reference value (2.1 cm). The lack of inhibition observed with the 500 mg/mL concentration would be due to a poor distribution of the extract during the impregnation of the extract on the disc. Thus, although this extract has activity on *Trichophyton violaceum*, it would be slightly higher than the reference.

4.4. For *Trichophyton interdigitale*

With *Albizia ferruginea* extracts, we obtained at 500 mg/mL an inhibition diameter of 3 cm, at 250 mg/mL a diameter of 1.5 cm and at 125 mg/mL a diameter of 2 cm. This value was lower than the reference value (2.1 cm). These values were lower than those obtained with the reference (4.5 cm). Thus, although this extract has activity on *Trichophyton violaceum*, it would be significantly lower than the reference.

With *Newbouldia laevis* extracts, we obtained at 500 mg/mL an inhibition diameter of 1.5 cm, at 250 mg/mL a diameter of 1.5 cm and at 125 mg/mL a diameter of 1 cm. This value was lower than the reference value (2.1 cm). These values were lower than those obtained with the reference (5.3 cm). Thus, although this extract has activity on *Trichophyton violaceum*, it would be significantly lower than the reference.

5. Conclusion

Our studies revealed that hydro-ethanolic and aqueous mixtures of *Albizia ferruginea* and *Newbouldia laevis* showed an activity on *Pseudomonas aeruginosa*, *Staphylococcus aureus* and three *Epidermophyton species*.

Trichophyton interdigitale, *Trichophyton rubrum* and *Trichophyton violaceum* were sensitive to the extracts. MIC was 12.5 mg/mL on *Staphyloccocus aureus* and 6.25 mg/mL on *Pseudomonas aeruginosa*; *Trichophyton rubrum*: 250 mg/mL for *Albizia ferruginea* and 250 mg/mL for *Newbouldia laevis; Trichophyton interdigitale*: 125 mg/mL for *Albizia ferruginea* and 125 mg/mL for *Newbouldia laevis; Trichophyton violaceum*: 125 mg/mL for *Albizia ferruginea* and 250 mg/mL for *Newbouldia laevis.*

This could be a reason why the traditional healer uses it traditionally to treat dermatosis. However further studies need to be made to see how to strengthen these activities.

Compliance with ethical standards

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Disclosure of conflict of interest

There is not any conflict of interest in this study.

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References

[1] ABONDO NR et *al*. Mapping of traditional medicine actors in Cameroon: case of the central region. Ethnopharmacologia. 2015; 53: 56-63

[2] ABONDO RNM et *al*. Pharmacognosy and phytochemical standardization of *Albizia ferruginea* (Guill and Perr) pulps. Journal of Pharmacognosy and Phytotherapy, 2018, 10(2), 27-33.

[3] MINYEM NAP et *al*. Standardization of *Newbouldia laevis* Powdered Pulps. HEALTH SCIENCES AND DISEASE, 2018, 19(4).

[4] Bauer A, Kirby W, Sherris JC, Turck M. Antibiotic susceptibility testing by astandardized single disk method. American Journal of Clinical Pathology. 1996; 4(5): 493-96

[5] Kareem SO, Akpan I and Ojo OP. Antimicrobial activities of Calotropi sprocera onSelected Pathogenic Microorganisms. African Journal of Biomedical Research 2008; p 105-110

[6] LANACOME. Procédure de conservation des souches microbiologiques. 2017

[7] Ghazghazia H et *al.* Comparison of polyphenol contents and antioxidant activity of methanolic extracts of four plants collected from northern Tunisia. Microbiology Hygiene. Alimentaire.2013; 25(73)

[8] Kuete V et *al*. Antimicrobial activity of the methanolic extract and of the chemicalconstituents isolated from *Newbouldia laevis*. Pharmazie. 2007; 62: 552–55