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Short Communication **Open Access**

In vitro antimicrobial activity of Mathesia® on bacterial isolates of wound infections in University Clinics and Hospital Centre of Mont Amba, Kinshasa, Democratic Republic of the Congo

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Abstract:

Background: Mathesia is a hydro-alcoholic emulsion, colourless and somewhat viscous, based on extracts of medicinal plants and containing saponins, polyphenols, tannins, and reducing sugars. In this study, we proposed to carry out an *in vitro* study of the antibacterial activity of phytomedicine Mathesia on strains of bacteria isolated from diabetic foot ulcers and chronic wounds of patients in care in the University Clinics and the Hospital Centre of Mont Amba in Kinshasa, Democratic Republic of the Congo.
Methodology: This *in vitro* study was carried out in the bacteriology laboratory of the Higher Institute of Medical Technology of Kinshasa from February to June 2022. The Kirby-Bauer disc diffusion method on Mueller-Hinton agar was used for antibacterial assay of different concentrations of Mathesia on 7 different Gram-positive and Gram-negative bacterial species isolated from chronic wounds. The minimum inhibitory concentration (MIC) of Mathesia for the isolates was determined by broth dilution method on Mueller Hinton broth media.
Results: The results from this study showed that Mathesia has an inhibitory effect upon the 7 bacterial species at MIC value of less than 100 µg/ml. The lowest MIC value of 1.95 µg/ml was obtained against *Staphylococcus aureus, Staphylococcus epidermidis* and *Proteus* species.
Conclusion: The results obtained in this study corroborate previous studies which demonstrated effectiveness of Mathesia on *Escherichia coli, Streptococcus pyogenes* and *Aspergillus* species. This activity could also be justified by the presence of phenolic acids, tannins and flavonoids which possess antibacterial properties.

Keywords: Phytomedicine; Mathesia; antibacterial activity; wound infection; multi-drug resistant bacteria

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Activité antimicrobienne *in vitro* de Mathesia® sur des isolats bactériens d'infections de plaies dans les Cliniques Universitaires et Centre Hospitalier du Mont Amba, Kinshasa, République Démocratique du Congo

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Résumé:

Contexte: Mathesia est une émulsion hydro-alcoolique, incolore et quelque peu visqueuse, à base d'extraits de plantes médicinales et contenant des saponines, des polyphénols, des tanins et des sucres réducteurs. Dans cette étude, nous avons proposé de réaliser une étude in vitro de l'activité antibactérienne de phytomédicament Mathesia sur des souches de bactéries isolées d'ulcères du pied diabétique et de plaies chroniques de patients pris en charge aux Cliniques Universitaires et au Centre Hospitalier du Mont Amba à Kinshasa, République Démocratique du Congo.
Méthodologie: Cette étude *in vitro* a été réalisée au laboratoire de bactériologie de l'Institut Supérieur des Technologies Médicales de Kinshasa de Février à Juin 2022. La méthode de diffusion sur disque Kirby-Bauer sur gélose Mueller-Hinton a été utilisée pour le dosage antibactérien de différentes concentrations de Mathesia sur 7 espèces bactériennes Gram-positives et Gram-négatives différentes isolées de plaies chroniques. La concentration minimale inhibitrice (CMI) de Mathesia pour les isolats a été déterminée par la méthode de dilution en bouillon sur milieu de bouillon Mueller Hinton.
Résultats: Les résultats de cette étude ont montré que Mathesia a un effet inhibiteur sur les 7 espèces bactériennes à une valeur CMI inférieure à 100 µg/ml. La valeur CMI la plus basse de 1,95 µg/ml a été obtenue contre les espèces *Staphylococcus aureus, Staphylococcus epidermidis* et *Proteus*.
Conclusion: Les résultats obtenus dans cette étude corroborent des études antérieures qui démontraient l'efficacité de Mathesia sur les espèces *Escherichia coli, Streptococcus pyogenes* et *Aspergillus*. Cette activité pourrait également être justifiée par la présence d'acides phénoliques, de tanins et de flavonoïdes possédant des propriétés antibactériennes.

Mots-clés: Phytomédicament; Mathesia; activité antibactérienne; infection de la plaie; bactéries multirésistantes

Introduction:

A wound is a physical injury involving a break in the continuity of the skin (1). The exposed subcutaneous tissues provide a favorable substrate of growth of a wide variety of micro-organisms; and if the tissues involved are devitalized and the host immune answering is compromised, the conditions become optimal for microbial growth (2). Indeed, the host immune answering process is an important factor in the occurrence of infection (3). Infection of a wound refers to a deposition and multiplication of bacteria on the tissue with reactions that can be classic signs like redness, pain, swelling and fever (4,5). The evolution of a wound to an infected state is dependent on a multitude of microbes and some factors include the type, location, and the depth of the wound, the extent of exogenous contamination, the general health of the wounded person, the immune status of the host, the microbial load, and the virulence combined with the types of micro-organism present (2).

The infection of the majority of wounds is polymicrobial with both aerobic and anaerobic bacteria. Aerobes often include *Staphylococcus aureus*, *Pseudomonas* and beta-haemolytic streptococcus which are frequently cited as a cause of prolonged wound healing (6-9). Tengrove et al., (10) reported that wound infection is not caused by a single micro-organism but by typical organisms including *Streptococcus* spp, *S. aureus, Pseudomonas* spp; *Escherichia coli, Klebsiella* spp, *Proteus* spp, *Bacteroides fragilis, Clostridium* spp, *Candida* spp and *Aspergillus*. These microorganisms are important in terms of mortality, morbidity, long hospital stay and delayed scarring from infections caused by them (10). Wound infections caused by multi-antibiotic resistant microbes are also costly to treat (11).

Another category of wounds that are more prone to severe infection or complicated by gangrene are the foot ulcers of diabetics. Indeed, more than a third of persons with diabetics develop foot ulcers, and half of these present with infections that result in amputation of foot or digit (12). Bacteria involved in such diabetic ulcers are both Gram-positive and Gram-negative bacteria. Of the Gram-positive bacteria, streptococcus (*S. agalactiae, S., pyogenes, S. mitis*), *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis* are frequently involved while among the Gram-negatives, members of the Enterobacterales such as *Escherichia coli, Klebsiella pneumoniae, Morganella morganii, Proteus mirabilis*, *Citrobacter,* as well as *Pseudomonas* and *Prevotella* spp frequently predominate (13).

Many studies have reported that alcoholic extracts of several plants are active against multi-drug resistant bacteria isolated from infected wounds, with minimum inhibitory concentrations ranging from 50 to 60 mg /ml (14-16). One *in vitro* efficacy study of a phytomedicine plant, Mathesia on multidrug resistant strains of tubercle bacillus (Koch's bacillus) by Kabedi et al., (17), showed that Mathesia was active against all the 33 strains isolated from TB patients, with a minimum inhibitory concentration of 30 µg/ml.

Mathesia is a hydro-alcoholic emulsion, colourless and viscous extracts of medicinal plants, containing saponins, polyphenols, tannins, and reducing sugars (18). This phytomedicine was developed by the team of Professor Mulenga Mbombo at the Faculty of Sciences of the University of Kinshasa in the Democratic Republic of Congo. We propose to conduct an *in vitro* study of the antibacterial activity of Mathesia on wild strains of bacteria isolated from diabetic foot ulcers and chronic wounds of patients hospitalized at the Cliniques Universities of Kinshasa and at the Mont Amba Hospital in the Democratic Republic of Congo.

Materials and method:

Study location and period:

The study was carried out at the bacteriology laboratory of the Higher Institute of Medical Technology of Kinshasa from February to June 2022.

Bacterial isolates:

The study was carried out on a collection of seven bacteria species isolated from different wounds of patients. These included 5 isolates of *Staphylococcus aureus* and *Staphylococcus epidermidis*, 6 isolates of *Escherichia coli*, and 1 isolate each of *Proteus* spp, *Klebsiella* spp, *Citrobacter* spp, and *Pseudomonas aeruginosa*.

Phytomedicinal plant Mathesia®:

Mathesia® was obtained from the Industrial and Technical Group (GITCO), in Kinshasa, DRC. The solution of Mathesia was prepared for different amounts of lyophilized Mathesia® with 14 different dilutions, ranging from 1000 µg/ml up to 0.121 µg/ml.

Determination of antibacterial activity of Mathesia by disc diffusion method:

The agar disc diffusion method was used to determine the antibacterial activity of Mathesia® extracts on the bacterial isolates. An inoculum of the overnight (~18 hours) culture of each bacteria isolate was made and standardized to match the turbidity of 0.5 McFarland standard which contains ~10^8 colony-forming units per milliliter (CFU/ml). The inoculum was spread on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 10 µl of Mathesia extract solution (1000 µg/ml). Prepared gentamicin (10 µg/ml) and norfloxacin (20 µg/ml) discs were used as reference discs.

The Mathesia and reference discs were placed on the inoculated plates and allowed to dry for 30 mins. The plates were then incubated aerobically at 37°C for 24hrs. The diameters of the inhibition zones were measured in millimeters (19), and the inhibition zones produced by Mathesia discs were compared with those of gentamicin and norfloxacin reference discs. The procedure was performed in triplicate and the mean diameter of inhibition zone (in mm) produced by Mathesia and the reference antibiotic discs was calculated for each bacterial isolate.

Determination of minimum inhibitory concentration (MIC) of Mathesia:

The minimum inhibitory concentration was determined by broth micro-dilution method as previously described (20-22). The inocula of the bacterial isolates were prepared from 24 hours old broth cultures. The absorbance was read at 600 nm and adjusted with sterile physiological solution to match that of 0.5 McFarland standard solution. From the prepared microbial solutions, other dilutions with sterile physiological solution were prepared to give a final concentration of 10^6 CFU/ml.

Stock solutions of the extracts were prepared in 0.1% (v/v) aqueous tween 80 (Fisher chemicals) at concentrations of 1 mg/ml. The two-fold serial dilutions in concentrations of the extracts were prepared in Mueller Hinton Broth (MHB), to give final concentrations ranging from 1000 to 0.121 µg/ml. An aliquot (10 µL) of a 10^6 CFU/mL overnight culture was added to wells of a sterile 96-well micro-plate titer. The positive control wells contained MHB + bacteria suspension without plant extract while negative control wells contained MHB only.

The MIC was determined as the lowest plant extract concentration at which no growth was observed after 24 hours. The viability of the bacterial growth was evaluated by the MTT (30 µl tetrazole in 0.01% aqueous solution) assay. For MBC determination, 10 µl was taken from each well of complete inhibition of bacterial growth after incubation and spot inoculated on freshly prepared MHB and incubated for 72 hours at 37°C.

Results:

Antibacterial effects of Mathesia by disc diffusion method on the bacterial isolates:

The sensitivity test result is shown in Table 1. According to Berche et al., (23), an extract is considered to have *in vitro* antibacterial effects when it produces an inhibition zone diameter ≥ 10 mm, hence Mathesia is active against all the tested bacterial isolates. The most susceptible isolates (measured by the mean diameter of inhibition zone) in

Table 1: Mean diameter of inhibition zone of Mathesia and reference antibiotics (gentamicin and norfloxacin) against the bacterial isolates

Bacteria isolates	Mean diameter of inhibition zone (mm)		
	Mathesia (1000 µg/ml)	Gentamicin (10 µg/ml)	Norfloxacin (20 µg/ml)
Staphylococcus aureus (n=5)	27.2±0.2[a]	18.22±0.608[b]	6.42±0.424[c]
Staphylococcus epidermidis (n=5)	26.08±0.46[a]	23.1±0.16[b]	7.16±0.27[c]
Escherichia coli (n=6)	21.7±0.4[a]	24.03±0.51[b]	17.5±0.67[c]
Klebsiella spp (n=3)	22.0± 0.0	20.0±0.0	17.5±0.0
Proteus spp (n=3)	23.0±0.0	21.0±0.0	20.7±0.0
Citrobacter spp (n=3)	19.0±0.0	20.5±0.0	19.5±0.0
Pseudomonas aeruginosa (n=3)	18.0±0.0	19.0±0.0	21.0±0.0

a, b, c: statistically significant difference from results of multiple comparisons tests (least significant difference test) between the bacterial isolates

Table 2: Minimum inhibitory concentrations of Mathesia against the bacterial isolates

Bacterial isolates	Concentrations of Mathesia (µg/ml)														MIC value (µg/ml)
	1000	500	250	125	62.5	31.25	15.62	7.81	3.9	1.95	0.97	0.485	0.242	0.121	
S. aureus (n=5)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
S. epidermidis (n=5)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
Proteus spp (n=1)	+	+	+	+	+	+	+	+	+	+	-	-	-	-	1.95
Klebsiella spp (n=1)	+	+	+	+	+	+	+	+	+	-	-	-	-	-	3.9
Escherichia coli (n=6)	+	+	+	+	+	+	+	+	-	-	-	-	-	-	7.81
Citrobacter spp (n=1)	+	+	+	+	+	+	+	-	-	-	-	-	-	-	15.62
P. aeruginosa (n=1)	+	+	+	+	+	+	-	-	-	-	-	-	-	-	31.25

+ = growth; - = No growth; MIC = Minimum inhibitory concentration.

descending order are *S. aureus* (27.2±1.2mm), *S. epidermidis* (26.08±0.46mm), *Proteus* spp (23±0.0mm), *Klebsiella* spp (22±0.0mm), *E. coli* (21.7±0.4mm), *Citrobacter* sp (19±0.0mm) and *P. aeruginosa* (18±0.0mm).

Compared to the reference antibiotics (gentamicin and norfloxacin) used, Mathesia demonstrated significantly higher antibacterial activity against *S. aureus, S. epidermidis, Proteus* spp, and *Klebsiella* spp (Table 1).

Minimum inhibitory concentration of Mathesia against the bacterial isolates:

The results of broth dilution MIC test of the different concentrations of Mathesia (1000 µg/ml to 0.121 µg/ml) on the bacterial isolates is presented in Table 2. The MIC of Mathesia was lowest (1.95 µg/ml) for *S. aureus, S. epidermidis* and *Proteus* spp, and highest for *Pseudomonas aeruginosa* (31.25 µg/ml).

Discussion:

From the results obtained in this study, it was clearly noticed that the phytomedicine Mathesia had inhibitory effects on the seven bacterial isolates tested, which was explained by the MIC values obtained that were less than 100 µg/ml for all the seven isolates (22,24). The lowest MIC value (1.95 µg/ml) was recorded for *S. aureus, S. epidermidis* and *Proteus* spp. The results obtained in this study corroborated our past research which showed *in vitro* antibacterial effects of phytomedicine Mathesia on *E. coli, Streptococcus pyogenes* and *Aspergillus* sp (18), and similar to those obtained by Bryskier (25).

The antibacterial activity of Mathesia on the seven bacterial isolates could be justified by some compounds present in Mathesia such as terpenes well known from their high antibacterial activity, especially the enumeration of the microbial membrane (18,26,27). This activity could also be justified by the presence of phenolic acids, tannins and flavonoids (18) which possess antibacterial properties (28,29).

Conclusion:

We showed in our study the broad-spectrum antibacterial activity of Mathesia on Gram-positive and Gram-negative bacterial pathogens isolated from chronic wound infections. This antibacterial activity could be attributed to compounds present in Mathesia such as terpenes, phenolic acids, tannins and flavonoids. The results of our study reaffirms that Mathesia could be classified as a natural broad-spectrum antibacterial plant that is useful in treating most infected wounds.

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Contributions of authors:

HNL, OK, and JPNKN conceptualized and designed the study; HNL, ZML, PNM, OLM, and CAI were responsible for data collection, analysis, and manuscript writing; JPNKN, MM, CMM, JMK, JKS, and TKM reviewed and edited the manuscript. All authors reviewed and approved the final manuscript submitted.

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Conflict of interest:

No conflict of interest is declared.

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