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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF PHYTOMEDECINE MATHESIA, A DRUG USE AGAINST BURULI ULCER IN REPUBLIC DEMOCRATIC OF THE CONGO (DRC)

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ABSTRACT

Buruli ulcer is a debilitating skin disease caused by Mycobacterium ulcerans. In recent years, the disease has become an important public health problem in an increasing number of tropical and subtropical countries. In this study the aims was to determine the phytochemical composition and to assess the antibacterial activity of Phytomedecine Mathesia. This study has place and duration in Faculty of Science, University of Kinshasa, between November 2012 and April 2013. The antibacterial activity of Mathesia extract was assessed using disc diffusion and micro-dilution methods respectively. The disc diffusion method was used to determine the antibacterial activity of extract while the micro-dilution method was performed to determine their Minimal Inhibitory Concentration. This study revealed presence of alkaloids reducing sugars, steroids, terpenes and total polyphenols like tannins, flavonoids and saponins in Mathesia extract. The results of this study revealed that extract of Mathesia possesses antibacterial activity. This study provides a scientific basis for the antibacterial activity of Mathesia extract. Isolation and purification of different phytochemicals may further yield significant antibacterial new leads compounds.

KEYWORDS: Phytomedecine Mathesia, Phytochemical and Antibacterial activity.

1. INTRODUCTION

Buruli ulcer is a debilitating skin disease caused by *Mycobacterium ulcerans*. In recent years, the disease has become an important public health problem in an increasing number of tropical and subtropical countries. There have been occasional reports of Buruli ulcer linked to international travel. The disease often occurs in localized and remote rural areas where populations have limited access to medical care.[1] This disease has emerged in recent times as an increasingly important cause of human morbidity around the world, partly due to environmental changes. Traditional medicine is the oldest method of curing and treating human diseases and various plants have been found as a source of effective chemotherapeutic agents against several human diseases in different parts of the world. Henceforth, there is a growing interest in the development of drugs from plant origin.[2-4] In Africa, traditional medicine is of great value and more than 70% of African communities refer to traditional healers concerning health issues.[3,5] Many studies reported the use of medicinal species for the treatment of various ailments as well as the evaluation of several biomedical properties of pharmaceutical relevance.[6-8] These properties that plants possess is due to its important phytochemical constituents like triterpenes, anthocyanins, glucids, coumarins, flavonoids, alkaloids and many others.[9,10] In order to scientifically validate the phyto-therapeutic wealth of the Democratic Republic of the Congo, our choice was focused on Mathesia, the hypothesis that she would contain secondary metabolites able of imparting to them the antibacterial activity. This phytomedecine is a hydroalcoolic solution of plant extracts Ahas the following secondary metabolites including Saponins, Polyphenols, Tanins and reducing sugars. The objective of the current study was to determine the phytochemical composition and to assess the antibacterial activity of Mathesia extract.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Mathesia

Mathesia was obtained from the Industrial and Technological Group (GITCO), Kinshasa, DR Congo. It

is a hydroalcoholic solution of plant extracts containing the following secondary metabolites.

2.1.2. Bacterial Strains

In the current study, three bacterial strains were used namely *Streptococcus pyogenes*, *Escherichia coli* and *Aspergillus sp*. These strains were provided by the Laboratory of Microbiology, Faculty of Sciences, University of Kinshasa.

2.2. METHODS

2.2.1. Chemical Screening of Mathesia

In this study, the phytochemical screening was carried out according to the standard protocol as previously described by Ngbolua et *al*.[11] and it can be performed in aqueous as well as in organic phases.[12]

2.2.1.1. Detection of Saponins

To two mL of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth (height: 1 cm) for 20 min.

2.2.1.2. Detection of Phenols

The aqueous extract (2 mL) was added few drop of Shinoda reagent in a test tube and the boiled. Formation of bluish black color indicates the presence of phenols.

2.2.1.3. Detection of Flavonoids

The aqueous extract (3 mL) was added few drop of Shinoda reagent in a test tube and the boiled. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

2.2.1.4. Detection of Alkaloids

The Alkaloids was detected by Dragendorff and Burchardat reagents. To five mL of the extract was added to two mL of HCl. To this acidic medium, 1 mL of Dragendorff and Burchardat reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

2.2.1.5. Detection of Anthocyanins

The presence of anthocyanins is revealed by a color change as a function of pH due to titration of the acidic aqueous solution with a solution of NaOH. If the solution turns a red color, the pH is less than 3, if against a blue color; the pH is between 4 and 6.

2.2.1.6. Detection of Tannins

Two methods were used to test for tannins. First, about 1 mL of the ethanol extract was added in 2 mL of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (gallic tannins) coloration. Second, 2 mL of the aqueous extract was added to 2 mL of water, a 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

2.2.1.7. Detection of Quinones

The presence of quinones was detected by Borntrager reagent. To 1 mL of organic extract was added few drops of Borntrager reagent (NaOH 10% or NH_4OH 10%) in a test tube. The solution was and then shaken vigorously. A sharp red or orange coloration indicates the presence of quinones.

2.2.1.8. Detection of Triterpenoids

Ten (10) mg of the extract was dissolved in 1 mL of chloroform; 1 mL of acetic anhydride was added following the addition of 2 ml of Conc. H_2SO_4. The formation of reddish violet colour indicates the presence of triterpenoids.

2.2.1.9. Detection of Coumarines

The coumarins were highlighted by the reaction on the lactone ring. 2 mL of ethanolic solution obtained from each residue is introduced into two test tubes. To one of the test tubes 0, 5 ml of 10% NaOH is added, then the test tubes are heated in a water bath to boiling. After cooling, 4 ml of distilled water is added to each test tube. If the liquid in the test tube to which the alkaline solution has been added is transparent or more transparent than the liquid in the control test tube (without alkaline solution), then the reaction is positive. When acidifying the transparent solution with a few drops of concentrated HCl, it loses its yellow colouring, becomes cloudy or a precipitate is formed.

2.2.1.10. Detection of Reducing Sugars

Reducing sugars were detected by Molish reagent. To 2 mL of aqueous extract was added few two drops of Ethanolic b-naphtol and concentrated sulfuric acid. A violet ring in contact with the phases indicates the presence of reducing sugars.

2.2.2. DETERMINATION OF ANTIBACTERIAL ACTIVITY

2.2.2.1. Disc Diffusion Method

The agar disc diffusion method was used to determine the antibacterial activity of Mathesia extracts as follow: A 1 mL of suspension of triplicate, and the developing inhibition zones were compared with those of reference discs.an 18 hours culture bacteria containing about 10^8 colony-forming units per milliliter (CFU/mL) were spread on Mueller Hinton agar medium using sterile swabs. Filter paper discs (6 mm in diameter) were soaked in 10 µL of extracts and placed on the inoculated plates and allowed to dry for 30 min, then incubated at 37 °C for 24 hours. The diameters of the inhibition zones were measured in millimeters.[19] Two controls were included in the test: the first was a control involving the presence of microorganisms but without the test extract sample and the last was two standard antibiotics (gentamycin disc, 10 µg/mL for gram-positive bacteria and ofloxacine disc, 20 µg/mL for gram-negative bacteria). Studies were performed in.

2.2.2.2. Determination of Minimum inhibitory concentration (MIC)

The Minimum inhibitory concentration was determined by broth micro-dilution method as previously reported.[13-15] The inocula of used microorganisms 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 a 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, to give final concentrations ranging from 250 to 1.95 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 were observed after 24 hours. MTT (30 µL) in aqueous solution (0.01%) was used to evaluate the micro-organism viability. 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.

3. RESULTS AND DISCUSSION

3.1. CHEMICAL SCREENING

The results of chemical screening of *Mathesia* are presented in Table 1.

Table 1: Results of chemical screening of *Mathesia.*

N°	***Search* groups**	**Reagent**	**Test**
01	Total polyphenols	Burton	+
02	Tannins	Stiasny	+
03	Flavonoids	Shinoda	+
04	Saponins	Foam test	+
05	Anthocyanins	HCl 20%	_
06	Coumarins	NaOH 10%	_
07	Quinones	Borntraëger	_
08	Alkaloids	Dragendorff	_
09	Steroids and ***Terpenes***	Liebermann	+
10	Reducing sugars	Molish	+

From the above figure, it can be seen that the chemical screening performed on the mathesia revealed the presence of Alkaloïdes, reducing sugars, steroids, terpenes and total polyphenols like tannins, flavonoids and saponins. The work of Kabedi et *al.*[15] showed the presence of these compounds in Mathesia extract. These secondary metabolites which are present in this phytomedecine are well known for their broad spectrum of pharmacological properties, including antimicrobial, antisickling and antioxidant activities Bongo et *al.*[12]; Mpiana et *al.*[16]; Pridmore.[14] All these secondary metabolites are endowed with remarkable pharmacological properties trying to justify the partial use of these plants in African traditional medicine against various infections.[17,18]

3.2. ANTIBACTERIAL ACTIVITY

Table 2 gives the results Antibiotic susceptibility test of bacteria after 24 hours of incubation.

Table 2: Antibiotic susceptibility test of bacteria after 24 hours of incubation.

Bacteria strains	**Mathesia**	**GN**	**Nor**	**Na**	**CTX**
E. coli (-	+	+	+	-	-
Streptococcus pyogenes	+	+	-	-	-
Aspergillus sp.	+	-	-	-	-

Legend: GN: Gentamycin; NOR: Norfloxacin; Na: Nalidin; CTX: Cefotaxime; +: sensitive straim to antibiotics; - : insensitive strain to antibiotics

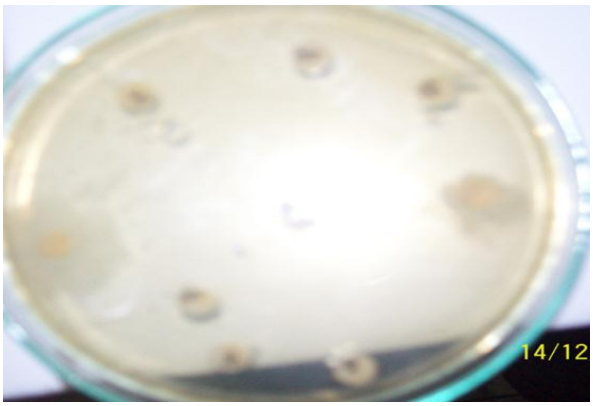

Figure 1: Antibiotic disks on different culture media inoculated with the isolated bacterial strains.

In view of the above table (2), the wild strain of three bacterials showed resistance to two antibiotics notably Nalidin and Cefotaxime. It was sensitive to the remaining antibiotics namely Gentamycin and Norfloxacin. This study indicates that Norfloxacin has an effect on Gram – bacteria, but also on Gram+ bacteria. Our results corroborate with those of Bryskier.[19] By comparing the different results we notice that Mathesia extract gives the good result compared to the antibiotics sold on the market. This shows that, the phytomedecine Mathesia has an effect on Gram- and Gram+ bacterial. These results corroborate with those of Kabedi et al.[15] who have demonstrated the antibacterial power of this phytomedicine use against Buruli ulcer in RDC.

Table 3 gives the results one determination of Minimum inhibitory concentration (MIC) of Mathesia.

Table 3: Determination of Minimum inhibitory concentration (MIC) of Mathesia.

Trains	**MIC (µg/mL)**
Least Virulent Strains	4,85
Most Virulent Strain	5,39

From the above table, it is observed that, the phytomedecine mathesia has the ability to inhibit the growth of virulent and non-violent microorganisms (Minimum inhibitory at 4,85 and 5,39 µg/mL). This would justify its ability to inhibit the production of mycolactones by *M. ulcerans*, the bacterium responsible for Buruli ulcer.

CONCLUSION

The main objective of this study was to determine the qualitative chemical composition and to assess the antibacterial activity of Mathesfia extracts used in Congolese traditional medicine for the management of Buruli ulcer. It was demonstrated that Mathesia tested contain various secondary metabolites such as alkaloids reducing sugars, steroids, terpenes and total polyphenols like tanins, flavonoids and saponins determination of their qualitative and quantitative chemical composition. It the anti-mycobacterial activity is certain in Mathesia and further research is needed to evaluate the extent and limits of its sensitivity in order to draw definitive conclusions.

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