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Antibacterial Potential and Phytochemical Analysis of *Momordica foetida* Extracts: A Prospective Study

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ABSTRACT

Momordica foetida, a medicinal plant from the Cucurbitaceae family, has been traditionally used across tropical Africa for its therapeutic properties. This study aimed to evaluate the antibacterial effects of extracts from different parts of *Momordica foetida* (leaves, stems, and fruits) against common bacterial pathogens and analyze their phytochemical composition. The extraction process involved cold water, hot water, and ethanol methods. Antibiotic susceptibility testing and minimum inhibitory concentration (MIC) assays were conducted against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Phytochemical screening was performed to identify the presence of alkaloids, tannins, flavonoids, quinolones, terpenoids, and saponins. Results showed significant antibacterial activity of ethanol stem extract against *Staphylococcus aureus*. Phytochemical analysis revealed the presence of various bioactive compounds across all extracts. The findings suggest the potential of *Momordica foetida* as a source of antibacterial agents, particularly against *Staphylococcus aureus* infections.

Keywords: Phytochemical Analysis; *Momordica foetida*; Antibacterial Activity; Wound Infections; Anti-bacterial agents

INTRODUCTION

Momordica foetida is a medicinal plant that belongs to the family Cucurbitaceae. It is widely distributed in tropical Africa, South Africa, and also found in the Flora of West Tropical Africa [1, 2]. The plant bears both male and female flowers [3] and has been shown to possess insecticidal properties [3]. Additionally, it is consumed as a vegetable in Gabon, Sudan, Uganda, Tanzania, and Malawi [4]. The drinking of aqueous leaf extracts of the plant to treat malaria is reported in East and Central Africa [5]. Other medicinal uses of extracts of this plant include the treatment of hypertension, peptic ulcers, diabetes mellitus, and as a purgative [4].

The earliest written records of therapeutic practices are found in the Ebers Papyrus, dating from the sixteenth century BC. This is historically valuable as it represents a compilation of earlier works containing a large number (877) of prescriptions and recipes. Many plants are mentioned in these records, including opium, cannabis, myrrh, frankincense, fennel, cassia, senna, thyme, henna, juniper, linseed, aloe, castor oil, and garlic [6]. The use of natural products with therapeutic properties is as ancient as human civilization, and for centuries minerals, plants, and animal products were the main sources of drugs [7]. Approximately 25% of drugs prescribed worldwide still come from plants, with 121 such active compounds currently in use [8].

In developing countries such as Uganda, where conventional drugs are scarce, some communities continue to use crude drugs of plant origin as their immediate remedy for common ailments. Even at the start of the twenty-first century, 11% of the 252 drugs considered basic and essential by the World Health Organization (WHO) are exclusively of flowering plant origin [9]

Most of Dioscorides' "De Materia Medica consists of plant medicines, while the remainder is divided more or less 10% mineral and 10% animal [10]. It is estimated that 60% of the antitumor and anti-infectious drugs already on the market or under clinical trials are of natural origin [11]. Eighty percent of the African population uses some form of traditional herbal medicine as their primary means of health care [12]. Little information is known about the antibacterial activity of the extracts of *Momordica foetida* and its phytochemical composition. With an increase in antibacterial resistance to conventional drugs, there is a need for research aimed at discovering and rationalizing new sources of antibacterial drugs. Additionally, there is a need to obtain information that justifies the use of extracts of *Momordica foetida* without delaying the process of seeking better-needed antibacterial therapy.

METHODOLOGY

Study Design

A short-term prospective-experimental study was conducted to predict and compare the anti-bacterial effects of extracts obtained from the leaves, stems, and fruits of *Momordica foetida*, using bacteria (Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Escherichia coli) as a model. The bacteria were treated with the extracts and then analyzed for antibacterial activity. Additionally, the phytochemical composition of the extracts was determined.

Study Population

Source of test microorganisms: Isolates of Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae from wound infections were obtained from the KIU-TH Microbiology Laboratory.

Sample Volume

The sample of each pathogen's pure culture was standardized to a known approximate number of microorganisms using the McFarland turbidity standard method. Therefore, the sample size for each organism was 108 cells per ml using the McFarland standard of 0.5.

Inclusion Criteria

Only pure colonies of bacteria that had been isolated and identified were used. Swabs taken from wounds were the only source of the bacteria.

Exclusion Criteria

Mixed species of bacteria were not considered. Any other microorganism other than the selected bacterial species were not considered for the study.

Data Collection Procedures

Collection and Preparation of the Plant Material

Fresh plants of *Momordica foetida* were collected from the Kizinda-Bwegiragye village area surrounding the university in Ishaka Municipality, Bushenyi District, Uganda, and taken for identification by a botanist at Mbarara University of Science and Technology. The plants were thoroughly washed, separated into different parts (leaves, stems, and fruits), and air-dried for two weeks. The dried leaves, stems, and fruits were then separately ground into powders using a blender. These powders were used to prepare liquid extracts for both antibacterial testing and phytochemical extraction.

Extraction of Plant Material

Cold Water Extraction:

100g of powdered sample was soaked in 1000ml of sterile distilled water, agitated manually, and allowed to extract for 48 hours. Each extract was then filtered using Whatmann No. 1 Filter paper. The filtrates were evaporated in a water bath at 50°C until dry, and the extracts were weighed and stored in a refrigerator at 4°C.

Hot Water Extraction:

100g of each powdered plant material was weighed, soaked in 1000ml of hot sterile water, and boiled for 30 minutes in a conical flask. After boiling, each extract was allowed to continue extracting for 48 hours. Each extract was filtered using Whatmann No. 1 filter paper and evaporated to dryness using a water bath at 50°C. The extracts were weighed and stored in a refrigerator at 4°C.

Ethanol Extraction

100g of each powdered plant material was weighed, soaked in 1000ml of absolute ethanol for 48 hours at room temperature with occasional stirring. The content was filtered and evaporated to dryness in a water bath at 50°C. The extracts were weighed and stored in a refrigerator at 4°C.

Sterility Test of the Plant Extracts

The ethanol and aqueous (cold and hot extract) extracts were tested for growth or contaminants. This was carried out by inoculating 1ml of each extract on sterile Mueller Hinton Agar and incubating at 37°C for 24 hours. No growth in the extracts after incubation indicated sterility.

Antibiotic Susceptibility Testing

The agar well diffusion method as described by [13], [14] and [15] was adopted for this experiment. Mueller Hinton Broth was prepared as specified by the manufacturer, autoclaved, and poured aseptically into sterile Petri dishes. Using a heat-sterilized loopful, the standardized bacterial cell suspension (108 CFU/ml) was evenly streaked on each agar plate. The extracts were reconstituted in distilled water (for water extracts) and 40% ethanol (for ethanol extracts) to obtain working concentrations of 25mg/ml. 200µl of each extract was inoculated into a well (6 mm diameter) previously bored with a sterile glass test tube in each plate. Negative controls were distilled water and 40% ethanol (200µl each), while the positive control was 5µg/ml of ciprofloxacin (200µl). The plates were allowed to stand for 30 minutes for prediffusion of the extracts before incubation at 37°C for 24 hours. The antibacterial activity of the extracts was determined by measuring the mean diameter zones of inhibition against the test organisms, recorded in millimeters using a transparent ruler.

Determination of the Minimum Inhibitory Concentration (MIC)

The plant extracts that demonstrated significant antibacterial activity by the agar well diffusion were subjected to MIC assay using the broth dilution method. One ml of a 24-hour culture of

test organisms (108 CFU/ml), adjusted to McFarland turbidity standard, was incubated in serial dilutions of 100, 200, 400, 600, 800, and 1000 µg/ml of plant extracts in physiological saline at 37°C for 24 hours. The concentration at which the lowest dilution with no detectable bacterial growth was considered as the minimum inhibitory concentration (MIC).

Phytochemical Screening of Extracts

The different extracts of *Momordica foetida* were tested for the presence of phytochemicals such as steroids, saponins, alkaloids, flavonoids, terpenoids, cardiac glycosides, and tannins using the standard procedures described by Trease and Evans,[16].

Test for Alkaloids

0.5g of the sample was accurately weighed and defatted with 5% ethyl ether for 15 minutes. The defatted sample was then extracted for 20 minutes with 5.0ml of aqueous HCl on a steam bath. The resulting mixture was centrifuged for 10 minutes at 3000rpm to remove the supernatant. 1.0ml of the filtrate was treated with a few drops of Mayer's reagent, and a second 1.0ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence of the presence of alkaloids.

Test for Saponins:

The ability of saponins to produce frothing in aqueous solution was used as a screening test for the sample. 0.5g of dried extract was shaken with water in a test tube, and frothing that persisted on warming was taken as evidence for the presence of saponins.

Test for Tannins

5.0g of dried extract was stirred with 10.0ml of distilled water. This was filtered, and ferric chloride reagent was

Data Analysis Procedures

The results were subjected to Microsoft Excel and presented in tables and graphs.

Ethical Considerations

Not applicable in this study

RESULTS

Results of the extraction process showed that the aqueous extracts gave the greatest amount of dried extract (stem 38.5g, leaf 38g) compared to ethanol, as shown in figure 1.

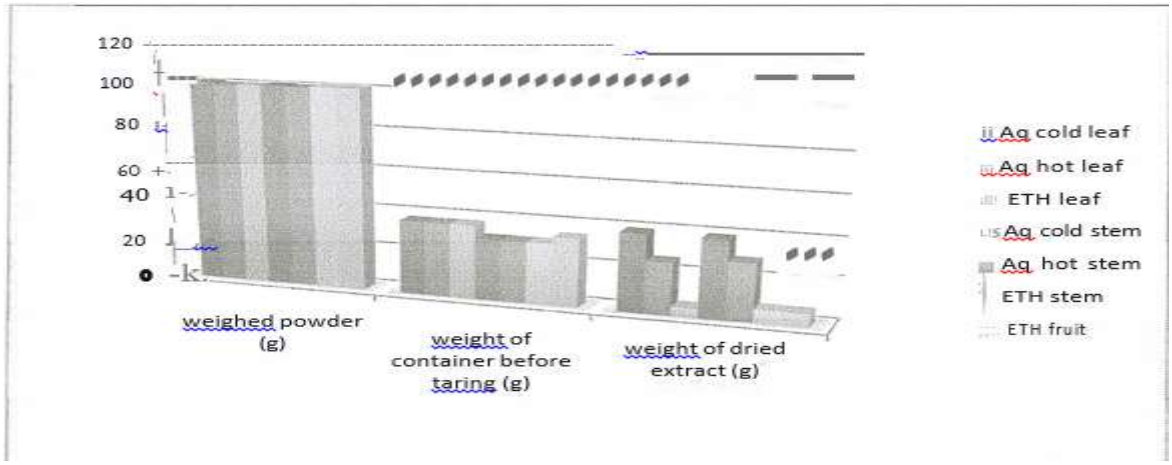

Figure 1: Shows the results for the weight in grams of the powder, the container in which the powder was weighed, and the dried extract obtained. Where 'Aq' represents aqueous and 'ETH' stands for ethanol.

The results obtained from the antibiotic sensitivity testing procedure were recorded, from which the diameter of the zones of inhibition was measured in millimeters (mm). The positive control of ciprofloxacin produced zones of inhibition in all the test isolates. These are shown in Tables 2 and 3 below. Additionally, as shown in Table 3, the ethanol stem extract was able to produce a zone of inhibition with a diameter of 21 mm.

Table 1: Diameter of Zones of Inhibition of the Test Bacterial Species to the Leaf Extracts and Controls

Test Isolate	Aq cold leaf 25mg/mL	Aq hot leaf 25mg/mL	ETH leaf 25mg/mL	CIPRO 5mg/mL	Ethanol 20%
P. auroginosa	Nil	Nil	Nil	25mm	Nil
E. coli	Nil	Nil	Nil	24mm	Nil
K. pneumonea	Nil	Nil	Nil	24mm	Nil
S. aureus	Nil	Nil	Nil	9mm	Nil

Where Aq is aqueous and ETH stands for ethanol. NiI is No Inhibition

Table 2: Diameter of Zones of Inhibition of the Test Bacterial Species to the Stem back Extracts and Controls

Test Isolate	Aq cold stem 25mg/mL	Aq hot stem 25mg/mL	ETH Stem 25mg/mL	ETH Fruits 25mg/mL	CIPRO 5mg/mL	Ethanol 20%
P. auroginosa	Nil	Nil	Nil	Nil	24mm	Nil
E. coli	Nil	Nil	Nil	Nil	24mm	Nil
K. pneumonea	Nil	Nil	Nil	Nil	25mm	Nil
S. aureus	Nil	Nil	21mm	Nil	9mm	Nil

Where Aq is aqueous and ETH stands for ethanol. NiI is No Inhibition

MIC test

Results of the MIC test of the stem ethanol extract showed growth in all the tubes at all the concentrations used (Table 3). Where + indicated the presence of growth.

Table 3: Results of the MIC test

Serial dilutions of ethanol stem-	l00mcg/ml	200mcg/ml	400mcg/ml	600mcg/ml	800mcg/ml	I000mcg/ml
Growth	+	+	+	+	+	+

Phytochemical analysis

Phytochemical analysis on *Momordica foetida* extracts revealed the presence of alkaloids, tannins, flavonoids, quinolones, and terpenoids in all extracts, while saponins were present in all except the hot aqueous stem and the ethanol stem extracts of *Momordica foetida*. This is summarized in Table 4 below.

Table 4: Showing the results of the phytochemical analysis

Phyytochemicals	Aq cold leaf	Aq hot leaf	ETH leaf	Aq cold stem	Aq hot stem	ETH stem	ETH fruit
Saponms	-	+	+++	+++	+++	-	+++
Terpenoids	+++		++	+++	+	+	++
Quinolones	+++	++	+++	+++	+	+++	++++
Favonoids	++	+	+	+	+	+	+
Tannins	+++	+++	++	++	++	+	
Alkaloids	++	+++	+	++	+	++	++

Where: - represented absence, + represented least presence, ++ represented abundant

+++ represented very abundant, ++++represented most abundant, Aq is aqueous and ETH stands for ethanol

DISCUSSION

In this study, the leaf, stem, and fruit were found to produce varying weights in the extracts of both aqueous (hot and cold) and ethanol. Figure I show that the aqueous cold extracts produced the highest weights, as seen in the stem and leaf extracts. This was followed by the aqueous hot extracts of the leaf and stem, while the ethanol extracts weighed the least. This difference possibly indicates varying extractive potentials for the solvents used, as the aqueous solvent exhibited a larger extractive ability. Consequently, one may easily generalize that the phytochemicals in this plant were more soluble in the aqueous solvent than in ethanol. Additionally, a difference in conditions of the aqueous solvent, with the cold extracts producing larger weights compared to the hot extracts, shows variations that may possibly be attributed to the loss of some components on heating, such as volatile oils. This could also be due to induction of chemical reactions that reduce solubility into the hot aqueous solvent[17].

Antibacterial susceptibility testing was able to show potential with activity against Staphylococcus aureus produced by the ethanol stem extract. This might indicate that this bioactive phytochemical mostly occurs in the stem and that it is highly soluble in ethanol compared to water[18, 19]. Also, the inhibitory activity of the ethanol stem extract is in direct agreement with a lack of growth in the sterility tests conducted on the extract, unlike the ethanol leaf extract, which did not subsequently show antibacterial activity. This probably means that, in the case of Staphylococcus aureus, the plant extracts did show promise, and the absence of activity in the other extracts might not be representative of a complete absence of the bioactive compounds. This is echoed in the views stated by [20] that, "The failure of some extracts to exert antibacterial effect on test organisms is not enough to conclude a lack of antimicrobial property because the potency of extracts depends on the solvent and method used to obtain the extract, the age of the plant when harvested, and the amount of the active constituent, which can vary in quality and quantity from season to season."

Phytochemical analysis of the extracts of *Momordica foetida* showed the presence of saponins, alkaloids, tannins, flavonoids, quinolones, and terpenoids in all extracts except for saponins, which were absent in the hot aqueous stem and the ethanol stem stems [21-40]. This might be because of an inability of the saponins to dissolve in either the hot aqueous or ethanol extracts of the stem, and also means that the plant had a variety of saponins, but in the case of the stem extracts, these were only the kind that were soluble in cold aqueous solvent, unlike those of the leaf and fruit extracts [41-46].

Because the phytochemical screening tests were carried out before the microbiological tests were done, the results gave the expectation of finding antibacterial activity against the test organisms. This idea is based on a number of findings about the activity of these phytochemicals. For example, [21, 22] stated that, "Flavonoids are phenolic structures containing one carbonyl group complexes with extracellular and soluble proteins and with bacterial cell walls, thus exhibiting antibacterial activity through these complexes." While [23] regarding tannins wrote that, "Tannins, on the other hand, have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of cell protein synthesis." The presence of cardiac glycosides and steroids has been documented to inhibit many bacteria and found to possess antioxidant potentials[24].

CONCLUSION

This study demonstrates promise for the use of *Momordica foetida* against infections caused by Staphylococcus aureus. Staphylococcus aureus has been implicated in soft tissue and wound infections, thus allowing the findings of this study to regard this plant as useful with respect to this bacterium.

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