Antibacterial Efficacy, Phytochemical Screening and Toxicological Analysis of *Momordica Charantia* Schaefer Fruits Extracts

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ABSTRACT

Momordica charantia commonly known as Bitter melon or Bitter gourd has long been used as phytopharmaceutics and it one of the abundance gift by nature. The antibacterial efficacy, bioactive constituents and toxicological studies of the root extracts of *Momordica charantia* was investigated in this present studies. The antibacterial susceptibility was done using modified agar – well diffusion methods. At concentration of 100mg/ml, ethanol and n hexane extracts inhibited the growth of all the tested bacteria, though with varying degree of the susceptibility of the bacterium. The diameter of zones of inhibition obtained ranged from 9.3 -16.1 mm to 7.0 mm and 15.0 mm for ethanol n hexane extracts respectively. The minimum inhibitory concentration (MIC) value ranged from 20.0 – 40.0 mg/ml for both ethanol and n hexane extracts. The minimum bacteriocidal concentration values ranged from 20.0 – 40.0 mg/ml for both ethanol and n Hexane extracts. The phytochemical screening result revealed the presence of Tannin, phenol, saponnins, alkaloids, flavonoids, Anthraquinones, steroids, carbohydrates and cardiac glycosides. The liver and the large intestine of the apparently albino rats that were fed with the fruits extract revealed normal histology of both. The result obtained in this study suggests that the root extracts of *Momordica charantia* may be used for prevention of infection caused by these bacteria

Keywords - *Momordica charantia,* antibacterial efficacy, phytochemical screening

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1. INTRODUCTION

Since antiquity, medicinal plants have been used as phytotherapeutics throughout the world. Generally, the bioactive constituents of medicinal plants are secondary metabolites which act as various pharmacological properties and they are used as the substances of modern drugs. The clinical success of plant-based drugs has rekindled interest in research into medicinal plants as potentials sources of new drugs. *Momordica charantia* leaves (Bitter gourd) has long been used as a food and medicine (Batran *et al*., 2006). The plant is called by different names since it grows in tropical regions such as India, Malaya, China, tropical Africa, Middle East, America (Kirtikar *et al*., 1993) and Thailand. Propagated by seed, bitter gourd vines flower in about 30 days, and produce mature fruits about 20 days. Medicinally, the plant has a long history of use by the indigenous people of the Amazon.

A leaf tea is employed for diabetes; as a carminative for colic; topically for sores, wounds, and infections; internally and externally for worms and parasites; as an emmenagogue; and as an antiviral for measles, hepatitis, and feverish conditions. It is antidotal, antipyretic tonic, appetizing, stomachic, anti-bilious and laxative (Nadkarni, 2007). It is also used in native medicines of Asia and Africa particularly for enhancing the digestion, metabolism, blood circulation, immunity and robustness. According to *Ayurveta*, and Indian system of medicine, bitter guard controls fever, blood impurities and jaundice. Moreover, this vegetable is beneficial in curing liver diseases, skin ailments and other windy complaints (Satyavati *et al*., 1987). Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aimed to carry out the in *vivo* antibacterial efficacy, phytochemical screening and toxicological studies of the fruits of *M. charantia* against some pathogens.

2. MATERIALS AND METHODS

2.1 Materials

Healthy fruits of *Momordica charantia*, Weighing balance, Mechanical grinder, Incubator, Oven, Petri-dishes, Sterile filter paper, Rotary evaporator, Cork borrer of 7 mm, Test organism, Conical flask, Beaker, Polythene bag, Autoclave, Nutrient agar, Mueller hinton agar and Sterile water.

2.2 Methods

SAMPLE COLLECTION

A healthy fruits of *Momordica charantia* was collected from the farm land in Omo-Owo area Offa, Kwara State. It was identified botanically and the identification was authenticated by a botanist in the department of Science Laboratory Technology, Offa, Kwara state, Nigeria. The leave was collected and transferred into a polythene bag immediately. The leave was transported to the site of experiment as soon as possible.

Sample Preparation

The fruits of *Momordica charantia* was cleaned and air dried under the shade at normal room temperature. After drying, the plant material was grounded using mortar and pestle to powder forms and kept in a moisture free, airtight container and kept under room temperature prior to the antibacterial assay.

Preparation of Ethanol and N Hexane Extract

100 g of dried powdered sample was soaked in 500 ml of 70% ethanol and n hexane in a different conical flask. Each flask were covered with cotton wool and then wrapped with aluminum foil and shaken vigorously at 5 hours interval for 48 hours at room temperature. After 48 hours, the crude extract was sieved using muslin cloth and whatman no 1 filter paper. The filtrate was evaporated to dryness using rotary evaporator. The dried extract was store in air tight sample bottle until it required.

Reconstitution of the Extract

For the antibacterial screening, the crude extract was reconstituted by dissolving 100 and 200mg of the extract in 1 ml of distilled water to obtain a concentration of 100 and 200 mg/ml.

2.3 Sterilization Techniques

Glass Ware
All glass ware washed in soapy water and sterilized in oven at 160^{0}C for 2 hours before use.

Culture Media
All media used were prepared according to manufactures specification and sterilized at 121^{0}C for 15 minutes.

Standardization of the Organisms
The organism was standardized using Mcfarland standard. To make original McFarland tube no 0.5 was prepared by mixing of 1.175% barium chloride ($Bacl_2$.) with 9.95 ml of 1% sulfuric acid (H_2SO_4) in distilled water in order to estimate bacterial density (Baron *et al.,* 1994). The tube was sealed and was used for comparison of bacterial suspension with standard whenever required.

Sources of Microorganism
Pure culture of *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* was obtained from General Hospital Ilorin, Kwara State and Lautech Teaching Hospital Osogbo, Osun State, Nigeria.

Antibacterial Activities
The antibacterial activity of crude extract was determined by modified agar-well diffusion method described by Irobi et al., (1994). All test organism were first grown in nutrient agar for 24hours before used and standardized to 0.5 McFarland standards (10^8 cfu/ml). The organism was inoculate in Mueller hinton agar plate. Sterile cork borer of 6mm was used to make four well in the Mueller hinton agar. 100mg/ml and 200mg/ml of the extract was filled in a separate well, sterile water was used as negative control and filled into third well. Chloramphenicol was used as positive control in the fourth well. All plates were incubated at 37^{0}C for 24 hours in an incubator. After 24 hours of incubation the plate were observed for zone of inhibition.

Determination of Minimum Inhibitory Concentration (MIC)
The estimation of MIC of the plant extract was carried out by using the method of Akinpelu and Kolawole (2004). Briefly, 10.0, 20.0, 40.0 and 50.0 mg/ml concentrations of the extract was prepared and introduce into each test tube containing 9 ml of the nutrient broth. 1 ml of the 18 hours standardize organism was also introduced into test tubes containing agar broth and extract. All the test tube was incubated for 24 hours at 37^{0}C. The least concentration of the extract that did not permit any visible growth in the broth was taken as the MIC. The MIC of the extracts was done for each test organisms.

Determination Of Minimum Bactericidal Concentration (MBC)
The MBC of the plant extracts was determined by the method of Spencer and Spencer (2004). 1 ml of broth were taken from the tubes with no visible growth in the MIC assay and was subcultured on a freshly prepared nutrient agar and later incubated at 37^{0}C for 48 hours. The MBC was taken as the concentration of the extract that did not show any growth on a new set of agar plates.

Experimental Animals
Eighteen (18) Wister rat of equal sex weighing between 140 -250g were kept in a cage in the botanical garden of Federal Polytechnic Offa, Kwara State, Nigeria.

Histopathology
Histopathological examination was carried out on the liver and large intestine of the apparently healthy albino rats

Phytochemical Screening
The extractions and all the qualitative methods have been done according to the most common and reliable methods described by Harbone (1998)

3. STATISTICAL ANALYSIS

The statistical analysis of the data obtained from antimicrobial activities was carried out using statistical package for social science (SPSS).

4. RESULTS

Table 1: Antibacterial activities of *Momordica charantia* fruits extract prepared at 100mg/ml

	Diameter of zones of inhibition in mm			
Test Organisms	Ethanol Extract	n hexane extract	Water	Chloramphenicol
Staphylococcus aureus	12.0 ± 1.1	10.0 ± 1.3	-	16.0 ± 0.2
Bacillus subtilis	15.2 ± 1.2	13.8 ± 0.4	-	17. 3 ± 0.4
Escherichia coli	16.1 ± 0.7	15.0 ± 0.3	-	17. 3 ± 0.6
Pseudomonas aeruginosa	9.3 ± 0.4	7.0 ± 0.2	-	12. 0 ± 0.8

Key: (-)=no activity

Table 2: Antibacterial activities of *Momordica charantia* fruits extract prepared at 200mg/ml

	Diameter of zones of inhibition in mm			
Test Organisms	Ethanol Extract	n hexane extract	Water	Chloramphenicol
Staphylococcus aureus	14.2 ± 0.4	12.5 ± 0.4	-	18.8 ± 2.0
Bacillus subtilis	16.9 ± 0.8	12.9 ± 0.6	-	18. 4 ± 1.3
Escherichia coli	17.9 ± 1.0	16.1 ± 0.4	-	18. 3 ± 0.8
Pseudomonas aureginosa	11.1 ± 0.6	11.4 ± 0.9	-	12.0 ± 0.5

Key (-)=no activity

Table 3: Minimum inhibitory concentration of the fruits extract of *Momordica charantia*

Bacteria	Concentration (mg/ml) Ethanol 80.0	60.0	40.0	20.0	n hexane 80.0	60.0	40.0	20.0
S. aureus	+	+	-	-	+	+	-	-
Bacillus Subtilis	+	+	-	-	+	+	-	-
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginosa	+	+	+	-	+	+	+	-

KEY: (-) = no growth, (+) = growth

Table 4: Minimum bactericidal concentration of the fruits extract of *Momordica charantia*

Bacteria	Concentration (mg/ml) Ethanol 40.0	20.0	n hexane 40.0	20.0
S. aureus	-	-	-	-
Bacillus subtilis	-	-	-	-
Escherichia coli	-	-	-	-
Pseudomonas aeruginosa	+	+	+	+

Key: = no growth
+ = growth

TABLE 5: Result of the phytochemical screening

S/N	PHYTOCHEMICALS	ETHANOL	N HEXANE
1	Tannins	+	+
2	Phenols	+	+
3	Saponins	+	-
4	Alkaloids	+	+
5	Flavonoids	+	-
6	Anthraquinones	-	-
7	Cardiac glycosides	+	+
8	Carbohydrates	+	+
9	Steroids	+	+

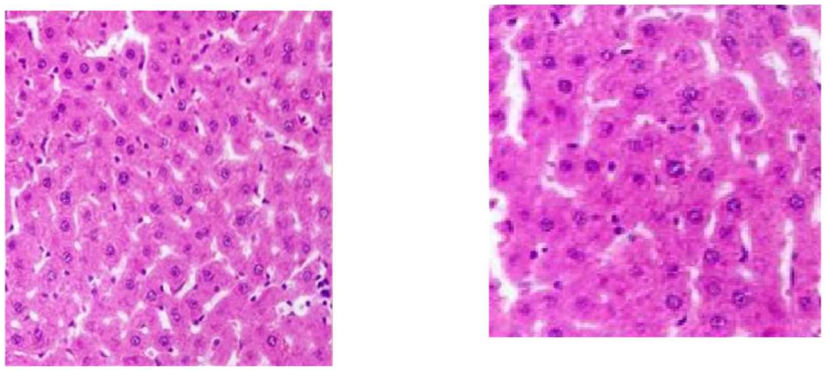

Plate 1 : Photomicrograph of histological examination of the liver of rat fed with leaf extracts of Momordica charantia (a) control rat (b) treated rat both showed normal histological structure of central vein and surrounding hepatocytes

5. DISCUSSION

The antibacterial efficacy, bioactive constituents and toxicological studies of the root extracts of *Momordica charantia* was investigated in this present studies against *Escherichia coli*, *Pseudomonas aeruginosa, Staphylolococcus aureus and Bacillus subtilis* using standard methods. The antibacterial susceptibility was done using modified agar – well diffusion methods. The result for antibacterial activity of the fruits extract of *Momordica charantia* revealed that both ethanol and n hexane extract exhibited varying degree of antimicrobial activities though with ethanol extract demonstrating highest activity against the test organisms. This is an indication that solvent system plays an important role in the solubility of the bioactive component and influence antimicrobial activity. However, the zone of inhibition for ethanol was low when compared with standard drug (Chloraphenicol). Chloramphenicol demonstrated the highest antibacterial activity when compared with both ethanol and n hexane extract. The highest activity was recorded with chloramphenicol because it is a standard antibiotic and it is in a pure state.

The bioactive constituents of the fruits extract of *Momordica charanta* indicated the presence of of Tannin, phenol, saponnins, alkaloids, flavonoids, Anthraquinones, steroids, carbohydrates and cardiac glycosides. These compounds are known to be biologically active and therefore aid the antibacterial activities of *Momordica charanta* fruits extracts. These secondary metabolites exert antibacterial activity through different mechanisms. The liver of the group of apparently healthy albino rats that were treated with the fruits extract showed normal histological structure of the central vein and surrounding hepatocytes and their large intestine showed regularly shaped villi lined by intact, moderately crowned columnar epithelium. This showed that the fruits extract is not toxic to the internal organs and can be safely administered for therapeutic purposes. The result obtained in this study suggests that the fruits extracts of *Momordica charantia* may be used for prevention of infection caused by these bacteria.

6. CONCLUSION

The fruits extract of *Momordica charantia* demonstrated antibacterial activities against Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus and Bacillus subtilis. In this study, ethanolic leaves extract exhibited higher zone of inhibition than n hexane extract. Also the plant demonstrated low minimum inhibitory concentration (MIC) values for both ethanol which are very important for evaluation of antibacterial activities. Therefore, plant could be used as a potential source for the development of an effective antibacterial agents against the tested bacteria. This investigation found that *Momordica charantia* fruits revealed the presence of Tannin, phenol, saponnins, alkaloids, flavonoids, Anthraquinones, steroids, carbohydrates and cardiac glycosides which could be responsible for the antimicrobial activity observed. Therefore, the extract can be used as antibacterial drug in the treatment of infections and diseases caused by these bacteria.

7. RECOMMENDATION

Further pharmacological and clinical studies should be done to understand the mechanism of action and the efficacy of the *Momordica charantia* in treating infection and diseases caused by these bacteria.

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