Antibacterial Properties of Clove (Syzygium aromaticum) and Tumeric (Curcuma longa) Plant Extract On Clinical Isolates

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ABSTRACT

Momordica charantia commonly known as Bitter melon or Bitter gourd belongs to the family of *Curcubitaceae* and commonly grown in the tropical and subtropical regions. It possesses many uses: antimicrobial, antidiabetic, anti helmintic and antioxidant. This present study investigated the antibacterial activity of Momordica charantia against Escherichia coli, Pseudomonas aeruginosa, Staphylolococcus aureus and Bacillus subtilis using standard methods. The antimicrobial activity was done using agar – well diffusion methods. The result showed that 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 10 -15 mm to 9 mm and 14 mm for ethanol n hexane extracts respectively. The minimum inhibitory concentration (MIC) value ranged from 30 – 40 mg/ml for both ethanol and n hexane extracts. The minimum bacteriocidal concentration values ranged from 30 - 40 mg/ml for both ethanol and n Hexane extracts. The phytochemical screening result revealed the presence of Tannin, Saponnin, Alkaloids and polyphenol. The liver and the large intestine of the apparently albino rats that were fed with the leaf extract revealed normal histology of the two internal organs. The result obtained in this study support the use of *Momordica charantia* in treating infection and diseases caused by these bacteria.

Keywords: Antibacterial, Properties, Clove (Syzygium aromaticum), Tumeric (Curcuma longa) Plant Extracts, Isolates.

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1. INTRODUCTION

Some plants are used medicinally in different countries and are source of many potent and powerful drugs (Srivastava *et al.,* 1996). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). The urgent need to discover new antimicrobial compounds with diverse chemical properties and novel mechanisms of action is on the increase, because of the alarming incidence of new and re-emerging infectious diseases. In addition, bacterial adaptations to antibiotic resistance over the past decades have generate a considerable worldwide public health problem (Anderson, 2003). Plants contain numerous biologically active compounds, many of which have antimicrobial activity (Cowan, 1999). Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds (Chah *et al.,* 2006). *Momordica charantia* (Karela) commonly known as bitter gourd, bitter melon or balsam pear is an economically important medicinal plant belonging to the family *Cucurbitaceae*.

Its fruit extract act as anti-diabetic agent in normal and alloxan-diabetic rats (Kolawole *et al.,* 2011). It is indigenous to tropical areas including India, Asia, South America and Nigeria and cultivated throughout South America as food and medicine. Various preparations of *M. charantia* extracts from fruit juice to dried fruit bits have been employed traditionally worldwide, particularly for blood-sugar lowering effects (Welihinda *et al.,* 1986; Raman and Lau, 1996). In addition, it has been reported to exhibit diverse biological activities such as antioxidant, antimicrobial, antiviral, antihepatotoxic and antiulcerogenic activities which are attributed to an array of biologically active plant chemicals including triterpenes, proteins and steroids (Grover and Yadav, 2004). Analyses of phytochemicals from *M. charantia* revealed the presence of active components like momorcharins, momordenol, momordicilin, momordicins, momordicinin,momordin, momordolol, charantin, charine, cryptoxanthin,cucurbitins, cucurbitacins, cucurbitanes, cycloartenols,diosgenin, elaeostearic acids, erythrodiol, galacturonicacids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol (Parkash *et al.,*2002).

In the view of ethnomedical reports, *M. charantia*is being used in folkloric medicine on various ulcers, diabetes and infections (Gurbuz *et al.,* 2000; Scartezzini and Speroni, 2000; Beloin *et al.,* 2005). Although, hundreds of plant species have been screened and tested for antimicrobial properties, the vast majority of the plants have not been adequately screened and evaluated (Gurbuz *et al.,* 2000). Considering the vast potentiality of plants as sources for antimicrobial drugs, the present research aimed to carryout phytochemical screening and evaluates the leaves of *M. charantia* for antimicrobial activity at elevated temperature and under acidic conditions. This present study is aimed to investigate the antibacterial effect of the leaves extracts of *Momordica charantia* against some pathogens.

2. MATERIALS AND METHODS

Materials

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

Methods

Sample Collection

A healthy leaves 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 leave 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.

Sterilization Techniques
Glass Ware: All glass ware were 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.

Antimicrobial Activity
The antibacterial activity of crude extract was determined by 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 (Farnsworth, 1996).

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: Antimicrobial activities of *Momordica charantia* extract prepared at 100mg/ml

	Diameter of zones of inhibition in mm			
Test Organisms	**Ethanol Extract**	**n hexane extract**	**Water**	**Chloramphenicol**
Staphylococcus aureus	10.0 ± 1.0	9.0 ± 0.6	-	13.0 ± 0.2
Bacillus subtilis	14.2 ± 0.4	13.3 ± 0.2	-	12. 3 ± 0.6
Escherichia coli	15.1 ± 0.3	14.0 ± 0.4	-	17. 9 ± 0.4
Pseudomonas aeruginosa	10.3 ± 1.1	8.2 ± 0.8	-	11. 0 ± 0.2

Key: (-)=no activity

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

	Diameter of zones of inhibition in mm			
Test Organisms	**Ethanol Extract**	**n hexane extract**	**Water**	**Chloramphenicol**
Staphylococcus aureus	11.2 ± 0.2	9.6 ± 0.4	-	13.3 ± 0.1
Bacillus subtilis	14.9 ± 0.8	12.6 ± 0.7	-	11. 3 ± 1.2
Escherichia coli	17.1 ± 0.2	14.5 ± 0.9	-	18. 0 ± 0.2
Pseudomonas aureginosa	11.9 ± 0.6	9.4 ± 0.2	-	11. 2 ± 0.1

Key (-)=no activity

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

	Concentration (mg/ml)							
	Ethanol				n hexane			
Bacteria	10.0	20.0	30.0	40.0	10.0	20.0	30.0	40.0
S. aureus	+	+	-	-	+	+	-	-
Bacillus subtilis	+	+	-	-	+	+	-	-
Escherichia coli	+	+	-	-	+	+	-	-
Pseudomonas aeruginosa	+	+	+	-	+	+	-	-

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

Table 4: Minimum bacteriocidal concentration of the leaf extract of *Momordica charantia*

	Concentration (mg/ml)			
	Ethanol		n hexane	
Bacteria	30.0	40.0	30.0	40.0
S. aureus	+	-	+	-
Bacillus subtilis	-	-	-	-
Escherichia coli	-	-	-	-
Pseudomonas aeruginosa	+	-	+	-

Key: = no growth
+ = growth

Table 5: Result of Phytochemical Screening of *Momordica charantia* Leave Extract

Test	Ethanol	N hexane
Tannin	+	+
Saponnin	+	+
Flavonoid	_	_
Alkaloid	+	+
Glycoside	_	_
Anthraquinones	_	+
Polyphenol	+	+

KEY

(-) Absent
(+) Present

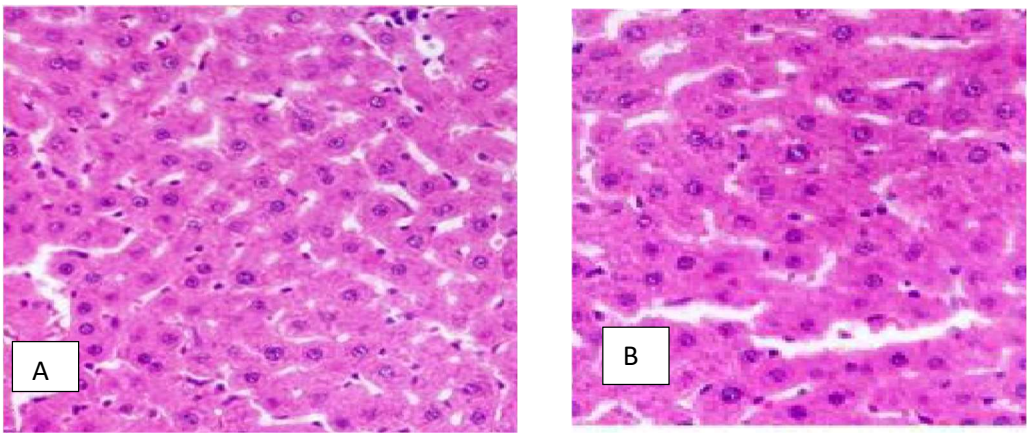

Plate 2: Photomicrograph of histological examination of the liver of rat fed with leaf extracts of *Momordica charantia* *(a)* control rat (b) treated rat both showed normal histological structure of central vein and surrounding hepatocytes

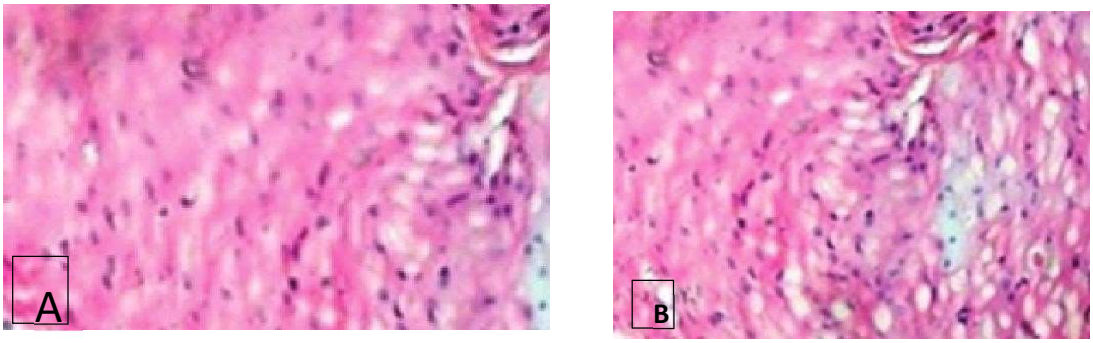

Plate 3 : Photomicrograph of the histological examination of large intestine of rat fed with leaf extracts of *Momordica charantia* (a) control rat (b) treated rat both showed regular shaped villi lined by intact and moderately crowned columnar epithelium

5. DISCUSSION

The result for antimicrobial activity of the leave 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. This work agrees with Sankaranarayanan and Jolly (2016) who have clinically demonstrated broad spectrum antimicrobial activity of leaf extracts of *Momordica charantia*

The phytochemical screening of the leaves extract of *Momordica charanta* revealed the presence of Saponins, Tannin, Alkaloid and Polyphenol in both ethanol and n hexane extract which agreed with work of Akinpelu (2004). These compounds are known to be biologically active and therefore aid the antimicrobial activities of *Momordica charanta*. These secondary metabolites exert antibacterial activity through different mechanisms. For instance, tannin have been found to form irreversible complexes with proline rich protein (Shimada 2006) result in the inhibition of cell protein synthesis (Parekh and Chanda 2007) reported that tannins are known to react with protein to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Dharmananda (2003) reported that tannins are used for treating disorders such as diarrhea.

These observations therefore support the use of leaves of *Momordica charanta* in curing some ailment caused by the test organisms. Alkaloids were also detected in the leave of *Momordica charanta*, alkaloids are toxic against cells of foreign organisms. These activities have been widely studies for their potential use in the elimination and reduction of human cancer cell lines. The liver of the group of apparently healthy albino rats that were treated with the leaf 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 leaf extract is not toxic to the internal organs and can be safely administered for therapeutic purposes.

The result of antimicrobial activities and phytochemical screening of *Momordica charantia* leave extract against the test organisms support the claims made by herbalist and local communities on the recommendation of *Momordica charantia* for use in treating many diseases.

6. CONCLUSION

The leaves extract of *Momordica charantia* demonstrated antibacterial activities on *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 antimicrobial activity. 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* leaves contain Alkaloid, Tannin and Saponnin 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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