

Antibacterial Activity of Chromatographically Separated Pure Fractions of Whole Plant of *Momordica Charantia* L (Cucurbitaceae)

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Abstract: Crude extract of whole plant of *Momordica charantia* was made to go through fractionation processes using thin layer chromatography (TLC) and the resultant fractions were tested for antibacterial activity against four pathogens. Chromatographic analysis yielded yellow fraction 690mg, dark green fraction 570mg while 740mg of the blue black fraction was obtained. At the concentration of about 40µg/ml fraction 1 (yellow fraction) was active against all test organisms except *Salmonella typhi*, but at the same concentration fraction 2 (dark green fraction) was active against two of the four test organisms *Staphylococcus aureus* and *Streptococcus pyogenes* while the third fraction (blue black fraction) was active against only one organism *Streptococcus pyogenes*. Results of Minimum Inhibitory Concentration (MIC) studies showed MIC between 40µg/ml and 60µg/ml for fractions 1 and 2 but 40µg/ml and 80µg/ml for fraction 3, while Minimum Bactericidal Concentration (MBC) studies revealed that MBC ranged from 40µg/ml-80µg/ml for fractions 1 and 2 but 60µg/ml-100µg/ml for fraction 3. The three fractions were active against the test organisms showing that a useful drug could be developed from this plant against some bacterial pathogens.

Key words: antibacterial activity, chromatographic fractions, whole plant, *Momordica charantia*.

Introduction

Before the discovery of antibiotics infectious diseases caused by microorganisms were known to unleash untold hardships on the people. Life expectancy was said to be very low world over as a result of the devastating effects of infectious agents. The coming of antibiotics in the 1930s was thought to bring an end to man's horrific experiences with infectious diseases. However, the development of resistance to the antibiotics soon after their discoveries became a nightmare

scenario and since then solutions are being sought one way or another.

The quest for solutions to the global problems of antibiotic resistance in pathogenic bacteria has often focused on the isolation and characterization of new antimicrobials from a variety of sources including medicinal plants [12].

In different parts of the world, a number of researches are ongoing to try to confirm the efficacies of these plant materials in medicine. It is hopeful, perhaps, these plants will be able to succeed where most synthetic and conventional

antibiotics have failed. Scientist have been able to ascertain in some cases, that crude extracts of some plants and some pure compounds from such plants can potentiate the activity of antibiotics *in vitro* [14]. For example epicatechin gallate from *Camellia sinensis* is known to potentiate Nofloxacin [6] while epigallocatechin gallate has been reported to potentiate lactams [15]. Plants are also known to be a source of efflux pump inhibitors that decrease the effectiveness of efflux pumps by blocking their activity thereby allowing for the accumulation of antibiotics inside the bacterial cell so that they can have access to their target sites. Efflux pumps are ubiquitous protein systems present in Gram negative bacteria.

They are either chromosomally or plasmid encoded [3]. They are largely responsible for the phenomenon of intrinsic antibiotic resistance found in certain bacteria [8]. Plants are believed to be natural reservoir of chemicals with medicinal values and a number of modern drugs are said to have been isolated from natural sources except the synthetic ones. Isolation of these phytochemicals is based on information provided by the locals on the medicinal uses of such plants [2]. Plant-derived products are potential sources of raw materials for the pharmaceutical industry.

Our present work on bioactivity of fractions from *Momordica charantia* was aimed at searching for its activity against the selected bacterial pathogens in order that we might develop useful drugs from the plant.

Materials and methods

Collection and Authentication of Plant Material:

fresh plant materials, (*Momordica charantia*) were collected from Bida, Niger State. Identification was carried out by local people and confirmed by a Botanist and Taxonomist in the Department of Botany, University of Ilorin, Nigeria.

Test Organisms:

Clinical strains of the organisms *Escherichia coli*, *Salmonella typhi*, *streptococcus pyogenes* and *Staphylococcus aureus* were isolated from clinical specimens obtained from Ahmadu Bello University (ABU) teaching hospital Chika, Zaria. Isolates were identified using their physical and biochemical characteristics (Buchanan and Gibbons, 1974).

Extraction and Preparation of Plants' Materials:

Voucher specimens of plant with number MFT1675 were deposited in the herbarium in Federal University of Technology, Minna and dried for two weeks until well dried. Dried plant materials were pound in laboratory mortar and pulverized to powdered form using blender. This was then followed by extraction of materials with alcoholic solvent.

Ethanol was used as solvent for the extraction of the plant materials. The method of Silva *et al.* [13] was adopted. Fifty (50) grams of ground sample of each plant part was suspended in 250ml of 95% ethanol for a period of about 120 hours. The extract was decanted and filtered and the filtrate evaporated in vacuo at 45°C. The extract was then reconstituted in 95% ethanol and reserved as stock concentration then stored at 4°C.

Chromatography:

The ethanolic extract (5g) was subjected to thin layer chromatography (TLC) over silica gel F254 using different gradient solvent systems. The extract was spotted on Merck silica gel (MSG) pre-coated TLC plate of thickness of about 0.2mm. The following solvents were used; n-Hexane-Chloroform, Ethanol-Methanol-Chloroform, Ethanol-Chloroform, Chloroform-Methanol, Hexane 100% and Chloroform 100%. The plates were developed in saturated chromatography tank and spots detected in iodine tank following development and drying of the solvent. Band of separated fractions were detected under UV light and in iodine vapour. The retention fraction (R_f) of each fraction or band was calculated and recorded. These were subjected to repeated thin layer chromatography (TLC) using n-Hexane-Chloroform (1:2) as solvent system. Fractions with similar spot characteristics and R_f -values were bulked to obtain 3 fractions (yellow, 690mg; dark green, 570mg and blue black, 740mg). Susceptibility testing of active fractions against test organisms.

The agar cup well technique as described by Silva *et al* [13] and Abalaka [1] was used. Three holes were bored on the surface of the agar medium equidistant from one another. The bottom of each hole was sealed with molten agar to avoid seepage. When solidified, each of the cups or holes made was filled with known volume and concentration of the prepared fraction solution and allowed to fully diffuse. The surface of the agar was streaked for confluent growth with an 18 hour culture of the test organism which has been previously standardized to approximately 10^6 cfu/ml and incubated at the temperature of 37°C in the incubator for 24

hours. Determination of Minimum Inhibitory Concentration (MIC). Using tube dilution method, the least concentration of fractions in which there was no turbidity was taken as the minimum inhibitory concentration (MIC) [5]. The MIC was determined by serially diluting fraction from 10^1 to 10^{10} . 1ml of each of the dilutions representing a known concentration of the fraction was introduced into 9ml of nutrient broth in the test tube. This mixture was then inoculated with 0.1ml culture of the test organism standardized to approximately 10^6 cfu/ml. This was then incubated at 37°C for 24 hours. The least concentration of the fraction in the test tube with no turbidity or cloudiness compared with the control was taken as the Minimum Inhibitory Concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC). Using tube dilution method, the least concentration of fractions in which there was no turbidity was taken as the minimum inhibitory concentration (MIC) [5]. The MIC was determined by serially diluting fraction from 10^1 to 10^{10} . 1ml of each of the dilutions representing a known concentration of the fraction was introduced into 9ml of nutrient broth in the test tube. This mixture was then inoculated with 0.1ml culture of the test organism standardized to approximately 10^6 cfu/ml. This was then incubated at 37°C for 24 hours. The least concentration of the fraction in the test tube with no turbidity or cloudiness compared with the control was taken as the Minimum Inhibitory Concentration (MIC).

Subsequently, those tubes that showed no turbidity were plated out on nutrient agar plates and absence of growth on incubation for 24 hours was confirmatory for Minimum Bactericidal Concentration (MBC). Phytochemical Screening of Fractions to evaluate the type of secondary metabolites.

Phytochemical analysis was also carried out as exactly as described by Abalaka *et al.* [2] to detect the presence of alkaloids, tannins, saponins etc.

Results:

Results obtained in Table 3 above revealed the least MIC of $40\mu\text{g/ml}$ with all fractions against *Streptococcus pyogenes*. The highest MIC of $60\mu\text{g/ml}$ and $80\mu\text{g/ml}$ were recorded against *Salmonella typhi* by all the fractions. Fraction 1 had stronger activity against test organisms compared to fractions 2 and 3. The least MBC of $40\mu\text{g/ml}$ was recorded against *Staphylococcus aureus* by fraction 2 and the highest MBC of $100\mu\text{g/ml}$ was recorded against *S. typhi* by fraction 3 Table 4.

Phytochemical tests performed on the fractions

revealed the results summarized on the table 5 above. Fraction 1 contained three different organic compounds while fractions 2 and 3 contained two secondary metabolites each at varying levels.

Discussion:

TLC was run with TLC plates to isolate appreciable quantities of the three fractions that showed activity. Centrifugation yielded 690mg, 570mg and 740mg of yellow, dark green and blue black fractions representing 23, 19 and 24.7% yield respectively (table 1). The yield of fractions in chromatography is dependent largely on the amount of the thin layer chromatography run on the extract, how meticulous one is in scrapping the gel after separation, centrifugation as well as carefulness in harvesting the resultant dried fractions.

Activities of fractions against organisms were expressed as clear zones of inhibition around the fractions at varying concentrations. Antimicrobial substances produce clear zones when in contact with microorganisms growing on microbiological plates Gislene *et al.* [7]. According to their work, if a plant extract demonstrates activity by zone of inhibition up to 7mm such an extract is considered active.

Table 2 revealed activity of the three fractions against test organisms. Fraction 1 showed the highest activity by demonstrating activity against three of the test organisms *Streptococcus pyogenes*, *Streptococcus pyogenes* and *Escherichia coli* at the concentration of $40\mu\text{g/ml}$. Fraction 2 followed next by showing activity against two of the four test organism while fraction 3 has the least activity being able to inhibit only *S. pyogenes* at the concentration of $40\mu\text{g/ml}$ and $50\mu\text{g/ml}$.

Susceptibility pattern of the organisms revealed that *S. aureus* is the most susceptible to the fractions at concentration as low as $40\mu\text{g/ml}$. others needed higher concentrations to be inhibited by the fractions. *E. coli* came after *S. aureus* in susceptibility, followed by *S. pyogenes*. *S. typhi* remained the most resistant organisms.

The responses by the organisms may be as a result of differences in the complexities of their cell wall components. This is because gram negative bacteria are known to possess more complex cell wall structure compared to the gram positive bacteria [11].

Activities of fractions compared fairly well with those of standard antibiotics (tetracyclines and ampicillin) used as controls in these experiments. According to Baker and Silverton [4] a chemical agent is said to be valuable as a chemotherapeutic agent if it produces zones of

inhibition wider than, or equal to or not more than 3mm lower than the control. In this work, the scenario described above was obtained. Fraction 1 at 10µg/ml produced a zone diameter of 25mm around *S. aureus* compared with 27mm of ampicilin. The fraction also produced a zone of inhibition of 26mm around *S. pyogenes* compared to 26mm produced by ampicilin (table 2).

The results of minimum inhibitory concentration (MIC) from table 10 suggest that fraction 1 had activity against three organisms at the concentration of 40µg/ml with activity against *S. typhi* at 60µg/ml. Fraction 2 has an MIC of 40µg/ml against *S. aureus* and *S. pyogenes* but 60µg/ml against *E. coli* and *S. typhi*. Fraction 3 had the least minimum inhibitory concentration. It produced MIC of 40µg/ml against *S. pyogenes*, 60µg/ml against *S. aureus* and *E. coli* but 80µg/ml against *S. typhi*. MIC is the least concentration of the fraction that can inhibit the growth of the organism (table 3).

The MBC (table 4) showed the least MBC of about 40µg/ml by fraction 2 against *S. aureus*. As for fractions 1 and 3 the least MBC was 60µg/ml. *S. typhi* was susceptible to fraction 3 at the concentration of 100µg/ml. The MBC is the

least concentration that completely kill all cells of a test organism. The lower the MIC and MBC of a given extract or fraction the higher the efficacy of such extract or fraction against the test organisms.

The general trend in these experiments is that lower concentrations of the fractions inhibited the organisms while higher concentrations were required to cause cidal effects on the organisms. Phytochemical screening revealed the presence of organic constituents such as alkaloids, tannins, glycosides and steroids either in abundance or trace in the fractions. Tannins are known to form irreversible complex with proline-rich-protein (Howard *et al.*, 1987) which would lead to inhibition of cell-wall-protein synthesis. The antimicrobial and resistance modulating potentials of natural occurring compounds have been reported [3]. Some of these compounds are known to improve the activity of some peptidoglycan inhibiting antibiotics by attacking some site in the cell wall [15]. This helps to overcome resistance of some organisms to antimicrobial agents. The presence of these secondary metabolites and antioxidants may have been responsible for the profound activity of these extracts and fractions from *Momordica charantia*.

Table 1: The yield of fractions and percentage yield

Fraction	Colour	Yield (mg)	Percentage yield (%)
F1	Yellow	690	23
F2	Darkgreen	570	19
F3	Blueblack	740	24.7

Table 1 the results of centrifugation of fractions which showed the yield of 690 for fraction 1, 570 for fraction 2 and 740mg for fraction 3. This represents 23, 19 and 24.7% for fractions 1, 2 and 3 respectively.

Table 2 :Ensitivity Analysis showing zones of inhibition (in mm) around fractions of ethanolic extract at varying concentrations

Conc. of extracts (µg/ml)	Fraction 1				Fraction 2				Fraction 3			
	<i>S.a</i>	<i>S.t</i>	<i>E.c</i>	<i>S.p</i>	<i>S.a</i>	<i>S.t</i>	<i>E.c</i>	<i>S.p</i>	<i>S.a</i>	<i>S.t</i>	<i>E.c</i>	<i>S.p</i>
30	0	0	0	0	0	0	0	0	0	0	0	0
40	9±1	0	8±3	9±1	8±4	0	0	9±2	0	0	0	1±1
50	12±2	0	9±3	15±0	10±3	0	0	10±2	0	0	0	4±0
60	15±1	9±3	10±2	19±1	13±2	8±1	14±2	16±2	16±2	0	18±2	7±1
70	16±1	9±1	12±2	20±1	15±2	10±1	16±2	18±2	17±2	0	19±2	19±1
80	17±1	10±3	13±2	22±1	16±2	12±1	17±2	20±2	18±2	11±0	21±2	21±1
90	19±1	11±3	15±2	23±1	17±2	14±1	19±2	20±2	20±2	14±0	23±2	21±1
100	25±1	14±3	19±2	26±1	20±2	18±1	24±2	28±2	26±2	15±0	27±2	28±1
Tetra (0.33mg/ml)	30±1	32±1	35±2	36±1	30±1	32±1	35±2	36±1	30±1	32±13	5±2	36±1
Amp (10µg/ml)	27±0	28±2	30±1	26±2	27±0	28±2	30±1	26±2	27±0	28±2	30±1	2 6 ± 2

Key: *S.a*=*Staphylococcus aureus*, *S.t*=*Salmonella typhi*, *E.c*=*Escherichia coli*, *S.p*=*Streptococcus pyogenes*.

Table 3: Minimum Inhibitory Concentration (MIC) of the fractions against test organisms

Extracts	Organisms	Concentration (µg/ml)											MIC	
		120	110	100	90	80	70	60	50	40	30	20		10
F ₁	<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	-	+	+	40
	<i>S. typhi</i>	-	-	-	-	-	-	-	+	+	+	+	+	60
	<i>E. coli</i>	-	-	-	-	-	-	-	-	-	+	+	+	40
	<i>S. pyogenes</i>	-	-	-	-	-	-	-	-	-	+	+	+	40
F ₂	<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	+	+	+	40
	<i>S. typhi</i>	-	-	-	-	-	-	-	+	+	+	+	+	60
	<i>E. coli</i>	-	-	-	-	-	-	-	+	+	+	+	+	60

Table 3: Continue

	<i>S. pyogenes</i>	-	-	-	-	-	-	-	-	+	+	+	40
F3	<i>S. aureus</i>	-	-	-	-	-	-	+	+	+	+	+	60
	<i>S. typhi</i>	-	-	-	-	+	+	+	+	+	+	+	80
	<i>E. coli</i>	-	-	-	-	-	-	+	+	+	+	+	60
	<i>S. pyogenes</i>	-	-	-	-	-	-	-	-	+	+	+	40

Table 4: Minimum Bactericidal Concentration (MBC) of the fractions against test organisms.

Extracts	Organisms	Concentration (µg/ml)												MBC
		120	110	100	90	80	70	60	50	40	30	20	10	
F ₁	<i>S. aureus</i>	-	-	-	-	-	-	-	+	+	+	+	+	60
	<i>S. typhi</i>	-	-	-	-	-	+	+	+	+	+	+	+	80
	<i>E. coli</i>	-	-	-	-	-	-	-	+	+	+	+	+	60
	<i>S. pyogenes</i>	-	-	-	-	-	-	-	+	+	+	+	+	60
F ₂	<i>S. aureus</i>	-	-	-	-	-	-	-	-	-	+	+	+	40
	<i>S. typhi</i>	-	-	-	-	-	+	+	+	+	+	+	+	80
	<i>E. coli</i>	-	-	-	-	-	+	+	+	+	+	+	+	80
	<i>S. pyogenes</i>	-	-	-	-	-	-	+	+	+	+	+	+	+80
F3	<i>S. aureus</i>	-	-	-	-	-	+	+	+	+	+	+	+	80
	<i>S. typhi</i>	-	-	-	+	+	+	+	+	+	+	+	+	100
	<i>E. coli</i>	-	-	-	-	-	+	+	+	+	+	+	+	80
	<i>S. pyogenes</i>	-	-	-	-	-	-	-	+	+	+	+	+	60

Key: F₁- Fraction 1 (yellow), Fraction 2 (dark green), Fraction 3 (blue black)
 - = there was activity, + = there was no activity

Table 5: Phytochemical screening of fractions for the presence of organic compounds.

Fraction	Test conducted	Result
Fraction 1 (Yellow)	Tannins	++
	Alkaloids	++
	Cardiac Glycosides	-
	Steroids	+
Fraction 2 (Dark green)	Tannins	-
	Alkaloids	++
	Cardiac Glycosides	+
	Steroids	-
Fraction 3 (Blue black)	Tannins	++
	Alkaloids	+
	Cardiac Glycosides	-
	Steroids	-

Key:
 ++ = Heavy
 + = Trace
 - = Absent

Conclusion:

Momordica charantia fractions showing strong activity against bacterial pathogens could be used to develop effective drugs against diseases caused by *S. aureus*, *S. pyogens*, *E. coli* and *S. typhi* which are fast becoming resistant to the once commonly known drugs of choice.

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