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ANTIBACTERIAL EFFECTS OF *Momordica charantia* LEAF AND ROOT FRACTIONS ON CLINICAL BACTERIAL ISOLATES

Alfa Suleiman, Maryam Umar Sadiq, and Aisha Bisola Bello
Department of Biological Sciences, The Federal Polytechnic Bida, P.M.B. 55, Bida
Suleiman.alfa@fedpolybida.edu.ng

ABSTRACT

This study focused on the antibacterial activities of ethanol extracts of leaves and roots of *Momordica charantia,* especially as emergence of antibiotic resistance becomes a major global health problem. The extracts were fractionated using column chromatography, and similar components were grouped with thin-layer chromatography (TLC) based on their R_F values. About 0.2 ml of the test cultures at 1.5 x 10^8 cfu/ml were exposed to the fractions at 20 mg/ml in comparison with ampiclox (30µg) using the agar well diffusion technique. *Escherichia coli*, *Salmonella Typhi*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were used in the study. The TLC analysis resulted into eight and six fractions of the leaf and root extracts respectively. Most leaf fractions showed strong antibacterial action with inhibition zones between 14.00±1.41 to 26.50±2.12 mm. The exception was fraction four, which had a smaller inhibition zone (9 mm) against *Klebsiella pneumoniae* and no activity against *E. coli* and *S. typhi.* The root fractions also demonstrated notable antibacterial effects, with inhibition zones ranging from 12.50±2.12 to 22.00±1.41 mm, though fractions F1 to F3 showed no effect on *S. aureus, E. coli,* and *S. typhi.* Leaf extracts of *Momordica charantia* showed more potent antibacterial activity than the root extracts, suggesting that leaf-derived compounds may be more effective for treating infections caused by these bacteria.

Keys: *Momordica charantia*, chromatographic analysis, Antibiotics, bacteria

INTRODUCTION

The widespread misapplication and overuse of antibiotics are fuelling instances of antimicrobial resistance (AMR) within microbial populations (Tariq et al., 2020). One of the major causes of death around the globe is infections caused by microorganisms, resulting from the emergence of resistance to the commercialized drugs. Multidrug resistant pathogens are projected to cause about 300 million premature deaths worldwide and up to $100 trillion loss to the global economy by 2050 (Huixue et al., 2019). The fact that bacterial infections can no longer be treated with antibiotics depicts an unknown future in healthcare (Chokshi et al., 2019). The effects and consequences of such phenomenon include treatments failure, relapse of infections, increase use and spread of antibiotic resistance, longer and more complicated stays in hospitals, high cost of healthcare, decrease societal productivity as well as increase morbidity and mortality rate (WHO, 2020).

Given the threat posed by drug-resistant bacteria, W.H.O. and numerous other groups and researchers agree that the spread of AMR is an urgent issue requiring a global, coordinated action plan to address (Mulani et al., 2019). In accordance with this, there is renewed interest in utilizing plant-based sources to identify potent novel antimicrobial agents that could serve as an alternative to fight against infections due to MDR bacteria (Bitchagno et al., 2020; Ngaffo et al., 2021).

Generally, plants have been contributing significantly to ecosystems by giving humans and other animals the vital services they need to maintain their health (Ajayi, 2019). According to the World Health Organization, more than 80% of Africans use plant products or herbal medicines to cure a variety of illnesses (Okaiyeto and Oguntibeju, 2021). In ethnomedicine, promising antimicrobial and chemotherapeutic drugs are derived from plants. Plant bioactive chemicals represent a different approach to lowering the occurrence of pathogenic diseases. In the realm of pharmaceuticals, substances originating from plants are regarded as an initial platform for research and the development of drugs (Hiremath et al., 2014). However, Plant materials are often prescribed in crude form without been refined. More acceptability and potency against pathogens would be achieved if the herbal drugs

are purified since impurities would have been removed (Abalaka et al., 2010).

The current study was undertaken to evaluate the antibacterial effects of fractions of *Momordica charantia* obtained from the chromatographic analysis

Sample Collection and Identification: Fresh samples of leaves and roots of *Momordica charantia* were collected separately from Bida town, 9º05'N 6º01'E / 9.083º N 6.017ºE of Niger State (Tiptopglobe.com, 2018). The plant material was identified by a local herbalist and confirms by a Botanist of Herbarium and Ethnobotany Department, National Institute for Pharmaceutical Research Development) and voucher specimen NIPRD/H/6910 was kept in the herbarium in National Institute for Pharmaceutical Research Development, Idu, Abuja.

Sample Preparation and Extraction of Phytocompounds: Method of extraction used by Ali et al. (2011) was adopted with some modifications. The roots and leaves of the plants were air dried at room temperature until well dried. The air-dried plant materials were crushed with mortar and pestle and milled into powder with a blender separately. Four hundred grams (400g) of the milled samples each was cold macerated in 1200 ml ethanol in a screw-cap bottle for 72 hours with intermittent stirring. The extract was filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The extracts were each concentrated in a water bath at 45 ºC, transferred into sample bottles and stored in refrigerator pending further analysis.

Extract Purification: Bioactivity-fractionation of the leaf and root extracts was performed using Column Chromatography (CC) as described by Sarker and Nahar, (2007). The extract was dissolved in 10ml of ethanol and preabsorbed with 2g of silica gel in order to make a smooth paste. The absorbed extract was then dried and pulverized into fine powder to give it a good surface area for solvents penetration. The column which was made of a sintered glass at the base was packed to two-third of its height with slurry made of silica gel and n-hexane (a non-polar solvent). The solvent was allowed to drain out gradually to allow the silica gel to be well packed in the column as stationary phase. The extract was then poured at the top of the silica gel which was blotted with cotton wool at the top to prevent distortion of the surface and allow uniform percolation of the solvents. The extract was then subjected to a gradient elution using hexane (1:0), hexane/Chloroform (1:1), Chloroform (1:0), Chloroform/Ethyl acetate (1:1), Ethyl acetate (1:0), Ethyl acetate /Ethanol (1:1) and Ethanol (1:0); 200ml each as the mobile phase.

Thin Layer Chromatography (TLC) of the Fractions: Silica gel coated TLC plates were used for the study. Two lines were drawn using a pencil about 5 mm from two ends of the plate indicating the spotting line and solvent front respectively. Marks were made on the spotting line for sample application. The fractions were spotted on the line with the help of capillary tube and allowed to dry. The plate was placed in the developing jar with the solvent. After the solvent reaches ¾ th of the TLC plate, it was taken out of the jar, examined under the Ultraviolent lamp and the spots were circled with pencil. The spots were labelled and their distances from the base line were measured. The Retention factor (R_f) values were calculated by the formula

$$R_f = \frac{Distance\ travelled\ by\ solute\ from\ origin}{Distance\ between\ the\ baseline\ and\ the\ solvent\ front}$$

Similar fractions identified on TLC plate were pooled together based on R_F values. This resulted into eight and six major fractions F1 to F8 and F1 to F6 for the leaf and root extracts respectively which were assayed for their antibacterial efficacies at the concentration of 20mg/ml in comparison with standard Beecham ampiclox (30µg).

Test bacteria: Clinical isolates of *Escherichia coli*, *Salmonella* Typhi, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from Microbiology Laboratory of the General Hospital Minna, Niger State. The isolates were authenticated by standard bacteriological method (Cheesbrough, 2006). The isolates were purified and maintained in slants and refrigerated until required.

Screening of the fractions for Antibacterial Efficacy: An in vitro test using the agar well diffusion method as described by Idu and Igeleke (2012) was adopted for the screening. Wells were bored unto the surfaces of Mueller Hinton agar using a sterile 6 mm cork borer aseptically and the bottom of each hole was sealed with a drop of molten agar. About 0.2 ml of the standardized (1.5 x 10^8 cfu/ml) cultures of each test organism was seeded evenly on the surfaces of the agar plates. Each well was filled differently with equal volume of the fractions at concentration of 20 mg/ml. The plates were allowed to stand for 30 minutes for pre diffusion of the extract to occur and then incubated at 37°C for 18hrs. Standard antibiotic (Ampiclox at concentration of 30µg/ml), was used as controls. The

diameter of the zones of inhibition was measured by calculating the difference between cork borer (6 mm) and the diameters of zones of inhibition to the nearest mm (Junaid et al., 2006). The means and standard deviations of triplicate results were taken. Values ≥ 10mm were considered active (Usman et al., 2007).

RESULTS AND DISCUSSION

Susceptibility Pattern of the test Organisms to the Fractions of Ethanol Leaf Extract of *Momordica charantia:*

Table 1 showed the antibacterial profiles of the eight (8) fractions of Ethanol leaf extract of *Momordica charantia* (MC^{LF}) on the test organisms, *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. Typhi*. The extents of the inhibitions were significant, which ranged from 12mm to 25mm except for fraction four (MC^{LF4}) with 9mm zone of growth inhibition against *K. pneumoniae*. Leaf fraction one (LF1) and five (LF5) had the highest inhibition against *S. aureus* while LF3, LF4 and LF8 had the least efficacy against the organism. *E. coli* was most susceptible to LF2 and less susceptible to LF3 and LF5. Furthermore, the organism was absolutely resistant to LF4 (0mm). LF1, LF7 and LF8 had the highest activity against *P. aeruginosa* while LF5 had the lowest activity against the organism. LF5 had the highest activity on *K. pneumoniae* while LF1 had the least activity against the pathogen. *Salmonella Typhi* is most susceptible to LF2 and LF5 and less susceptible to LF3 and LF1. LF4 was not active against *S. Typhi*. The standard antibiotic (Ampiclox) was observed to be more efficacious than the fractions against the test organisms except LF2 which was as active as the control against *E. coli* while LF1, LF7 and LF8 were observed to be more effective than the control against *P. aeruginosa*.

Susceptibility Pattern of the test Organisms to the Fractions of Ethanol Root Extract of *Momordica charantia:*

Table 2 revealed the antibacterial activities of six (6) fractions of Ethanol root extract of *M. charantia* (MC^{RF}) on the test organisms. The organisms were significantly susceptible to the fractions except *S. aureus* and *E. coli* which were resistant to RF1, RF2 and RF3 while *S. typhi* was resistant to RF1. RF4, RF5 and RF6 had the same activity against *S. aureus*. RF5 had the highest activity against *E. coli* while RF6 had the lowest activity. *P. aeruginosa* was most susceptible to RF5 and RF6 and least susceptible to RF1 and RF3. RF4 had the highest activity against *K. pneumoniae* and *S. typhi* while RF1 and RF3 had the least activity against *K. pneumoniae* and *S. typhi* respectively. All the fractions were observed to be less active compared to the standard antibiotic except fraction 5 and 6 which were as effective as the standard antibiotic (Ampiclox) against *P. aeruginosa*.

Table 1: Susceptibility Profiles of the test Organisms to the Fractions of Ethanol Leaf Extract of *Momordica charantia*

Fractions (20mg/ml)	Inhibition zones (mm) *Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella Typhi*
LF1	26.50 ± 2.12^{b}	19.75 ± 1.06^{b}	26.75 ± 1.06^{a}	17.50 ± 0.71^{d}	20.00 ± 1.41^{c}
LF2	20.50 ± 0.71^{de}	24.50 ± 2.12^{a}	19.00 ± 1.41^{bc}	20.50 ± 0.71^{bc}	23.00 ± 1.41^{b}
LF3	15.50 ± 2.12^{f}	15.00 ± 1.41^{c}	18.00 ± 1.41^{bc}	20.50 ± 0.71^{bc}	17.50 ± 3.54^{c}
LF4	16.50 ± 0.71^{f}	0.00 ± 0.00^{d}	17.25 ± 1.76^{c}	9.50 ± 2.12^{e}	0.00 ± 0.00^{d}
LF5	27.50 ± 2.12^{b}	18.00 ± 1.41^{c}	14.00 ± 1.41^{d}	21.50 ± 0.71^{b}	23.00 ± 1.41^{b}
LF6	25.50 ± 0.71^{bc}	20.00 ± 0.71^{b}	16.50 ± 0.71^{c}	19.50 ± 0.71^{bc}	20.50 ± 2.12^{bc}
LF7	18.50 ± 0.71^{ef}	20.50 ± 0.71^{b}	24.50 ± 0.71^{a}	20.00 ± 1.41^{bc}	19.50 ± 0.71^{bc}
LF8	22.50 ± 0.71^{c}	20.75 ± 1.06^{b}	26.00 ± 0.00^{a}	18.50 ± 0.71^{cd}	19.00 ± 1.41^{bc}
Ampiclox (30µg)	35.00 ± 0.00^{a}	26.00 ± 0.00^{a}	20.00 ± 0.00^{b}	32.00 ± 0.00^{a}	35.00 ± 0.00^{a}

Amplx = Ampiclox.

a,b,bc,c,cd,d,de,e,ef and f are ranks due to means of treatments separation by Duncan's Multiple Range Test
Values are means ± standard deviation. Values with the same letter along the column are not significantly different (P> 0.05). Values ≥ 10mm are considered active (Usman et al., 2005; Usman et al., 2007).

Table 2: Susceptibility Profiles of the test Organisms to the Fractions of Ethanol Root Extract of *Momordica charantia*

Fractions (20mg/ml)	Inhibition zones (mm) *Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Salmonella Typhi*
F1	0.00±0.00^{c}	0.00±0.00^{e}	12.00±2.82^{c}	14.00±1.41^{e}	0.00±0.00^{d}
F2	0.00±0.00^{c}	0.00±0.00^{e}	15.00±1.41^{bc}	18.00±0.00^{cd}	19.50±2.12^{bc}
F3	0.00±0.00^{c}	0.00±0.00^{e}	12.50±2.12^{c}	17.50±0.71^{d}	17.00±1.41^{c}
F4	18.00±1.41^{b}	18.00±0.00^{c}	18.50±0.71^{ab}	22.00±1.41^{b}	21.50±0.71^{b}
F5	17.50±0.71^{b}	21.50±0.71^{b}	20.00±1.41^{a}	20.50±2.12^{bc}	19.5±2.12^{bc}
F6	19.50±2.12^{b}	16.25±0.35^{d}	20.50±1.41^{a}	18.00±0.00^{cd}	20.00±1.41^{bc}
Ampiclox (30µg)	35.00±0.00^{a}	26.00±0.00^{a}	20.00±0.00^{a}	32.00±0.00^{a}	35.00±0.00^{a}

Amplx = Ampiclox
a,ab,b,bc,c,cd,d,and e are ranks due to means of treatments separation by Duncan's Multiple Range Test
Values are means ± standard deviation. Values with the same letter along the column are not significantly different (P> 0.05). Values ≥ 10mm are considered active (Usman et al., 2005; Usman et al., 2007)

Findings from this research study have clearly shown that the leaves and roots extracts of *Mormodica charantia* herb contained different phytocompounds with different Rf values when eluted with various solvents on TLC analysis. According to Nanna et al. (2013), Rf values represent relative migration only, whereas absolute values depend on various environmental parameters (e.g. temperature, humidity) which may vary depending on location. Mahmood and Doughari, (2008) reported that different phytochemicals possess different degree of solubility in different type of solvents depending on their polarity.

According to Usman et al. (2007), Plants extracts with values of antimicrobial activities of equal or greater than 10mm are considered effective. Higher zones of inhibition were observed with the fractions of ethanol leaf extract of *Momordica charantia*. The apparent increased in spectrum of activity in the fractions may signal a possible gain in potency in the event of purification of the plant components. According to Abalaka et al. (2010), more and better activity could be observed, if plant extracts are refined. However, the lesser zone of inhibition observed in fraction could also be due to the differences in diffusability of the fractions in agar. Accordingly, higher zones of inhibitions were equally observed with the fractions of ethanol root extract of *Momordica charantia.* The disparities in the susceptibility and resistant pattern observed with the organisms could be attributed to the type or amount of the active compounds present in the fractions or the extra outer membrane possessed by the gram-negative bacteria in addition to the acetyl muramic acid (AMA) or acetyl glucose amine (AGA) components of the bacterial cell walls. The Ampiclox used as control showed a consistent higher activity on the test organisms than the fractions (Tables 1 and 2). This is not surprising because standard antibiotics are well refined industrial products so there is no doubt their activity will be more compared to crude extracts (Abalaka et al. 2010).

CONCLUSION

It was shown from the study that not all the fractions of *M. charantia* extracts were active against the test organisms. However, it has become increasingly clear that some fractions were as active against the test organisms as the referenced drug and even more. Purifying the Ethanol extract of this plant may lead to the discovery of a very active drug that will have wide spectrum of activities over a number of bacterial pathogens. Generally, the antibiograms indicated that the leaf fractions of the extract have better activities than the root fraction, which can be interpreted to mean that *M. charantia* leaves fractions may be preferably used for the treatment of various disease caused by these test organisms.

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