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INVITRO ANTIMICROBIAL ACTIVITY OF BITTER MELON LEAF EXTRACT USING WELL DIFFUSION METHOD

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Abstract

The aim of present study examines the invitro antimicrobial properties of leaf removes from Momordica charantia, commonly known as bitter melon. Utilizing different extraction techniques, we arranged separates and considered their viability in contrast to a scope of pathogenic microorganisms, including microbes and growths. Using a paper plate dispersion test, its antimicrobial viability against Staphylococcus aureus, Salmonella typhi, methicillin-resistant Staphylococcus aureus (MRSA), Escherichia coli, and Pseudomonas aeruginosa was assessed using its methanolic extracts. The Agar Well-Dispersion Strategy ensured adequate antifungal efficacy against specific strains of Penicillium and Aspergillus. The base inhibitory focus (MIC) was determined using the Stock Weakening method and the Agar Well-Dispersion method. Antimicrobial examines, including agar dissemination and least inhibitory fixation (MIC) tests, were directed to decide the viability of the concentrates. The outcomes exhibited huge antimicrobial properties, with fluctuating levels of hindrance across various microbial strains. These discoveries propose that Momordicacharantia leaf separates have likely remedial applications as normal antimicrobial specialists, featuring the requirement for additional investigation into their dynamic mixtures and instruments of activity. This study contributes to the growing interest in plant-derived substances for combating microbial resistance.

Keywords: Invitro, antimicrobial, pathogenic microorganisms, Momordicacharantia.

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Introduction

Over 80 rate of world's populace depends on therapeutic herbs for their fundamental healthcare prerequisites, illustrating how critical plants are to human civilization [1-5]. A common fixing in nourishment and medication, severe melon is a therapeutic plant that has a place to the family Cucurbitaceae and found around the world in tropical and subtropical regions. Including India, South America, and Asia [6]. Common names for it incorporate severe gourd in English, kakarakaya in Telugu, hagalakayi in Kannada, Karela in Hindi and Bengali, and Paakharkaa in Tamil [7]. It is an yearly vine that climbs meagerly and has long-stalked takes off. The single yellow blossoms are carried in the leaf afflicts of the male and female plants. The title Momordica comes from the Latin word for "to nibble, alluding to the

battered edges of the takes off that see like teeth [8]. Numerous diverse compounds are found in Momordica charantia, such as goyaglycosides, goyasaponins, elaeostearic corrosive, erythrodiol, galacturonic corrosive, gentisic corrosive, momorchanins, momordenol, momordicilin, momordicius, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitacins, cucuritanes, cycloartenols, diosgenin, elaeostearic corrosive, erythrodiol, galacturonic corrosive, cycloartenol, charantin, charantin, charine [9, 10]. Alkaloids, glycosides, compounds taking after saponins, rennin, and an fragrant unstable oil mucilage are found in the natural product and takes off. Biting gourd is broadly utilized in medication for its effective antimicrobial properties, making a difference to treat different conditions such as wind nibbles, diabetes, disease, jaundice, and

hemorrhoids. The natural products and clears out of severe gourd show assortment of organic characteristics like anti-inflammatory, anti-tumor, anti-diabetic, anti-rheumatic, anti-ulcer effects [11, 12]. A substance that murders or moderates down the development of microorganisms like infections, microbes, organisms, and parasites is called an antimicrobial, enveloping antibacterial, antifungal, and antiviral activities. In expansion to being utilized as an antiviral pharmaceutical against the hepatitis and measles infections Moreover, it is utilized to treat diabetes, evoke feminine cycle, and kill gas in the intestines [13, 14]. The material's antibacterial properties against *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Bacillus subtilis* were reviewed utilizing the Mixes up plate scattering responsiveness method and well dispersal strategies (agar strategy). In living things, free radicals are significant to the improvement of tissue work and pathogenic processes [15, 16]. It has been illustrated that higher admissions of natural products and vegetables has been connected to diminished threatening growth [17, 18]. The cancer prevention agents show in natural products and verdant nourishments are at risk for this impact [19–21] Natural product extricates from *Momordica charantia* have been appeared to have chemoprotective and antioxidant properties by Asli et al [22]. Methanol and water extricates of *Momordica charantia* were assessed for their antibacterial adequacy and antioxidant movement to help in the advancement of novel, inventive drugs.

Momordica Charantia

Momordica charantia, frequently alluded to as sharp melon, has been considered for its antimicrobial properties. Considers uncover that its natural products, seeds, and takes off have extricates that are dynamic against an assortment of maladies, counting organisms and bacteria. Important investigate has illustrated that its bioactive components such as phenolic acids, flavonoids, and saponins contribute to its antibacterial activity. For occasion, a ponder that was distributed in the Diary of Ethnopharmacology illustrated that biting melon extricates stops the improvement of *Escherichia coli* and *Staphylococcus aureus*.²³

Taxonomy

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoliopsida

Subclass: Dilleniidae

Order: Violales

Family: Cucurbitaceae

Genus: *Momordica*

Subject: *Momordica charantia* L.

Morphology

Momordica charantia Linn., often known as Karela, is a cucurbitaceae flowering climber. The tendril-bearing,

herbaceous plant can reach a height of six meters or more. The leaves have 3–7 deeply spaced lobes and a diameter of 4–12 cm. The lobes have a few tiny border tips, but they are usually blunt. There are no conditions. Flowers are always unisexual and actinomorphic. The epigynous zone of the perianth is brief to protracted, and it is yellow on the short-lived male or female peduncles. Fruits are ovoid, ellipsoid, or spindle-shaped, and they frequently have an oblong shape and a visible exterior of warts. It has a cross-section that is hollow, and its central seed chamber is stuffed with large, flat seeds and pith and a comparatively thin layer of flesh surrounding it. 24 8–13 mm long, compressed seeds with a corrugated edge and two faces that are sculptured [25].



Phytochemical study of Momordica charantia

Momordica charantia is known to contain major phytochemicals that have anti-microbial movement, especially against microbes and organisms. These incorporate charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucuritanes, cycloartenols, diosgenin, elaeostearic acids, erythrodilol, galacturonic corrosive, gentisic corrosive, goyaglycosides, goyasaponins, and multiflorenol [26].

Material and Methods

Chemicals Required

Sigma Chemicals Co., St. Louis, England, supplied all of the analytical-grade chemicals used in this study.

Collection of Leaves

In April 2012, Leaf of *M. charantia* L. gathered from Mada Village, Gussau, Zamfara State, and placed in polythene bags. The leaves were identified and given a voucher number (VN/528/2012) by Mr. U.S. Gallah from the Department of Biological Sciences, Herbarium Unit at Ahmadu Bello University in Zaria, Nigeria.

Preparation of Leaves

M. charantia leaf that had just been picked were cleaned, then allowed to air dry at room temperature in the shade. Following drying, the plant material was crushed into smaller pieces with a pestle and mortar, and an electric blender was used to combine it into a powder. From that point onward, 100 grams of the powdered example was set in impermeable containers and kept up with at room temperature until required.

Collection of organisms tested

Microorganisms being studied acquired from Ahmadu Bello University. Teaching Hospital's Department of Bacteriology and Parasitology in Zaria, Nigeria. *Staphylococcus aureus* and *Bacillus subtilis* are examples of Gram-positive bacteria, while *Escherichia coli* and *Pseudomonas aeruginosa* are examples of Gram-negative bacteria. These bacteria were cultivated in the nutrient broth and kept at 4°C on slants of nutrient agar.

Preparation of water and ethanol extracts

A 500 ml flask holding 250 ml of refined water was filled with 20 grams of the dried powdered test. The flask was brutally shaken three hours separated for 48 hours at room temperature after a cotton plug was set interior and aluminum thwart was wrapped around it (El-Mahmoud, 2009). After a 48-hour period, the rough extricate was blended and sifted through channel paper and muslin cloth. The illustrations were at that point cleaned utilizing a Millipore channel and dried with a rotational evaporator set to 40°C. The extricate had a abdicate of 13.4%. The unadulterated concentrate was put absent in an impervious case bottle until required. 100, 200 and 300 mg of the reconstituted watery extricate were broken down in 1 ml of refined water to get ready a concentration of 100, 200, and 300 mg/ml for antimicrobial screening, separately. Ethanol extricates were made in a way that was comparable to past endeavours [27].

Phytochemical examination of plant extracts

Phytochemical tests are conducted to detect plant secondary metabolites in the extracts. Methods described by Baker and Thormsberg (1983), Trease and Evans (1989), Harborne (1973), Baker and Thormsberg (1983), and AOAC (1990) were used to identify tanins, saponins, steroids, alkaloids, and glycosides.²⁸⁻³¹

Preparation of media for microbial growth

The antimicrobial experiment carried out using nutrient agar prepared according to the manufacturer's instructions. A 500 ml sterile conical flask was used to dissolve 7 g of nutrient agar in 250 ml of pure water. The medium was then autoclaved for 15 mins, 121°C. About 20 ml of the sterilized media added to sterile Petri plate and left the gel once the media had cooled to 45°C.

Table 1. two leaf extracts of *M. charantia* yield phytochemical compounds.

Yes: Presence; No: Not detected

PHYTOCONSTITUENTS	WATER EXTRACT	ETHANOL EXTRACT
Alkaloids	YES	YES
Tannins	YES	YES
Saponins	YES	YES
Flavonoids	YES	YES

Cardiac glycosides	YES	YES
Steroids	YES	YES

Anti-Microbial Activity

The crude extracts had antibacterial properties that were evaluated using the agar-well diffusion method by Irobi et al. (1994). Prior to testing, the bacterial isolates were cultivated for 18 hours in nutrient broth to standardize them to 0.5 McFarland standards (10^6 cfu/ml). Wells were then filled with 500 µl of ethanol and aqueous extracts at concentration of 100 mg/ml and incubated for 24 hours, 37°C. Parallel setups included positive controls (Streptomycin at 10 mg/ml) and negative controls (water and ethanol). After incubation, the plates were examined zones of inhibition [32].

Minimum inhibitory concentration (MIC) determination

The plant extracts MIC was determined using Akinpelu and Kolawole (2004) method. In a nutshell, the extracts were prepared in varying concentrations (6.25, 12.5, 25, and 50 mg/ml) and added to 9 ml of nutritional broth in test tubes. The 18-hour culture was diluted to 10^6 cells/ml with approximately 0.1 ml, and it was cultured for 24 hours, 37°C. The lowest concentration of extract that prevented discernible growth in the broth was deemed to be the minimum extract concentration (MIC). The MIC of the extracts examined in triplicate for each test organism [33].

Minimum bactericidal concentration (MBC) determination

The MBC of the plant extracts was determined using the method of Spencer and Spencer (2004). Briefly, 200 µl of broth was taken from plates that showed no growth in MIC test, subcultured onto nutrient agar plates that had just been made and incubated at 37°C for 48 hours. The MBC was the concentration of the extract that did not grow on the new agar plates [34].

Impact of pH on the activity of extracts

In order to achieve a concentration of 100 mg/ml in individual sterile test tubes, 5 milliliters water and ethanol were separated from 500 milligrams of powdered plant material. This mixture was then thoroughly mixed. 1 N HCl was added to the first test tube dropwise until pH 2 was achieved. Using a pH meter to check the adjustment, 1 N NaOH was added drop by drop until pH 10 was attained in the second test tube. A pure solvent solution used as negative control, while positive control was third test tube, which was unaffected by acid or alkali. After that, 1 milliliter of bacterial culture solution was added, and the previously mentioned the antibacterial activity of the samples was evaluated using the agar well diffusion method.

Impact of temperature on the activity of extracts

By dissolving 500 mg of the powdered plant material in 5 ml of solvent, the extract was reconstituted, thoroughly mixing it to achieve a convergence of 100 mg/ml in two separate sterile test tubes. 1 milliliter of

culture was incubated for thirty minutes at 10°C in a water bath, while another tube incubated for thirty minutes at 100°C. After treatment, test tubes were left at room temperature for an additional half hour to stabilize. As a positive control, third test tube was left untreated at room temperature, while a solution of the pure solvent served as the negative control. Subsequently, 1 ml of contents from each tube added to wells created in a Mueller Hinton agar plate. The wells were produce for 24 hours at 37°C, and diameters of the resulting inhibition zones were measured.

Results and Discussion

The extraction utilizing ethanol and water made disclosures illustrated that ethanol was a more reasonable dissolvable, fulfilling a abdicate of 15.6%, whereas water had a abdicate of 13.4%. This illustrates unequivocally that the dissolvable framework influences antibacterial action and is vital to the dynamic principle's solvency. Hence, both the water and ethanol extricates of *M. charantia*'s takes off included saponins, steroids, tannins, glycosides, alkaloids, and flavonoids, agreeing to phytochemical screening (Table 1). Since these substances are recognized for their physiological movement, they contribute to the antibacterial properties of *M. charantia*. Distinctive strategies permit these auxiliary metabolites to apply their antibacterial activity. For illustration, it has been found that tannins and proline-rich proteins can frame irreversible complexes that square the creation of unused proteins in cells (Shimada, 2006). As per Parekh and Chanda (2007) illustrated that tannins interface with proteins to make trademark tanning affect, which is basic for mending ulcerated tissues. Concurring to Dharmananda (2003), diarrhea and the runs are among the conditions that tannins are utilized to remedy. These discoveries loan bolster to the utilize of *M. charantia* takes off in treating certain ailments caused by the microscopic organisms examined. Moreover, the takes off of *M. charantia* found to contain alkaloids, which are noxious to remote life form cells. The potential application of these exercises in the diminishment of human cancer cell lines and their destruction have been broadly examined (Nobori et al., 1994). Fair et al. (1998) famous that saponins have an inhibitory impact on provocative cells; this finding loans prove to the plant's potential utility in the administration of aggravation. Quinlan et al.'s (2000) investigate included steroidal extricates determined from different therapeutic plants that appeared antibacterial properties against a assortment of bacterial strains. Also, Neumann et al. (2004) confirmed that steroids had antiviral properties Agreeing to Hodek et al. (2002), Flavonoids have been appeared to have antioxidant, cytostatic, anti-allergic, antibacterial, anti-inflammatory, anti-angiogenic, pain relieving, and anti-allergic properties. These auxiliary metabolites found in *M. charantia* takes off bolster the

neighborhood communities' claims that this plant can be utilized to mend a assortment of sicknesses in Mada town. The discoveries with respect to the antimicrobial action *M. charantia* clears out uncovered that both ethanol and watery extricates had shifting degrees of antibacterial action. At a concentration of 100 mg/ml, ethanol extricates were the most viable. For *E. coli*, the hindrance zones measured 17 mm, for *S. aureus*, 16 mm, for *B. subtilis*, 15 mm, and 14 mm, separately. (Figure 1).

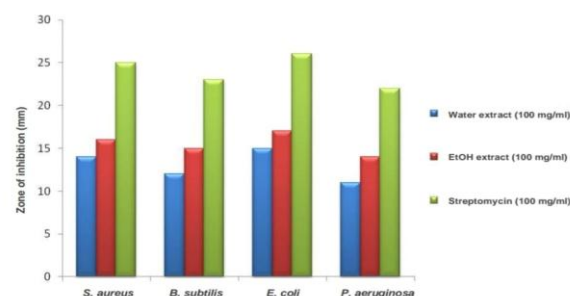


Figure 1. Antibacterial activity profile of two extracts from the leaves of *M. charantia*.

However, when compared to the usual medication (streptomycin) and ethanol extract, which act as positive controls, The aqueous extract had a smaller inhibition zone. In comparison, streptomycin demonstrated the strongest antibacterial activity, with inhibition zones measuring 25 mm for *E. coli*, 23 mm for *S. aureus*, 22 mm for *B. subtilis*, 26 mm for *P. aeruginosa*. Overall, streptomycin exhibited the highest level of activity since it is a common antibiotic medication that is pure. The MIC and MBC are the fundamental parameters used to assess the in vitro activity of antimicrobial drugs having antibacterial potential. The minimum inhibitory concentration (MIC) for the various pathogenic bacteria tested in the ethanol and aqueous extracts ranged from 6.5 to 12.5 mg/ml and from 12.5 to 50 mg/ml, respectively. The ethanol extract's minimum bactericidal activity (MBC) for the bacterial species tested ranged from 12.5 to 25 mg/ml, while the aqueous extract's MBC ranged from 50 to 100 mg/ml. (Figures 2 and 3).

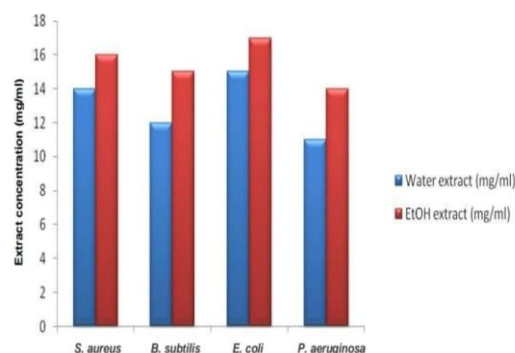
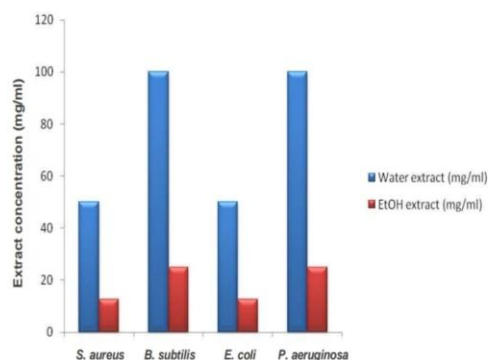
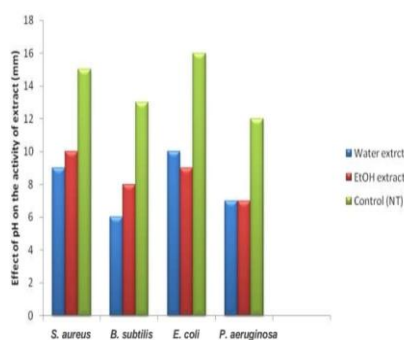
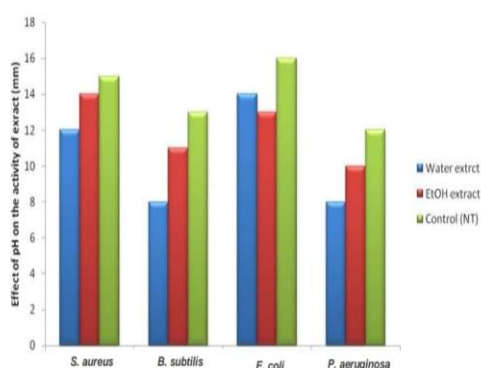


Figure 2. The MIC of leaves extracts of *M. charantia*.

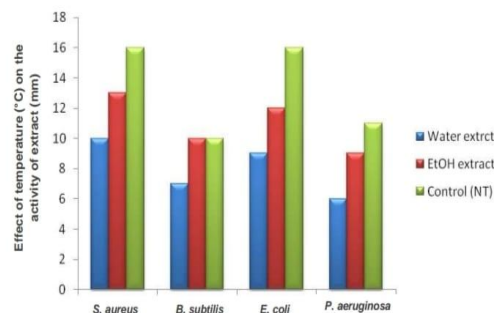
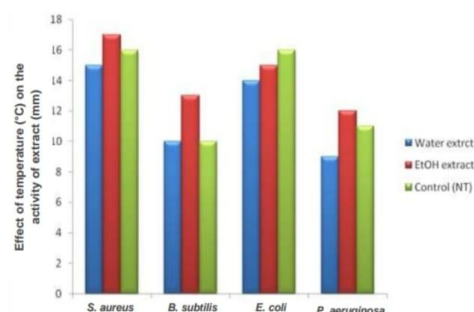
Figure 3. The MBC of leaves extracts of *M. charantia*.

Waterextract's antibacterial movement was negligible and second rate to that extricate of ethanol. This may be clarified by presence of dissolvable polyphenolic, phenolic chemicals, which are less demanding to extricate utilizing non-polar solvents like ethanol than polar solvents like water. The plant may be considered for medicine improvement as a potential remedy for ailments brought on by these pathogenic microscopic organisms due to the extricate of *M. charantia*'s leaves' inhibitory activity against pathogenic bacterial strains.

Figure 4. Effect of pH 2 on the activity of leaves extracts of *M. charantia*.Figure 5. Effect of pH 10 on the activity of leaves extracts of *M. charantia*.

The impacts of ethanol and water extract of *M. charantia* leaf on their antibacterial action at pH 2 and 10 were shown in Figures 4 and 5. The alter towards alkalinity had a minor negative effect on the extracts' antibacterial properties, agreeing to the comes about. For illustration, at pH 2, the ethanol extricates illustrated antibacterial properties against *P. aeruginosa*, *S. aureus*, *E. coli*, and *B.*

subtilis, creating hindrance zones distances across of 14, 13, 11, and 10 mm, separately. Be that as it may, the diminished *P. aeruginosa*, *S. aureus*, *E. coli*, and *B. subtilis* action to 10, 9, 8, and 7 mm at pH 10. In spite of the fact that, in differentiate to the ethanol extricate, the

Figure 6. Effect of temperature (10°C) on the activity of leaves extracts of *M. charantia*.Figure 7. Effect of temperature (100°C) on the activity of leaves extracts of *M. charantia*.

antibacterial action was negligible. The considered animals were more sensitive to the acidic pH of the plant isolates than to the dissolvable pH, based on watched impacts of pH on their movement. This proposes that *M. charantia* leaf extricates may be able to withstand be utilized orally to treat sicknesses connected to the bacterial species beneath examination. The wide extend antibacterial movement of *M. charantia*'s takes off extricate in both watery and ethanolic shapes was steady with Jagessar et al.'s (2008) findings. Additionally, Ankita et al. (2012) found wide run antibacterial activity by *Praecitrullus fistulosus* (tinda) and *M. charantia* against a number of hurtful microbes Figures 6 and 7 portray the affect of temperature on the leaf extracts' movement. Ethanolic extracts stopped *S. aureus* from growing at 10°C, as shown by the formation of zones with diameter of 13 mm at 10°C and 17 mm at 100°C; At 10°C, *E. coli* showed zones with diameter of 12 mm, while at 100°C, they were 15 mm; At 10°C, *B. subtilis* displayed zones with diameter of 10 mm, while at 100°C, they were 13 mm; and zones of 9 mm at 10°C and 12 mm at 100°C were observed in *P. aeruginosa*. temperature effects of aqueous extract showed little antimicrobial activity when compared to ethanol extract. The current study concluded that an increase in the extracts' temperature causes an increase in their antibacterial activity. This may indicate why local communities and traditional healers boil plant extracts before giving them to patients. 35-43

Conclusion

This study found that *M. charantia* takes off contain auxiliary metabolites that may be in charge of the antimicrobial movement seen, counting alkaloids, tannins, saponins, flavonoids, and glycosides. Due to the solvent's impact on antibacterial action, the ethanol extricate illustrates more noteworthy power compared to the water extricate. Also, plant shown low MIC and MBC values for the water & ethanol extricates, separately, which is significant for an assessment of antibacterial adequacy. Subsequently, plants may valuable source for the creation of capable antibacterial specialists. Moreover, the extricates appeared incredible action in acidic and high-temperature situations. As a result, the plant may withstand the stomach's corrosiveness and be made into a pharmaceutical that is utilized to treat respiratory, gastrointestinal, and urinary tract diseases.

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