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In vitro antimicrobial and antioxidant activity of *Momordica charantia* leaves

Sonal
Research Scholar, Department of Centre for Medical Biotechnology (CMBT), MDU, Rohtak, Haryana, India

Abstract
The goal of this study was to compare the *in vitro* antibacterial and antioxidant activities of water- and methanol-prepared Momordica charantia leaf extracts. Initial phytochemical analysis identified several different bioactive chemicals, including glycosides, alkaloids, phytosterols, saponins, phenolic compounds, proteins, lipids and fixed oils, and flavonoids; these results were confirmed by thin layer chromatography. Antimicrobial activity was measured against Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae, and Bacillus subtilis using the stokes disc diffusion and well diffusion methods. Methanolic plant extract showed the largest zone of inhibition against Escherichia coli using the disc method, whereas it was more efficient against Bacillus and Klebsiella using the well diffusion method. Antioxidant activity was determined by utilising both the DPPH and ABTS methods using ascorbic acid and gallic acid as standards. Furthermore, IC50 values were calculated.

Keywords: antimicrobial, antioxidant, *Momordica charantia*

Introduction

Vegetation plays an important part in human civilization. Eighty percent or more of the global population uses some kind of herbal medicine as their main source of healthcare1. The cucurbitaceae family includes tropical and subtropical countries such as India, Asia, and South America, where Momordica charantia thrives. Bitter gourd translates to "bitter cucumber" in English, "Paakharkaai" in Tamil, "Karela" in Hindi and Bengali, "kakarakaya" in Telugu, and "hagalakaya" in Kannada. The Latin name for this plant, Momordica, means "to bite," which is an apt descriptor for the way its leaves look. 4

Mucilage, alkaloids, glycosides, saponin-like compounds (rennin), and a fragrant volatile oil (from the leaves and fruit) have all been isolated from Momordica charantia. Other chemical constituents include momordenol, momordicilin, momordicius, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitns, cucurit Due to its strong antibacterial activity, bitter gourd may be useful in the treatment of a wide variety of conditions, such as piles, leprosy, jaundice, diabetes, and even snake bites. Its fruit and leaf extracts have been shown to have antimicrobial, antifungal, antiviral, and antiparasitic properties (anti parasitic activity)

Researchers investigated the antibacterial effects of a leaf tea against Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Bacillus subtilis using the stokes disc diffusion sensitivity technique and the well diffusion techniques. The tea is also used to combat diseases like measles and hepatitis, cure diabetes, and stimulate menstruation.

The fruit of the Momordica charantia tree has been studied for its antioxidant and chemoprotective properties, as described by Asli *et al*. This research set out to determine whether extracts of Momordica charantia fruit were effective against bacteria and other microorganisms. Pathological processes and tissue damage are both influenced by free radicals in living beings. Fruits and vegetables' antioxidants are responsible for this impact.

Phytochemical screening

Both water and methanol extracts of Momordica charantia were tested for the existence of bioactive substances. Glycosides, phytosterols, proteins, alkaloids, flavonoids, tannins, saponins, fats and fixed oils, gums and mucilages were some of the components found in these foods.

Test for glycosides

Hydrolysis of the extract was carried out in a hot water bath with hydrochloric acid (HCL) for a few hours, and then the hydrolysate was subjected to the Fehling's, Benedict's, and Barfoed's tests.

Test for alkaloids

Wagner's reagent (potassium iodide solution), Hager's reagent (saturated solution of picric acid), Mayer's reagent (potassium mercuric iodide solution), and Dragendorff's reagent (potassium bismuth iodide solution) were all used to identify the presence of alkaloids (iodine and potassium iodide solution).

Test for phytosterols

Lieberman-Burchard and Salkowski tests were used to identify phytosterols. Initially, the residue was dissolved with a few drops of acetic acid, followed by the addition of three drops of acetic anhydride and a few drops of concentrated sulfuric acid. The development of a bluish-green hue indicates the presence of phytosterol.

Test for fixed oils and fats

Small amounts of extract were combined with 0.5N alcoholic potassium hydroxide and phenolphthalein. The combination was warmed in a water bath for one to two hours. Soap formation or a partial neutralisation of alkalinity are both indicators of the presence of solid oils and lipids. An oil spot test was also conducted.

Thin Layer Chromatography (TLC)

For this study, TLC plates with a silica gel layer were acquired. A line was created on the TLC plate, 2 cm from

the bottom, and spots were indicated for sample application along the line. The sample was distributed down the drying line at predetermined intervals using a capillary tube. We placed the plate on top of the growing jar, which contained the mobile phase. The TLC plate is taken out of the jar and the solvent front is withdrawn when the solvent level reaches three-quarters. The plates were dunked in the iodine solution, jiggled, and then taken out of the jar. We looked them over with a UV/Vis light and made notations of any imperfections we found. Given that the points were labelled, their distance from the gridlines could be calculated. Rf values were determined by formula:

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by solute from origin}}{\text{Distance travelled by solvent from origin}}$$

Antimicrobial Activity

Microorganisms

The aqueous and methanolic extracts were tested for antibacterial activity using four different microorganisms: Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Bacillus subtilis. The biochemistry lab at Administrative Management College in Bangalore, India is where we obtained each microorganism. This was investigated using the stokes disc diffusion sensitivity method and the well diffusion methods.

Stokes disc diffusion method

After 24 hours incubation at 37 °C, the soaked Stokes discs (10 mm in diameter) were placed on the cultured plates to enable the bacteria to disseminate uniformly across the disc. The solvent alone, without any extracts added for comparison, was employed. Streptomycin, the prototypical antibiotic, was employed at a 10 g dose for evaluation purposes. After 24 hours of incubation, the size of the inhibition zone was used to determine how effective the substance was against the bacteria. Comparisons were then made between the zone of inhibition diameters of the samples and the reference antibiotic discs. In each experiment, the diameter of the inhibitory rings was evaluated three times.

For the purpose of detecting antibacterial activity, the well diffusion technique was adopted. The four test organisms were infected with 0.1 ml of a standard inoculum each, and then spread out over nutrient agar plates. The inoculum was dispersed on the plate using a loop or a sterile glass spreader. The inoculation plates were incubated for 20 minutes at 37 degrees Celsius. When the incubation period was through, wells of a typical cork order and a diameter of 6 mm were cut consistently into the surface of the nutritional agar medium. The plates were incubated at 37 degrees Celsius for 24 hours after adding 10 microliters of extracts to each well (mm). The inhibition zones' normal distributions and standard deviations were calculated.

Assessment of *In Vitro* Antioxidant Activity

Free radical scavenging activity

Extracts of Momordica charantia were tried out for their antioxidant properties in water and methanol on a petri dish. Antioxidant scavenging activity was evaluated using the free radical scavenger 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Test solutions of different concentrations in 0.1 ml were added to a methanol solution of 0.1 mM DPPH until the final volume reached 0.9 ml. For the sake of comparison, the control group in this research was given 0.1 ml of methanol. How many free radicals were neutralised after a 30-minute room-temperature incubation was measured by measuring the absorbance at 517nm. The level of ascorbic acid was used as a standard. Each sample's scavenging effectiveness was related to the amount of DPPH it quenched. Ascorbic acid was utilised as a control for comparison.

The percent inhibition was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample) /Absorbance control] x100

To get the IC50 values, a dose-response curve was drawn. In this context, "IC50" refers to the concentration needed to accomplish 50% of the maximum scavenging capacity. All investigations and experiments were done three times independently, and the average result was used.

ABTS radical scavenging assay

At ambient temperature and in the dark for 16 hours, ABTS (7 mM) and potassium persulfate (2.45 mM) react to create ABTS radical cations. Phosphate-buffered saline (PBS) was then used to dilute the resultant solution to an absorbance of 1.000. The 1 ml of final volume in the test tube was obtained by mixing 50 l of the test sample (at different concentrations) with 950 l of the ABTS working solution. The absorbance was measured instantaneously at 745 nm. Gallic acid was used as a precision standard. A percentage of inhibition was calculated using the following formula:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

To get the IC50 values, a dose-response curve was drawn. In this context, "IC50" refers to the concentration needed to accomplish 50% of the maximum scavenging capacity. All investigations and experiments were done three times independently, and the average result was used.

Discussion

Two spots were found in the TLC fingerprint profiles of both the aqueous and methanol extracts. Two Rf values of 0.60 and 0.34 are found, with the other two values being 0.56 and 0.34. The extracts showed broad-spectrum antibiotic activity by preventing the growth of the test microorganisms. When the methanolic extract (at a concentration of 100 mg/ml) was administered to E. coli, a 6 mm inhibitory zone was seen using the disc diffusion method. However, when tested against Bacillus subtilis and Klebsiella pneumonia, the same concentration showed a maximum zone of inhibition of 8 mm when subjected to the well diffusion method. Using the DPPH and ABTS *in vitro* antioxidant tests, methanolic leaf extracts outperformed those prepared using water.

Conclusion

Momordica charantia leaves have been used for their curative properties for thousands of years. Thanks to its high concentration of secondary metabolites such glycosides, alkaloids, phytosterols, proteins, saponins, and phytosterols, it has been shown invitro to have antimicrobial properties. This led us to test the efficacy of leaf extracts in both

methanol and water against various microorganisms. The antibacterial activity of water-extracted Momordica charantia leaves was lower than that of methanol-extracted leaves, indicating that methanol-extracted leaves contain a higher concentration of the active antimicrobial compounds (including alkaloids, glycosides, and volatile oils) that are more common in this species. A comparable investigation into the impact of plant extract and phytochemicals on antibiotic-resistant bacteria in livestock was conducted by Gislene *et al*. (2000). The study's authors decided that any chemical with an inhibitory zone size of 7 mm or more qualified as an ac. It is evident that Momordica charantia leaves exhibit broad-spectrum antimicrobial characteristics due to their antibacterial activity against E. coli and Pseudomonas when extracted in water, ethanol, and methanol. It has been shown that extracts of both the leaves and the fruit may kill Helicobacter pylori and Entamoeba histolytica, respectively. In addition, it has been used as a treatment for GI infections, a method of controlling blood sugar in diabetics, and a stimulant of hunger. Some kinds of cancer and viral infections have been suggested as potential uses. Newly identified ribosome inactivating proteins (RIPs) in Momordica charantia, such as alpha- and beta-momorcharin and MAP-30, have been demonstrated to inhibit HIV *in vitro* (Momordica anti-HIV protein). Since studies are only beginning, however, we don't know which active chemicals have therapeutic potential. Phytochemicals in Momordica charantia leaves show free radical scavenging effect, and further study of these compounds is needed.

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