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Investigation and Therapeutic potential activities of *Momordica charantia* L. (Bitter Gourd) extract

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Abstract

The herbal climber *Momordica charantia Linn.* (karela) is a member of the Cucurbitaceae family and is grown in tropical and subtropical climates. From ancient times to the present, *Momordica charantia* (Karela) has been used to treat a wide range of illnesses. It is inexpensive, harmless, and has been employed in many different processes. It is also widely utilized for its antioxidant, antibacterial, and anti-inflammatory properties. The strong oxidizing power of *Momordica charantia* L's aqueous extract, which produces free radicals, encourages the inactivation of bacteria by slowing their growth. Extract from leaves in water using the Soxhlet technique. This study assessed the synthetic extract's anti-inflammatory, antioxidant, and antibacterial properties. The bovine serum albumin/anti-denaturation assay was used to test for anti-inflammatory activity; DPPH was used to test for antioxidants in vitro; and the Agar well diffusion method was used to test for antibacterial activity. The aqueous extract of *Momordica charantia* L showed a significant degree of antioxidant qualities in the DPPH test. Using the albumin denatured state method, the aqueous extract of *Momordica charantia* showed good anti-inflammatory capabilities when compared to the popular drug Diclofenac. Several quantities of *Momordica charantia* aqueous extract (50, 100, and 150 mg/mL) were tested against harmful bacterial strains, and they showed strong antibacterial activity by inhibiting both Gram-positive and Gram-negative bacteria. The study's overall conclusions recommended extracting the sample for potential therapeutic uses, such as its antibacterial and anti-inflammatory properties.

Key words : Antibacterial activity; anti-inflammatory activity; DPPH assay; diclofenac.

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The previously 90 groups and over 700 species that make up the Curcubitaceae family are mostly found in tropical and subtropical regions (Asia, Amazonia, Oriental Africa, and Caribe). Temperate climates are also home to the species. Many species, such as West Indian gherkin (*Cucumis anguria* L.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.), and pumpkin (*Cucurbita* sp.), are grown for their edible qualities. *Momordica charantia* L., another member of this family, is widely distributed throughout various parts of Brazil. This species is a liana with blooms and yellow fruits that, when ripe, yield crimson seeds. It has numerous uses and is popularly called "melão-de-são-caetano," "melão am argo," or "cabaço-amargo." These include acting as an antidiabetic, carminative, antihelmintic, antimalaral, antimicrobial, antiviral, anticancer, colon cleansing, immunostimulant, laxative, antioxidant, and insecticidal. Additionally, it is used to treat skin conditions such as eczema, acne, mycoses, scabies, hemorrhoid, and furuncules[1].

An essential component of human civilization is plants. More than 80% of people on the planet rely on medicinal plants to meet their fundamental medical needs. Momordica charantia is a medicinal plant that is widely used for food and medicine. It is a member of the Cucurbitaceae family and is found in tropical and subtropical regions of the world, including South America, Asia, and India. Bitter gourd in English, Paakharkaai in Tamil, Karela in Bengali and Hindi, Kakarakaya in Telugu, and Hagalakayi in Kannada are some of its frequent names[2]. It is a long-stalked, slender climbing annual vine with yellow, solitary male and female flowers that are carried in the leaf ails. Momordica, a member of the Cucurbitaceae family, is a slender, climber monoecious plant with yellow-colored individual male and female flowers that emerge in the leaf axils and long-stalked leaves. Tendrils are either unbranched or two-branched, and leaves are simple or alternating, measuring 4–10 cm with 3–6 deeply separated lobes[3]. The leaves' sharp edges, which give the impression that they have been bitten, are referred to by the Latin word Momordica, which means "to bite."

Alkaloids, glycosides, saponin-like compounds, rennin, and an aromatic volatile oil mucilage are found in the fruit and leaves. Bitter gourd has strong antibacterial properties and is used in medicine to cure a variety of illnesses, including diabetes, jaundice, leprosy, piles, and snake bites. In addition to being utilized as an antiviral drug against hepatitis and measles viruses, leaf tea is used to cure diabetes, eliminate intestinal gas, and encourage menstruation. Using the Stokes disc diffusion sensitivity technique and well diffusion methods, its antibacterial capabilities were examined against *E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Bacillus subtilis*. In living things, tissue development and disease processes are significantly influenced by free radicals. Increased consumption of fruits and vegetables has been shown to lower the risk of cancer. Antioxidants found in fruits and vegetables are responsible for this. According to Asli *et al*., fruit extracts from *Momordica charantia* have chemoprotective and antioxidant properties[4]. In order to aid in the creation of new, innovative medications, the current study was conducted to assess the antibacterial efficacy and antioxidant activity of aqueous extract of *Momordica charantia* L.

Preparation of MC Powder :

After properly washing the fresh *M. charantia* leaves in tap water for ten minutes to get rid of any dust particles, they were quickly rinsed in deionized water. 10 g of cleaned and finely chopped leaves were combined with 100 ml of deionized water in a 250 ml Erlenmeyer flask, and the combination was boiled at 60 C for five minutes to create the plant leaf broth solution[5]. Whatman No. 1 filter paper was used to filter the mixture after it had boiled (Fig. 1).

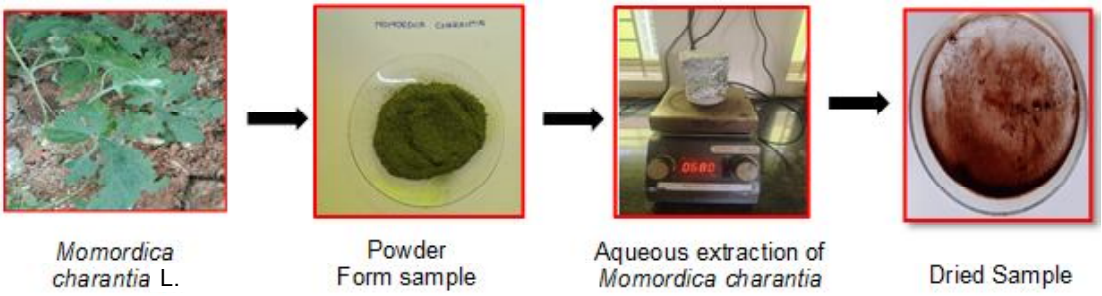

Fig. 1. Schematic representation of *Momordica charantia* L.

Antibacterial activity :

The effectiveness of *Momordica charantia* L. aquoues extract against harmful bacterial strains, namely *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumonia*, was assessed. Mueller Hinton Agar and Broth (Himedia) were used to develop the bacterial cultures. Diffusion disc plates on agar were used to measure antibacterial activity; roughly 0.1 mL of each bacterial culture was applied to the surfaces of the agar plates. All bacterial strains were plated on Mueller Hinton Agar (Himedia) for agar diffusion tests after being cultured in Mueller Hinton Broth (Himedia) for a full day at 37°C for the antibacterial assay[6]. To absorb 100 µL of the sample, paper discs with a diameter of 6 mm were placed on the agar medium. After being incubated at 37°C for 24 to 48 hours, inhibition diameters were measured. Gentamycin (100 µg/ml) was used as the positive control in this investigation, while DMSO was used as a negative control to evaluate the bacterial culture's sensitivity. To evaluate antibacterial activity, various quantities of identically produced water extracts from chosen samples are used[7].

Antioxidant activity :

DPPH free radical scavenging methods were used to evaluate the extracts antioxidant effectiveness. *Momordica charantia* leaf extract and ascorbic acid were dissolved in water to create a stock solution containing 1 mg/mL. Diphenyl-2-picryl hydrazyl radical, at 1 mM 100 microliters of leaf extracts in concentrations of 20, 40, 60, 80, and 100 microliters were mixed with three milliliters of 1 mM DPPH in water[8]. Ascorbic acid was used as a positive control in the DPPH solution in methanol, whereas methanol by itself was used as a blank. The color of the sample changes from deep purple to pale yellow when

DPPH interacts with the antioxidant. A UV-visible spectrophotometer was used to measure the absorbance at 680 nm after the solution had been incubated for 30 minutes at room temperature. Every test was carried out three times[9,10]. The following formula was used to calculate each plant extract's percentage of radical scavenging activity at different dilutions:

Scavenging activity (%) = (Abs control – Abs sample)/ Abs control × 100

Anti-inflammatory activity :

A 1% aqueous solution of bovine albumin fraction and test extracts at various concentrations (20, 40, 60, 80, and 100µl/ml) made up the reaction mixture. A tiny amount of 1N HCl was used to change the pH of the reaction mixture [11]. The typical medication was diclofenac sodium. After 20 minutes of incubation at 37°C, the samples were heated for 30 minutes at 57°C. Turbidity was determined spectrophotometrically at 660 nm after the samples had cooled. Three duplicates of the experiment were conducted. The following formula was used to determine the percentage inhibition of protein denaturation:

$$\text{Percentage Inhibition} = \frac{(\text{A of Control} - \text{A of Sample})/\text{A of Control}}{\text{Concentration of proteins}} \times 100$$

Antibacterial activity :

People have used plants as medicine since the dawn of human civilization. The issues with synthetic antibiotics make it obvious that the use of plants to treat illnesses was unavoidable. Numerous studies used a range of polar chemical solvents, including high (aqueous), to study *Momordica charantia* L. [12]. The conventional agar well diffusion method was used to assess the antibacterial activity of leaf extract preparations. When aqueous extracts were compared to the well diffusion method, the largest inhibitory zones against *Bacillus subtilis, Escherichia coli*, and *Klebsiella pneumoniae* were 11 mm, 22 mm, and 22 mm, respectively. *Momordica charantia* L. demonstrated its antibacterial activity against the pathogens examined when dissolved in DMSO (300 mg/mL) at concentrations of 50, 75, and 100 g/mL (Figure 2, Table-2).

Fig. 2 Antibacterial Activity of *Momordica charantia L* (A) *Bacillus subtillis* (B) *Escherichia coli* (C) *Klebsiella pneumoniae*

Table-2. Antibacterial activity of aqueous *Momordica charantia* L. extract

S. No	Organisms	NC (DMSO)	PC (Gen)	Zone of Inhibition		
				50 µg/ml	75 µg/ml	100 µg/ml
1.	*Bacillus subtillis*	_	5 mm	7 mm	10 mm	11 mm
2.	*Escherichia coli*	_	7 mm	16 mm	18 mm	22 mm
3.	*Klebsiella pneumoniae*	_	15 mm	17 mm	20 mm	22 mm

Gram-positive as well as gram-negative bacteria are susceptible to their antibacterial properties. This study used three different kinds of organisms: *Klebsiella pneumoniae, Bacillus subtilis*, and *Escherichia coli*[13, 14]. For this experimental study, *Escherichia coli* was identified as gram-negative bacteria and *Bacillus subtilis* and *Klebsiella* as gram-positive bacteria.

Antioxidant activity :

Antioxidants shield cells from harm caused by free radicals. It has been demonstrated that antioxidants prevent or lessen the oxidation of other substances. They have the ability to stop chain reactions and prevent oxidation processes by eliminating radical inte rmediates and going through self-oxidation. The body has chemicals that can either prevent the production of free radicals or lessen the harm they cause. Natural antioxidants are widespread in edible materials and medicinal plants. These leaves have a variety of biological effects as polyphenols and carotenoids. It is essential to effectively extract and precisely evaluate antioxidants from food and medicinal plants in order to investigate prospective sources of antioxidants and promote their use in functional foods, medicines, and food additives[15,16].

The aqueous extract of MC powder showed IC_{50} values of 37.4 µg and 57.4 µg for the control ascorbic acid and aqueous extract, respectively, according to the DPPH radical scavenging activity data. Fig. 6 shows

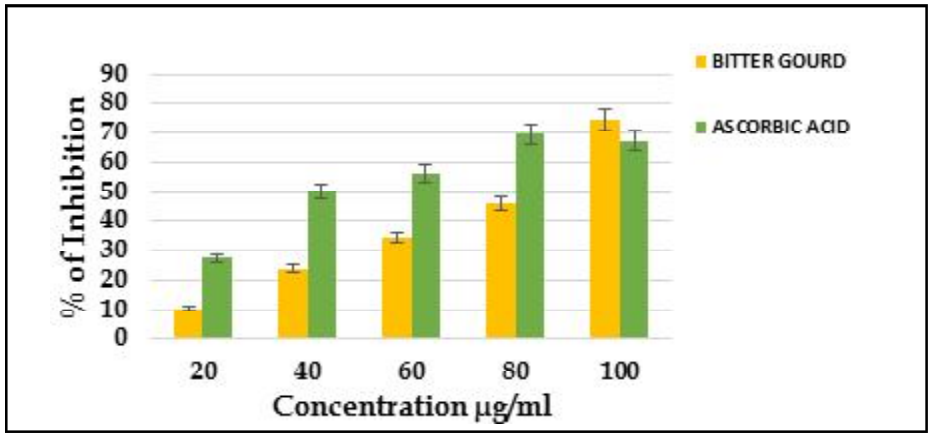

Figure 3. Graphical Representation of aqueous *Momordica charantia* L extract.

antioxidant activity. These extracts have proven to be quite effective at scavenging DPPH radicals. To determine the IC_{50} values, Kovendan, *et al*.,[13] used the concentration of the sample and standard that produced 50% inhibition of free radicals. The DPPH radical scavenging method was used to evaluate the antioxidant effectiveness of *Momordica charantia* leaf extracts. A frequent free radical compound used to evaluate the scavenging activity of different free radical samples is DPPH [16-19]. The approach offers immediate data on the test system's total antioxidant capacity in addition to the benefits of speed, simplicity, and affordability.

Anti-inflammatory activity :

Diclofenac, *Momordica charantia* L. concentrations ranging from 20 to 100 µg/mL, and their interactions were evaluated spectrophotometrically at 660 nm for the prevention of bovine albumin denaturation. Bovine albumin protein denaturation was decreased by *Momordica charantia* L at the lowest dose of 10 g. As sample concentration rose, the proportion of denaturation inhibition grew steadily[20-22]. Diclofenac was shown to have a denaturation-inhibiting effect on the bovine albumin fraction at different doses, while the produced aqueous extract of the source had the same effect (Table-3 Fig. 4).

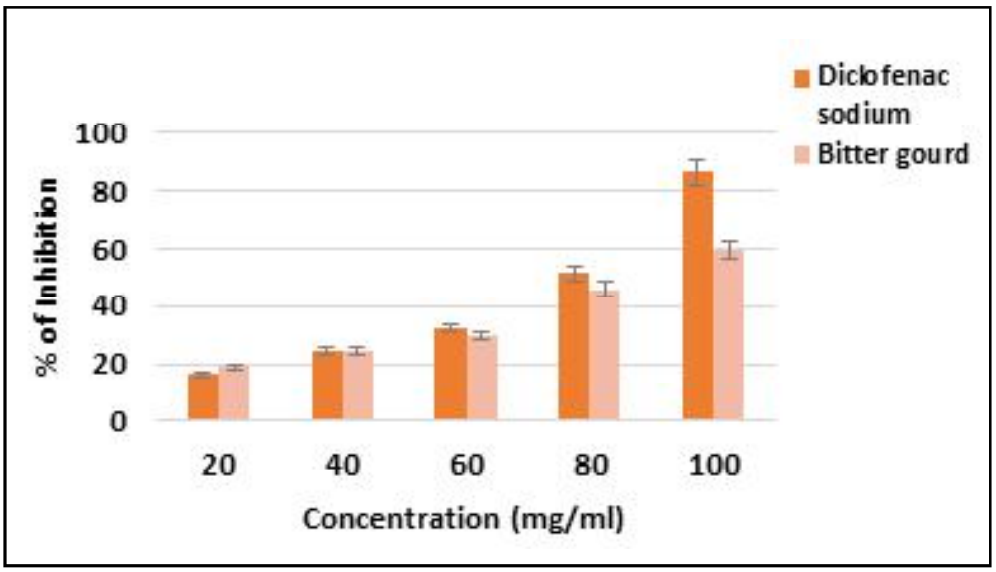

Fig 4. *In-vitro* Anti-inflammatory activity of *Momordica charantia* L.

Momordica charantia L showed a 50% inhibition (IC_{50}) of heat-treated bovine albumin at about 35 µg/ml. Diclofenac sodium treatment showed similar protection against heat-treated bovine serum albumin denaturation; IC_{50} values of 42 µg/ml are shown in Table-3. Perenz *et al.*[22] *used* when compared to conventional diclofenac sodium, Table-3 demonstrated much higher anti-inflammatory activity in a dose-dependent manner. Protein denaturation is 45–90% inhibited by the extract at volumes of 20–100 µl[23].

Table-3. Anti-inflammatory activity of *Momordica charantia* L

Concentration (µg/ml)	Percentage of Inhibition (%) Bitter gourd	Diclofenac sodium
20	18.9	16.21
40	24.3	24.32
60	29.7	32.43
80	45.9	51.3
100	59.4	86.4

In conclusion, Aqueous extract of *Momordica charantia* synthesized using soxhlet method. This Aqueous extract of *Momordica charantia* exhibits potent antioxidant ability by scavenging free radicals and exhibit strong anti-inflammatory effect against protein denaturation. Furthermore these extract exhibits strong antibacterial activity against various microorganisms, including both gram-positive and gram-negative bacteria. And they all showed good activity. Further optimization of this study helpful its potential usage in biomedical applications and environmental remediation.

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Author contributions :

Leelavathy S: Investigation, Methodology, Writing-Original draft, Supervision, Acquisition, Jayathsenayai A: Conceptualization, Investigation, Sheela K: Methodology, Writing review & editing.

Declarations

Conflict of Interest

The authors of this study would like to declare no conflict of interest.

Ethical approval

The experiments carried out in this study did not involve human tissue.

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