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"In Vitro Assessment and Evaluation of the Phytochemicals Analysis and the Pharmacological Activities of methanolic leaf extract of Bangladeshi *Momordica charantia*"

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Abstract
As food and medicinal ingredient, Bitter gourd (Momordica charantia) (belonged to the Cucurbitaceae family), has long been used in several Asian countries. To assess and evaluate the phytochemicals analysis and pharmacological activities of methanolic extract of dried leaves of Momordica charantia, the present study has been conducted, in where we have been used Folin-Ciocalteu's colorimetric assay, aluminium chloride colorimetric assay, Ferrous-ferrozine complex inhibition assay, and agar disc diffusion method. The maximal phenolic contents got was 20.22±0.27 mg of GAE/g of dry extract and the total flavonoid contents got was 38.78±0.74 mg of CE/g of dry extract. The ferrous ion chelating activity was determined by Ferrous-ferrozine complex inhibition assay, by virtue of which we obtained 262.05 µg/ml. In the early situation, this chelating activity rose with the increment extract concentrations, subsequently, it becomes flatter. On the other hand, the Antibacterial efficacy of leaves of Momordica charantia determined against eleven different bacteria, which we obtained in the range of 7-8.5 mm zone of inhibition. Especially, this leaf extract shows a satisfactory antibacterial efficacy on S. paratyphi, ETEC, V. cholerae N-16961, and EPEC. Finally, our findings provided evidence that the crude methanol extract of our tested plant contains medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases, and can even be a brand-new source for several drug discoveries.

Keyword: *Momordica charantia, phytochemicals analysis, pharmacological activity, Folin-Ciocalteu's colorimetric assay, agar disc diffusion method.*

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I. Introduction

The exemplary sources of medicine are plants, that contain many active compounds, which are used to generate several drugs [1]. In recent years, research has been going on to find out the relationships between the bio-activity of plants and the prevention of several diseases including, Cholera, Salmonellosis, Cardiovascular disease, Cancer, Diabetes, Thalassemia, etc., which has been increasing sharply. Researchers have been trying to isolate many active compounds of medicinal plants, which can be used as some therapeutic agents. These active compounds are known as phytomedicinal or secondary plant products, which are found in nature. According to their chemical classes, these can be classified into several groups including phenolics, flavonoids, chelating agents, alkaloids, terpenoids, steroids, glycosides compounds, etc. The concrete significance of these compounds is indispensable, due to their antioxidants, antimicrobials [2, 3], anti-cardiovascular diseases, anti-viral, anti-cancer, anti-mutagenic, anti-inflammatory, metal ion chelating, anti-analgesic, anti-neurodegenerative disease, anti-diabetic activities etc. Flavonoid compounds act as anti-cancer, antioxidant, anti-allergic, anti-viral, anti-inflammatory [4], antimicrobial, and anti-cardiovascular diseases agents, etc. In the same way, phenolic

compounds show antioxidant, anti-mutagenic, anti-carcinogenic, anti-inflammatory, hormonal action, enzyme stimulator, antiaging activities [5, 6] *etc.*

However, thalassemia which has ultimately happened by the Oxidative stress [7-9]. To improve both qualities of life and survival, thalassemia patients always require blood transfusions but a human cannot eliminate the excess iron from the body, which is further deposited as ferritin and hemosiderin in the spleen, liver, myocardium, and endocrine organs. Tissue damage is happened by the accumulation of toxic quantities of iron, which leads to complications such as heart failure, endocrine abnormalities like hypothyroidism, diabetes, liver failure, and ultimately early death [10-12]. Several conditions like anaemia, iron overload, potentiating of ROS, and damage to major organs (especially the cardiovascular system) characterize thalassemia major [13]. Iron chelators can form soluble and stable complexes with tissue irons, which are then excreted by the urine, or faces. Additionally, the dysregulation of brain iron, and its association with amyloid precursor protein plaque formation that is involved in Alzheimer's Disease (AD) pathology. Because of that, iron chelation can be considered as a rational therapeutic strategy for AD [7, 13]. In reducing iron-related complications, Chelation therapy can be used and which will improve quality of life, and overall survival but its lower oral bioavailability, short plasma half-life, and severe side effects are still not optimal [8, 9, 14-18].

Microbes have recently become resistant to the common antimicrobial drugs, which are getting life-threatening to humans day by day. To prevent the growth of genetically resistance microbes, the development of new drugs is a burning question in the whole world. Moreover, compared to commercial antibiotics, natural phytochemicals are used as an alternative remedy for the treatment of various diseases due to their more effective and lower side effects. Motivated by these needs, we should develop antimicrobial ingredients from medicinal plant sources to overcome these situations. Searching for antimicrobial properties in plant products has recently intensified, along with phytochemicals are used as antimicrobial agents in the medical field right way [18, 19]. Our plant extracts were conducted to investigate the antibacterial activity against selected pathogenic microbes that cause human skin, and diarrhoea disorders.

As food and medicinal ingredient, Bitter gourd (*Momordica charantia*) (belonged to the Cucurbitaceae family), has long been used [20], that's known as by completely different names since it grows in tropical regions. In the treatment of Anorexia, Blood Impurities, Diabetes Mellitus, Diarrhoea, Skin Infections, ulcer, gout, rheumatism, Respiratory Problems, & Pyorrhoea, the bitter gourd has been reported to use as a drug [21], and also used as antidiabetics, carminative, anti-colics, antibilious, emetic, and anthelmintic agent. [22] However, there has been very little info regarding the quantitative analysis of phytochemicals, Ferrous ion chelating, and antimicrobial activities of it in methanolic extracts.

In ancient civilization, Plant extracts were used to the treatment of various ailments. Even today, these materials stay a vital resource for combating diseases. Because of that, the present study will conduct to investigate the Phytochemicals Analysis (total phenolic and total flavonoid contents) and the Pharmacological Activities (antibacterial efficacy and ferrous ion chelating activity), by using several in vitro methods.

II. Materials and methods

2.1 Collection of Plant Materials

The leaves of *Momordica charantia*(MC) [Bitter gourd or Bitter melon] were collected from the local area (Santosh in Tangail district in Dhaka province of Bangladesh).

2.2. Chemicals and Reagents

Folin-Ciocalteu's (FC) phenol reagent [Merck KGaA, Germany], Gallic acid, Catechin hydrate [Japan], Sodium carbonate anhydrous, Aluminium chloride [Mumbai, India], Sodium nitrite [China], Sodium hydroxide [Mumbai, India], Methanol [Merck KGaA, Germany],Mueller Hinton Agar (MHA) [Becton, Dickinson and Company, USA],Mueller Hinton Broth (MHB) [Himedia, India],Standard antibiotic disc (Ampicillin, Azithromycin, Kanamycin) [Himedia, India]**,**Ferrozine monosodium [India]**,** Ferrous (II) sulfate [Mumbai, India]**,** Ethylenediaminetetraacetic acid (EDTA), Ethanol [Merck KGaA, Germany],deionized distilled water and Dimethyl Sulphoxide (DMSO) [Merck KGaA, Germany]used for the assessment of total phenolic and flavonoid contents, Ferrous ionchelating activity, and antimicrobial efficacy. All of these used were of analytical grade.

2.3. Preparation of leaf Extracts

2.3.1. Preparation of powder: Consideringthe uniform size and colour of leaves, bitter gourd (MC) leaveswere chosen, which were manually removed from the stem and washed in tap water, and then dried under the shade at RT for 15 days. Then, the dried samples were crushed by using an electric blender. To an average particle size of 107 µm, crushing samples were sieved, and stored in an airtight container prior to the extraction process and kept under normal RT until required.

2.3.2. Preparation of leaf extracts in methanol solvents: Each 40 g of MC powder was subjected to solid-liquid extraction by using 100% methanol solvent, in where they were maintained with solid to the solvent ratio 1: 10 (w/v) and kept at RT for 1 day. After 1 day, the solvent extracts were continuously swirled at 150 rpm in an orbital shaker for 2 hours at RT, and then transferred into falcon tubes for good rupturing the cell wall of cells, and well dissolved the components into the solvents, by centrifuge machine at 8000 rpm for 15 minutes. Using What man filter paper (120 mm) number 1, the sample extracts were filtered, and further re-filtered twice following the same procedure. The collected filtrates were concentrated by rotary evaporator, removing the entire methanol solvent. Finally, the crude extracts were formed and then stored at -20 °C until further analysis.

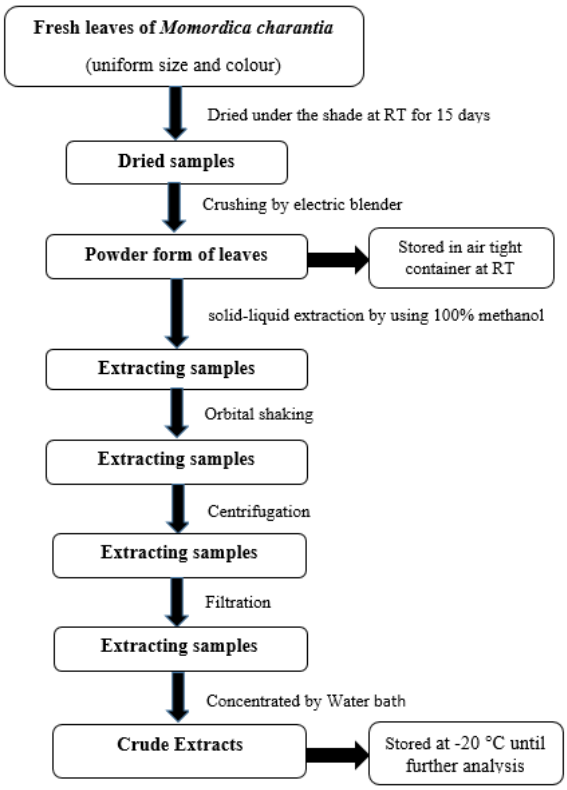

Figure 1. Schematic outline of the extraction protocols used in this study

2.4. Quantitative Analysis of Phytochemicals

2.4.1. Determination of total phenolic contents (total reducing capacity) in the extract

Singleton and Rossi described Folin Ciocalteu's (FC) reagent method [23], which was used to evaluate the total phenolic contents of crude extracts. Firstly, the stock solutions of plant extract and standard samples were prepared in methanol solvent (2 mg/ml). To get the concentration of 75 µg/ ml, MCleave extracts were diluted with methanol. Then, 1 ml of the diluted MeMC sample was added to a 15 ml test tube.To that 5 ml of FC reagent (10%) and 4 ml of 7% Na_2CO_3 were added to get a total of 10 ml solution. The mixture showed a blue colour that was shaken vigorously and then incubated for 30 minutes at 40 º C in a water bath. Finally, the absorbance was measured at 760 nm against a blank (containing all reagents except sample or standard). The total phenolic contents were calculated by the use of standard calibration curves of Gallic acid and Catechin. All the reaction was conducted in three times. The results were expressed as mg of GAE (Gallic equivalents)/g of dry extract or mg of CE (Catechin equivalents) /g of dry extract (mg/g).

2.4.2. Determination of total flavonoids content in the extract

Acharya P. described aluminium chloride colorimetric assay [24], which was used to determine the total flavonoid contents of crude extracts. Firstly, the stock solutions of plant extract and standard samples were prepared in methanol solvent (2 mg/ml). To get the concentration of 400 µg/ ml, MC leave extracts were diluted with methanol. Then, 1 ml of the diluted MeMC sample was added to a 10 ml test tube (containing 4 ml of double-distilled water). At the zero time, 0.3 ml, 5% sodium nitrite was added to the flask. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added to the flask. At 6 minutes, 2 ml of 1 M NaOH was added to the mixture. Immediately, the total volume of the mixture was made up to 10 ml by the addition of 2.4 ml double-distilled water and mixed thoroughly. The mixture showed a pink colour that was shaken smoothly. Finally, the

absorbance was measured at 510 nm against a blank (containing all reagents except sample or standard). The total phenolic contents were calculated by the use of standard calibration curves of Catechin. All the reaction was conducted in three times. The results were expressed as mg of CE (Catechin equivalents) /g of dry extract (mg/g).

2.5. Pharmacological activities

2.5.1. Determination of Ferrous ion chelating activity

Carter *et. al* [25]and Yan *et. al.* [26] described the iron (II)– ferrozine complex method, which was used to evaluate the ferrous ion chelating activity of crude extracts at 562 nm. Firstly, solutions of 0.125mM and 0.3125 mM ferrozine were prepared. The stock solutions of plant extract and standard EDTA were prepared in the concentration of 0.50 mg/ml in a volumetric flask. To get the concentration of 20-800 µg/ ml, MC leave extracts were diluted with methanol. Then, 1 ml of the diluted MeMC sample was added to a 10 ml test tube, and then it was with 1 mL of 0.125 mM $FeSO_4$ and 1 mL of 0.3125 mM ferrozine. Then, the mixturewas shaken vigorously, and then incubated for 10 minutes at RT. Finally, the absorbance was measured at 562 nm against a blank (containing the same components as stated above but the extracts were replaced with deionized distilled water). Under the same conditions as the test samples, the blank was incubated. EDTA was used as a positive control. All the reaction was conducted in three times.

The percentage inhibitions of ferrozine (Fe2+) by the extracts & standard were determined using the following expression.

$$\textbf{Chelating Effect (\%) or Rate} = \left(\frac{\textbf{Acontrol}-\textbf{Bsample}}{\textbf{Acontrol}}\right) \times 100$$

2.5.2. Antimicrobial susceptibility assay

2.5.2.1. Test microorganism

In antimicrobial efficacy, the crude extract of MeMC was nominated and tested individually against eleven microorganisms (Table 1), which were gram-positive and the remaining were gram-negative. All bacterial strains were obtained from the Department of Biochemistry & Molecular Biology, Mawlana Bhashani Science and Technology University, Santosh, Tangail, Bangladesh. The following bacterial strains were used in our antimicrobial study.

Table 1. Name of the Test microorganisms used in the experiment

Gram positive bacteria	Gram negative bacteria
1. Streptococcus mutans *2. Staphylococcus epidermidis* *3. Staphylococcus aureus*	*Salmonella typhi* *Salmonella paratyphi* *Pseudomonas aeruginosa* *Klebsiellapneumoniae* *Vibrio cholerae N-16961* *Vibrio cholerae C6706* *Enterotoxigenic Escherichia coli (ETEC)* *Enteropathogenic Escherichia coli (EPEC)*

2.5.2.2. Preparation of fresh culture and Test plates

100 µl of each test organisms (previously prepared) was transferred to different MHB slants (containing 2 ml) in aseptic condition, and then were incubated 150 rpm at RT for 24 hours to assure the growth of test organisms, which were latterly used for the Antimicrobial Susceptibility Assay. To give a uniform depth of approximately, the agar plates were prepared, which contained 30 ml of MHA medium.

2.5.2.3. Preparation of MeMC sample with DMSO solvent.

100 mg/ml stock solution was prepared, in which 100 mg of sample extracts and 1ml of pure DMSO was present, which was mixed by the vortex machine. To get the concentration of 25 mg/ ml, MC leave extracts were diluted with DMSO.

2.5.2.4. Determination of inhibitory effects

The antimicrobial activities of MeMC leaves were tested by agar disc diffusion bioassay [27]. In the disc diffusion method, bacteria were cultured in MHB overnight and spread with a sterile cotton swap into agar plates, and then filter paper discs (5mm in diameter) impregnated with the plant extract were placed on the cultured plates and incubated at 37°C for 24 hours. Each of the discs contained 25 µl of sample or DMSO. Kanamycin, azithromycin & Ampicillin were applied as standard antibiotics. In measuring the diameter of zones

of inhibition, the antimicrobial activity was assessed at cross angles after incubation and then compared with the zone of inhibition of the standard antibiotics. The experiment was done twice.

2.6. Statistical Analysis

Using Microsoft Excel 2016, the statistical mean was calculated with ± SD and regression analysis was performed to calculate the dose-response relation between the extracts. Then, to find out the Pearson's correlation coefficient, Linear regression analysis was performed. Finally, the statistical significance of the assessments was evaluated based on 95% of the confidence limits employing P-value, which was declared at $p < 0.05$.

III. Result

3.1. Quantitative analysis of phytochemicals

3.1.1. Evaluation of total phenolic contents (total reducing capacity) in the sample: The leaves of *Momordica charantia*display the total phytochemical constituents like phenolic and flavonoid, which was carried out by our research work. Folin-Ciocalteu's colorimetric method was used to determine the total phenolic contents in plant extract, where standard calibration curves of Gallic acid & Catechin (Figures 2 & 3) used for getting the concentration value of the extract. The results of the total phenolic contents of leaf extracts are shown in Table 2 and Figure 5.

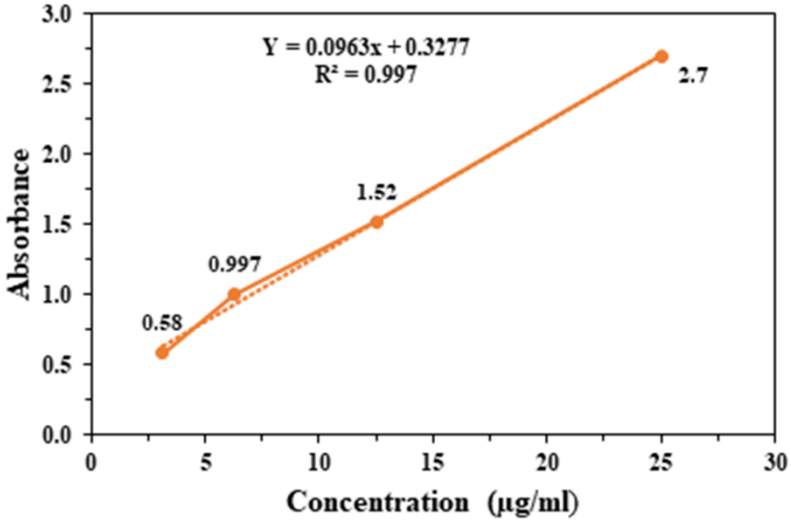

Figure 2. Standard calibration curve of Gallic acid for the assessment of total phenolic. R^2 values represented mean data (n = 3) at concentrations of 3.125 - 25 µg/ml. Means were significantly different at the level of P [0.001] ≤ 0.001.

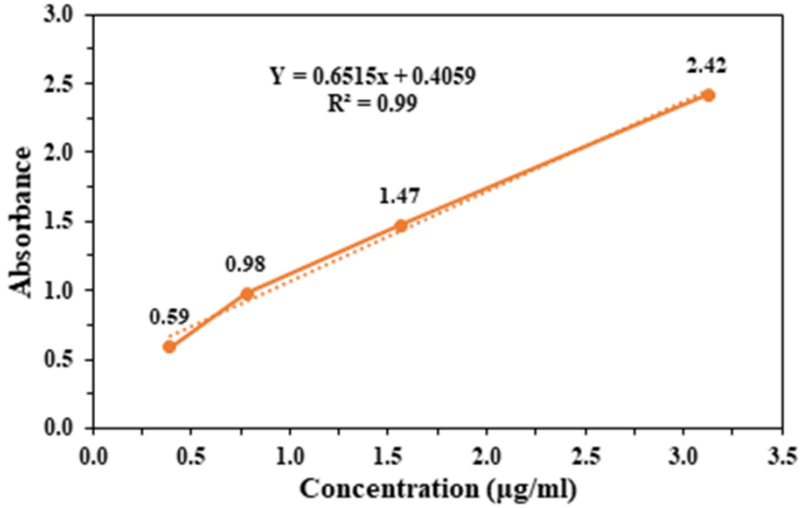

Figure 3. Standard calibration curve of Catechin for the assessment of total phenolic. R^2 values represented mean data (n = 3) at concentrations of 0.391-3.125 µg/ml. Means were significantly different at the level of P [0.003] < 0.005.

From the regression equationY=0.0963x+0.3277, R^2=0.997 of Gallic acid and Y= 0.6515x+0.4059, R^2=0.99of Catechin, the concentration of phenolic contents in MeMC leaves were calculated individually. The total phenolic contents were calculated using the following expression and expressed as mg Gallic acid equivalents (GAE) per g of extract (in mg/g) and mg Catechin equivalents (CE) per g of extract (in mg/g).

$$TPC=\frac{C\times V}{M}$$

Here, TPC= total phenolic contents in mg/g (mg GAE/ g or in mg CE /g). C= concentration of sample extracts established from the calibration curve in mg/ml. V= the volume of extract in ml & M= the weight of plant extract in g.Finally, the TPCs of MeMC are gotten as 20.22±0.27 mg of GAE/g of dry extract &1.48±0.04 mg of CE/g of dry extract.

3.1.2. Evaluation of Total Flavonoid Contents in the sample: Aluminium chloride colorimetric assay were used to determine the total flavonoid contents in plant extract, where the standard calibration curve of Catechin (Figure 4) used for getting the concentration value of extract. The results of the total flavonoid contents of leaf extracts are shown in Table 2 and Figure 5.

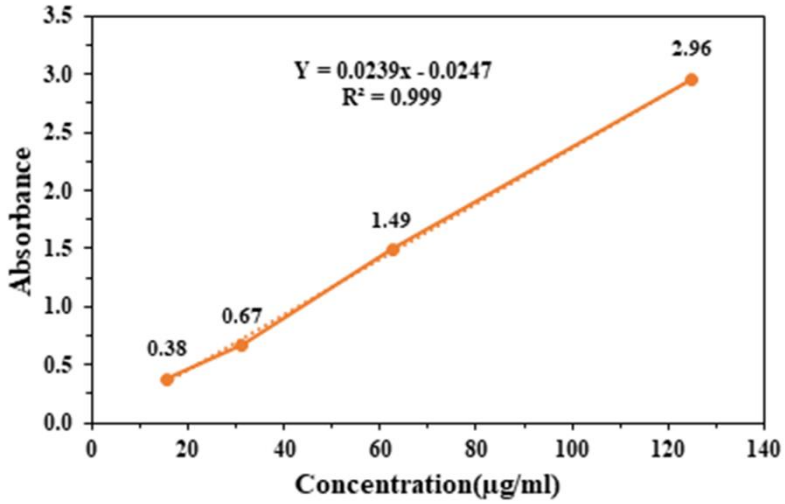

Figure 4. Standard calibration curve of Catechin for the assessment of total flavonoids. R^2 values represented mean data (n = 3) at concentrations of 15.63-125 µg/ml. Means were significantly different at the level of P [0.0005] ≤ 0.0005.

From the regression equation Y=0.0239x-0.0247, R^2=0.999 of Catechin, the concentration of flavonoid contents in MeMC leaves was calculated individually. The total flavonoid contents were calculated using the following expression and expressed as mg Catechin equivalents (CE) per g of extract (in mg/g) as we mentioned earlier.

$$TFC=\frac{C\times V}{M}$$

Finally, the TFCs of MeMC are gotten as 38.78±0.74 mg of CE/g of dry extract.

Table 2. Total phenolic and flavonoid contents in the extracts

Plant parts	**Total Phenolic contents**		**Total Flavonoid Contents**
	100% methanol mg GAE/ g of dry extract (Mean TPC± S.D)	**100% methanol mg CE/ g of dry extract (Mean TPC± S.D)**	**100% methanol mg CE/ g of dry extract (Mean TFC± S.D)**
Leaves extract of MeMC	**20.22±0.27**	**1.48±0.04**	**38.78±0.74**
Values are mean ± S.D, n=3			

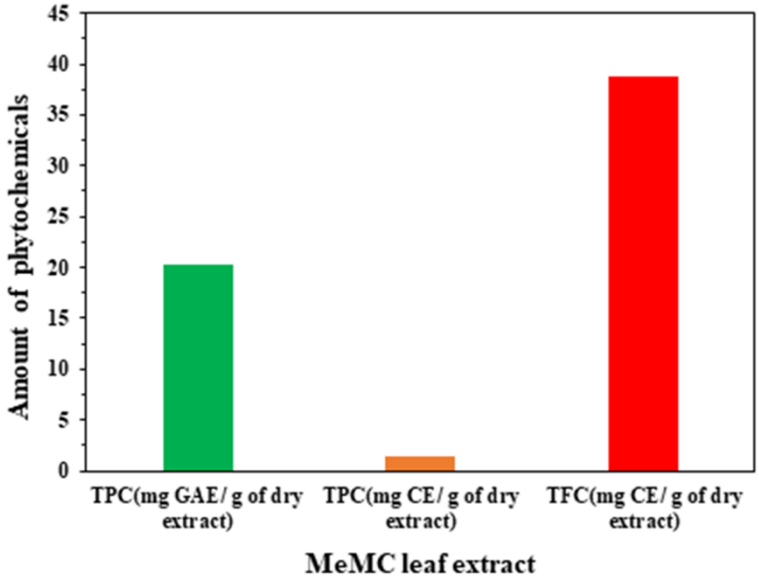

Figure 5: Comparison of total phytochemical constituents of MeMC leaf extract. Values are expressed as mean with standard error (n = 3) which were significantly different at the level of P < 0.01.

3.2. Pharmacological activities

3.2.1. Ferrous ion chelating activity of the extracts:

Iron chelating activity by the extract was assessed by Dinis *et al*. [28]. To evaluate the chelating effects MeMC leaf extracts, the chelating rate was investigated, according to Carter *et. al.* [25]and Yan *et. al.* [26]. The results of ferrous ion chelating activities standard EDTA and MeMC leaf extract are shown in (Figure 7, and Table 3).

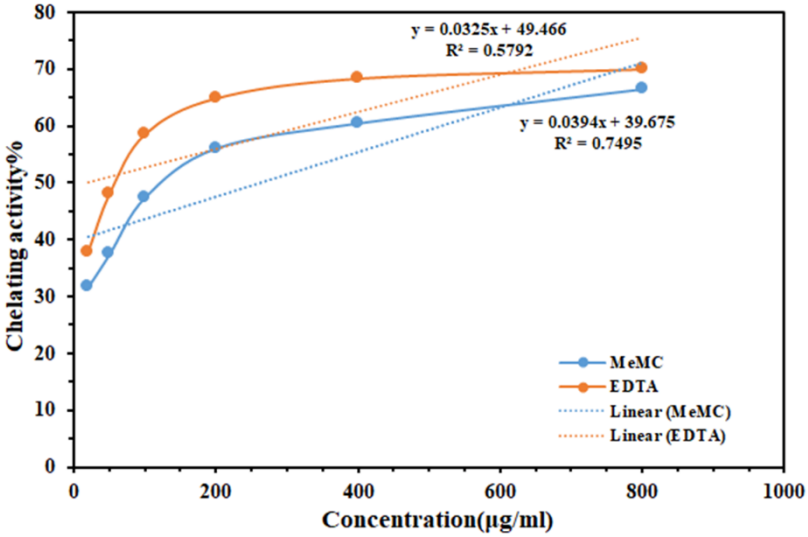

Figure 6: Comparison of Ferrous ion chelating activity of MeMC & EDTA as a reference standard at concentrations (20-800µg/ml).

The relative activities of Ferrous ion chelating of leave extracts of bitter gourd against the control (EDTA) are shown in Figures 6 and 7. Table 3 and Figure 7 are shown the result of IC_{50}values. This result suggests that MeMC is the active extracts interfered with the formation of ferrous and ferrozine complex, and captures ferrous ion as an iron chelator. And the IC50 value is 262.05µg/ml for MeMC and for standard chelator EDTA has the value 18 µg/ml.

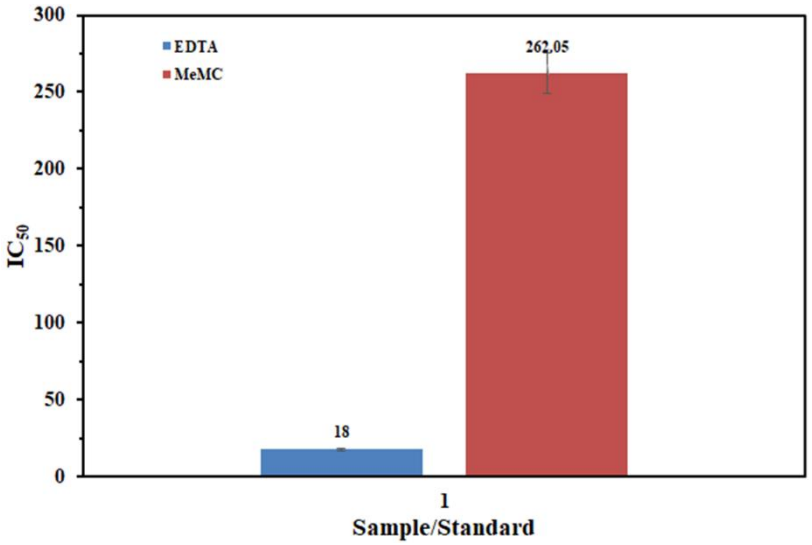

Figure 7: Comparison of IC_{50} of MeMC leaf extract and standard EDTA. Results were expressed as the mean with standard error (n = 3) at concentrations of 20- 800 µg/ml.

Table 3. Chelating activity of methanol solvent extracts of the leaf sample of MC.

Specimen	Solvent	Concentration (µg/ml)	Inhibition % (Mean±SD)	Linear Equation (Y=mx+C, R^2)	IC_{50}(µg/ml)
Leaf of ***Momordica charantia***	Methanol	20	31.74±0.24	y = 0.0394x + 39.675 R^2 = 0.7495	**262.05**
		50	37.53±0.14		
		100	47.51±0.49		
		200	56.10±0.37		
		400	60.51±0.42		
		800	66.49±0.28		

Values are mean ± S.D, n=3. Means which were significantly different at the level of P[0.04] < 0.05

3.2.2. Antibacterial efficacy

In antimicrobial efficacy, the crude extract of MeMC was tested individually against eleven bacteria at concentrations 625 µg/ disc. Standard antibiotic disc Kanamycin (30µg/disc), Ampicillin (25µg/disc) & Azithromycin (30µg/disc) were used for comparison. The results obtained are shown in the Table: 4. From Table 4, the zone of inhibition for MeMC against bacteria *S. paratyphi, ETEC, V. cholerae N-16961, EPEC, S. typhi, V. cholerae C6706, S. epidermidis, P. aeruginosa, and K. pneumoniae* are gotten 8.5,8.5,8.5,8,7.5,7.5,7.5,7 and 7 mm respectively. However, there is no significant antibacterial activity shown against gram positive bacteria such as *Streptococcus mutans, Staphylococcus aureus*. species.

Table 4. In vitro antimicrobial activity of MeMC leaves & standard antibiotics against gram positive and gram negative pathogens.

Name of Bacterial Strain	Zone of inhibition of sample extract/ standard antibiotic disc (mm)				
	Sample of MeMC leaves	DMSO	Standard Antibiotics		
	Conc. (625 µg/disc)		K (30µg/disc)	AZM (30µg/disc)	AMP (25µg/disc)
S. paratyphi	8.5±0.5	7	15.5±0.5		Resistant
S. typhi	7.5±0.5	6	18±0		Resistant
V. cholerae C6706	7.5±0.5	5.5	16±0		Resistant
V. cholerae N-16961	8.5±0	7	15.5±0.5		Resistant
ETEC	8.5±0.5	6.5	16.5±.0.5		Resistant
EPEC	8±0	6	15.5±0.5		Resistant
S. aureus	7±0	7		19±1	Resistant
S.epidermidis	7.5±0	5.5		20.5±0.5	Resistant
S. mutans	7.5±0.5	7.5		21.5±0.5	Resistant
P. aeruginosa	7±0	5.5		19.5±0.5	Resistant
K.pneumoniae	7±0	6.5		18.5±0.5	Resistant
Values are mean ± S.D, n=2					

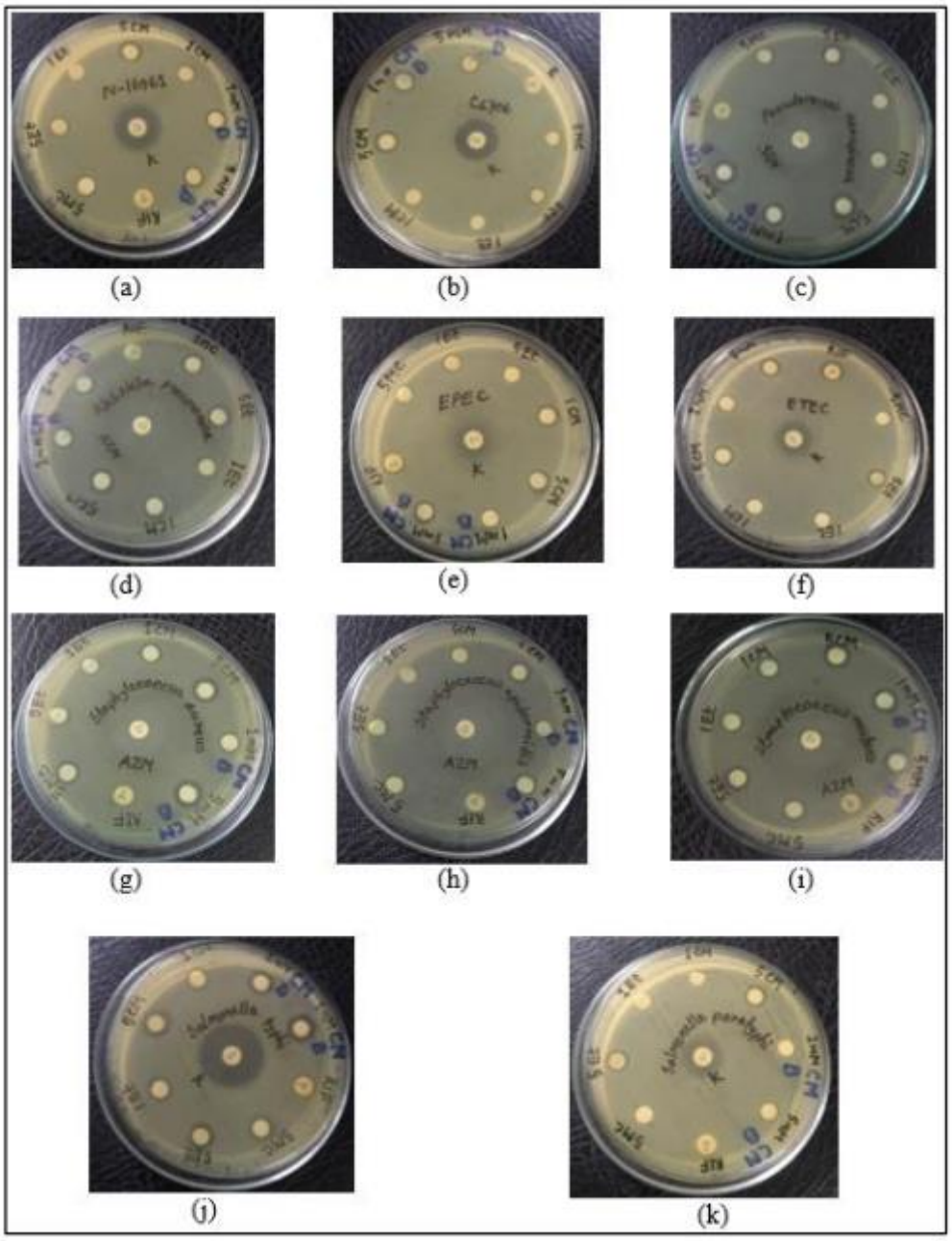

Figure 8: Antimicrobial activity of MEMC leavesagainst gram (–) & gram (+) strain. JL= *Citrus maxima*, KL=*Momordica charantia*, ERY=Erythromycin, AMP=Ampicillin, DMSO=Dimethyl sulfoxide, K= Kanamycin

IV. Discussion

Phenolic compounds and flavonoids are the important components of *M. charantia* [29, 30]. There are varieties of phenolic acid constituents and those constituents were distributed in various amounts for each phenolic acid among a variety or parts of tissues. In M. charantia flesh, the main phenolic acids were gallic acid, gentisic acid, catechin, chlorogenic acid and epicatechin, and ranged from 8.04 to 39.76, 16.99 to 32.39, 23.06 to 82.45, 4.55 to 15.83, and16.14 to 44.28 mg/100 g dry material [31].

Catechin and epicatechin are the two most common flavonoids in plants. Budrat and Shotipruk [32] revealed that catechin is the chief phenolic acid contained in bitter melon (46.16 mg/g dry weight, 72–86% of the total phenolic contents) from the extracts obtained by subcritical water extraction, followed by gentisic acid (4–12%), gallic acid (0.25–0.87%) and chlorogenic acid (0–0.26%), respectively [33]. In our research we have also found that catechin contents is high in *M. charantia* that is 38.78±0.74 mg of CE/g of dry extractfor the

assessment of total flavonoids and it's gallic aciid contents is also high that is 20.22±0.27 mg of GAE/g of dry extract for the assessment of total phenolic acid.

Ferrozine monosodium can form a complex with Fe^{2+} in quantitatively but in the presence of chelating agents the complex formation is disrupted, as a result, the red colour of the complex is decreased. The estimation of the chelating activity of the co-existing chelator is allowed by the measurement of colour reduction. The transition metal ion (such as Fe^{2+}), which has the ability to move a single electron, can start with relatively non-reactive radicals, and latterly allow the formation and propagation of many radical reactions [34]. The IC_{50} value for the chelating activity of the extract is 262.05 µg/ml, which is lower than the positive standard EDTA (IC_{50} = 18 µg/ml). In the early situation, this chelating activity rose with the increment extract concentrations, subsequently, it becomes flatter. The IC_{50} values revealed that MeMC is the active extracts interfered with the formation of ferrous and ferrozine complex and captures ferrous ion as an iron chelator (Table 3). The IC_{50} value for chelating activity of the extract is lower than the positive standard EDTA (Figure 7) but the chelating activity increases with extract concentrations.

On the other hand, the aqueous extract from M. charantia seed exhibited significant antimicrobial activity against several bacteria in the following ascending order: *P. multocida*, *S. typhi*, *S. epidermidis* and *L. bulgaricus*. As for the ethanolic extract, the sequence was S. aureus, M. luteus, E. coli, S. epidermidis and *L. bulgaricus*, while n-hexane and petroleum ether extracts were effective against S. aureus [35]. *M. charantia* pulp extract has been proven to have broad-spectrum antimicrobial activity [36], the same as the hydrophilic leaf extracts, which exhibited antibacterial activities against *E. coli*, *Staphylococcus*, *Pseudomonas*, *Salmonella* and *Streptobacillus*. This may be attributed to 5-a-stigmasta-7, 25-dien-3-b-ol, elasterol and lanosterol[37]. Ethanol extracts of *M. charantia* leaves exhibit inhibition on *B. cereus* and *S. aureus*. The ethanol fraction has no apparent effect on *E. coli*, which is in contrast with the treatment of the ethyl acetate extracts [38]. Methanolic extract from M. charantia leaves showed the strongest antibacterial activity amongst several organic solvent extracts, with a significant inhibitory effect on *E. coli* and *S. aureus* [39]. On the other hand, in our study the MeMC leaf extract shows a satisfactory antibacterial efficacy on *S. paratyphi, ETEC, V. cholerae N-16961, and EPEC*. Table 4 and Figure 8 indicates that the different cultures respond to MeMC leaf extract in a variable manner, resulting in zones of inhibition of 7–8.5 mm. On the other hand, the standard antibiotic disc of Kanamycin shows more susceptible to *Salmonella typhi, Salmonella paratyphi, V. cholerae N-16961, V. cholerae C6706, ETEC, EPEC*. Likewise, Azithromycin shows susceptible to *S. aureus, S. epidermidis, S. mutans, P. aeruginosa and K.pneumonia* but zones of inhibition are vague. However, Ampicillin is resistant to those bacteria.

V. Conclusion

In the present studies, the significant amount of phytochemicals (flavonoid, phenolic), which can be responsible for the activity of antibacterial and also have significant amount of ferrous ion chelating of the leaf extract of *Momordica charantia*. Additionally, these findings allow us to aggregate that *Momordica charantia* leaf could be a sensible natural source of flavonoid, phenolic, antibacterial, and ferrous ion chelator. Likewise, the flavonoid contents of this leaf may be a good substitute for synthetic ones. Bitter gourd blossom does exhibit some significant antimicrobial activity against a few tested microorganism cultures including *S. paratyphi, ETEC, V. cholerae N-1696,* and *EPEC* so, it can act as antimicrobial agents to some selected microorganism. Further experiments are necessary to do on these extracts to isolate active compounds and verify the relationship between chemical composition, and these activities, by virtue of which could be treated against some infectious diseases.

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Author's contribution

Hossain Md. Amjad,Shamima Afroze, and Rahaman MFoysol collected the data and performed the statistical analysis and also prepared the figure and table. The whole manuscript, figures and tables were again checked and corrected by Nishat Akther, Khairul Islam and Mohammad Nazmul Hossain. NA conceived the study and designed the experimental procedures. NA supervised the study andHossain MAmjadwrote the manuscript. All authors read and approved the manuscript.

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