Original Article

Extraction, Formulation And In-Silico And In-Vitro Antimicrobial Evaluation Of Ethanolic Extract Of Plant Momordica Dioica

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Abstract

In the treatment of infectious diseases, the rapid emergence of microbial resistance to traditional antibiotics has been a major source of concern. Phytochemicals have demonstrated potential antimicrobial effects against both susceptible and resistant microorganisms. Momordica dioica ethanolic extract was tested against a wide range of pathogenic microorganisms like Escherichia coli (2931), Bacillus cereus (2217), Staphylococcus aureus (5345) and Salmonella typhi (2501) to establish their antimicrobial activities and shown moderate activity as compare to ciprofloxacin as a standard against most of the tested microorganisms. The phytoconstituents found present in the plant extract are Alkaloids, Tannins and Phenols. The molecular docking studies of momordicine, an active constituent of extract was executed with PDB ID-4PSS, and found the similar affinity of momordicine towards the receptor, like Cocrystallized ligand and standard ciprofloxacin. The antimicrobial activity of ethanolic extract of Momordica dioica at concentrations (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) in the present study showed a prominent zone of inhibition against B. cereus, S. typhi, E. coli, and S. aureus are and the found IC_{50} values are 29.27**,** 41.35, 43.76, and 46.14 respectively. The MIC for B. cereus, S. typhi, E. coli, and S. aureus are 1µg/ml, 2µg/ml, 4µg/ml, and 16µg/ml, respectively. The activity against all the pathogenic bacteria was found at every concentration of the test solutions where the least activity was shown against S. aureus and most activity was shown by B. cereus. The Momordica dioica ethanolic extract formulations (lotion and ointment) were prepared and evaluated. The stability of the lotion and ointment were confirmed using TLC and IR spectroscopy.

Keywords: antimicrobial activity, Momordica dioica, Ciprofloxacin.

1. Introduction

Momordica dioica, sometimes referred to as Kantola, spiny gourd, or teasle gourd, is a dioecious perennial cucurbitaceous climbing creeper. It has a substantial population in Bangladesh and India and is of Asian heritage. It has been used for numerous years as a vegetable high in nutrients as well as a preventative and therapeutic agent for a number of diseases. Kakrol is viewed as an underappreciated vegetable despite having better nutritional content than many commonly consumed vegetables and an excessive amount of certain chemicals.[1-4]. Additionally, because it is a classic medicinal plant, there is still a chance that its phytochemical components will raise the need for a more thorough assessment to support its other therapeutic functions. As a result, this endeavor will be beneficial to scientists who want to reveal Momordica dioica's unjustified phytotherapeutic significance. Hederagenin, momordicine, momordicaursenol, momordicafoetid, cucurbitacins, and cucurbitane make up the majority of its chemical makeup. The leaves of it are anthelminthic, aphrodisiac, anti-hemorrhoidal, hepatoprotective, anti-bronchitis, antipyretic, anti-asthmatic, and antipyretic, whereas its fruits are diuretics, laxative, hepatoprotective, antivenomous, antihypertensive, anti-inflammatory, anti-asthmatic, antipyretic and anti-leprotic[5-7]. Ciprofloxacin and other newer quinolone antibacterial treatments outperform older equivalents such as nalidixic acid in terms of potency and frequency of spontaneous bacterial resistance[8].

2. Experimental

2.1. In-silico studies

A type of computational simulation of complexes created by the interaction of a number of molecules is known as molecular docking. Based on the binding characteristics of the involved ligand and target molecules, it anticipates the three-dimensional arrangement of adducts. The score function in the molecular docking tool's software ranks and groups the various potential candidate structures that molecular docking creates into categories. Based on the system's total energy, docking simulations forecast an optimum docked conformer[9]. Predictions of protein-ligand binding made with computer assistance are helpful while looking for new drugs. A grading mechanism and an algorithm for searching are often the two basic components of protein-ligand docking algorithms. Independent of conformational investigation, it is interesting to assess the intrinsic effectiveness of scoring functions in order to identify their advantages and disadvantages and make suggestions for improvement[10]. A molecular docking analysis of the drug extract was carried out on Intel Core i3 11th Generation CPU, with 8 GB RAM and SSD system, to learn more about its possible mechanism of action. All of the sketched compounds were prepared for docking investigations using the UCSF Chimera program. The ligands were then desalted, and tautomer were created. Certain chirality's were preserved, and stereoisomers were produced for each ligand. The 3D structure of the produced ligand molecules was changed into a 2D structure that reduced energy and was then used for docking. The protein verified as an antimicrobial target's X-ray crystal structures were obtained from the protein data library (PDB). The antibacterial receptor used is 4PSS. The receptor was chosen among variety of receptors acting on certain bacteria used in the present study.

Momordicine was found to resemble a co-crystallized ligand that exhibits interaction with the MET1 amino acid residue by hydrogen bonding with MET1 residue, which is how the molecule primarily connects with the target enzyme (see Figs. 3 and 4). Additionally, Van der Waals and hydrophobic interactions were implicated. The docked target compound's binding affinity value was discovered to be -12.7 kcal mol^{-1}. The findings showed that the acetyl's -C=O, the ring's Ar-OH substituents, and the pyran ring's 'O' exhibit significant hydrogen bonding interactions with the residues of amino acids of the protein 4PSS's A chain, which demonstrates its superior antibacterial efficiency. These findings suggest that momordicine exhibits antibacterial properties in a manner that is probably similar to the co-crystallized ligand. The momordicine exhibits a higher binding score than the co-crystallized ligands, which may inhibit several bacterial proteins, according to the docking analysis.

Sr. No.	Docking Score (kcal/mol)		
	Ciprofloxacin	Momordicin	Co-crystallized Ligand
1	-12.8	-12.7	-12.4

Table No. 1: Docking Scores

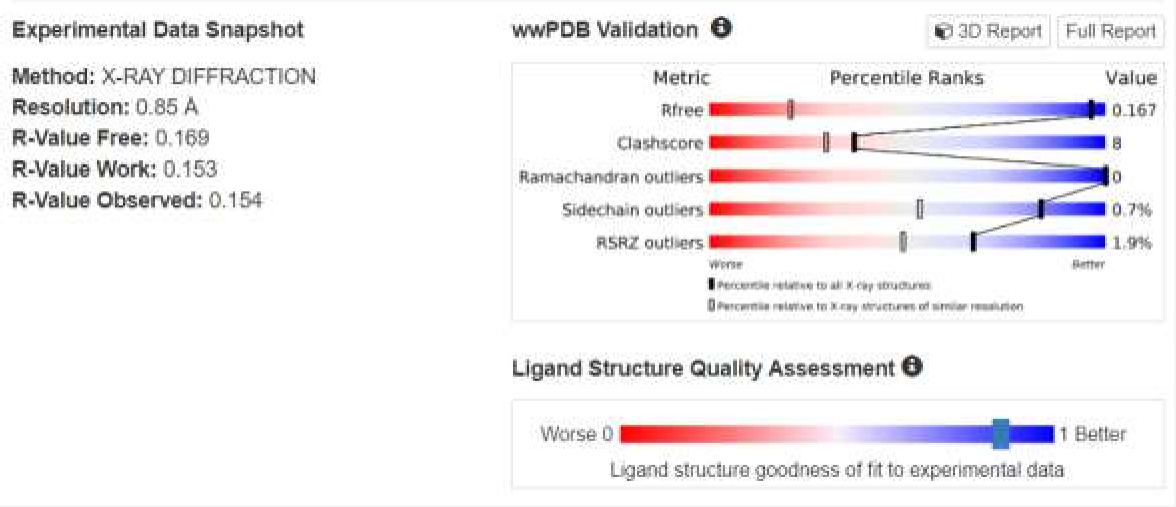


Fig.1: Representation of Ram-Chandran plot and Resolution for 4PSS

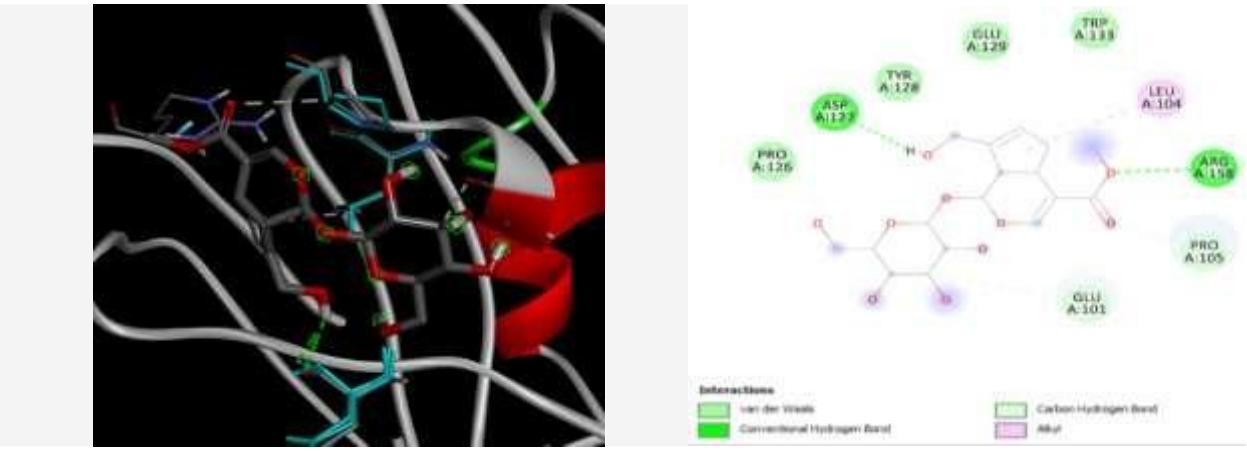


Fig.2: Images showing 2D and 3D interaction between ciprofloxacin with PDB ID-4PSS

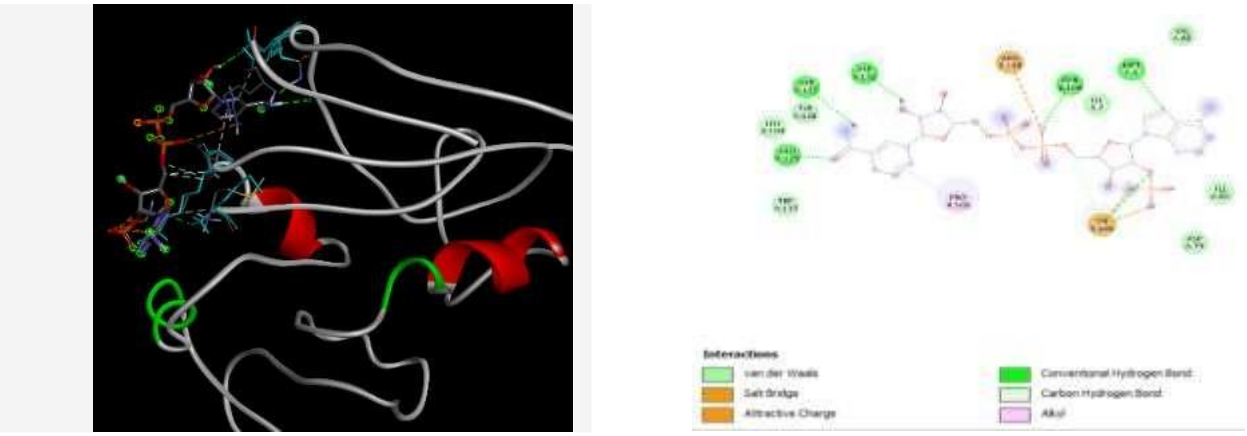


Fig.3: Images showing 2D and 3D interaction between co-crystallized ligand and PDB ID-4PSS

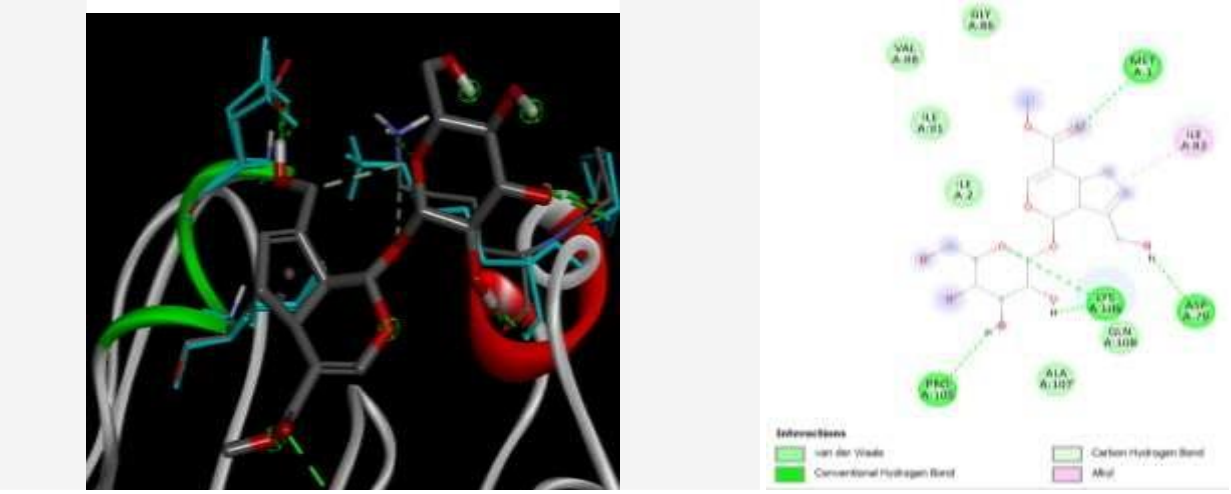


Fig.4: Images showing 2D and 3D interaction between momordicine and PDB ID-4PSS

Prediction of ADME Using pkCSM Software

Pharmacokinetics, toxicity, and efficacy must all be present for a medicine to be effective. The characteristics of an agent's ADME (absorption, distribution, metabolism, and excretion) are described in its pharmacokinetic profile. The ability of a new medicine to reach the target site in sufficient concentrations to produce its physiological impact safely is equally important as ensuring that it has excellent binding properties to the therapeutic target. A significant decrease in the number of drugs that underperformed in clinical studies due to weak ADMET properties has been seen as a result of the inclusion of ADMET qualities in early-stage drug discovery as a result of the awareness of their importance.[11-13]

Sr. No.	Descriptor	Value
	CODE	Momordicine
1	MW	472.71
2	HB^d	3
3	HB^a	4
4	PlogP(o/w)	5.4556
5	Intestinal absorption	94.307
6	Skin Permeability	-3.223
7	BBB Permeability	-0.604
8	PSA	206.464
9	Rule of 5	0
10	LD50	3.336

Table No. 2: Determination of pharmacokinetic properties using pkCSM software

2.2. Phytochemical tests[14].

Sr. No	Phytochemicals	Test	M. dioica ethanol extract
1.	Alkaloids	Dragendorff's Test	**Positive**
		Wagner's Test	**Positive**

2.	Carbohydrates	Barfoed's Test	Negative
		Molisch Test	Negative
3.	Glycosides	Legal Test	Negative
		Brontrager Test	Negative
4.	Tannins	Gelatin Test	**Positive**
		Bromine Water Test	**Positive**
5.	Steroids	Salkowski Test	Negative
		Libermann-Burchards Test	Negative
6.	Phenols	Iodine Test	**Positive**
		Ferric Chloride Test	**Positive**
7.	Flavonoids	Alkaline Reagent Test	Negative
		Shinoda's Test	Negative

Table No. 3: Phytochemical Tests.

2.3. Antimicrobial Activity

2.3.1. Preparation of Extract

In vitro antimicrobial screening was performed with ethanol extract of leaves of Momordica dioica, against pathogenic bacteria. For extraction, the first step was to run a cycle through Soxhlet Apparatus using petroleum ether as a solvent in order to washout and remove all the lipid and fat substances from the plant material. The second cycle included ethanol (95%) as solvent as it is more potent and less toxic for extraction[15].

2.3.2. Preparation of subculture media for inoculum

13g of nutritional broth powder was mixed with 1 liter of sterilized water in a conical flask. It was thoroughly blended to dissolve. The dissolved media is then autoclaved for 15 minutes at 121°C and 15lbs of pressure. The conical flask is removed once the autoclaving procedure is finished and chilled to a temperature of around 40–45°C. Microorganisms were transferred in loops from an agar slant culture maintained in the lab into a conical flask containing sterile nutrient broth medium. At 37°C, they were left to incubate for 24 hours. While making the plates, a series of dilutions were further prepared for these bacteria[16].

2.3.3. Preparation of nutrient agar and spread plates

28g of nutritional agar powder and 1 liter of sterile water were combined in a conical flask. It was thoroughly blended to dissolve. The dissolved media is then autoclaved for 15 minutes at 121°C and 15lbs of pressure. The conical flask is removed once the autoclaving procedure is finished and chilled to a temperature of around 40–45°C. For proper dispersion of a microbial suspension on solid agar, a glass spreader is employed in the spread plate method. A sterile nutrient agar plate was treated with 0.1ml of prepared subculture. These dilutions were then disseminated using a glass spreader that had been sterilized by flame after being dipped in alcohol and allowed to cool. The antimicrobial drug and extract solution is then added to the well in a volume of 0.1–0.3ml at the desired concentration after an 8mm-diameter well is aseptically bored with a borer. Agar plates are then incubated for 24 hours at 37°C. The antibiotic ingredient spreads across the agar media and stops the tested microbial strain from growing. Three 8mm wells were aseptically punched into each of the prepared petri plates. Three wells were prepared for each concentration, and their mean was computed. The concentration of the standard drug was reduced, while simultaneously concentration of the test drug was increased in the proportion of 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml where the first number in ration represents the concentration of the drug[17].

Symbol	S	T_1	T_2	T_3	T_4	T_5
Concentration (mg/ml)	0.01	5	10	15	20	25

Table No. 4: Different concentrations of standard and test solution

2.3.4. Preparation of Ciprofloxacin standard solution:

Weigh 1g of the standard drug (a small amount of the drug was dissolved using DMF). Dissolve weighed quantity in 1000 ml of water and shake for 5 minutes. (Concentration achieved - 1mg/ml). Pipette out 10 ml of solution in a 100 ml volumetric flask and make up the volume using water (Concentration achieved – 1mg/ml). Pipette out 10 ml of this solution and make up the volume in a 10 ml volumetric flask. (Concentration achieved – 10ug/ml).

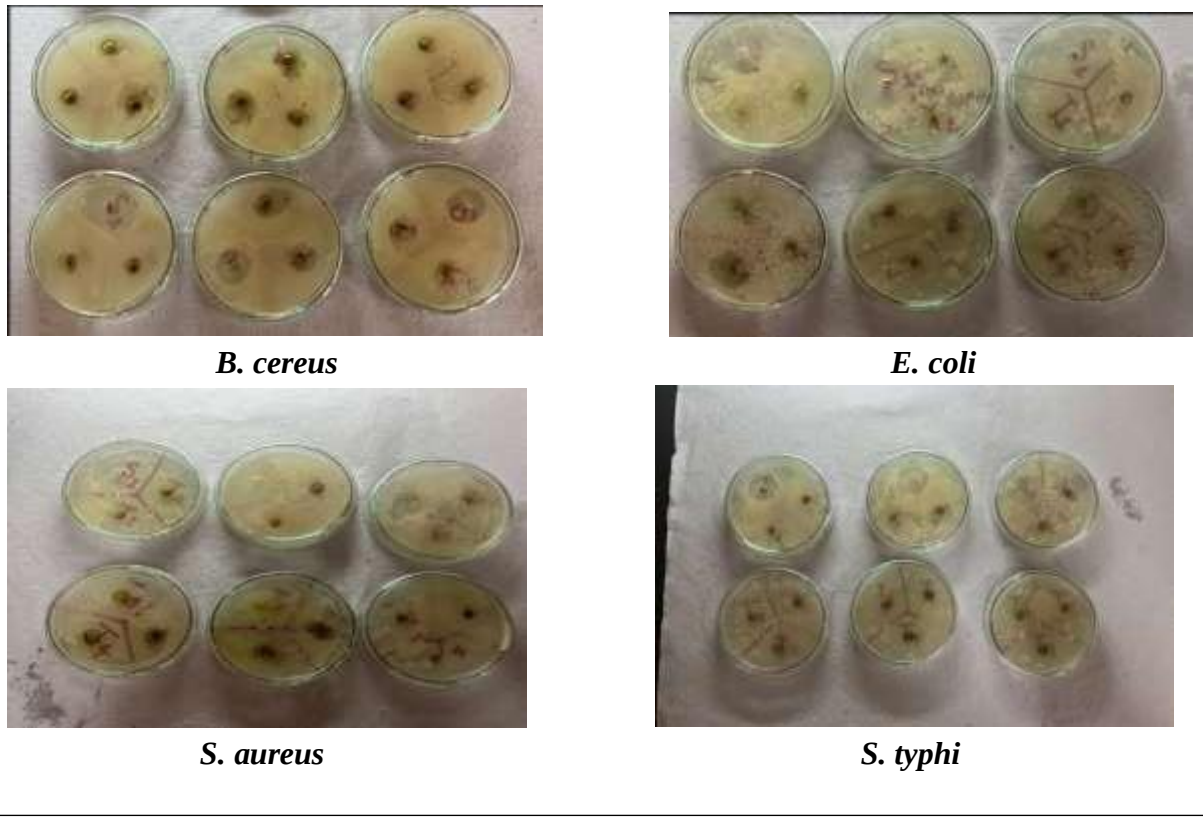
2.3.5. Preparation of Test Solution:

Weigh 0.05g, 0.1g, 0.15g, 0.2g and 0.25g of conc. extract and label them individually. Dissolve weighed quantity in ethanol and make up the volume till 10ml (Concentration achieved – 5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml respectively).
Minimum Inhibitory Concentration

Compound	S.aureus(5345)	B. cereus(2217)	E. coli(2931)	S. typhi(2501)
M. dioica	16µg/ml	1µg/ml	4µg/ml	2µg/ml

Table No. 5: Observation Table showing MIC of herbal extract

Fig. 05: Zone of Inhibition



Zone of Inhibition, Standard Error Mean and IC_{50}

Compound	Conc (mg/ml)	B. Cereus (2217)						S. aureus (5345)					
		ZOI (mm)			Mean	SD	SEM	ZOI (mm)			Mean	SD	SEM
Control Positive (Ciprofloxacin)	0.01	27	32	30	29.7	2.52	1.45	12	10	18	13.333	4.1633	2.404
M. dioica	5	6.9	7.3	6.4	6.87	0.45	0.26	5.2	6.1	4.6	5.3	0.755	0.436
	10	10.8	11.4	11.4	11.2	0.35	0.2	8.9	8.2	7.8	8.3	0.5568	0.321
	15	14.6	15.3	14.3	14.7	0.51	0.3	10.6	10.2	10.1	10.3	0.2646	0.153
	20	16.8	16.5	15.6	16.3	0.62	0.36	11.3	12.6	12.1	12	0.6557	0.379
	25	21.5	22.9	22.5	22.3	0.72	0.42	12.8	15.1	14.1	14	1.1533	0.666
IC_{50}		29.27						46.14					

Compound	Conc (mg/ml)	E. coli (2931)						S. typhi (2501)					
		ZOI (mm)			Mean	SD	SEM	ZOI (mm)			Mean	SD	SEM
Control Positive (Ciprofloxacin)	0.01	20	19	20	19.67	0.58	0.33	22	22	23	22.3	0.58	0.333
M. dioica	5	4.8	5.3	4.9	5	0.27	0.15	11.2	11.2	10.6	11	0.35	0.2
	10	8.7	9.1	9.2	9	0.27	0.15	11.5	12.5	12.9	12.3	0.72	0.416
	15	10.6	10.2	12.2	11	1.05	0.61	13.8	12.8	12.4	13	0.72	0.416
	20	11.8	12.3	10.5	11.53	0.93	0.54	16.3	16.8	16.7	16.6	0.27	0.153
	25	16.8	17.1	16.3	16.73	0.40	0.23	17.6	20.2	18.9	18.9	1.3	0.751
IC_{50}		43.76						41.35					

Table No. 6: Zone of Inhibition, Standard Error Mean and IC_{50}

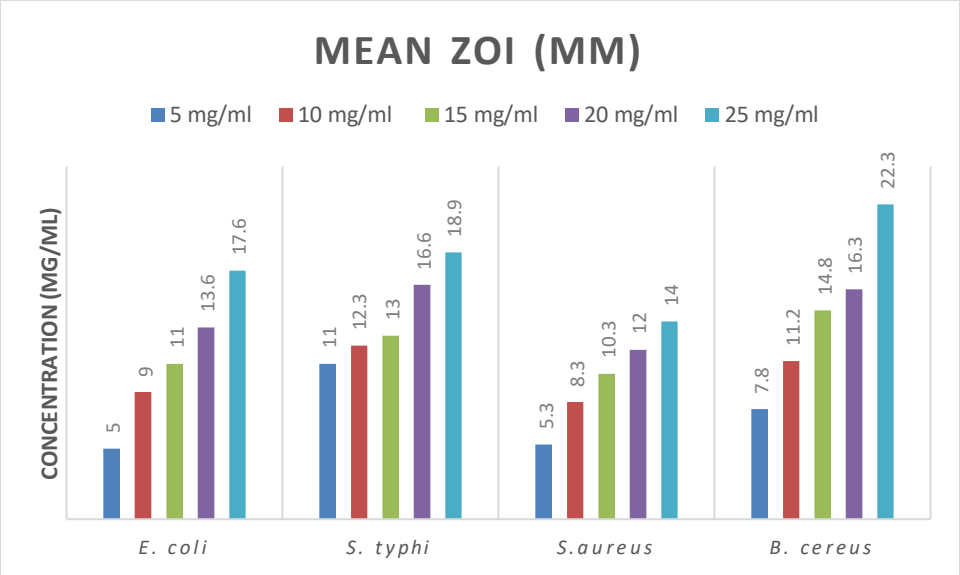


Fig.06: Graphical representation of different cultures and their activity at various concentrations

2.3.6. Formulation Studies[18-19].

Sr. No.	Ingredients Required	Quantity Required
1	Extract	0.8g
2	Zinc oxide	2.5g
3	Bentonite	1.5g
4	Sodium citrate	0.25g
5	Liquified Phenol	0.25ml
6	Glycerol	2.5ml
7	Sterilized water	q.s.

Table No. 7: Formula for lotion.

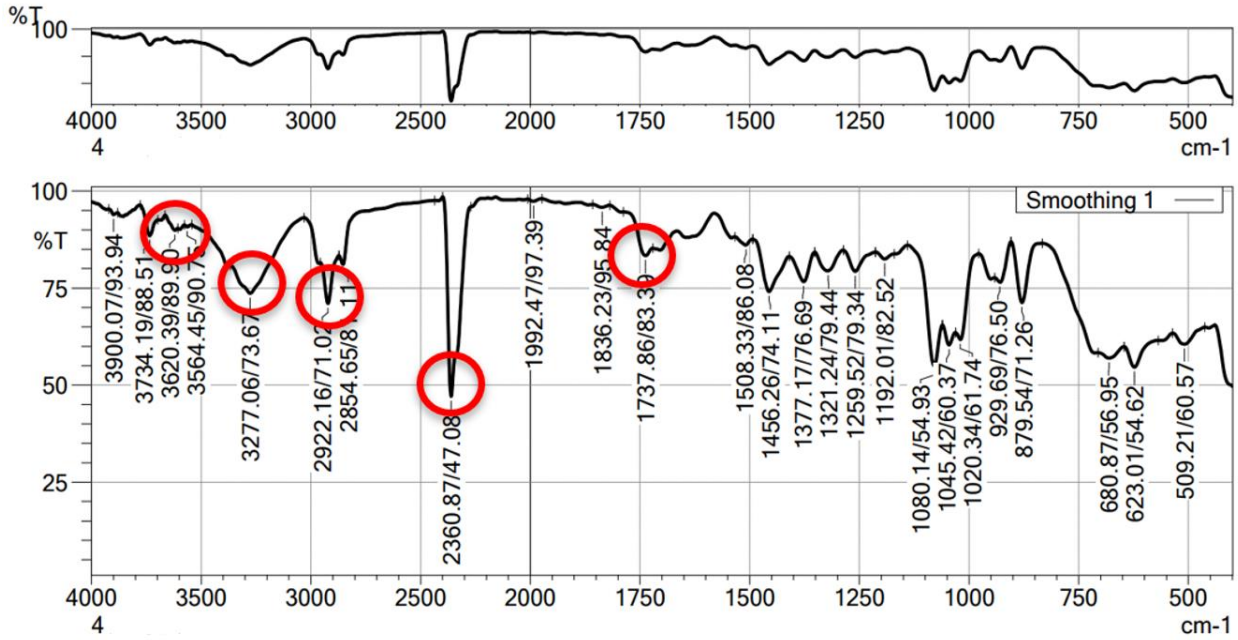


Fig.07: IR of Momordica dioica pure extract.

FTIR (cm^{-1}): 3277.06 (O-H stretch), 2922.16 (aliphatic C-H stretch), 2360.87 (CO2 stretch), 1737.86 (C=O stretch of acid), 1508.33 (C=C aromatic stretch)

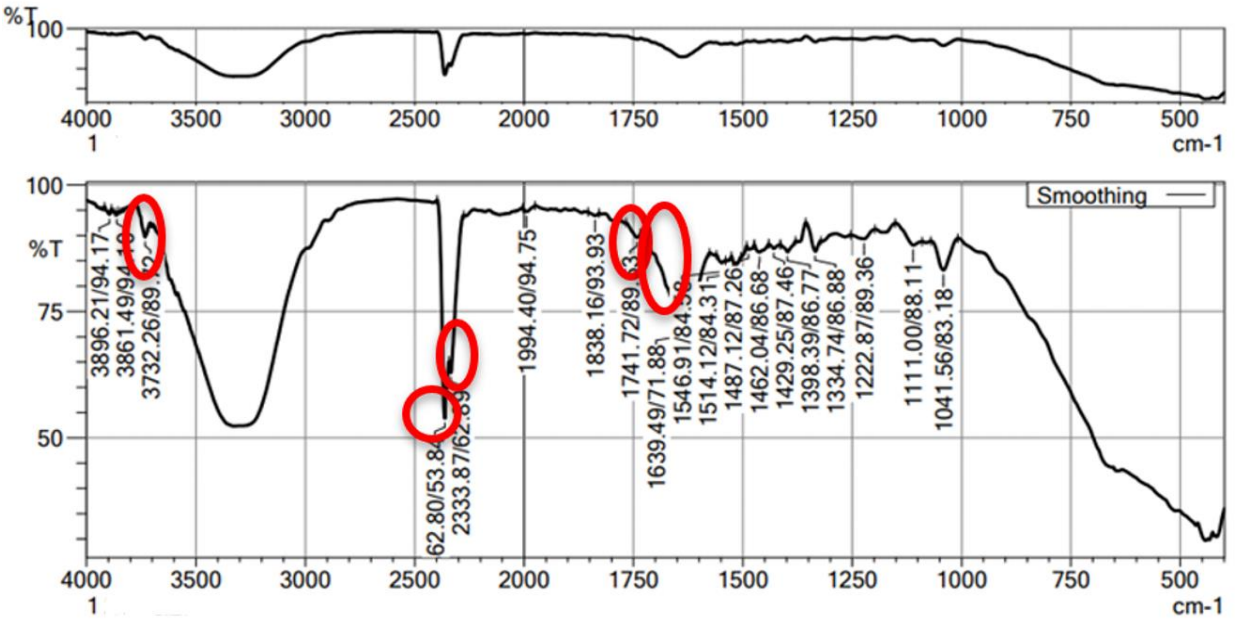


Fig.08: IR of Momordica dioica Lotion.

FTIR (cm^{-1}): 3732.26 (O-H aromatic stretch), 2662.80 (aliphatic C-H stretch), 2333.87 (CO2 stretch), 1741.72 (C=O stretch of acid), 1639.59 (C=C aromatic stretch)

Sr. No.	Ingredients Required	Quantity Required
1	Extract	0.8gm

2	Wool fat	1.2gm
3	Cetostearyl alcohol	1 ml
4	Hard paraffin	1 gm
5	White soft paraffin	16 gm
6	Perfume	0.05 ml

Table No. 08: Formula of ointment preparation.

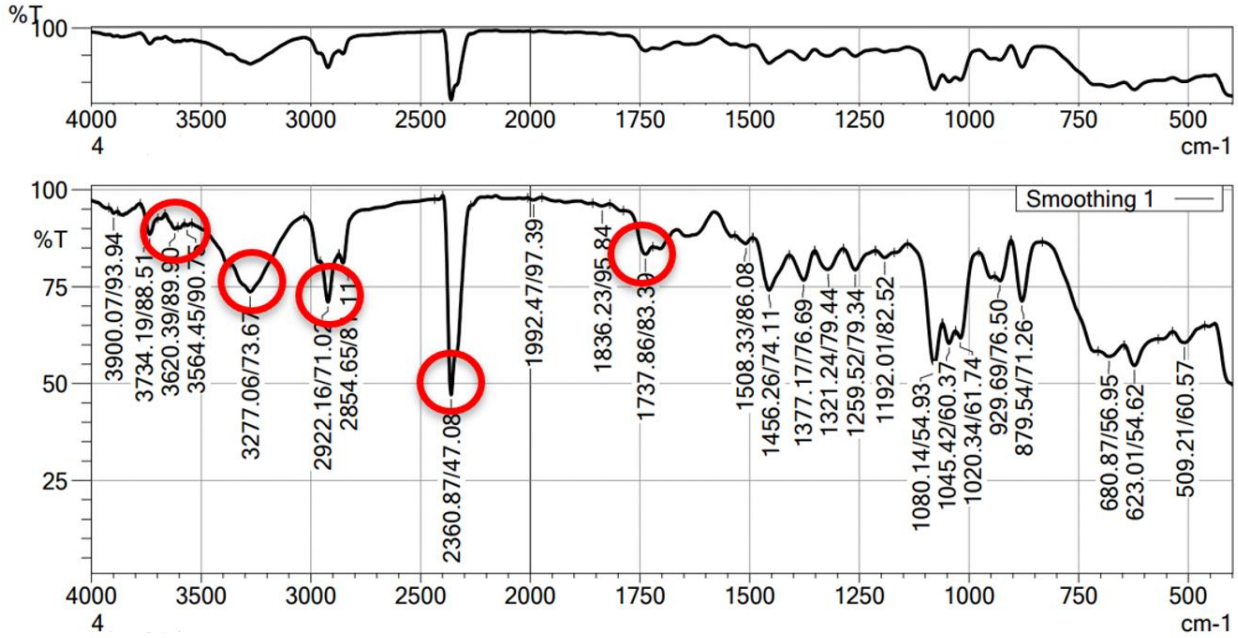


Fig.09: IR of Momordica dioca pure extract.

FTIR (cm^{-1}): 3277.06 (O-H stretch), 2922.16 (aliphatic C-H stretch), 2360.87 (CO_2 stretch), 1737.86 (C=O stretch of acid), 1508.33 (C=C aromatic stretch)

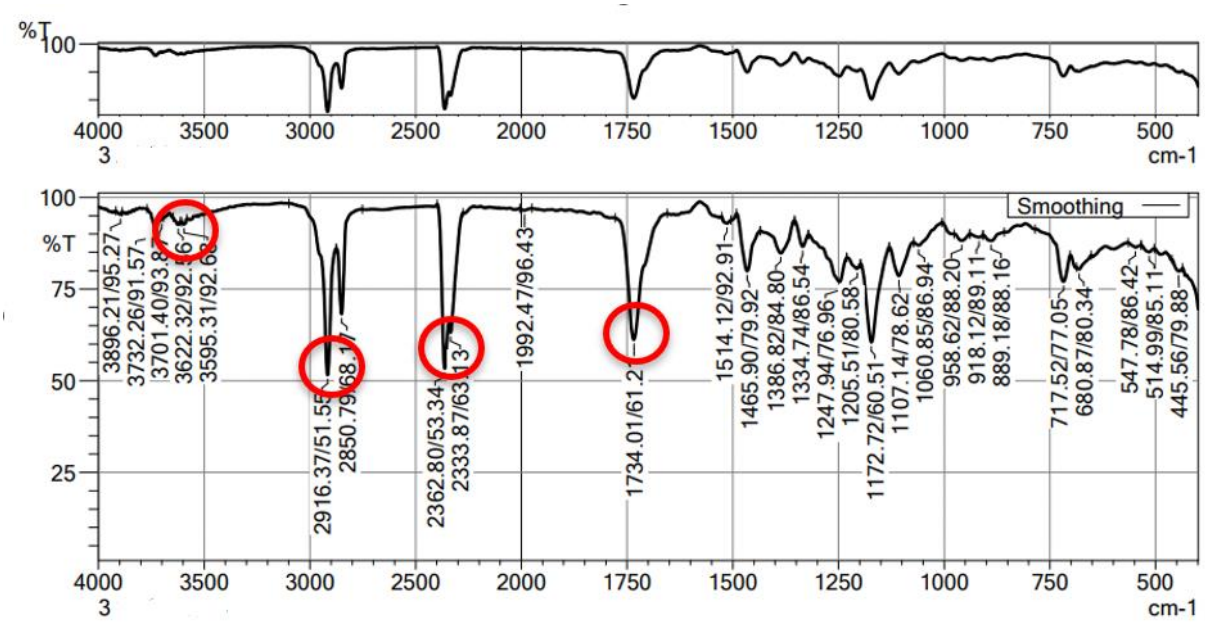


Fig.10: IR of Momordica dioca Ointment.

FTIR (cm^{-1}): 3622.32 (O-H aromatic stretch), 2916.37 (aliphatic C-H stretch), 2333.87 (CO2 stretch), 1734.01 (C=O stretch of acid)

Sr. No.	Lotion		Ointment	
	Parameter	**Values**	**Parameter**	**Values**
1	pH	7.92	pH	7.25
2	Viscosity[41]	0.183kg/ms	Ash value[46]	16.02mg
3	Density[43]	1.135g/cc	-	-
4	Loss on Drying[39]	2.10%	-	-
5	UV Vis. Spectroscopy(λmax)	666nm	-	-

Table No. 09: Evaluation parameters of lotion and ointment

3.0 Result and Discussion

The extract was dark green in color and weighed around 5.34g. The phytoconstituents found present in the plant extract are Alkaloids, Tannins and Phenols. The antimicrobial activity of ethanolic extract of Momordica dioica at concentrations (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) in the present study showed zone of inhibition against Escherichia coli, Bacillus cereus, Staphylococcus aureus and Salmonella typhi. The antimicrobial activity of ethanolic extract of Momordica dioica at concentrations (5mg/ml, 10mg/ml, 15mg/ml, 20mg/ml and 25mg/ml) in the present study showed prominent zone of inhibition against B. cereus, S. typhi, E. coli, and S. aureus are and the found IC_{50} values are 29.27**,** 41.35, 43.76, and 46.14 respectively. The MIC for B. cereus, S. typhi, E. coli, and S. aureus are 1µg/ml, 2µg/ml, 4µg/ml, and 16µg/ml, respectively. The activity against all the pathogenic bacteria was found at every concentration of the test solutions where the least activity was shown against S. aureus and most activity was shown by B. cereus. The formulations (lotion and ointment) prepared were assessed for various evaluation parameters where it shows good stability.

4.0. Conclusion

The in-silico studies proposed a good binding affinity of momordicine with receptors showing a more binding score of 12.7 than Co-crystallized ligand. It was observed that the extracts of Momordica dioica showed antimicrobial activity against all the microorganisms. Based on the aforementioned finding, it was shown that Momordica dioica demonstrated considerable antibacterial activity against Escherichia coli, Bacillus cereus, Staphylococcus aureus, and Salmonella typhi at different concentrations when compared to the antimicrobial medication Ciprofloxacin. The different levels of sensitivity of the organism being tested can be attributed to the microorganisms' inherent tolerance as well as the variations in chemical composition and constituent structure. The formulations were prepared and evaluated which also shows good stability. In the end summarize by saying that the extract of Momordica dioica showed antimicrobial effect similar to as of Ciprofloxacin against various bacterial species.

Conflict of Interest

None

Acknowledgement

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