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Evaluation of Anti-Oxidant Potential and Novel Synergism Effect of *Citrullus colocynthis* against MDR pathogenic Strains

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ABSTRACT

Citrullus colocynthis belong to family Cucurbitaceae is a medicinal plant traditionally used for the treatment of various diseases [1][2] including diabetes, bacterial infections, constipation and many other diseases. Various parts of plant individually produced anti-microbial and anti-oxidant potential. This is the first study reporting 1. The Novel synergism effect of Citrullus colocynthis, Fruit, Seed and root extract against MDR pathogenic strains in the least concentration of various organic solvents extracted compounds (first study reporting anti-microbial activity of Butanol extracted compounds) 2. Inhibitory concentration (IC50) of these compounds against MDR 3. Novel Synergism of C. colocynthis fruit, seed and root showing anti-oxidant potential. According to findings, Citrullus colocynthis can be used as medicinal plant as its various compounds isolated through organic solvents showed antimicrobial activity against MDR-pathogens. Maximum activity against MDR pathogens of Pseudomonas Aeurignosa, Staphylococcus aureus (MRSA), Klebsiella pneumoniae, Stenotrophomonas maltophilia, Salmonella typhi, Salmonella paratyphi, Acinetobacter baumanni and Enterococcus faecalis was shown by Butanol extract followed by Chloroform, Acetone, Ethanol, Hexane and DMSO extract. The plant also showed 92% anti-oxidant potential in the least concentration of 93µg/mL.

1.1 INTRODUCTION

Plants are named as medicinal plants due to presence of pharmacologically beneficial compounds and are being used worldwide from ancient time [1][2]. Almost 80% population worldwide use them as these plants exhibits medicinal properties due to presence of anti-oxidant [3] and antimicrobial properties [4][7] and many other properties due to secondary metabolites (alkaloids, glycosides, essential oils and terpenoids) [5] [6] which constitute the major portion of modern medicine by history of 50 years research [8]prepared from selection to grinding in consumable form under the supervision of experts[9], furthermore, these compounds are identified by GCMS and UPLC [10]

Citrullus colocynthis, member of Cucurbitaceae family has been traditionally used as medicinal plant from ancient time to aid the treatment of oedema [13], bacterial or fungal infection [14] constipation diabetes [20] and cancer [32].The mature fruit possess 90% moisture with 30% protein, 10% carbohydrate 4% ash cover and 3% fiber content [14][36][37][39] ensuring the major portion of glycosides by the production of elataricin B, E and dihydroelataricin B [60][61].

Fig Citrullus Colocynthis Fruit (mature and immature)

Due to high temperature resistance property subtropical the plant is found in tropical and areas [59][22].

Regional language	Common Names
English	Colocynth or Vine-of-Sodam
Hindi	Indaryan or Ghorumba
Telugu	Eti-puchcha
Malayalam	Paikummatti
Tamil	Paedikari- Attutummatti
Kannada	Hamekkae, harmekkikayi
Arabic	Handhal
German	Bitter-melone or koloqnite
Spanish	Alhandhal or coloquintida
Sanskrit	Indarvani or Brihadvani
Bengali	Makhal, Indaryan, Panjot, Indrabuni
Gujrati	Indarayan
Marathi	Kaud-Indarvani
Punjabi	Kaudtumba
Urdu	Hanzal, Indaryan, Shahmehinzal
French	Coloquinte
Portuguese	Colocintida
Swedish	KoloKvint

The family shows genetic diversity due to extreme temperature resistance and efficiently grow in alkaline soil [55][56]. The water melon similar plant exhibit perennial structure due to herbaceous vine plant with yellow flower on axial site with apple sized extreme bitter fruit having white spongy flesh filled coriaceous peel with brown and white ovate seeds inside [57][58][59]

Fig Morphological characteristics of Citrullus colocynthis fruit seed and pulp

KINGDOM	**PLANTAE**
Sub-kingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Mangoliopsida
Sub class	Dilleniidae
Order	Cucurbitales
Family	*Cucurbitaceae*
Genus	*Citrullus*
Specie	*Colocynthis (L.) Schard*

The plant shows pharmacological importance due to its secondary metabolites [15] with the treatment of various conditions including hyperlipidemia, analgesic ailment and diabetes [29][30][31]. The plant shows anti microbial [16][83][45], analgesic [24][32][28], anti cancerous [45][46][48], hypolipidimic [5][51], hypoglycemic [62][17][23][41][39][47][40], Larvicidal [52][53][54] anti alopecia [63][64] properties with the contrast of toxicity by due to its over consumption [25] leading severe condition [26] to various parts with possible cases of colitis, rectorrhagia and bloody diarrhea [34][35]. The plant shows its adverse affects due due to its anti fertility [64][65][66][67].

Resistance towards various antibiotics is a serious health care problem and is not only restricted to hospital environment as earlier due to over consumption of multi broad spectrum dosage [68][69] these include gram positive S.aureus and Enterococcus and gram negative ESBLs are being reported as major threat to develop antimicrobial resistance [69][70][71][72]. These MDR strains include Pseudomonas Aeurignosa MDR and XDR strains [73][74][75][76][77][78], Stenotrophomonas maltophilia [81][82][84], Acinetobacter baumanii [79][80], Staphylococcus aureus (MRSA) [85][86][87][89], Salmonella typhi [90][91][92][93][94], Salmonella paratyphi [95][96][97], Klebsiella pneumoniae with it MDR-hvKP strains [99][100][101] with poor MDR strains of Enterococcus faecalis [102][103][104][105]

The study was designed to evaluate whether Citrullus colocynthis can inhibit the growth the of MDR pathogenic strains as reported in earlier studies which include its antimicrobial property against ATCC strains and other lab strains as well as evaluation of their least effective dose working as IC50 against them using the Novel synergism of its own fruit seed and root which individually shown the anti microbial property.

Materials

Bacterial MDR clinical isolates, Sterile swabs, Petri dishes, Wire loop, Bunsen burner, Eppendorf tubes, Glass tubes, Surgical gauze, Micro pipette 100-200µL, Micro pipette 500-100µL, Sterile tips, Glass tubes, Beaker (50, 100, 250, 500 and 100)ml, Conical flask (500 and 1000ml), Spatula, Separating funnel, Watman filter paper no.41, Aluminium Foil, Water bath, Safety cabinet, Micro titer plates, Incubator, Shaking incubator, Weighing Balance (0.000g) , Marker (permanent), Match box, Fridge for storage, Sanitizer, Cotton, Sterile Gloves, Measuring Cylinder, Paraffin tape, Wooden sticks, MacConkey agar, Manitol salt agar, Mueller Hinton agar, Nutrient agar, Glycerol, Distilled water, Trypton, NaCl, Beef extract, Yeast extract, Normal Saline (0.5% NaCl), 70% ethanol, Cefexime, DPPH, DMSO, n-Hexane, Butanol, Methanol, Ethanol, Acetone, Ethyl Acetate, Cat-ion adjusted MH Broth

Methodology

Plant Preparation

Plant material was collected from tropical region of Pakistan and the sample was identified by the head of botany department Minhaj University Lahore. After identification the plant was sterilized after washing with deionized water and allow drying in hot air oven for 3-7 days within temperature of 35-37ºC.

Plant Extraction

After drying the plant extract was grinned into fine powder and was measured using weighing balance was mixed with 1000ml methanol allowing extraction at 25ºC for 15-20 days

Crude preparation

Using a surgical gauze the extract was filter out as pure solution of plant metabolic compounds in methanol using a surgical gauze and watman filter paper no. 41 and allowing crude preparation by standing on water bath at 60ºC to get semisolid crude extract

Fractionation

Fractionation was done using various organic solvents by 2 solvent fractionation by using 1:2 by using Chloroform. Ethanol, DMSO, Butanol, n-Hexane and Acetone. After extraction in various organic solvents all the solvents were allowed to dry on water bath by keeping the temperature range below than their boiling point

Collection of Bacterial Isolates

MDR strains were collected from Sheikh Zayed Hospital Lahore under the supervision of Head of microbiology Department Sheikh Zayed Hospital Lahore the MDR strains of Staphylococcus aureus (MRSA), Klebsiella Pneumoniae, Acinetobactor Bummaniae, Salmonella Typhi, Salnonella Paratyphi, Enterococcus faecalis, Pseudomonas aeurignosa and Stenotrophomonas Maltophilia were collected using sterile swabs

Isolation of Pure colonies

pure colonies of these isolates were obtained using manitol salt agar and macConkey agar plates for gram positive and negative respectively. The safety cabinet was sterilized with 70% ethanol and leave under UVLight for 15 minutes after and prior the work and place the

Evaluation of antibiogram

For the confirmation MDR strain, anti-biogram of these MDR strains was evaluated by using Disc Diffusion method and their resistance pattern toward various antibiotics was documented

Table : Antibiogram data of MDR pathogens collected from Diagnostic Cultures at Shiekh Zayed Hospital Lahore

antibiotic	p.aeurignosa	S.aureus	k.pneumoniae	S.Maltophilia	S.typhi	S.paratyphi	A.baumani	E.faecalis
AK	R	R	-	R	-	-	R	I
AMC	-	-	R	-	-	-	-	-
AMP	-	-	S	-	R	R	R	-
ATM	S	-	R	R	-	-	-	-
AZM	-	-	-	-	S	S	-	-
C	-	I	S	-	R	S	R	R
CAZ	R	-	R	R	-	-	R	-
CIP	R	I	R	S	R	R	S	S
CN	R	R	S	S	-	-	I	S
CRO	-	-	R	-	-	-	-	-
CXM	-	-	S	-	-	-	S	-
DA	-	R	-	-	-	-	-	S
E	-	S	R	-	-	-	R	S
FD	-	R	R	-	-	-	-	R
FOT	-	-	R	-	-	-	-	-
FOX	-	R	-	R	-	-	-	S
IPM	R	-	R	S	-	-	R	-
LEV	-	-	-	S	-	-	-	-
MEM	R	-	S	S	S	S	S	-
P	-	R	-	-	-	-	-	R
PB	S	-	-	R	-	-	R	-
SXT	R	R	S	R	-	-	R	S
TE	-	S	R	-	-	-	-	S
TEG	-	-	R	-	-	-	R	-
TOB	R	-	-	S	-	-	I	-
TZP	R	-	S	S	-	-	-	-
VA	-	S	-	-	-	-	-	R

Preparation of Culture Broth

Culture broth was prepared for the purpose of storage as well as for the purpose of MIC work. Freshly prepared nutrient broth was used

Storage of Bacteria

Bacteria were stored as they were collected in different periods using 50% glycerol solution freshly prepared in lab. Using sterile eppendorf tubes all the organisms were stored in their glycerol stock and stored at -80ºC for the future use.

Preparation of Antibiotic Stock Solution

To Determine the antimicrobial activity and MIC it was necessary to obtain liquid drugs antibiotic stock solution were prepared by adding 3mg of semisolid extract in their respective solvents producing 3mg/ml antibiotic stock solution of each antibiotic solvents of ethanol, Butanol, chloroform, acetone, n-Hexane and DMSO. All the solution were kept in fridge and taken out at least 30 minutes prior to application

Preparation of Nutrient Agar

Nutrient agar was only prepared to obtain the fresh growth from freeze stock microbes stock. These organism were streak on nutrient agar using sterile wooden sticks

Setting Up MIC apparatus

MIC set up was done by using Micro titer plate labeled with the applied antibiotic stock on the top of all rows the concentration was mentioned in the range between 300 to 1.17µg/ml using 2 fold serial dilution while the first column serve as antibiotic control (Cephalosporin 200mg/ml). and the 2nd last column served as growth control while the last column served as sterility control. Cation adjusted Mueller Hinton agar was used as media. Using the MIC method each well was served with 100µL of Mueller hinton broth. Wells of 1st row were served with antibiotic control while the other 9 columns showed the descending concentration of test solvents. The growth control column only consist of bacterial isolate and Mueller hinton broth confirming the test free from false positive results while the sterility column contain only broth confirming test free from environmental contamination.

After applying all the setup each plate was coverd with sterile lid and incubated at 37ºC with 5% CO2 for 24 hours.

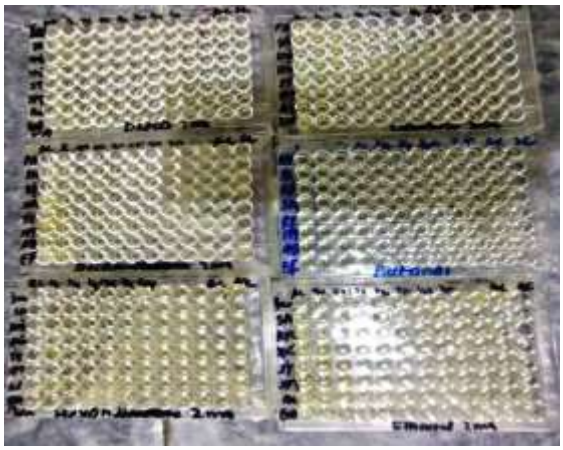


Fig Micro dilution Method of Evaluation of MIC of MDR Strains

After the completion of incubation period the results were determined using ELISA plate reader. Triplicates of reading were taken and data was analyzed to determine the percentage inhibition by using the formula of

$$\frac{\text{Absorbance of control (Ac)} - \text{Absorbance of sample (As)}}{\text{Absorbance of control (Ac)}} \times 100$$

These percentage were further analyzed in comparison with one another

Preparation of Crude for Anti-oxidant potential determination

For this purpose the methanolic extract was deleted into 5 different concentrations of 1500, 750, 375, 187.5 and 93.75 by two fold serial dilutiuon method in order to check the radical scaving potential against DPPH free redicals determining its inhibitory potential ratio at various concentration

DPPH Preparation

0.1mM DPPH was prepared by dissolving 2mg DPPH in 250ml methanol which was further homogenized in ultrasonic bath for approximately 30 sec and was stored in dark (Aluminum Foil wrapped flask)due to its light sensitivity

DPPH Assay

Using an ELISA plate DPPH assay was tested to evaluate anti-oxidant potential of Synergism of C.colocynthis each well was marked with the methanolic concentration used in it i.e. 1500, 750, 375, 187.5 and 93.75 µL/mL present in the volume of 100µL. after incubation with 0.1mM DPPH in the period of 30 minutes results was evaluated with ELISA plate reader of BioTEK 800 TS. And results were documented in the percentage inhibition using the standard formula after taking triplicate of reading.

$$\frac{\text{Absorbance of control (Ac)} - \text{Absorbance of sample (As)}}{\text{Absorbance of control (AC)}} \times 100$$

RESULTS

Concentration (µg/mL)	Inhibition (%)
1500	93.87±0.25
750	93.50±0.93
375	93.38±0.58
187.5	92.84±0.46
93.75	92.76±0.35

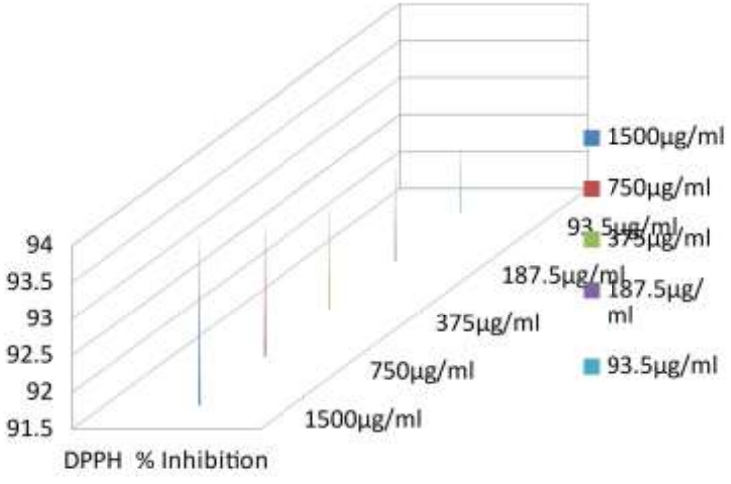


Fig Graphical representation of Maximum %inhibition of Methanol extract of C.colocynthis against DPPH

Table : Mean percentage inhibition of Citrullus Colocynthis Ethanol extract Against MDR Pathogens

Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	***P.aeurignosa***	***S.aureus***	***K.pneumoniae***	***S.maltophilia***	***S. typhi***	***S.paratyphi***	***A.baumanni***	***E.faecalis***
300	56.82±0.54	62.31±0.15	75.73±0.41	60.83±0.55	59.86±1.09	52.97±0.62	75±0.08	59.43±1.01
150	55.08±0.35	56.92±0.42	73.34±0.64	57.14±1.50	54.71±0.87	51.14±1.67	71.06±1.69	56.44±1.57
75	52.55±.1.28	55.41±1.92	72.42±0.69	53.41±1.52	51.85±1.36	48.47±0.31	68.34±0.93	51.29±1.54
37.5	50.16±0.36	53.38±0.74	70.46±0.32	50.55±0.99	44.89±1.03	46.95±0.83	63.44±0.71	44.61±1.90
18.75	49.74±0.66	50.25±0.42	67.83±0.57	45.80±0.86	41.26±1.18	43.83±0.73	57.04±1.50	39.42±1.53
9.37	47.10±0.98	47.02±0.62	63.90±0.78	42.22±0.62	35.26±0.99	39.95±0.93	48.39±2.21	30.26±0.41
4.68	42.10±0.12	43.85±0.53	59.65±1.99	40.20±0.50	32.05±0.26	38.91±0.36	42.02±1.89	28.85±1.30
2.34	41.20±2.70	39.59±1.2	54.78±1.2	35.80±1.2	29.31±1.2	33.50±1.2	30.43±1.2	21.93±1.2
1.2	35.20±1.2	25.22±0.22	48.33±0.78	30.45±1.24	20.16±1.56	28.33±1.23	21.25±1.23	16.51±0.85

Mean percentage inhibition of Citrullus Colocynthis Hexane extract A

Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	*P.aeurignosa*	*S.aureus*	*K.pneumoniae*	*S.maltophilia*	*S. typhi*	*S.paratyphi*	*A.baumanni*	*E.faecalis*
300	49.88±0.34	60.16±1.67	45.74±0.1	42.08±0.3	40.52±1.0	54.53±1.2	64.0±1.0	50.0±1.5
150	48.42±0.54	58.99±1.31	42.18±0.42	38.02±0.97	33.13±2.9	49.48±1.3	59.01±2.2	44.41±1.7
75	45.05±0.54	55.35±1.01	37.5±1.7	32.68±1.0	30.62±2.25	46.63±1.1	56.83±0.5	40.17±1.14
37.5	41.73±0.69	53.88±0.82	34.98±0.66	31.70±0.36	25.44±0.7	40.88±2.2	52.18±1.2	31.32±1.0
18.75	40.38±0.38	51.94±1.13	32.04±1.5	28.25±0.65	19.76±0.81	38.53±0.9	48.42±0.8	28.10±1.14
9.37	37.21±0.31	46.58±1.38	27.91±1.07	21.38±0.86	18.71±1.0	33.74±1.19	44.09±1.86	24.01±1.01
4.68	34.26±1.35	44.92± 1.5	26.07±0.46	16.50±1.6	13.22±0.6	27.40±0.3	38.48±2.6	18.55±0.74
2.34	32.82±0.01	40.10±1.2	24.11±0.8	15.29±0.6	7.23±102	23.38±1.2	30.68±1.2	17.01±0.42

Table Mean percentage inhibition of Citrullus Colocynthis DMSO extract Against MDR Pathogens

Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	*P.aeurignosa*	*S.aureus*	*K.pneumoniae*	*S.maltophilia*	*S. typhi*	*S.paratyphi*	*A.baumanni*	*E.faecalis*
300	52.50±0.4	61.68±0.53	34.55±0.72	50.19±0.17	52.24±0.55	36.41±0.66	56.08±0.56	54.44±0.44
150	50.19±0.34	57.42±0.40	31.98±0.45	41.99±0.44	45.78±1.27	22.38±1.86	46.63±0.22	51.10±0.0.29
75	44.35±0.55	50.25±2.99	27.05±0.47	36.88±1.13	40.44±0.17	17.46±0.57	43.52±1.01	48.03±0.60
37.5	39.85±0.9	40.47±2.38	25.82±0.66	30.07±1.12	35.42±0.70	16.11±0.36	30.40±3.51	42.76±0.64
18.75	38.11±0.46	36.05±0.69	22.08±0.23	26.0±0.24	30.39±0.58	11.64±0.51	29.97±0.73	40.48±0.75
9.37	36.34±0.42	34.39±0.28	18.22±1.18	20.67±0.62	19.56±0.65	8.24±1.63	24.92±0.73	33.88±1.95
4.68	34.02±0.21	26.19±0.05	14.98±0.93	12.56±1.68	13.30±3.19	6.13±0.20	17.56±1.82	27.63±1.65
2.34	30.80±1.23	18.48±1.03	4.74±0.42	1.46±1.08	2.47±2.01	1.24±0.57	11.76±0.75	17.21±0.35
1.2	24.44±1.35	12.36±1.15	3.45±0.58	resistant	resistant	resistant	2.74±1.25	10.23±0.74

Table: Mean percentage inhibition of Citrullus Colocynthis Chloroform extract Against MDR Pathogen

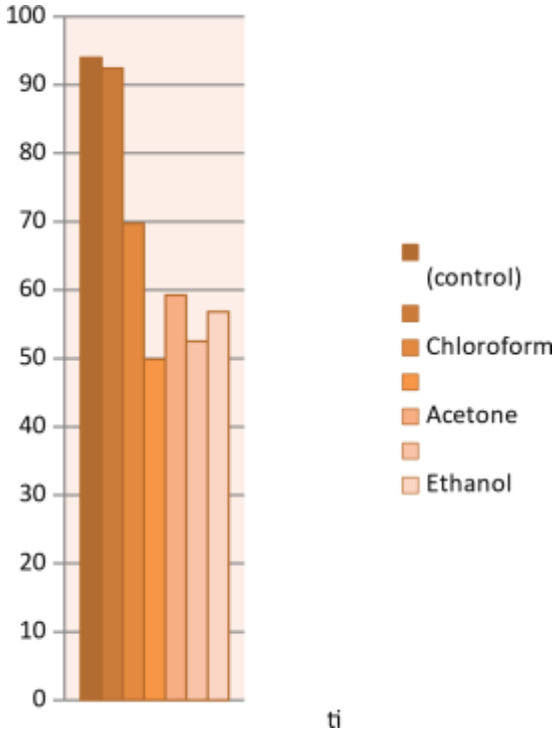
Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	*P.aeurignosa*	*S.aureus*	*K.pneumoniae*	*S.maltophilia*	*S. typhi*	*S.paratyphi*	*A.baumanni*	*E.faecalis*
300	69.75±1.79	54.38±0.29	64.0±1.08	65.20±0.24	61.29±1.81	64.91±0.62	71.99±0.38	59.39±2.19
150	66.633±0.35	53.97±1.68	60.41±2.11	61.03±0.19	54.17±0.61	59.39±1.14	68.24±0.53	52.90±0.18
75	64.89±1.01	50.40±2.54	54.81±0.59	57.09±1.20	50.41±1.05	56.47±0.42	63.69±2.23	50.0±0.54
37.5	59.69±0.75	45.39±1.29	52.69±1.04	51.69±0.99	42.42±0.74	51.31±1.04	58.47±1.08	46.14±1.42
18.75	57.50±0.52	41.51±1.76	47.64±1.45	48.04±1.28	39.94±1.79	49.61±2.58	56.18±1.55	42.45±1.24
9.37	54.15±1.65	40.57±1.27	44.76±0.15	42.38±0.77	33.72±3.76	46.15±0.31	49.21±1.79	39.11±1.55
4.68	50.05±0.25	35.58±1.22	40.59±1.10	39.87±1.04	26.37±3.46	41.82±1.74	46.17±1.77	36.04±1.75
2.34	42.74±0.65	30.88±0.25	36.12±1.12	35.12±0.12	19.05±1.87	38.0±0.02	40.37±1.35	30.71±1.04

Table : Mean percentage inhibition of Citrullus Colocynthis Acetone extract Against MDR Pathogens

Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	*P.aeurignosa*	*S.aureus*	*K.pneumoniae*	*S.maltophilia*	*S. typhi*	*S.paratyphi*	*A.baumanni*	*E.faecalis*
300	57.24±0.10	60.40±1.55	51.07±0.28	65.82±1.80	51.35±1.04	55.61±2.04	58.86±0.56	53.10±0.33
150	56.21±0.43	57.70±0.58	46.13±0.97	61.52±0.33	43.07±0.4	52.14±0.33	57.43±0.55	48.89±2.61
75	53.09±0.92	55.45±1.13	42.49±0.78	57.29±0.84	39.59±0.87	48.40±1.78	49.78±1.50	41.86±0.82
37.5	50.25±0.36	52.94±0.35	37.28±0.34	53.02±0.70	34.22±1.33	42.65±1.47	43.95±1.03	36.94±1.44
18.75	48.87±0.39	48.87±0.52	34.46±0.95	49.80±1.12	31.01±0.82	38.11±0.42	36.98±0.48	28.30±1.39
9.37	44.15±1.06	38.15±1.98	27.78±1.13	42.77±1.03	24.12±0.99	33.71±2.42	35.58±0.43	19.10±1.0
4.68	40.97±0.08	26.0±0.63	24.54±1.03	38.93±1.20	18.75±0.46	27.26±0.83	32.40±0.70	15.72±0.29
2.34	33.95±0.50	20.73±1.05	18.13±0.34	33.0±0.99	11.05±1.74	21.41±1.98	28.21±2.57	6.80±0.59
1.2	19.98±1.42	8.33±2.75	6.04±1.58	21.25±0.33	5.36±0.23	12.82±0.45	16.86±1.78	resistant

Table: Mean percentage inhibition of Citrullus Colocynthis Butanol extract Against MDR Pathogens

Mean% Inhibition Of MDR Pathogens								
Conc. µg/ml	***P.aeurignosa***	***S.aureus***	***K.pneumoniae***	***S.maltophilia***	***S. typhi***	***S.paratyphi***	***A.baumanni***	***E.faecalis***
300	92.46±1.06	92.10±0.93	92.70±0.377	90.75±0.75	89.44±0.71	91.71±0.42	91.55±0.528	91.11±0.24
150	88.33±0.45	81.89±0.56	86.82±0.47	86.62±0.51	85.61±2.79	84.61±0.64	82.72±0.67	84.59±2.7
75	86.93±0.46	79.38±1.41	83.85±.47	79.45±1.66	82.63±0.48	79.97±.26	80.07±1.22	76.49±0.18
37.5	83.50±0.42	77.13±.81	82.23±1.06	75.97±1.52	79.69±1.53	77.68±1.19	80.90±.53	70.95±1.30
18.75	81.15±1.01	68.79±1.38	78.79±1.24	71.19±0.67	75.32±1.40	72.79±1.02	77.25±0.70	65.60±0.44
9.37	70.26±1.11	63.69±1.41	73.28±0.70	65.13±1.12	68.94±1.28	70.16±0.74	72.42±1.23	63.01±1.42
4.68	66.30±0.17	60.15±.80	68.36±0.93	60.97±0.20	65.54±0.61	67.15±0.68	69.31±1.39	57.94±1.41
2.34	57.39±0.71	51.78±0.95	63.05±0.59	57.94±2.65	62.37±1.24	63.99±2.02	66.66±2.12	55.30±0.04
1.2	51.23±1.02	41.23±1.23	54.26±0.48	49.23±2.56	54.01±0.56	55.87±1.58	57.82±2.87	48.56±0.63



Cephalosporin

Butanol

Hexane

DMSO

Pseudomonas aeurignosa % inhibi on

ti

Fig.4.2: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Pseudomonas Aeurignosa.

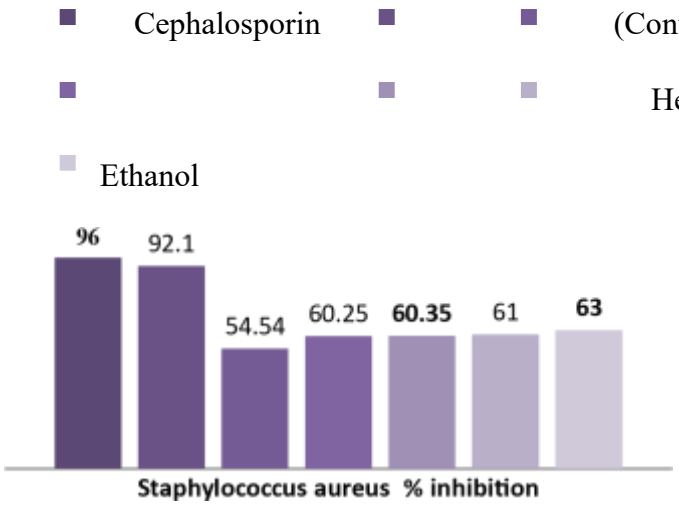


Fig.4.3: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Staphylococcus Aureus (MRSA).

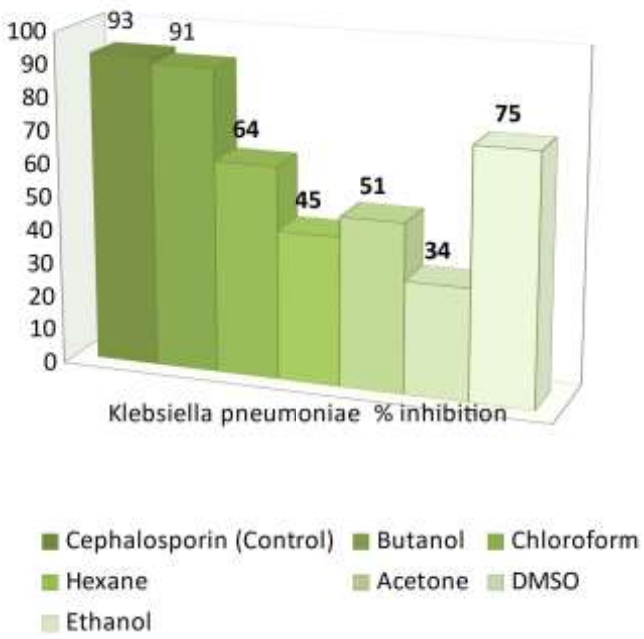


Fig.4.3: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Klebsiella pneumoniae.

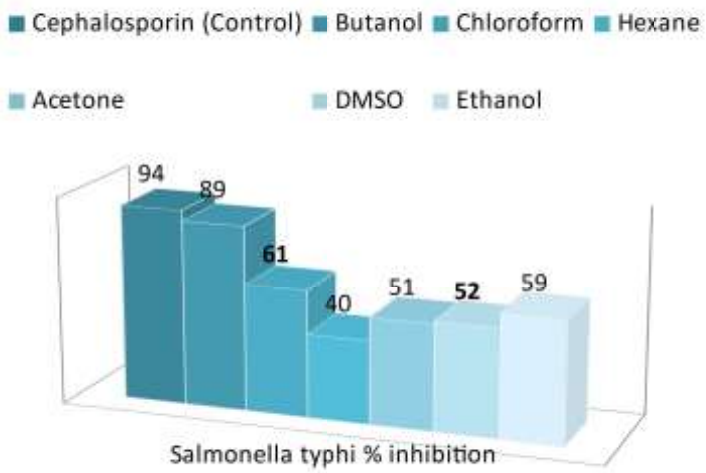


Fig.4.4: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Salmonella Typhi.

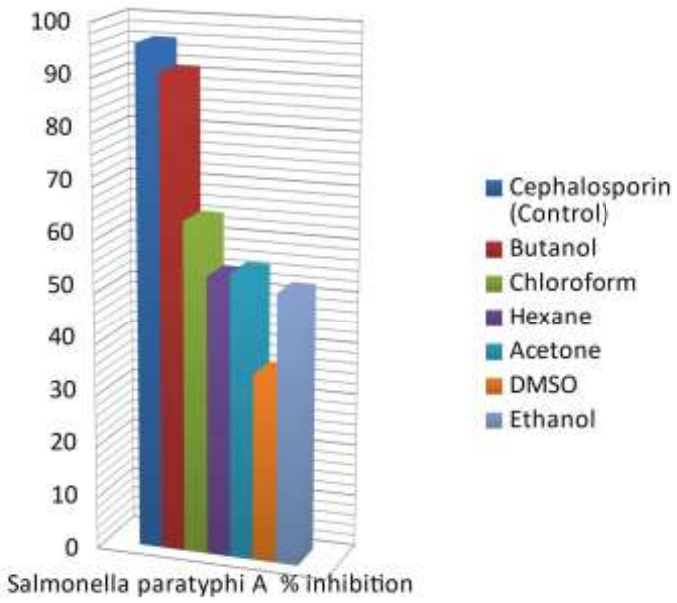


Fig.4.5: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Salmonella Paratyphi A.

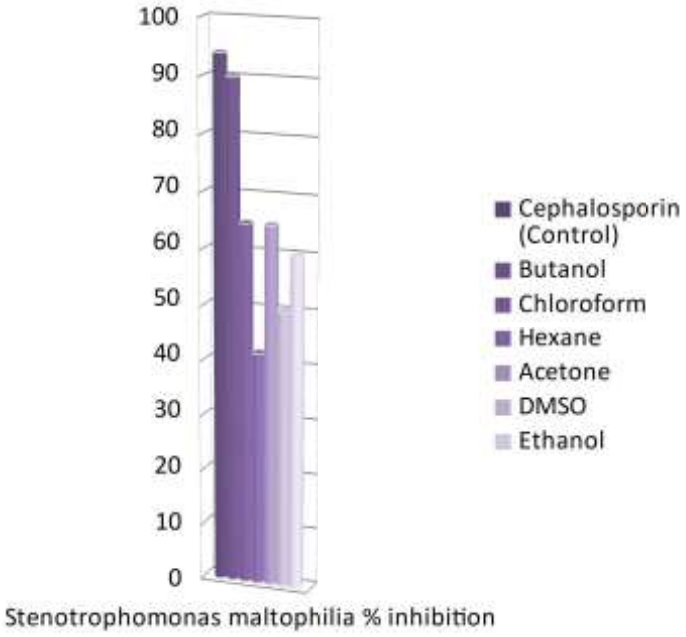


Fig.4.6: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Stenotrophomonas maltophilia.

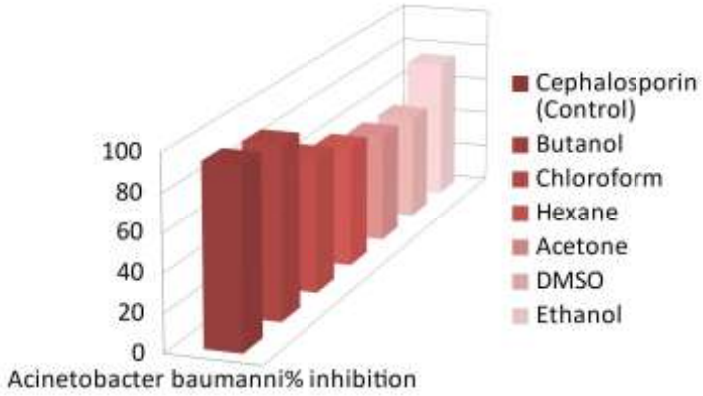


Fig.4.7: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Acinetobacter baumanni.

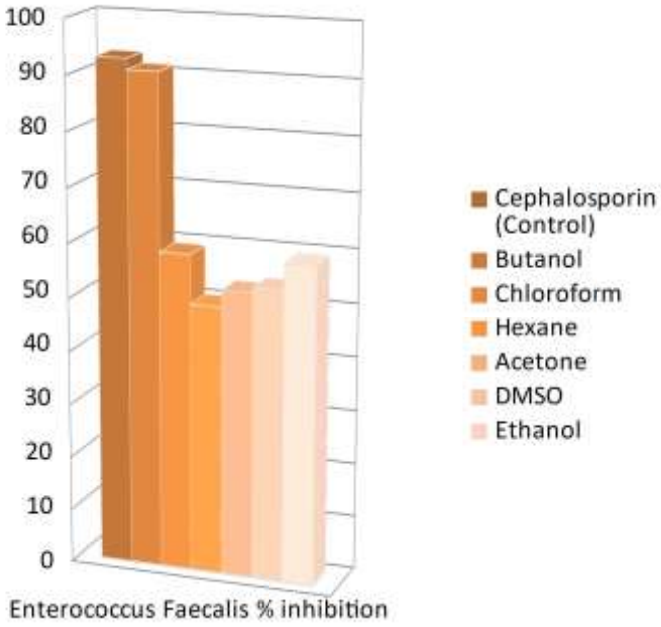


Fig.4.2: Graphical representation of Maximum %inhibition of all solvents extract of C.colocynthis against Enterococcus Faecalis.

Table 4.8: Mean growth inhibition (%) of MDR strains by Control (third generation Cephalosporin)

MDR Strains	Inhibition (%)
Pseudomonas Aeurignosa	94±0.35
Staphylococcus aureus(MRSA)	96±0.96
Klebsiella Pneumoniae	93±0.74
Stenotrophomonas Maltophilia	94±1.2
Salmonella Typhi	91±1.32
Salmonella Paratyphi A	96±0.03
Acinetobacter baumanni	94±0.26
Enterococcus Faecalis	93±0.99

Discussion

The study proves the enhanced or effective result of synergism effect of Citrullus colocynthis root, seed and fruit in both ways including anti-oxidant and anti microbial activity as they show both phenomenon. The goal of the study was to evaluate the least effective concentration used to inhibit the growth by keeping in mind the Plant`s lethal dose. For anti-microbial activity,The novelity of the work is that the compounds isolated in Butanol organic solvent showed the maximum inhibitory affect of 91% against MDR strains in the least concentration of 1.2µg/mL ensuring its pharmaceutical importance. Not all the solvent extracted compound showed anti-microbial potential but they showed different results against different organisms. Butanol extracted compounds showed anti-microbial potential against all MDR strains including Gram +ve and -ve strains. The results of synergism effect of all parts evaluate that, In the used concentration of C.colocynthis all the factors individually produced less anti- oxidant potential as compared to the synergism effect that not only shown the maximum inhibition but give maximum result in minimum concentration of 93µg/mL.

The maximum DPPH inhibitory concentration of Citrullus Colocynthis methanolic root extract is 23.63% alone (Ahmned, et al., 2019) while in the synergism effect with other parts including peel, seed and pulp it gave 93.75% DPPH scavenging activity with the same methanolic extract. The maximum inhibitory effect of fruit was 88.8% according to (Benariba, et al., 2013), while with synergism with its own root can produce 92.76% inhibition with the concentration of 93µg/ml indicating the synergism effect to be more effective even in the less concentration.

The Chloroform extract of Citrullus colocynthis fruit cannot produce antimicrobial activity according to (Priyavardhini, S., Vasantha, K., Umeadevi & M., 2009) while the study reveal all the tested strains of various pathogenic species showed sensitivity towards the same chloroform extract of the Citrullus colocynthis fruit, seed, peel and root and some resistant pathogens produce significant results.

The minimum inhibitory concentration of Citrullus colocynthis fruit chloroform extract can produce antimicrobial activity against ATCC strains of Staphylococcus aureus (MRSA) by MIC of 23.375µg/ml and MBC of 40.376 µg/ml and against Pseudomonas aeurignosa with MIC of 25.375 µg/ml and MBC of 50.750 µg/ml (Belsem, et al., 2012) while the multi Drug resistant strain of Staphylococcus aureus (MRSA) can be inhibited by using MIC with IC50 of 150µg/ml and for Pseudomonas aeurignosa was 300 µg/ml.

Conclusion

Resistance towards multi drugs is considered as one of the major risk for Health Care System, it is necessary to device new ways in order to prevent the spread of these pathogens.. According to findings, Citrullus colocynthis can be used as medicinal plant as its various compounds isolated through organic solvents showed antimicrobial activity against MDR-pathogens. Maximum activity against MDR pathogens of Pseudomonas Aeurignosa, Staphylococcus aureus (MRSA), Klebsiella pneumoniae, Stenotrophomonas maltophilia, Salmonella typhi, Salmonella paratyphi, Acinetobacter baumanni and Enterococcus faecalis was shown by Butanol extract followed by Chloroform, Acetone, Ethanol, Hexane and DMSO extract. The plant also showed 92% anti-oxidant potential in the least concentration of 93µg/mL. Suggestions

- Evaluation of antimicrobial activity of C.colocynthis against XDR can be include.
- Evaluation of other organic Solvents Extracts antimicrobial activity can perform.
- Evaluation of the responsible compounds and their classification as broad spectrum and narrow spectrum antibiotics.

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