

## In Vitro Antifungal, Antibacterial, Antioxident Activity of Bitter Apple (*Citllus Colo Cynthes*) Plant Parts in Acetone

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### Abstract

In this article we studied the dimensionsof anti-microbial and anti-fungal, antioxidantconsequences of *Citrullus colocynthis*(bitter apple) towards a few trendy pathogens. Acetoneextract of fruit, leaf, root and seeds of *Citrullus colocynthis* have been observed and demonstrated asinfluential aninsufficientmicroorganism and fungi.2,2-diphenyl-1-picrylhydrazyl and lipid peroxidation radical approaches were used to estimate the anti-oxidant activities. Ethyl acetate performed the best was shown by these experiments. Anti-microbial properties of this plantwere assessed by testing the different extracts in contrast to foodborne, infective, and decomposition bacteria and fungi. Acetone extract showed anti-bacterial and anti-fungal activities. The outcome of this work demonstrated that bitter appleextracts might be utilized as an anti-oxidant and anti-microbial operator in conservation and nourishment of human health.The studies determined, this plant would be a beneficial supply for antimicrobicantifungal mediators and as well as antioxidant action.

**Keywords:***Citrullus colocynthis*, Antimicrobial and Antifungal, Antioxidant, Acetone

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### 1 Introduction

The survey of World Health Organization found around the 7080% of globally inhabitants relies on the non-traditional medicines, from plant bases, for theirhealth care.Creating nation where the fetched Western-style specialist and the fetchedpharmaceutical surpassmost individuals (Ali AlSnafi's.,2016).Plants are known around the world wheretraditional medication, constitute the

main module of the material utilized in pharmacy; recognized by the WHO studies. Plant exhibited a broad spectrum of pharmacological activities including antimicrobial, antioxidant, anticancer, and many other pharmacological effects (Aljabry A. Set al., 2019). Bitter apple could be fruitless region plant, a supply of abundant bioactive compounds in conjunction with vital fats, alkaloids, fatty acids, glycosides, flavonoids, carbohydrate, protein, and separated amino acid. Medicinal flowers enhance the immune system. The natural product has been deliberated outstandingly for its anti-inflammatory, anti-microbial, and anti-oxidant activities. Seed powder of *C. colocynthis* has been utilized as an emulsifier, fats, and flavoring (Qin-Yuan Li, et al., 2022).

The extract of bitter apple own extensive against bacterial action towards the microorganism examined *C. colocynthis* towards infective microorganisms and determined that *C. colocynthis* confirmed extensive spectrum antimicrobial activity towards fifteen microorganisms, which include microorganism Viz., *Bacillus subtilis* (ATCC6633), *Escherichia coli* (ATCC25922), *Lactobacillus acidophilus* (ID1099), *Staphylococcus aureus* (ATCC25923), *Streptococcus cricetus* (ATCC19642), *Salmonella typhi* (food isolate), *Salmonella enterica* (ATCC14028), *Enterococcus faecalis* (ATCC29212), *Bifidobacterium bifidum* (DSM 20215), *Listeria monocytogenes* (F2365), *Enterococcus eurekaensis* (CCUG61259), *Aspergillus flavus* (NRRL21882), *S. Cerveisea*, *Aspergillus Niger* (CTM1009) and *Rhizopus* (RA99-880). No correlation changed into discovered among sensitivity, draining to anti-biotic resistance and plant extract. Extensive anti-microbial activity of active extracts was differentiated from the average Tobramycin (5-10 mcg/mL). +The scavenging ability of 2,2-diphenyl-1-picrylhydrazyl from aqueous dilute acetone extracts was strong minded from different parts. All organ extricates affirmed a dose-relying antioxidant movement; however, outcomes had been selecting and depending on the extricates. The found outcomes powerfully help the utilization of *C. colocynthis* as a vital supply of herbal antioxidant agents. (Z. Marzouk, B. Marzouk et al., 2010)

## 2. Materials and Methods

Bitter apple develops on desert region of Rahim Yar Khan. Fruits of this plant parallel to the watermelon, however are tiny. It has a very bitter taste; Therefore, it was traditionally utilized only for treating some diseases.

**Arrangement of basic extricates:** Fruits and seeds of this plant were discussed, dried out into powder employing on mortar and pestle. 100-gram powder at room temperature for 4 to 5 days was removed progressively with acetone. The extricates were to begin with sifted through Whatman filter sheet.

## Culture Media:

**Nutrient Broth:**It consist of yeast extract,peptone, and sodium chloride.13 grams of medium dissolves in one liter of distilled water to produce this broth, then autoclaved at 121degrees Celsius for 15 minutes and pH was set to 7.4 and then filled into 5 ml screw cap bottles.

**Mueller Hinton Agar:** 38 gramspowderwas weighed, poured into 1-liter distilled water and soaked, placed for dissolving, shaken then autoclaved at 121degrees Celsius,airconditionedat 47degrees Celsius, mix well, and then transferred into sterilized Petri dishes.

**Sabouraud Dextrose Agar:**62 grams powder was weighed, poured into1 liter water, soaked for 10 minutes, shaken to mix, autoclaved at 121 degrees Celsius, and airconditionedat 47degrees Celsius, mix well and then transferred into sterilized Petri dishes.

**Antimicrobial Activity:** Test Strains and Culture Media: Microorganisms strains were used and obtained from the Lab of University of Lahore. The bacterial species used: *Bacillus subtilis* (ATCC6633), *Lactobacillus acidophilus* (ID1099),*Staphylococcus aureus* (ATCC25923), *Streptococcus cricetus*(ATCC19642), *Enterococcus faecalis* (ATCC29212),*Bifidobacteriumbifidum*(DSM20215)Gram negative bacteria; *Salmonella enterica* (ATCC14028), *Listeria monocytogenes* (F2365), *Salmonella typhi* (ATCC6539),*Escherichia coli* (ATCC 25922), *Enterococcus eurekaensis*(CCUG61259). The fungal species were *Aspergillus flavus* (NRRL21882), *S. Cervisea*and *Rhizopus*(RA99-880), *Aspergillus Niger*(ATCC9763).

**Antibacterial Assay:** Anti-bacterial actionwasassessedthrough disk spreadingtechnique with some modifications. 20, 10 and 5 mg/ml Concentration solutions of extracts were organizedthrough dilution and 5% dimethyl sulfoxide. Filter sheet disks with a diameter of 6.0 milli meter were soaked with 10 µl of crude extracts after solidificationand then dried. DMSO was used as a negative control.While tobramycin (5-10mL)used as a positive control.Petri dishes incubated at 37 degrees Celsius for 24 h, anti-bacterial discs were spread on the inoculated agar plates.

**Antifungal Assay:** Similartechniqueused for bacteria to assessment anti-fungal activity, by using Sabouraud dextrose agar. Inoculated medium for *Candida albicans* was incubated at 25 degrees Celsius for two days, three days for *Aspergillus Niger*(Aljabry, A.S *et al.*, 2019).

**Antioxidant Assay:**The scavenger activity was firm by the spectrophotometric technique supported the reduction of a DPPH solution in methanol. experiment or standard control (Alayed, A.Set *al.*,2012).The reaction mixture of 3ml DPPH in 100 %methanol, antioxidant resolutionas an experiment or customary regulator and left at room temperature. This formula  $100 - (\text{Abs of test} / \text{Abs of con}) \times 100$  was used to calculate the percentage of DPPH decolorization activity (purification)(Akteret *al.*,2020).

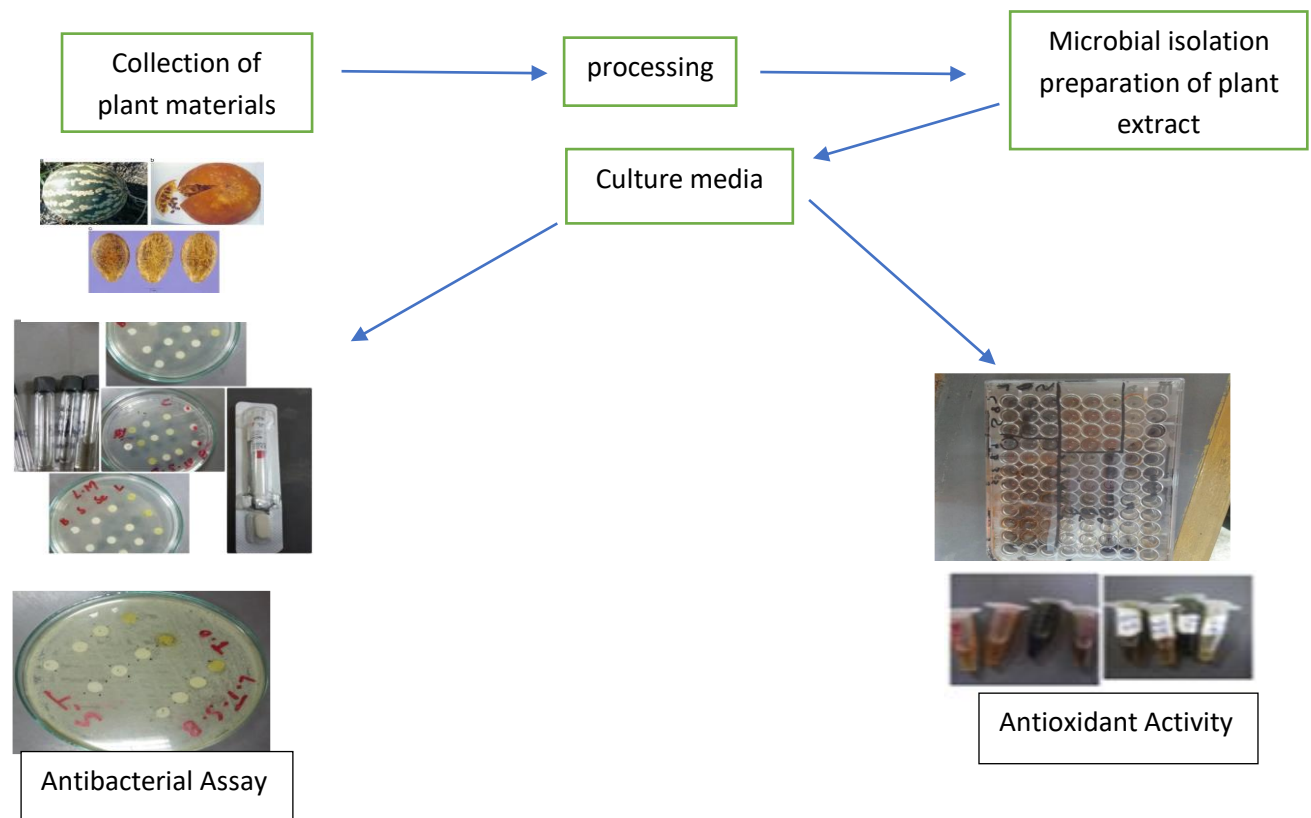


Fig:1

### 3.Results

Microbial infections are a major problem in world. Although bitter apples are known as drugs, these are no published reports on the effect of acetone extract on clinical isolate in the local areas of Lahore. In this study antibacterial of antifungal and antioxidant activity on seed and fruit and leaves of acetone extract was investigated.

**Table3.1Representation of Antibacterial Activity (zone of Inhibition)**

Strains	CC.F.C	CC.ST.C	CC.L.C	CC.SE.C
<i>Escherichia coli</i> (ATCC 25922)	10	13	14	15
<i>Salmonella typhi</i> (ATCC6539)	8	8	10	9
<i>Salmonella enterica</i> (ATCC14028)	8	9	9	8
<i>Listeria monocytogenes</i> (F2365)	13	12	14	12
<i>Enterococcus aurekensis</i> (CCUG61259)	9	10	14	10

<i>Bacillus subtilis</i> (ATCC6633)	9	8	8	11
<i>Lactobacillus acidophilus</i> (ID1099)	8	8	10	8
<i>S. aureus</i> (ATCC25923)	6	8	7	8
<i>S. cricetus</i> (ATCC19642)	16	12	11	18
<i>E. faecalis</i> (ATCC29212)	14	14	14	15
<i>Bifidobacterium bifidum</i> (DSM20215)	8	8	7	8

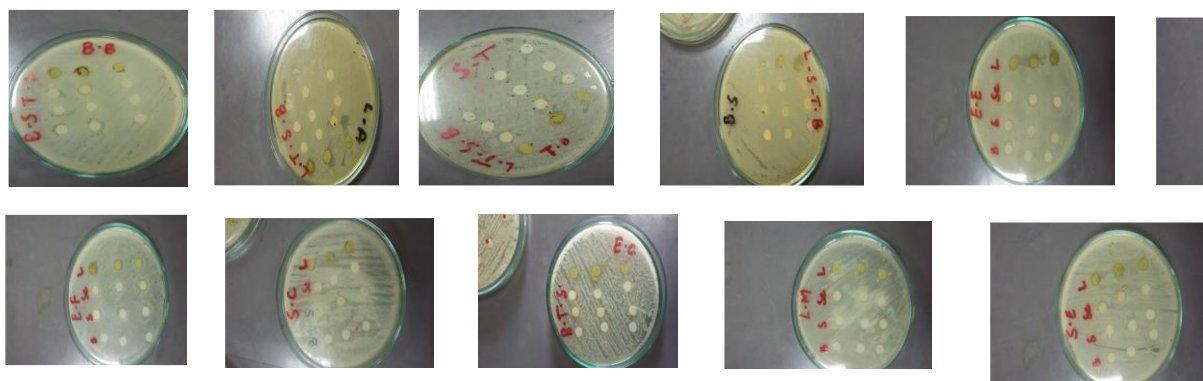
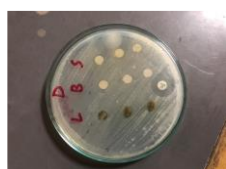


Fig:2

### Representation of Antifungal Activity (zone of Inhibition)

Table3.2

STRAINS	CC.ST.C	CC.F.C	CC.SE.C	CC.LE.C
<i>Aspergillus Niger</i> (clinical)	0	0	0	0
<i>A. flavus</i> (ATCC200026)	10	21	16	26
<i>Rhizopus</i> (ATCC52813)	10	12	8.8	9
<i>S. cerevisiae</i> (ATCC9763)	0	0	0	0



A) *Aspergillus niger* (ATCC52813)



B) *S. cerevisiae* (ATCC9763)



C) *Rhizopus* (ATCC52813)



D) *A. flavus* (ATCC200026)

Fig:3

Representation of Antioxidant Activity

Table3.3

PLANT PART ( <i>C. COLOCYNTHIS</i> )	% OF SCAVENGER+SD 100MG/ML
Leaves	1.91± 0.114984
Fruit	0.4±0.156673
Stem	0.307± 0.160594
Seed	0.529±0.118374



Fig:4

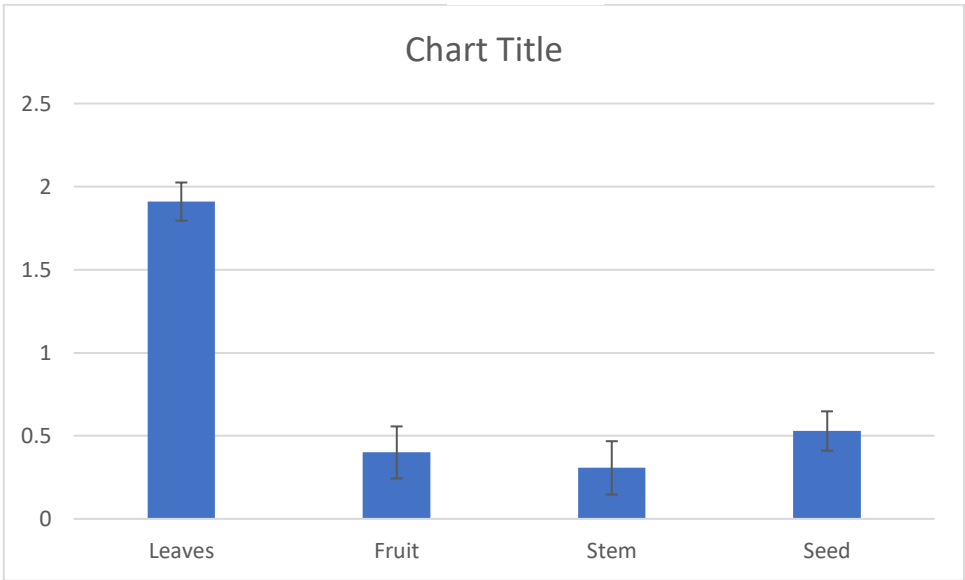


Fig:5Scavenger effect of *C. colocynthis* extracts at different concentrations on the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH).

#### 4. Discussion:

This experiment contrasts as of the earlier ones by circumstance the prior instructions used fruit with a single solvent; while stem, leaf, seeds and fruits were used as substrate and acetone solvents were used with at adjustable meditations in this study. The outcomes of the recent study demonstrate that *C. Colocynthis* has anti-bacterial, antifungal actions in contradiction of all microorganisms tested (Table 1) along with antioxidant possessions (Figure 2). Antioxidant activity is initiated in four extracts; fruit, seed, stem, and leaf. Results are reliable by Ali AlSnafi's efficient assessment, which evaluated *C. colocynthis* as an auspicious therapeutic plant through an inclusive range of pharmacological activities that would be used in various medicinal claims due to its potency and protection. (Ali AlSnafi's., 2016) Another study consistent with the recent one is that of S. Shayamala *et al.* accomplished who decided that the leaf extract of anti-bacterial compassion was detected via the diffusion disc technique through measuring the diameter of the region of inhibition. *C. Colocynthis* chloroform acetone extract displayed important anti-bacterial activity in contrast to all bacteria tested (S Shyamala *et al.*, 2009). A topical study by Ilham *et al.* Anti-bacterial action of *C. colocynthis* in contradiction of different species of bacteria, exhibited that *C. colocynthis* is an appreciated plant source of curatively beneficial compounds. The fruit extracts were a virtuous basis of bioactive ones. Compounds that revealed virtuous anti-microbial properties (Ilham Bnyan *et al.*, 2013). Different biochemical assays were used to measure antioxidant potential of *C. colocynthis* extracts by Anis Ben HSOUNA *et al.* DPPH free radical searching activity, reservation of lipid peroxidation through the carotene-linoleate structure. From the predisposition of the numerous extracts to permitted activists, it can be decided that the *C. colocynthis* ethyl acetate extract has a tougher and comprehensive spectrum of antioxidant movement associated to the other bitter apple extracts. In reality, contemporary study exhibited, acetone extract had pointedly advanced DPPH searching activity. This consequence establishes the hydrogen-donating capacity, subsequently properties of anti-oxidants on scavenging DPPH radicals are believed to be due to their hydrogen-donating capacity and concluded that the *C. colocynthis* acetone extract has a good antioxidant action associated to the other *C. colocynthis* extracts. Recent study also illustrates the existence of various Phytochemicals along with organic activity that can be of treasured beneficial catalogue. The results demonstrate that *C. colocynthis* plant amusing in tannins and phenolic complexes have anti-bacterial and antifungal activities against a range of microorganisms, as well as antioxidant activity. The present study justifies the declared use of *C. colocynthis* leaf, fruit, seed and stem extracts in the outmoded medicine system for the action of numerous infectious illnesses caused by the microbes.



### Conclusion:

Among the extracts tested, only acetone extract showed bactericidal, fungicidal and antioxidant actions. To our acquaintance, this is the first statement on the chemical conformation, antioxidant and anti-microbial activity, anti-fungal of acetone extracts from *C. colocynthis* seed, fruit, stem and seed. The consequences of this exertion point to that bitter apple extracts could be utilized like an anti-oxidant, anti-microbial agent in nutrition conservation and social health. Bitter apple plant consists glycosides, flavonoids, alkaloids, and fatty acids, carbohydrate, protein, separated amino acid. tannins, phenols, and numerous additional biochemical collections. It influenced anti-oxidant, antibacterial, antifungal and several additional properties. This article will highlight the chemical ingredients and pharmacological properties of *C. colocynthis*.

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