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EVALUATING THE ANTI-BACTERIAL, ANTI-FUNGAL, ANTIOXIDANT ACTIVITY OF WILD PLANT *CITRULLUS COLOCYNTHIS* AGAINST MULTI DRUG RESISTANCE PATHOGENS USING DIFFERENT CHEMICAL EXTRACTS.

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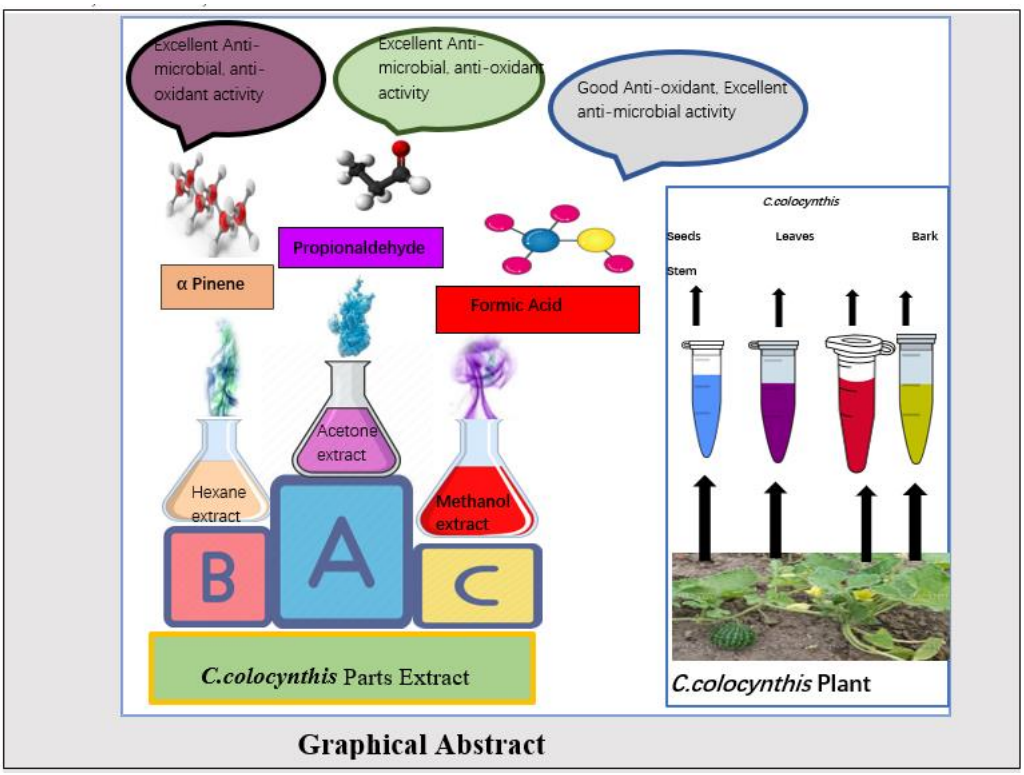
Abstract:
Citrullus colocynthis leaves, fruits, stem, and seeds have been found to offer high potential for producing plant protection products with a wide range of biological activities. As a result, in this investigation, we describe the possible antibacterial and anticancer characteristics of *C. colocynthis* seeds extracted with different polarity solvents, such as methanol (ME), hexane (H.E.), and chloroform (A.E.). *C. colocynthis* seed, fruit, stem, and leaf extracts were tested for antibacterial activity against gram-positive and gram-negative bacteria and fungus. All extracts exhibited notable antibacterial activity with minimum inhibitory concentrations (MICs) ranging from 70% against Lactobacillus acidophilus (ATCC4356) and Streptococcus equinus (ATCC 9812); At the same time, they were found to have moderate activity (MIC 80%) against *Enterococcus faecalis* (ATCC29212) and *Enterobacter sakazakii* (ATCC29544) strains and had the highest antibacterial activity against *Enterobacteriaceae* (ATCC25922, *Bacillus Alcalophilus* (ATCC27647)) and *Listeria monocytogenes* (ATCC 13932) with an MIC value of 100%, which is equal to the standard dose of the positive control drug tobramycin in this study. While for the antifungal drugs hexane and methanol , all extracts had the lowest MIC (0%) against *S. Cervisiae* (ATCC 9763) and moderate activity (MIC 60%) against *Rhizopus spp* (ATCC 52813). The extract was spectrophotometrically investigated using radical scavenging with 1, 1-diphenyl-2-picryl hydrazyl. *Citrullus colocynthis* extracts in methanol, acetone, and n-hexane inhibited the most. Because of the seed extract's high antioxidant ability, the 1, 1-diphenyl-2-picryl hydrazyl technique yielded 0.15% and hydrogen peroxide yielded 10_50 g mL-1, respectively. As a result, the primary goal of this thorough study is to offer an overview of the data on the beneficial and detrimental effects of *C. colocynthis* ingestion on human health. This narrative piece will continue to discuss studies on the potential of this

intriguing natural fruit and its bioactive chemicals in human medicine as nutraceuticals and superior therapeutic medications. To the best of our knowledge, all of the plant components found in this investigation were extracted for the first time from *C. colocynthis*.

Keywords: Plant extracts, *Citrullus colocynthis*, Antimicrobial activity, Antioxidant activity, n-Hexane, Methanol, Acetone.



1. Introduction:

Folk medication has long been subordinate on plants, which are considered as a vital source of bioceuticals for the treatment and avoidance of endless maladies for eras (Rhulani Makhuvele *et al*.,2020). Though colossal advance in cutting edge pharmaceutical, a tremendous chunk of populace over the globe, and especially, individuals of a moo- wage category, are still subordinate on normal product-based conventional strategies of treatment for curing a assortment of sicknesses (Zhenyu Zhao *et al*.,2021). The overwhelming utilize of these treatment strategies is basically determined by antiquated information, neighborhood assurance, adequacy, and low-cost (Solomon Tesfaye *et al*.,2020). In fact, the later rise of pandemics has enormously reestablished interface within the application of characteristic items, counting plant-based materials and their compounds as nutraceuticals (Alessio Alesci *et al*.,2022).Plant components are often dried, pulverised, or extracted to create goods known as botanical solutions, homemade pharmaceuticals, or phytotherapeuticals (Salehi B *et al*.,2018).As a result, a variety of commercial medications have been created from plant sources, and in reality, in the discovery of new pharmaceuticals, plant materials provide a few advantages due to their abundance in nature and extensive geographic conveyance (S Mohamed Musthaba *et al*.,2009). By the by, present day medicate disclosure strategies are still to a great extent subordinate on the method of extraction from common items, alteration of as of now connected phytotherapeuticals, plan and blend of particles imitating phytoconstituents, etc (Gordon M Cragg *et al*.,2013).

Notably, phytochemicals are secondary metabolites produced by plants to defend themselves from pathogenic bacteria, insects, and other dangerous species (Vishnu D. Rajput *et al*., 2021). Because

most phytochemicals have antibacterial capabilities, they can protect humans and animals from infectious illnesses caused by germs or poisons (M. Asimuddin *et al*., 2019). Phytochemicals comprise a wide range of substances such as phytosterols, terpenoids, flavonoids, alkaloids, phenolic compounds, carotenoids, organic acids, and protease inhibitors, among others.

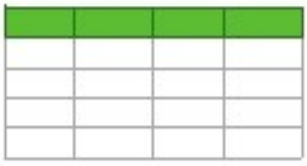


Table 1.1. Nutritional and fatty acid composition of different parts of bitter apple

Conventional medications are used to treat 80% of human ailments in the world's major countries, according to the World Health Organization (Nandhini. VS and Viji S. BG., 2015). Regarding to ethnopharmacy as a means of bio-active compounds has expanded around the world, especially to explore anti-inflammatory drugs (Marazouk B *et al*., 2011). The study of medicinal plants as a source of chemical compounds with pharmacological activity is becoming more popular worldwide. Lemon Grass, Licorice, and *Syzigium cumini* are aromatic medicinal plants that produce essential oils that are used to fight antimicrobial activity. Herbal medicines made from S. cumini plant components are thought to be safer than synthetic ones and have a variety of therapeutic properties. The extract from S. cumini leaves exhibits antibacterial and antifungal action against several pathogenic microorganisms, making it useful in the treatment of skin wounds (Hajra Sohail and Hafiza Ayesha Andleeb *et al*., 2023). A traditional Chinese herb called *Cymbopogon citratus* (DC) was used in a prior study. In Brazilian folk medicine, stapf, also called lemongrass, was used to treat H. pylori. According to Hafiza Ayesha Andleeb *et al*. (2022), it was an aromatic plant. Licorice is primarily used in traditional medicine and medical education to treat HCV, liver damage, influenza, and cardiovascular diseases. According to a prior study (Hafiza Ayesha Andleeb *et al*., 2023), Licorice metabolites have anticancer, anti-inflammatory, antiviral, anti-atherosclerosis, and antibacterial properties.

Citrullus colocynthis is a desert plant that contains a variety of bioactive compounds such as essential oils, glycosides, flavonoids, alkaloids, and fatty acids. *C. colocynthis* also has excellent pharmacological properties such as laxative and purgative properties, as well as anti-diabetic, anti-inflammatory, antihelmintic, and anti-cancerous properties.

While the extract demonstrated anticancer, antibacterial, and antioxidant properties, regular use of antibiotics can lead to drug resistance in animals and people, compromising health. As a result, the European Union prohibited antibiotics as growth promoters in 2006 (Qin-Yuan Li *et al*., 2021).

Solvent extraction is the most often used approach for extracting medicinal secondary metabolites from plants and other natural sources because it is successful and simple to utilize (Gusthinnadura Oshadie De Silva *et al*., 2018). The concentration and yield of secondary metabolites in this approach vary depending on the solvent employed for extraction. This is owing to the solvents' differing polarities and other physicochemical features (Neliswa A Matrose *et al*., 2021).

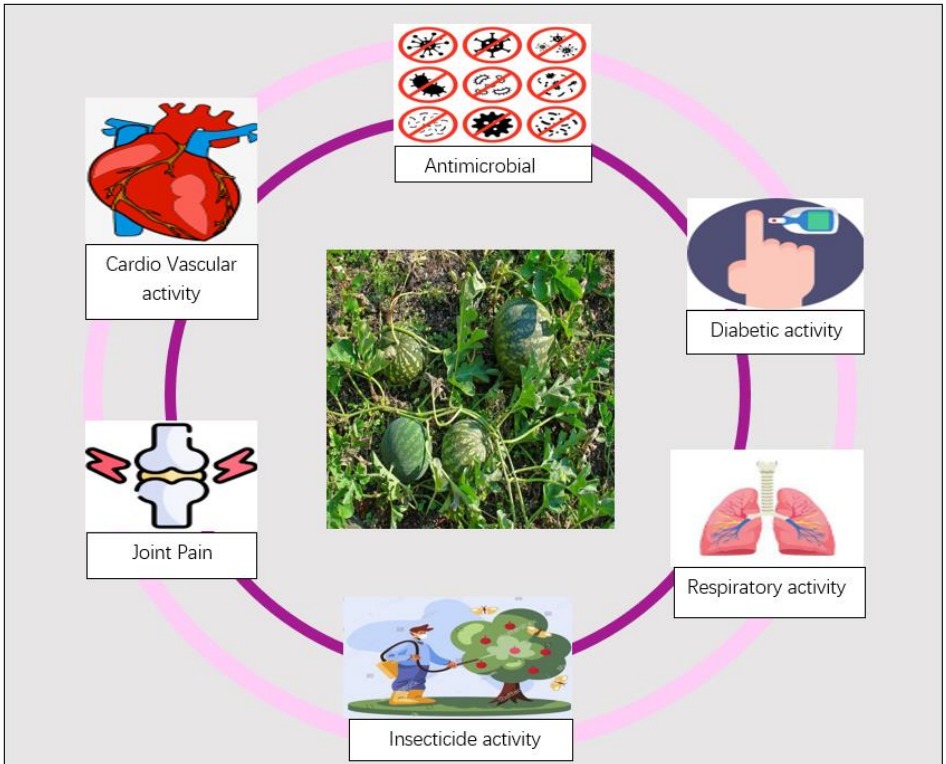


Fig 1.1. Applications of *Citrullus Colocynthis* Plant

Previous research by Hafiza Ayesha Andleeb *et al*. *C. colocynthis* and its different parts (fruit, leaves, stems, and seeds) were soaked in n-hexane and methanol as solvents. *Citrullus colocynthis* n-hexane methanol extract has been tested against various pathogenic microorganisms (Hafiza Ayesha Andleeb *et al*., 2023). For example, Chiavaroli *et al*. Rhizophora racemosa G. Different solvents and extraction procedures were used to obtain and study Mey leaf and bark extracts (Annalisa Chiavaroli *et al*.,2020). Methanolic leaf and bark extracts were produced using both homogenizer-assisted extraction and maceration extraction procedures.

The effect of solvents on the biological potential of *C. colocynthis* is investigated in this study. Plant materials were extracted using several solvents, including methanol (M.E.), hexane (H.E.), and acetone (A.E.). Furthermore, the antibacterial and antioxidant activities of each extract were assessed separately against a variety of microorganisms.

2.Materials and Methods

2.1. Plant Substance:

C. colocynthis aerial pieces were found in May 2019 from the Rahim Yar Khan district of Pakistan's Punjab province. The identifications of *C. colocynthis* were verified by Dr. Naureen Zahra of The University of Lahore's IMBB department. The IMBB department at The University of Lahore has a bacterial and fungal sample (24,531) of C. colocynthis.

2.2. Chemicals:

JK Enterprises, a chemical business in Lahore, Pakistan, supplied all of the chemicals, including methanol, chloroform, and n-hexane. Hexane is a non-polar solvent, whereas acetone dissolves easily in water; nonetheless, methanol is entirely miscible in water and is utilized for comparative analysis.

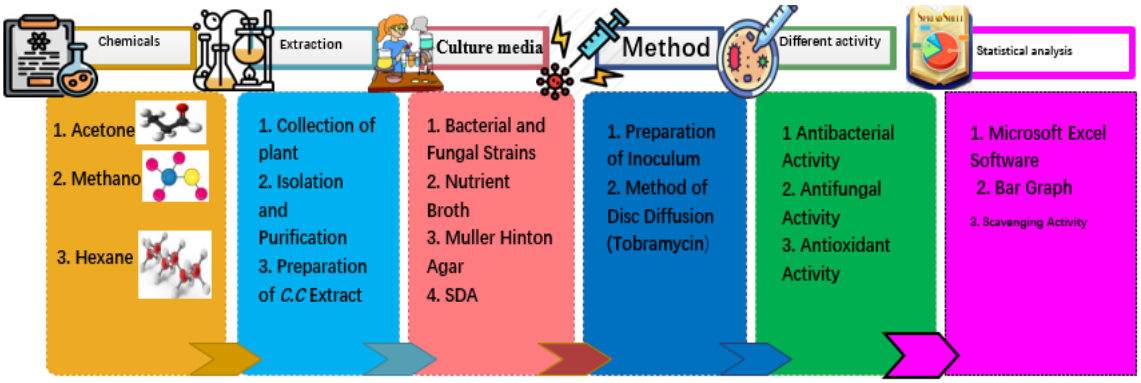


Fig 2.1. Flow Chart of Methodology of Antimicrobial and Antioxidant activity

2.3. *C. colocynthis* extract preparation:

The root, stem, leaf, and fruit of *C. colocynthis* were ground into powder using an electric blender; the seeds were carefully removed from the fruits and ground using a grinder; 30 grammes of the ground powder from each part of the plant were then dissolved in 300 milliliters of n-Hexane, acetone, and methanol in a blue cap bottle, shaken vigorously by hand, and left to stand for five to six days before being filtered through filter papers and transferred into petri plates. After the extract was evaporated and the plates were left exposed to dry, the sticky extract was produced. The evacuation procedure takes two or three days in total. After the extracts have dried, store them in an Eppendorf container. Lastly, store the extracts at room temperature or in the freezer. The antioxidant and antibacterial properties of these dried extracts of n-hexane, acetone, and methanol were examined independently.

Strains used

- **Bacterial strains:**

Total 8 bacteria were strained to examine antibacterial activity. The microorganisms are gram negative: *Enterobacteriaceae (ATCC25922, Salmonella enterica (ATCC 14028), Enterobacter sakazakii* (ATCC29544), *Listeria monocytogenes (ATCC 13932) gram positive: Bacillus Alcalophilus (ATCC27647), Enterococcus faecalis (ATCC29212), Lactobacillus acidophilus (ATCC4356), Streptococus equinus* (ATCC 9812)

- **Fungal strains:**

To test antifungal activity, we used the two fungi listed below.
Rhizopus spp. (ATCC 52813), *S.cervisiae* (ATCC 9763).
These microorganisms can be found at the University of Lahore's microbiology lab 407.

2.4. Media preparation:

The culturing media were chosen for their ability to support the growth of a diverse range of bacterial and fungal strains; 8 strains of microscopic bacteria and 2 strains of fungi were used. Mueller Hinton agar was used; it is a microbiological growth medium commonly used for anti-microbial weakness testing. It was used for bacterial and fungal development, and the rest of the systems were also carried out on similar media; it was designated as the primary requirement for our work. It was initially designed for the separation of pathogenic *Neisseria spp*., but it is now commonly used for vulnerability testing via the Kirby-Bauer plate dissemination procedure.

2.5. Antibiotic drug used:

As a positive control, the drug tobramycin was used.

3.7 Culture media

- **Nutrient broth:**

This medium contains sodium chloride, yeast extract, and peptone. To manufacture it, one litter of distilled water was mixed with thirteen grammes of the medium. After bringing the medium's pH down to 7.4, it was separated into ten milliliter screw-capped bottles and autoclaved for fifteen minutes at 121°C.

- **Mueller Hinton agar (MHA):**

Mueller Hinton agar powder, 38 grammes, was weighed, diluted in one liter of distilled water, and then allowed to soak for ten minutes. Following a 15-minute autoclave at 121°C, the medium was dissolved in a water bath, mixed well, and then cooled to 47°C before being transferred onto sterile Petri plates.

- **Sabouraud Dextrose agar (SDA):**

Following weighing and a 10-minute soak, sixty-two grammes of powdered Sabouraud dextrose agar were combined, chilled to 47°C, and autoclaved for 15 minutes at 121°C to sterilize the mixture. The mixture was then put onto sterile Petri plates.

2.6. Methods

- **Inoculum preparation:**

Microscopic organisms were inoculated in nutrient broth and normal saline. In the case of bacteria, 9 test tubes were taken and each test tube was loaded with 10ml of nutrient broth. In the case of fungi, however, 3 tubes of 10ml normal saline were prepared. As a negative control, one extra tube prepared in both cases was used.

- **Disc diffusion method:**

Muller-Hinton agar sanitized in a flask and cooled at room temperature was applied to sterilized petri plates for microorganism testing. The organized test inoculums were swabbed on the coagulated media's highest point. The discs were put on the media's surface using sterile forceps. The experiments were conducted using various extract concentrations (100l). The extract was loaded into a 100-liter pipette. The disc had a 6 mm diameter. The plates were incubated for a whole day. Millimeters (mm) were used to measure the restriction zones.

2.7. Evaluation of Antimicrobial and Antoxidant Activity

- **Antibacterial Activity**

A panel of four pathogenic bacterial strains, including gram negative *Enterobacteriaceae* (ATCC25922, *Salmonella enterica* (ATCC 14028), *Enterobacter sakazakii* (ATCC29544), *Listeria monocytogenes* (ATCC 13932), and gram-positive *Bacillus Alcalophilus*, were tested for the antimicrobial activity of *C. colocynthis* seeds, fruit, leaf, and stem extracts using the well diffusion method. The Mueller-Hinton agar Petri plates were seeded with 0.1 mL of previously prepared microbial suspensions containing 1.0 107 CFU/mL (equal to 0.5 McFarland standard) to hold the eight pathogenic reference strains. Using a cork borer, 6.0 mm diameter wells were created on the media plates. The produced test extracts were then applied to each well in a sterile laminar air flow chamber, with a dose range of 250-0.48 g/well. A positive control was established using tobramycin standard antibiotic solution, which was employed at a dosage range of 250-0.48 g/well, along with an extract that included dimethyl sulfoxide (DMSO). After the plates were incubated for 24 hours at 37 degrees Celsius, the well with the lowest concentration that displayed the inhibition zone was thought to have the minimal inhibitory concentration (MIC). All trials were conducted in duplicate, and the means are displayed.

- **Antifungal assay:**

Using Sabouraud Dextrose Agar and the same protocol as outlined for testing bacteria, the antifungal activity was evaluated. Two milliliters of the sterile, molten Sabouraud Dextrose Agar (45 to 50°C) were thoroughly combined with 20 milliliters of each fungal stock suspension before

being divided among sterile Petri dishes and allowed to set. The plates were then incubated for 24 to 48 hours at 37°C while standing upright. After the incubation periods, the diameter of the inhibition zones was measured, and the average results were tabulated.

- **Antioxidant Activity:**

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging test was used to assess the levels of free radicals in the stem, leaf, and seeds of C. colocynthis fruit, using a procedure that was slightly modified from. To put it briefly, 3.95 mg of DPPH powder were dissolved in 50 mL of pure methanol to produce 0.15% mL of DPPH reagent, which had a final concentration of 0.08 mg/mL. The extracts of methanol, acetonic, and hexane, as well as the positive control material, butylated hydroxytoluene (BHT), were dissolved in methanol and used at different concentrations (10–50 g/mL). DPPH was mixed with methanol as a negative control. All of the extracts and the controls were added to a 96-well plate in triplicate. The DPPH reagent was mixed with 50 milliliters of each extract. The DPPH reagent was mixed with 50 milliliters of each extract. After that, the plate was covered with foil and left in the dark at RT for sixty minutes. The reaction endpoint indication was the change in colour of the DPPH reagent from dark violet to bright yellow following response with the antioxidants in the sample. The absorbance at 515 nm was measured quantitatively using a microplate reader. The antioxidant activity was expressed as IC50 (g/ml), which is the quantity of antiradical that must be present to inhibit free radicals by 50%. Vitamin C was the norm.

DPPH assay (diphenyl-1-picrylhydrazyl):

DPPH, or 2, 2-diphenyl-1-picrylhydrazyl, is a stable, cell-porous free radical that is commonly employed to measure the antioxidant activity of tissue fragments and assess a compound's capacity to function as a hydrogen donor or free radical scavenger. When an antioxidant or depleting chemical combines with DPPH, hydrazine DPPH2 (absorbance at 515–528 nm) is generated. This reaction results in a hue shift from purple to yellow.

2.8. Analytical statistics

After the data were collected, Microsoft Excel was used to make graphs. Bar graphs displaying the data were created using the mean standard error (SE). Excel was used to analyze the data, and the following formula was used to determine the percentage of free radical scavenging activity, which was expressed as a drop in DPPH absorbance.

%RSA= (Abs of control)-(Abs of sample)/(Abs of control)×100

3. Results and Discussion:

3.1. Chemical Profiling of Various Extracts

Bioactive secondary metabolites are essential to human and plant physiology because they protect against oxidative stress by acting as antioxidants (Peter Cavazos *et al*.,2021). In this regard, a substantial body of research has been documented in the literature describing the vital biological characteristics of secondary metabolites, including their anti-oxidant and anti-microbial capabilities (Dikdik Kurnia *et al*.,2021). Thus, an investigation of the phytoconstituents of different extracts of the seeds, leaves, bark, and stem of *C. colocynthis* is conducted in terms of chemical characterization. Solvents having different polarity, such methanol (M.E.), hexane (H.E.), and acetone (A.E.), are used to extract these extracts. The biological potentials of each extract, including its antioxidant and antibacterial qualities, were also evaluated. The solvent was extracted from the seeds, stem, leaves, and bark of *C. colocynthis* from Pakistan using the standard percolation method at room temperature.

Different ranges of seed, stem, leaf, and bark extract were obtained in M.E., H.E., and A.E., respectively, by using different solvents for the extraction process. First, 1.98 g of stem, 2.13 g of bark, 2.16 g of seed, and 2.13 g of leaf were extracted from *C. colocynthis*. Notably, the extraction processes yielded extracts that were approximately the same color—dark brown—but differed

slightly in quantity and kind based on the secondary metabolites that were isolated. Higher yields with C.E. extraction, for example, have been observed due to the high solubility of a wide variety of phytoconstituents, including medium-polar and polar chemicals in the acetone solution. Table: 3.1. Seed, stem, bark, and leaf extract concentrations in M.E., A.E., and H.E.

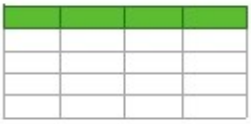


Table: 3.1 Extracts obtained from *C. colocynthis* plant parts

3.2. Antibacterial activity of *C. Colocynthis* in acetonic, methanolic, hexane extract

Citrullus Colocynthis plant extract has been tested for its antibacterial activity against a variety of harmful bacteria, including gram-negative *Enterobacteriaceae* (ATCC25922, *Salmonella enterica* (ATCC 14028), *Enterobacter sakazakii* (ATCC29544), *Listeria monocytogenes* (ATCC 13932), and gram-positive *Bacillus Alcalophilus* (ATCC27647), *Enterococcus faecali* Fruit, stem, seed, and leaf extract all demonstrated the largest zone of inhibition (100%) against *Enterobacteriaceae* (ATCC25922) and the lowest zone of inhibition (80%) against *Salmonella enterica* (ATCC 14028) in A.E, which is identical to the tobramycin antibiotic's (100%) commercial potency. While also tested against *Enterococcus faecalis* (ATCC29212) (80%), hexane extract was less effective (at 70%) against *Lactobacillus acidophilus* (ATCC4356). fig:3.1. Additionally, antibiotics table:3.2. demonstrated the elevated zone of inhibition (100%) against *Bacillus Alcalophilus* (ATCC27647). The methanol extract, however, only showed a very slight amount of activity against the other three bacterial species. It exhibited the lowest zone of inhibition against *Streptococus equinus* (ATCC 9812) (70%), a moderate zone of inhibition against *Enterobacter sakazakii* (ATCC 29544) (80%), and a high zone of inhibition against *Listeria monocytogenes (ATCC 13932* (100%).

Table 3.2: Anti-bacterial activity of Acetone Extract

Strains	**CC.F.C**	**CC.St.C**	**CC.L.C**	**CC.Se.C**	Mechfrank stand end
Enterobacteriaceae (ATCC25922)	9	10	14	10	100%
Salmonella enterica (ATCC 14028)	8	10	9	8	80%

Table 3.3: Anti-bacterial activity of Hexane Extract

Strains	**CC.F.C**	**CC.St.C**	**CC.L.C**	**CC.Se.C**	Mechfrank stand end
Bacillus Alcalophilus (ATCC27647)	10	7	15	14	100%
Enterococcus faecalis (ATCC29212)	10	9	8	8	80%
Lactobacillus acidophilus (ATCC4356)	8	9	10	8	70%

Table 3.4: Anti-bacterial activity of Methanol Extract

Strains	**CC.F.C**	**CC.St.C**	**CC.L.C**	**CC.Se.C**	Mechfrank stand end
Streptococus equinus (ATCC 9812)	9	9	8	17	70%
Listeria monocytogenes (ATCC 13932)	9	12	10	10	100%
Enterobacter sakazakii (ATCC29544)	9	9	9	9	80%

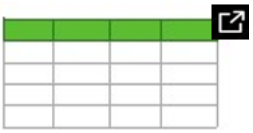


Fig: 3.1. Disc diffusion method for Acetone, n_Hexane, Methanol, extracts of the leaf, fruit, seed and stem against different bacteria.

3.3. *Citrullus Colocynthis'* antifungal properties in methanolic and hexane extract

Hexane extract denoted by (A) was found to have a higher zone of inhibition (17) and a lower zone of inhibition (0) against strains of *Rhizopus spp.* (ATCC 52813), respectively. The 0 zone of inhibition was observed against *S. cerevisiae* (ATCC 9763) strains in the case of the methanolic extract indicated by (b). Hexane demonstrated greater antifungal activity against *Rhizopus spp.* (ATCC 52813) strains in plant parts compared to methanolic extract, while hexane demonstrated greater antifungal activity than methanolic extract against *Rhizopus spp.*

Table: 3.5. Antifungal activity of hexane, methanol extract

Strains	CC.L.N	CC.F.N	CC.St.N	CC.Se.N	Linear growth of extract & control
Rhizopus spp. (ATCC 52813)	**17**	**0**	**12**	**14**	**60%**
S. Cervisiae (ATCC 9763)	**0**	**0**	**0**	**0**	**0%**

3.4. Results of the antioxidant activity in the extract of hexane, acetone, and methanol

The data shown below demonstrates the scavenging capacity of *C. colocynthis* extracts in n-hexane, acetone, and methanol. Of all the sections of *C. colocynthis*, the fruits exhibit the greatest levels of antioxidant and free radical scavenging activity (Hafiza Ayesha Andleeb et al., 2023).

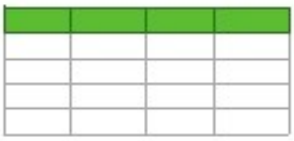
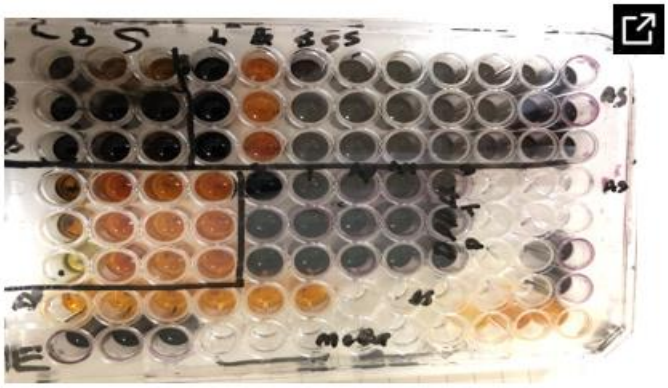


Fig: 3.2. Disc diffusion method for methanol, n_Hexane extracts of the leaf, fruit, seed and stem against different fungi.



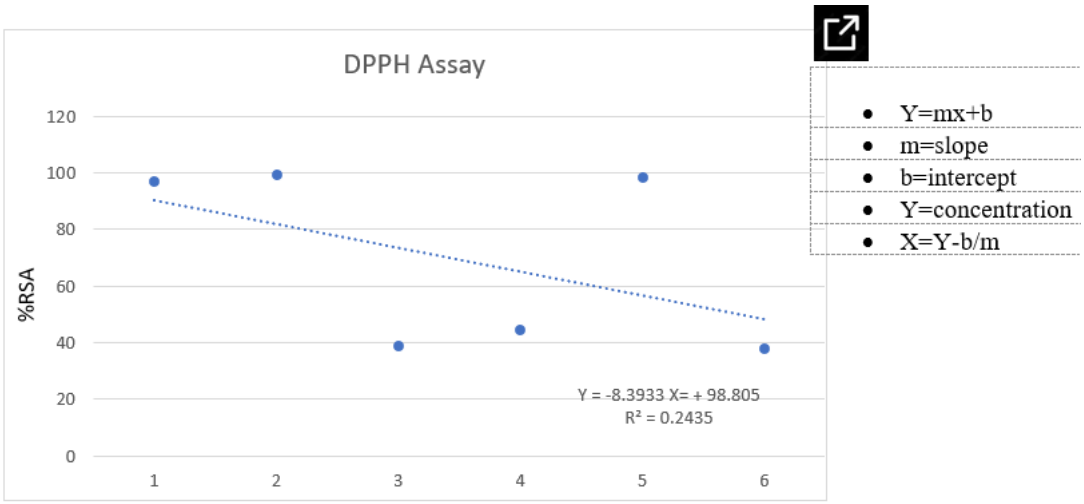
96 well plate used for antioxidant activity

Table:3.6. Results of Antioxidant Activity

Calculation of % Radical Scavenging and IC50 from DPPH Assay						
Absorbance Measurement Data						
		Concentration (µg/ml)	Control	Sample	%RSA	IC50
Acetone	Leaves	10	68.5	1.91	97.21167883	10.58046299
Acetone	Fruit	20	68.5	0.4	99.41605839	9.389036493
Methanol	Stem	30	68.5	41.8	38.97810219	8.197609998
Methanol	Seed	50	68.5	37.99	44.54014599	5.814757009
Hexane	Leaves	10	68.5	0.98	98.56934307	10.58046299
Hexane	Fruit	20	68.5	42.57	37.8540146	9.389036493

Chart showing antioxidant activity results

This graph displays the values of the bitter apple's leaf, fruit, stem, and seed, which are also listed in the table above. These numbers show their antioxidant capacity. When used as a standard, ascorbic acid exhibits 68.5% antioxidant activity. The standard value is used to compare all other plant components. Antioxidant activity is shown by the leaves (1.91), fruit (0.4%) in acetone, stem (41,8%), and seed (37.99%) in methanol, leaves (0.98), fruit (42.57) in hexane. Fruit in hexane extract has the highest potential for antioxidants.



% inhibition of n-hexane, methanol, acetone *Citrullus colocynthis* extract

Conclusion:

The present investigation investigated the impact of the extraction solvents' polarity on the phytochemical composition and biological potential of extracts from the fruit, seeds, stem, and leaves of *C. colocynthis.* In order to achieve this, the phytoconstituents of the examined plant material were separated using three distinct solvents: M.E., A.E., and H.E. The major components of all the studied extracts varied greatly; for example, the M.E. extract showed formic acid and contained one carbon, three hydrogen atoms, and one oxygen atom, while the H.E. and A.E. extracts showed α-pinene and propionaldehyde as their major constituents. The main components and biological potential of the H.E. and C.E. extracts were evidently different from those of the other extracts. Specifically, the A.E. extract—which contains propionaldehyde—showed better antibacterial activity against the majority of the strains under investigation. It showed outstanding antibacterial activity that was nearly as potent as the antibiotic that is sold commercially. Hexane extract, however, exhibits strong antioxidant activity in the fruit portion. Thus, the extracts of *C. colocynthis* seeds, fruit, stem, and leaves may provide a range of commercial entities, food products, and phytopharmaceuticals in the form of pure phytomolecules that are biologically active, like propionaldehyde and α-pinene.

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