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THE PHYTOCHEMICAL PROFILE AND ANTI-MICROBIAL EFFICACY OF CITRULLUS COLOCYNTHIS PULP EXTRACT

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ABSTRACT

This article provides a comprehensive overview of the antibacterial properties of *Citrullus colocynthis* pulp extract, a medicinal herb traditionally used for numerous health conditions, was evaluated for antibacterial properties using the Cup plate Agar diffusion method. The extract exhibited broad-spectrum antimicrobial activity against bacterial strains and there was no observed relationship between the micro-organisms resistance to antibiotics and their susceptibility of the plant extract. Phytochemical analysis of the bioactive extracts revealed the presence of alkaloids, glycosides, flavonoids, Carbohydrates. It possesses many pharmacological properties such as anti-oxidant, anti-microbial, diuretic, hypolipidemic, anti-cancerous activity. When compared to standard antibiotics-amoxicillin, the extract showed notable antimicrobial effects. These findings indicate the potential use of *Citrullus colocynthis* in managing infections caused by the tested pathogens.

KEYWORDS: *Citrullus colocynthis*, Antibacterial activity, Phytochemical analysis, Cup plate Agar diffusion method.

INTRODUCTION

Plants are widely acknowledged as fundamental sources of nutrition and therapeutic agents for humans. Phytochemicals or biologically active compounds derived from plants are often associated with fewer adverse reactions compared to synthetic drugs. *Citrullus colocynthis* a species within the Cucurbitaceae family, It is known by various regional names, such as coloquinte in French, “bitter gourd”, “bitter apple”, “bitter cucumber” in English and Koloquinthe in German. These vernacular names highlight the plant’s notably bitter flavor, which is primarily attributed to Colocynthine, its major active constituent.[1] It is a perennial herb and in India most usually found in sandy lands of Northern West region such as Sind, Punjab, Central region, Southern region and Coromandel costal area.[2] *Citrullus colocynthis* are widely known for their diverse pharmaceutical, medicinal, and nutraceutical benefits.[3]

Botanical description

Citrullus colocynthis is a perennial herbaceous vine known for its small, pale yellow flowers and distinct fruit. The plant features angular, rough stems covered with coarse hairs. These fruits are green with undulating yellow stripes, turning fully yellow upon drying. The fruit is hard, bitter and encased in a rind, containing approximately 200-300 seeds per gourd. Seeds are small (about 6mm long), ovoid, compressed, smooth and brownish when riped.[4] Leaves are alternatively arranged on petioles, rough to the touch, 5-10cm long and 1.5-2cm wide, deeply 3-7 lobed. Flowers are solitary, monoecious, yellow, pedunculated, and located in leaf axils. Each plant produces 15-30 globular fruits, measuring 7-10cm in diameter.[5]

Vernacular names

English	Colocynth, Bitter apple, Bitter cucumber
Hindi	Indrayan
Sanskrit	Indra Varuni
Kannada	Hamekkae, haramekkikayi
Telugu	Eti-puchcha
Tamil	Paedikari Attutummatti
Malayalam	Paikummatti

Taxonomical classification

Kingdom	Plantae
Sub kingdom	Tracheobionta
Super division	Spermatophyta

Division	Magnoliophyta
Class	Magnoliopsida
Sub- class	Dilleniidae
Order	Cucurbitales
Family	Cucurbitaceae
Genus	Citrullus
Species	Colocynthis

Pharmacological activities

Citrullus colocynthis exhibits a wide range of biological activities. Its phenolic compounds show antioxidant properties, while saponins contribute to anti-hyperlipidemic and antidiabetic effects. A reduction in cholesterol levels has been linked to its anti-fertility activity. Flavonoids, Saponins, Tannins and Alkaloids are reported to possess anti-ulcer properties, whereas alkaloids, tannins and Flavonoids demonstrate antimicrobial effects. Additionally, tannins, steroids, flavonoids, alkaloids and iridoids have been associated with antibacterial activity.[6]

MATERIALS AND METHODS

Collection and identification of plant material

The dried *Citrullus colocynthis* fruits were collected from the local market Bengaluru. The identification of study fruit was authenticated by the Department of Botany at Central Ayurvedic Research Institute.

Fig no:1; Dried *Citrullus colocynthis* fruit pulp.

Method of extraction

Prior to extraction preparation, the dried fruit pulps were finely ground using a grinder and it is sieved. Subsequently, powdered pulp were soaked in sufficient volume of water and ethanol (20:80) for 24hrs.Then the mixture is filtered and subjected to rotary evaporator to get the crude extract.[7]

Fig no:2; Maceration

Fig no:3; Extract.

Phytochemical analysis[8]

Phytochemical tests were carried out using ethanol extract of *Citrullus Colocynthis* using standardized procedures to identify the constituents as described by the Harborne.

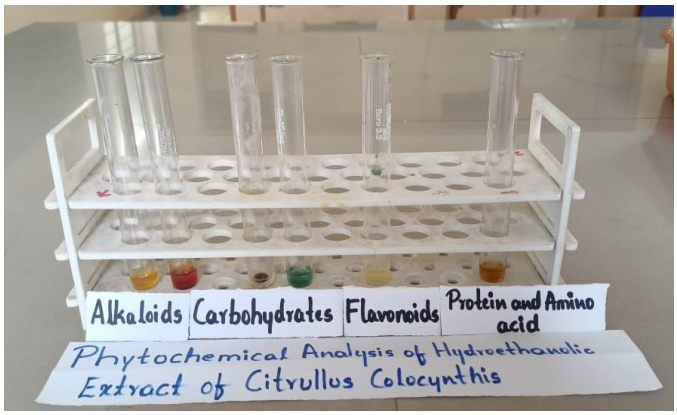


Fig no:4; Phytochemical analysis.

a) Alkaloids - Extracts were dissolved individually in dilute hydrochloric acid and filtered		
Test	**Procedure**	**Observation**
Mayer's test	Few ml filtrate + 1-2 drops of mayer's reagent	Yellow precipitate
Dragondroff's test	Few mL filtrate + 1-2 drops of dragondroff's reagents	Brown/Reddish precipitate
Wagner's test	Few mL filtrate + 1-2 drops of wagner's reagent	Brown/reddish precipitate
Carbohydrates - Extracts were dissolved in distilled water and filtered		
Molish's test	Few mL filtrate + 2 drops of α naphthol + con H_2SO_4	A violet ring
Barfoed's test	Few mL filtrate + barfoed reagent + heated for 2min	Orange Red precipitate
Flavonoids		
Alkaline reagent test	Extract + few drops NaOH solution	Yellow colour
Lead acetate test	Extract + few drops lead acetate solution	Yellow precipitate
Protein and Amino acid		
Ninhydrin test	Extract + 2 drops of ninhydrin solution +boil for	Blue colour

	few minutes	
Xanthoprotein test	Extract + few drops of Conc. Nitric acid	Yellow colour
Phytosterols		
Salkowski's test	Extract + few drop of chloroform +filtrates+ Conc. H_2SO_4, shaken well and allowed to stand	Golden yellow colour

Antibacterial activity

Antimicrobial resistance is a serious global health challenge that calls for the discovery of new treatment strategies. The excessive use of antibiotics, along with their careless release into the environment, has accelerated the rise of resistant microorganisms. Many modern medicines have their roots in plant extracts, which have long been studied for their pharmacological potential. Plants produce a wide range of secondary metabolites such as alkaloids, phenols, terpoids that help them adapt and survive in their natural environment. These diverse phytocompounds serve as promising sources of bioactive molecules. Combining plant derived compounds with conventional antibiotics could offer a powerful approach to tackling antimicrobial resistance and improving the treatment of infectious diseases.[9]

Procedure

Antibiotic sensitivity test was performed using Agar well diffusion method. Nutrient agar medium was prepared and sterilized. 20ml of nutrient agar was transferred into culture tubes. Transfer 1ml of the inoculum to the nutrient agar medium after cooling to 40˚C. Pour the agar medium into a sterile petridish. Prepare the solution of known concentration of antibiotics to be tested. Bore cavities in the agar medium using a sterile cork borer under aseptic conditions. Transfer a known volume of the antibiotic into the cavity. Same procedure was carried out for plant extract. Leave the plates at room temperature for 1 hr to allow diffusion of antibiotic and plant extract into the medium and then both were incubated at 37 ˚C for 18hrs. After incubation, the antibiotic and plant extract plates were examined for zones of inhibition and diameters of the zones were measured and recorded.

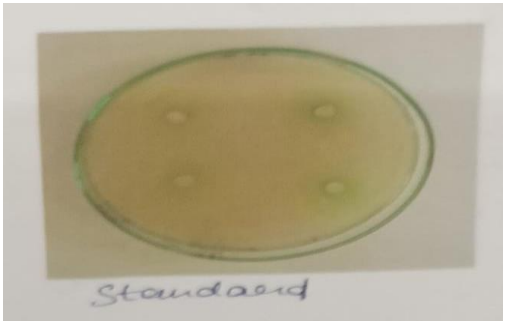
Standard Amoxycillin Preparation

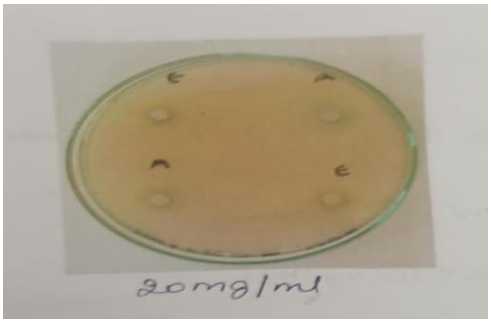
Equivalent to 30mg of powdered capsules diluted in 50ml of double distilled water. Further serial dilution was carried out to obtain final concentration of .50 microgram per ml concentration.

Plant Extract Preparation

1gm of *Citrullus Colocynthis* Extract is diluted in 10 ml of double distill water (100mg/ml), in that 1ml is diluted in 10ml of double distilled water(10mg/ml), again 1ml is taken and diluted in 10ml of double distilled water(1mg/ml), in 100 mg/ml 2ml is taken and diluted in 10 ml of double distilled water(20mg/ml).

RESULTS





Standard/Plant Extract	Zone of Inhibition-1 (mm)	Zone of Inhibition-II (mm)	Average
Amoxicillin(20mg/ml)	1.1	0.9	1.0
Amoxicillin(100mg/ml)	1.8	1.3	1.55
C.C Extract(20mg/ml)	0.3	0.6	0.45
C.C Extract(100mg/ml)	0.6	0.8	0.7

DISCUSSION

The present study aimed to evaluate the phytochemical profile and antimicrobial activity of the Citrullus colocynthis pulp extract using agar well diffusion method. The phytochemical analysis revealed the presence of key bioactive compounds as alkaloids, flavonoids, carbohydrates, proteins and phytosterols, which are known to contribute to the pharmacological potential of medicinal plants. These constituents are widely reported to possess antimicrobial, antioxidant and anti-inflammatory activity. The hydroethanolic extract of Citrullus colocynthis demonstrated a dose-dependent inhibitory effect against the tested

bacterial strains and the zones of inhibition produced by the plant extract exhibits measurable antimicrobial properties. This suggests that Citrullus colocynthis contains secondary metabolites capable of inhibiting bacterial growth, which aligns with previous studies highlighting its broad pharmacological spectrum.

The antimicrobial efficacy observed may be attributed to the synergistic effects of multiple phytochemicals present in the extract. These compounds possibly interfere with bacterial cell wall synthesis, enzyme function by inhibiting bacterial proliferation. The moderate antibacterial activity recorded at higher concentration (100mg/ml) indicates that the extract is less potent than synthetic antibiotics, but it still holds potential as a complementary therapeutic agent.

CONCLUSION

The findings of this study demonstrates that citrullus colocynthis pulp extract possesss significant phytochemical constituents and exhibiting promising antibacterial activity. The presence of alkaloids, flavonoids and other bioactive compounds underscores its potential as a natural source of antimicrobial agents. Although its inhibitory effect was lower than that of standard antibiotic amoxicillin, the results validate the traditional use of citrullus colocynthis in managing infectious diseases. Further studies focusing on purification, compound isolation and mechanism of action are necessary to enhance its therapeutic applications and to explore its potential in developing new plant based antimicrobial drugs

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