Section: Research Paper

Studies on antimicrobial activity of *Citrullus vulgaris* (Watermelon) Seeds Extract on selected Bacteria

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Abstract:

Herbs are natural remedies for various diseases with higher safety and efficacy profile. India is gifted with varieties of large number of medicinal herbs because of variety of climatic conditions and seasons favourable for growth of many species of plants. Amongst the large number of herbal drugs existing in India, very few have been studied systematically so far. Citrullus vulgaris is a highly valuable drug and is one of the essential ingredients in the most of the compound preparations included in Ayurvedic literature. It is well known for number of pharmacological activities like hepatoprotective activity, anti-inflammatory, wound healing, Antidibetic activity, hypotensive effect, immunomodulating property, bronchial catarrh, dysentery, diarrhea and to prevent falling of hair, promotes the growth of hair, and antimicrobial activity against both gram-positive and gram- negative bacteria. Its fruit juice active constituents shows antiseptic, insecticidal and parasiticidal properties, against conjunctivitis and is used also to check hemorrhage from cuts, bruises and wounds insect repellent. It is also used as bioadsorbent for chromium. Here we try to make attempt for focusing on antimicrobial activity of Citrullus seeds extract. Their antibacterial activities were evaluated in vitro against clinical bacterial isolates. In effort to identify novel bacterial agents, this study was initiated to evaluate the antimicrobial properties of Citrullus seed extract against E. coli and Staphylococcus aureus by using Agar well-diffusion assay. The seed extracts demonstrated good antimicrobial activity against these bacteria tested with inhibition zones. The minimal inhibitory concentration (MIC) values of extract against the tested bacteria were found to 47.3 mg/ml and 52.20 against E.coli and Staphylococcus aureus respectively. The methanolic extract of seeds exhibited a pronounced activity against E. coli and Staphylococcus aureus. The minimum inhibitory concentration was found in methanolic Extracts of Citrullus vulgaris against test organisms.

Introduction:

Watermelon (Citrullus lanatus) is a flowering plant species of the Cucurbitaceae family and the name of its edible fruit. A scrambling and trailing vine-like plant, it is a highly cultivated fruit worldwide, with more than 1,000 varieties.

Watermelon is grown in favorable climates from tropical to temperate regions worldwide for its large edible fruit, which is a berry with a hard rind and no internal divisions, and is botanically called a pepo. The sweet, juicy flesh is usually deep red to pink, with many black seeds, although seedless varieties exist. The fruit can be eaten raw or pickled, and the rind is edible after cooking. It may also be consumed as a juice or an ingredient in mixed beverages.

The watermelon is an annual that has a prostrate or climbing habit. Stems are up to 3 metres (10 feet) long and new growth has yellow or brown hairs. Leaves are 60 to 200 millimetres (2+1⁄4 to 7+3⁄4 inches) long and 40 to 150 mm (1+1⁄2 to 6 in) wide. These usually have three lobes that are lobed or doubly lobed. Young growth is densely woolly with yellowish-brown hairs which disappear as the plant ages. Like all but one species in the genus Citrullus, watermelon has branching tendrils. Plants have unisexual male or female flowers that are white or yellow and borne on 40-millimetre-long (1+1⁄2 in) hairy stalks. Each flower grows singly in the leaf axils, and the species' sexual system, with male and female flowers produced on each plant, is (monoecious). The male flowers predominate at the beginning of the season; the female flowers, which develop later, have inferior ovaries. The styles are united into a single column.

The sweet watermelon was first described by Carl Linnaeus in 1753 and given the name Cucurbita citrullus. It was reassigned to the genus Citrullus in 1836, under the replacement name Citrullus vulgaris, by the German botanist Heinrich Adolf Schrader[1]. (The International Code of Nomenclature for algae, fungi, and plants does not allow names like "Citrullus citrullus".)[2]

The species is further divided into several varieties, of which bitter wooly melon (Citrullus lanatus (Thunb.) Matsum. & Nakai var. lanatus), citron melons (Citrullus lanatus var. citroides (L. H. Bailey) Mansf.), and the edible var. vulgaris may be the most important. This taxonomy originated with the erroneous synonymization of the wooly melon Citrullus lanatus with the sweet watermelon Citrullus vulgaris by L.H. Bailey in 1930. Molecular data, including sequences from the original collection of Thunberg and other relevant type material, show that the sweet watermelon (Citrullus vulgaris Schrad.) and the bitter wooly melon Citrullus lanatus (Thunb.) Matsum. & Nakai are not closely related to each other. A proposal to conserve the name, Citrullus lanatus (Thunb.) Matsum. & Nakai, was accepted by the nomenclature committee and confirmed at the International Botanical Congress in 2017[3].

In 2020, global production of watermelons was 101.6 million tonnes, with China (mainland) accounting for 60% of the total (60.1 million tonnes). Secondary producers included Turkey, India, Iran, Algeria and Brazil – all having annual production of 2-3 million tonnes in 2020[4].

Watermelon fruit is 91% water, contains 6% sugars, and is low in fat (table)[56]. In a 100-gram (3+1⁄2-ounce) serving, watermelon fruit supplies 125 kilojoules (30 kilocalories) of food energy and low amounts of essential nutrients (see table). Only vitamin C is present in

appreciable content at 10% of the Daily Value (table). Watermelon pulp contains carotenoids, including lycopene[5].

Materials and Methods:

Plant:

The fresh seeds of *Citrullus vulgaris*(Cucurbitaceae)[6] were collected in the months of march from the garden located in campus of M.A.M College Of Pharmacy, Narasaraopet, Andhra Pradesh state, India, and authenticated by the authority of botany department, Acharya Nagarjuna University, Guntur.

Bacteria:

Pathogenic strains of *Staphylococcus aureus* (MTCC 3160), and *Escherichia coli* (MTCC 293) were obtained from obtained from Institute of Microbial Technology, Shanthi Path, 39A, Sector 39, Chandigarh-160036. and were maintained on agar slant medium(HiMedia) at 4 ºC for further experiments.

Culture Media:

Different types of media were required for carrying out this study, Nutrient agar (biolife) and Mueller-Hinton agar (HiMedia). Also methanol was used for traction process. All the chemicals were procured from Qualigens fine Chem, Mumbai and Himedia Lab, Mumbai.

Antibiotics:

Gentamycine antibiotic (Eye Drops) purchase potency 0.3% w/v in 10 ml and 1 ml contain 300 unit /ml purchased from Lifespring medical store located in Narasaraopet, manufactured by Allergan India Pvt. Limited shows antibiotics potency. The standard concentration of gentamycin is 10 µg/ml

Preparation of Seeds Extract:

Seeds of *Citrullus vulgaris* plant were collected and kept for shed drying for optimal period. After complete drying they were subjected for grinding into coarse powder by the mechanical means. The powdered seeds were kept for maceration in methanolic medium for 96 Hrs at room temperature and filtered·

Assessment of Activity:

By using well diffusion method:

The antibacterial activity was performed by agar well diffusion method. In this method Mueller –Hinton (MH) agar petridishes is used (by using autoclave at 121^0C for 15 min).The PH level of the agar was maintained between 7.2 and 7.4 by adding NaOH and HCl. MH agar at 37ºC was inoculated with a MH broth culture of each bacterial species and poured over the agar plates to form a homogenous layer. Three to four well (holes) were made in the plates (of about 6mm diameter) using a sterile cork borer and seed extract was tested in triplicate with gentamycine (10µg/ml) well as a reference or positive control. [8] The plates were evaluated after incubation at 37^0C for 24 hours after which the zone of inhibition around each was measured by using a scale in millimeters (mm). The ratio between the diameter of inhibition zone (mm) produced by seed extracts and the inhibition zone around the well with gentamycin (mm) was used to express antibacterial activity.

Results:

Table 1: Phytochemical screening of Methanoilc extract of seedsof *Citrullus vulgaris*

Tests	Methanolic Extract
Carbohydrate	
Molish's	+ve
Fehling's	+ve
Benedicts	+ve
Cardiac glycoside	
Legal test	+ve
Saponin	
Foam test	-ve
Flavonoids	
Shinoda test	+ve
Lead acetate test	+ve

Table 2: Inhibition zone (mm) by Methanolic extract of seeds of *Citrullus vulgaris* against *E. coli* at different concentration

Species	Concentration	Zone of inhibition (mm) mean.
E. Coli	47.3 mg/ml	8.5 ± 1.378
	150 mg/ml	25.16 ± 0.7528
	Std 10µg/ml	18.83 ± 0.23

Table 3: Inhibition zone (mm) of methanolic extract of seeds of *Citrullus vulgaris* against *S.Aureus* at different concentration

Species	Concentration	Zone of inhibition in(mm) mean
S. Aureus	52.20 mg/ml	09.5 ± 0.4528
	100mg/ml	15.27 ± 0.3528
	Std 10 µg/ml	28.16 ± 1.23

Images of Activity: Images of Anti-bacterial activity of *Citrullus vulgaris* seeds extract at various concentrations, against to *E.coli* and *Staphylococcus aureus*

Discussion:

Resistance in micro-organisms to many antimicrobial agents has resulted in morbidity and mortality from treatment failure and increased health care costs and increasing capability of microbes to develop multidrug resistance has encouraged search for new, safe and effective bioactive agents of herbal origin[8]. It has been reported that *Citrullus vulgaris* medicinal plants have been used in the treatment of different diseases. Effect of methanolic extract on different micro-organisms i.e *S. aureus* and *E. coli*[9]. The methanolic extract shows zone of inhibition in well diffusion method after incubation of plates for 24 hours at 37^0C. As per the results, *E.coli* showed maximum zone of inhibition by methanolic extract compared with *S.aureus* On the basis of the antibacterial assay of this study *E.coli* was found more (susceptible to the employed seeds extracts) than *S.aureus*. The methanolic extract of seeds of *Citrullus vulgaris* was evaluated for their MIC against E. coli and S. aureus at 47.3 mg/ml and 52.20 mg/ml respectively[10]. The methanolic extract of *Citrullus vulgaris* showed significant antimicrobial activity against positive *Staphylococcus aureus* and on *E. coli* gram negative bacterial strains. Gram positive (*S. aureus*) bacteria and gram negative bacteria (*Escherichia coli*), showed a reduction in their growth on treatment with the different

concentration of extracts of *Citrullus vulgaris*[6]. The degree of inhibition was measured by the well diffusion method, reported that the more zone of inhibition in gram negative when compare with gram positive bacteria[11].

Conclusion

As per the day by day increasing need of Antibiotics, Research scope and area also expanding accordingly. Our effort is also one of the trials to identify new anti bacterial agent which is also getting from edible fruit seeds (*Citrullus vulgaris* seeds extract). We used to found that the seeds extract was rich in Carbohydrates, Glycosides and flavonoids but lack of saponins. Surprisingly the seeds extract showed significant antimicrobial activity. The methanolic extract of seeds of *Citrullus vulgaris* has significant antibacterial activity against S. Aureus and E. coli. But *E.coli* i.e. gram negative bacteria are more susceptible than *S.aureus* i.e. gram positive bacteria.

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