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Evaluation of the antibacterial activity of citrullus colocynthis extracts (In vitro study)

Authors names	Abstract
<p>a. Hayder yousif falih b. Zahraa F Shaker c. Hayder Hamed Abed</p> <p>Article History Received on: 26/6/2021 Revised on: 15/7/2021 Accepted on: 25/7/2021</p> <p>Keywords: Citrullus, E.coli, Extract, Staphylococcus aureus, Antibacterial activity</p> <p>DOI: https://doi.org/10.29350/jops.2021.26.4.1400</p>	<p>The plant of c. colocynthis is perennial herbal of the Cucurbitaceus types that is used as a traditional medicinal plant on a Mediterranean tub and equatorial nations. Studies on the antibacterial properties of Citrullus colocynthis leaf extract, Medicinal plants have conventional been used in abortifacient and treats constipation, edema, Infection caused by bacteria, cancer and diabetes problems. The antibacterial efficacy of citrullus colocynthis (L.) was investigated in this work using five species of pathogenic bacteria isolated from urinary tract infections: E. coli, Klebsiella pneumoniae, Streptococcus spp, Psudomonas auroginosa, and Staphylococcus aureus. For the antibacterial test, water and ethanol extract were employed. The ethanolic extract showed inhibitory activity against Escherichia Coli at (100 °C, 80 °C, 60 °C) while Staphylococcus aureus at (100 °C, 80 °C), Streptococcus spp. at (100 °C). Water extract exhibited less or no activity against all types of bacteria. The findings of this investigation indicate that C. colocynthis might be utilized to treat disorders caused by the test organisms.</p>

Introduction

Medicinal plants provide a variety of active ingredients that have different medicinal benefits. They are source of chemicals having medicinal characteristics, ex: (tannins, flavonoids, saponins, resins, and alkaloids), that are typically unavailable from manufactured chemical compounds (9, 11). Medicinal herbs are used in many of the African population for basic health care. Diabetic condition is treated using a variety of medicinal herbs in traditional medicine (4). Cucurbitaceae is among the most ecologically varied family of food plants, with edible fruits found all across the world. There are found alot of species in the Cucurbitaceae family. Climbing herbaceous plants make up the majority of this family. Cucumber, gourd, bitter apple, melon, and pumpkin are the most significant Cucurbitaceae species (13). Cucurbitaceae is a plant that belongs to the Cucurbitaceus types C. Colocynthis are endemic to dry deserts in tropical and

subtropical locations, and that extensively dispersed throughout Africa's Sahara-Arab region, the Mediterranean tub and portions of asia (8). In the Arabic area, anyway fruits of *C. colocynthis* have many names as bitter apple, bitter watermelon, colocynth and Handhal (7). Each *C. colocynthis* plant produces about 15–30 smooth, almost sphere-shaped fruits. When mature, *C. colocynthis* fruits contain seeds into a compact shape, smooth and brownish. In general, seeds account for around 75% of dry fruits (5). *Citrullus colocynthis* This fruit is well-known for its vast variety of traditional medical applications, including diabetes, asthma, gastrointestinal ailments, and other microbial infections (1, 12). The fruit of a well-known bitter, acid, cooling, cathartic, carminative, antipyretic, and anthelmintic, the fruits of *Citrullus colocynthis* are used to treat hypoglycemia, tumors, asthma, urethra, dyspepsia, constipation, splenomegaly and other (6). Medicinal plants contain a range of active compounds of great importance and therapeutic properties as most studies indicate the increasing activities of antimicrobial plants by detecting areas targeted by antibiotics by plant extracts against drug-resistant microbial pathogens (10).

Figure 1: Fruit and seeds of the citrullus colocynthis plant

Methods and materials

Plant material collection

Citrullus colocynthis (Cucurbitaceae) fruits were obtained and identified in N.H Abu - Gheriab from a small market in Baghdad, Republic of Iraq. The fruit was washed and dried at shade before being stored at room temperature until they were needed again (Fig.1).

Extract preparation

In 250 ml of solvent, a powder (100 gm) was extracted. Separate extracts were made using water and ethanol as solvents. The mixture was filtered using Whatman no.1 filter paper and stored incubator (37) degrees Celsius until all of the ethanol had evaporated. Antimicrobial activity was tested using the extracts (19).

Bacterial preparation

The test included both gram negative and gram positive bacteria. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are gram negative bacteria, while *Staphylococcus aureus* and *Streptococcus* spp. are gram positive bacteria. Freshly made blood agar and MacConkey agar were used to keep all bacterial strains alive.

Antibacterial activity

The antibacterial susceptibility test was performed using the agar diffusion technique. From the stock culture, a loopful bacterium was extracted and dissolved in 0.1 ml saline. All of the experiments were carried out by inoculating the MacConkey agar and blood agar surfaces with 10 ml of MHA liquid medium before putting the disk (6 mm diameter) soaked with (200 µl) extracts microorganisms that are gram negative and gram positive (3).

Bacterial isolates

The isolated pathogenic bacteria which used in this study from urinary tract infection samples, were patients collected suffered from different problems with UTI and many clinic symptoms. *E. coli*, *Pseudomonas aeruginosa*, *Streptococcus* spp., *Klebsiella pneumoniae* and *Staphylococcus aureus* were extracted from the urine of the patient.

Culture and the media

There are several cultured media that are used to grow various harmful bacteria, such as blood, MacConkey, chocolate agar, and other media that are created according to the Hi-media –India Company's recommendations

Results

Pathogenic bacteria	Citrullus colocynthis extract					
	Aqueous extract inhibition zone (mm)			Ethanol extract inhibition zone (mm)		
	100°C	80°C	60°C	100°C	80°C	60°C
<i>Staphylococcus aureus</i>	1	0	0	9	6	0
<i>Streptococcus</i> spp.	0	0	0	2	0	0
<i>E. coli</i>	2	0	0	17	9	3
<i>Pseudomonas aeruginosa</i>	0	0	0	0	0	0
<i>Klebsiella pneumoniae</i>	0	0	0	0	0	0

Table 1: Antibacterial activity of *Citrullus colocynthis* aqueous and ethanol extracts

Figure 2 shows the impact of aqueous extract on several bacteria species

Figure 3: The impact of ethanol extracts on several bacterium kinds

Discussion

Citrullus colocynthis is a medicinal plant that contains beneficial bioactive compounds and has been utilized in a variety of applications. Its extracts were an excellent source of physiologically active chemicals with beneficial therapeutic qualities (15, 17). The obtained data indicated that aqueous 100 °C had activity against *E. coli* and *Staphylococcus aureus*, whereas alcoholic extraction had activity against *E. coli*, *Streptococcus spp.*, and *Klebsiella pneumoniae*. However, *E. coli* bacteria showed the highest activity of both extracts. Table (1) shows the Effectiveness of

bacterial of *Citrullus colocynthis* aqueous and ethanol extracts. The extraction procedure was applying for 90 minutes. The elevation of the extraction temperature improves extraction efficiency for bioactive material with increasing the lethal efficiency. *Citrullus colocynthis*' biological effect on bacterium cells might be attributed to a fast drop in the amount of the p34CDC2/cyclin B1 complex protein, which is required for the control of G2 presence and mitosis (2). *Citrullus Colocynthis* extracts, both aqueous and ethanoic, have excellent Effectiveness of bacterial against *E.coli* and *Staphylococcus aureus*, but had less impact against *pseudomonas aeruginosa* and *Klebsiella pneumoniae* (14). The reason behind this low activity could related to its ability to acquire resistance, via mutations, to all relevant treatments (16,18) (fig. 2 & 3).

In conclusion: water extraction is suitable to extract active ingredients *Citrullus Colocynthis* at 100 °C for the time period of three or four hours at least. The *Citrullus Colocynthis* alcoholic extract at pervious conditions was a very effective inhibitor to *E.coli*, *Staphylococcus aureus*, *Streptococcus* spp. While, watery extract were effective against *E.coli* *Klebsiella pneumoniae*. This extract was low cost, simple and sophisticated for treatments.

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