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Assessment activity of Crocus sativus L. extract against bacteria causes Urinary Tract Infection

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ABSTRACT

Urinary tract infection (UTI) is the invasion and growth expansion of bacteria in a part of the urinary tract system, most of the time (about 80% to 85%) it caused by *Escherichia coli*. *E.coli* are one of the most core sources of the disease and infect peoples of all ages. This infection could lead to many difficult issues, including the most attention-grabbing issue which is the increasing of microbial resistance to antibiotics. Nowadays, the increased bacterial resistance to antibiotic has make room to herbal therapies with less side effects supersede the conservative medicines. Consequently, this study focused on the antibacterial effects of methanolic extract of saffron petal against *E.coli* strains.The method used to evaluate the extract effect is the disc diffusion method. The result showed that the crude extract has a very effective antibacterial activity against the mentioned bacteria causing UTI. More studies should be conducted to verify the safety of the extract, and then it could be considered as an alternative for antibiotics used in UTI treatment.

Keywords: Medicinal plants, Antimicrobial effects, Pathogenic bacteria, saffron, UTI.

1. Introduction

1.1 Crocus sativus L.

Crocus sativus L. (Saffron) is a perpetual plant belong to the Iridaceae family [(1)]. It is currently being cultivate intensively in India ,Iran, Azerbaijan, Spain, Greece, Italy, Turkey, France, Pakistan, Switzerland, China, Egypt, Japan, Iraq, Afghanistan as well as in Australia[(2)].Saffron was used in unorthodox medication as an eupeptic, pain reliever, antispasmodic, anti-catarrhal,nerve sedative, diaphoretic, gingival sedative,carminative, expectorant, emmenagogue, aphrodisiac and stomachic [(3)]. It is further used in the field of cooking to flavour food in innumerable parts of the world and as an agent for treating a range of diseases [(4)]. It has been confirmed by several scientific studies from the previous several decades that the growth of both stain gram negative and positive bacteria, mold and yeast can be prevented by an assortment of species [(5)]. For a long time now, antibiotics have been used to treat a variety of infections, but the continued evolution of bacteria, generation after generation, has revealed the problem of bacterial resistance to antibiotics, which is an increasing problem. Drug-resistant bacteria generate extra cases of sickness, longer healing times and needless death that necessitate the search for original and efficient antimicrobial compounds.This circumstances take attention of scientists to investigate for new

antimicrobial substances from different plants which have good quality of antimicrobial chemotherapeutic agents [6].A range of biological activities like antimicrobial and antioxidant activities have been credited to extracts of *Crocus sativus* L [7,8].The activity of saffron spatially antimicrobial effect have been reported due to safranal and crocin compounds. These substances participate in microbial death through being volatile and/or readily soluble in water, which makes it feasible for them to reach the harmful microorganism [9,10].Therefore, the main target of the current study was to inspect the effect of saffron as antimicrobial agent against *E. coli* causing UTI bacteria.

1.2 Urinary tract infection (UTI)

It is one of the most widespread hospital-acquired infection, accounting for beyond 30% hospital-borne of infections, and it is furthermore the second prevailing reason of bacteraemia in nosocomial patients [11].Pathogens involved in the causality of UTI are., *Staphylococcus, Klebsiella sp., saprophyticus ,Enterobacter sp., Enterococcus sp ,Proteus-Morganella-Providencia sp., sp.,Citrobacter* plus the most common causative bacteria which is *Escherichia coli* (*E. coli*) [12,13] . *E. coli* is a very varied species of bacteria found inherently in the intestine of all humans and numerous other animal species. *E. coli* can cause enteric/diarrhoeal disease, and a different division cause extra-intestinal diseases, one of them is the UTI [14].*E. coli* financial records for as many as 90% or more of all urinary tract infection identified in the ambulatory populace and by their late 20s, about half of all women had experienced a UTI. [15,16].Different findings report greater than before resistance of *E. coli* in UTI, particularly to beta-lactam antibiotics and fluoroquinolones separately or even in mixture [17,18].

UTI is complicated, not just in that of the great number of infections that occur year by year, but moreover by reason of that the identification of UTI is not always in a straight line[19].As it has become known, the expand of antibiotic-resistant among plenty of pathogens is a main communal health threat, and microbes included in resistant are *Enterobacteriaceae* in particular, that can endure lethal dosages with a range of chemical compositions and modes of action[20].For instance, the resistance rates of uro-pathogenic *E. coli* to different antibiotics have been approved as (57.4%)of beta-lactams, (48.5%) of co-trimoxazole, (74.5%)of quinolones, (58.2%)of gentamicin, (33.4%)of amikacin, (56%) of cefuroxime, (77.7%) of nalidixic acid [21,22,23,24,25] .

Materials and Methods

In this study,an extract was made by soak 60grams of dried saffron petals with 300 ml of 90% ethanol as solvent for 24 hours. This solvent was leave for evaporation at room temperature.From it, the concentrated extract was extracted.Agar Well Diffusion method was used to establish Minimum Inhibitory Concentration (MIC). Bacterial isolates were collected from urine of patient from different hospitals in Baghdad , both sexes, different ages and in period between September 2020 and February 2021.Characterization and identification if isolates as *E.coli* was performed by growth on selective media (MacConkey and EMB agar), physiological and biochemical testes, Vitek 2 system as well as EPI system. In order to prepare a microbiological suspension, 4-5 bacterial culture colonies were moved to Mueller Hinton Broth (MHB) in order to modify the turbidity of the microbial suspension in accordance with the standard 0.5 McFarland tube.The microbial suspension was diluted to 0.01 to achieve a concentration of 1.5 × 106 cfu/ml. so as to assess the effect of antibacterial of ethanol extract on *E.coli* , 4 concentrations 150 mg/ml,112 mg/ml,75 mg/ml,37 mg/ml of ethanolic extract were prepared in 5% DMSO solvent. For agar well diffusion method

preformed, 500 ml of microbial suspension was moved onto Mueller Hinton Agar (MHA) medium and cultured in 3 directions by sterilized swab. Then wells at 6 mm in diameter were arranged.100 µl of 150 mg/ml,112.5 mg/ml,75 mg/ml,37.5 mg/ml concentrations of saffron ethanol extract were put into each well. Gentamicin antibiotic was utilized as the positive control and 5% DMSO was used as the negative control. After that, plates were incubated for 24 hours at 37°Cand inhibition zone were measured in millimeters.

Results and Discussion

Along with Table 1, The ethanol extract of saffron petals' antibacterial properties shown that the extract significantly inhibited the growth of *E.coli* bacteria. As the ethanolic extract concentration increased, the inhibitory effect increased. 150 mg/ml concentration has higher antibacterial effect ,while 37.5 mg/ml concentration have no significant effect on *E.coli*, 75 mg/ml concentration in some isolate have significant effect .Isolate number 3 resistance to gentamicin but sensible to saffron petal extract.Minimal inhibitory concentration is112.5 mg/ml.These results demonstrate that there was a considerable variation in the sensitivity between the tested bacteria to saffron petal extract ($p < 0.05$).

Table 1. Mean diameter of non-growth zone of ethanolic extract of saffron petal against *E.coli* in millimeters (mean ±standard deviation)

No.of specimen	150 mg/ml constration	112.5 mg/ml constration	75 mg/ml constration	37.5 mg/ml constration	Negative control	posative control
1	12mm	10mm	10mm	----------	----------	20mm
2	10mm	7mm	----------	----------	----------	20mm
3	22mm	13mm	----------	----------	----------	-------
4	15mm	11mm	8mm	----------	----------	25mm
5	15mm	12mm	----------	----------	----------	25mm
6	15mm	12mm	10mm	----------	----------	25mm
7	22mm	18mm	15mm	----------	----------	25mm

Researches on the establishment of herbal medicines have greater attention than before as a result of the rise in resisting of pathogenic bacteria to a range of antibiotics, in addition to the resulting societal and cost-effective issues. Composition that can hold back the invasion of pathogenic microorganisms or destroy them with low toxicity to cells body are assess as contender for the production of new antimicrobial drugs. Therefore, there is a greatly need to give substitute to ordinary antibiotics from natural source[26].The domino effect of this study demonstrate that the extract of saffron petal has an inhibitory effect on the Gram-negative *E.coli* isolated from UTI patients.

In 2010,study by Vahidi *et al*., investigated the antimicrobial property of saffron extract obtain fr om several parts against *E.coli*, *S.epidermidis*,micrococcus and fungi and the end result mentioned their antimicrobial effects[27].According to Pintado *et al*. 2011,saffron's antibacterial effect is accredited to safranal, crocin, and interrelated compounds[28].Differences in susceptibility of bacterial species to different antimicrobial agents have identified by many studies. Because of the diversity in composition of the wall

of different microorganisms case variation in susceptibility to antimicrobial agents. As a result of the faty layer of the outer membrane ,gram-negative bacteria are more impermeable which increase their resistance to antimicrobial agents[29].In 2003., Razaghi *et al*. were study the saffron stigma's antimicrobial effects on three types strains *P.aeruginosa*, *S.aureus* and *E.coli* and the results display that safranal compound repressed the growth of *S.aureus* and *E.coli* strains[30].Tajalli 2008, investigated saffron petal methanolic extract's antioxidant properties and the results exhibit that saffron petals were a natural and easy resource of antioxidant with the top concentration of inhibitory extract at 300pM[31]. In 2008 ,Islam *et al*. in their study shows that effect of saffron plant extract's against gram-negative bacteria were less effectual than gram-positive bacteria[32]. Nasab, 2019 stady the antibacterial activities of saffron petal's hydroalcoholic extract on some pathogenic bacteria, the results display that hydroalcoholic extract of saffron petals and antibiotics were an effectual on all tested strains of bacteria and the majority effective antibacterial properties has been observed on *L. monocytogenes* followed by *E.coli*[33].

Conclusions

Extract of Saffron petals exhibits significant promise as an antimicrobial component toward microorganisms.Therefore, it can be utilized to the healing of infectious diseases caused by resistant microbes.Additionally, the combination of an antibiotic and saffron extract has a synergistic impact that helps combat resistant bacteria, opening up new treatment options for infectious diseases.This effect permits the use of the appropriate antibiotic during therapeutic treatment when it is no longer effective on its own.

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