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Evaluation of Antimicrobial Activity of Safflower (Carthamus tinctorius) and its Synergistic Effect with Antibiotic

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Abstract

Introduction: Safflower (*Carthamus tinctorius*) is a herbaceous, like annual medicinal plant found in many in many Arabic countries including the Kingdom of Saudi Arabia. It is grown for the production of oil from its seeds.

Aim: To investigate the antibacterial activity of Safflower and its synergistic effect with antibiotics against various harmful bacterial species.

Method: The synergistic effect between plants and extraction of antibiotics was evaluated using the disk diffusion method. In addition, the minimal inhibitory concentration (MIC) of the plant extracts against the tested bacteria using microdilution method.

Results: The results indicated that the Safflower plant has antibacterial properties against the tested bacteria with various degrees. In addition, the zones of inhibition of bacteria came about because of plant extract and antibiotics blends were more noteworthy than those came about because of antibiotics alone.

Conclusion: The findings of this study support the traditional utilization of Safflower which can be recommended for utilization as antimicrobial agents in combination with antibiotics for the therapy of infectious disease caused by pathogens.

Keywords: *Medicinal Plants; Inhibition Zone; Escherichia coli; Staphylococcus aureus*

Introduction

Utilization of substances with antimicrobial properties is known to have been the basic practice for no less than 2000 years. Old Egyptians and old Greeks utilized particular forms and plant concentrates to treat contamination. More recently, microbiologists observed antagonism between some bacteria and discussed the benefits of controlling these interactions in medicine. Today, various antimicrobial agents exist to treat a wide range of infectious diseases [1].

The particular function of numerous phytochemicals has been as yet hazy; however, a significant number of studies have demonstrated that they are engaged with the plants/pests/diseases. Antimicrobial screening of plant extracts and phytochemicals, at that point, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the advancement of new and complex methods. These techniques assumed a noteworthy role in the search for additional resources of raw material for pharmaceutical industry [2].

Medicinal plants have immunomodulatory and cancer prevention agent properties, prompting antibacterial activities. They have versatile immunomodulatory activity by stimulating both non-specific and specific insusceptibility [3].

The utilization of plant extracts and phytochemicals, both with known antimicrobial properties, can be of awesome significance in remedial medications. Over the most recent couple of years, various studies have been to demonstrate such effectiveness. Many plants have been utilized because of their antimicrobial, which are because of compounds synthesized in the secondary metabolism of the plant [4].

Carthamus tinctorius L. is regularly known as Safflower. *C. tinctorius* extracts and oil are vital in drug development with various pharmacological activities on the planet. This plant is developed primarily for its seed, which is utilized as edible oil. *C. tinctorius* has been utilized as a part of customary medications a laxative, pain relieving, antipyretic and an antitoxin to poisoning [5].

It is a valuable plant in difficult menstrual issues, baby blues discharge and osteoporosis. Moreover, *C. tinctorius* has antioxidant, pain relieving, anti-inflammatory and antidiabetic properties [5]. Carthamidin, isocarthamidin, hydroxysafflor yellow A, safflor yellow A, safflamin C and luteolin are the fundamental constituents which are accounted for from this plant. Caryophyllene, p-allyltoluene, 1-acetoxytetralin and heneicosane were recognized as the significant parts for *C. tinctorius* flowers essential oil. Because of the simple gathering of the plant and being widespread and also remarkable biological activities, this plant has become an important source of both food and medicine in many parts of the world [6].

Materials and Methods

Collection of samples

Plant materials employed in this study included Safflower (*Carthamus tinctorius*) which was collected from various sites in Hail territory. While the tested bacteria included: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumonia*), Acinetobacter baumannii, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*) and *Salmonella* spp. These bacteria were obtained from the microbiology laboratory of the Department of Biology, Faculty of Science, University of Hail, and from King Khalid Hospital, Hail. The bacterial isolates were maintained in Brain Heart Infusion (BHI) agar medium (HiMedia) at 4°C pending analysis.

Preparation of plant extract

For aqueous extraction, 20g of air-dried plant powder were added to 150 ml of distilled water and boiled on slow heat for 2 hours. Then it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 minurtes, and the supernatant was collected. This method was repeated twice; after 6 hours, the supernatant was collected at an interval of 2 hours and concentrated to make the final volume one-fourth of the original volume

Preparation of culture media, reagents and antibiotics

The bacterial growth media of the study were purchased from a private company at Quassim city included: Brain Heart Infusion broth, Nutrient agar (biolife) and Mueller-Hinton agar (HiMedia). Also, methanol and distilled water were used as solvents for the extraction process. The antibiotics utilized in the study were purchased from pharmacies in Hail city and included: Vancomycin, Cefotaxime, Tetracyclines, Chloramphenicol, Ampicillin, Neomycin, Erythromycin and Penicillin.

Preparation of plant extracts solutions

One gram of each aqueous extract and alcohol pre-prepared (each separately) were taken and the aqueous extract was dissolved in 5 grams sterile distilled water, while alcoholic extracts was dissolved in 5 ml of Dimethyl Sulfoxide (DMSO). Thus 200 mg/ml of stock was obtained as a standard concentration of aqueous and methanol extracts. Aqueous extracts were sterilized and methanol extract was pasteurized for 15 minutes at temperature 62°C.

Preparation of inoculum

Stock cultures were maintained at 4°C on nutrient agar slants for bacteria. Active cultures for experiments will be prepared by transferring a loopful of culture to 5 ml of Brain Heart Infusion broth and incubated at 37°C for 24 hours.

Antibiotics activity assay

For antibiotics activity assay, CLSI [7] developed method was used. Antibiotic discs were placed on the surface of a Mueller-Hinton agar that had been inoculated with test microorganisms. During incubation, the antibiotics diffuse outward from the discs creating a concentration gradient. After 18-24 hours, the zone diameter of inhibition was measured and reference tables were used to determine if the bacteria are Sensitive (S), Intermediate (I) or Resistant (R) to the antibiotic.

Plant extracts activity assay

To evaluate the plant extract activity against the tested bacteria, the paper disk diffusion assay was used according to Kumar., *et al* [8]. A suspension of bacteria being tested was spread on Muller Hinton Agar (MHA) medium. The filter paper discs (5 mm in diameter) were placed on the agar plates which were inoculated with the tested microorganisms and then impregnating with 20 µl of plant extract (concentration 200 mg/ml). The plates were subsequently incubated at 37℃ for 24 Hrs. After incubation the growth inhibition zone was quantified by measuring the diameter of the zone of inhibition in mm.

Determination of MIC of plant extract

A microdilution technique using commercially available media and materials was developed and used to determine the minimal inhibitory concentrations (MICs) of Vancomycin, Cefotaxime, Tetracyclines, Chloramphenicol, Ampicillin, Neomycin, Erythromycin and Penicillin. The 96-well plates were prepared by dispensing 50 µl of Mueller–Hinton broth for bacteria, into each well. An aliquot of 50 µl from the stock solution of testing extracts (concentration of 200 mg/ml) were added into the first row of the plate. Then, two-fold; serial dilutions were performed by using a micropipette.

The obtained concentration range was from 100 to 0.1953 mg/ml, and then 10 µl of inoculum was added to each well except a positive control inoculum was adjusted to contain approximately 1.5 X 108 CFU/mL. A plant extract with media were used as a positive control and inoculum with media were used as a negative control. The test plates were incubated at 37°C for 18h. After 18h 50 µl of a 0.01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plates were incubated for another hour. Since the colorless tetrazolium salt was reduced to red colored product by biologically active bacteria, the inhibition of growth was detected when the solution in the well remained clear after incubation with TTC. MIC was defined as the lowest sample concentration showing no color change (clear) and exhibited complete the inhibition of growth [9,10].

Synergism between plant extract and antibiotics

Brain heart infusion (BHI): is a nutrient-rich which can be used to culture a variety of fastidious organisms, was used in the synergism test according to Betoni JE., *et al* [11]. The bacterial cultures were grown in BHI broth at 37°C. After 4h of growth, each bacterium was inoculated on the surface of Mueller-Hinton agar plates. Subsequently, the antibiotic disk (diameter = 5 mm) was placed on the surface of each inoculated plate and then added 20 µl of plant extract, to identify the synergistic effect between the plant extract at a concentration of 200 mg/ml and antibiotics. The plates were incubated at 37°C for 24h. The diameters of clearing zones will be measured.

Results and Discussion

Antibacterial activity of Safflower plant extract

The utilization of medicinal herbs as, known therapeutic agents in modern health care has been increasing over the past three decades. The pursuit for medicinally useful plants and the way to create their active principles and ingredients now incorporates extensive research program and even entire scientific institutes.

The present study investigated the impact of Safflower (*Carthamus tinctorius*) water (aqueous) and methanol extracts on the growth of the tested bacteria was investigated. The inhibition zone diameters of the tested bacteria against the various extracts are shown in table 1. The tested bacteria included: *P. aeruginosa*, *E. coli*, *Acinetobacter baumannii* and *K. pneumonia*. From the results it appears that aqueous extracts of Safflower did not inhibited growth of most of the tested bacteria with exception to *Acinetobacter baumannii* bacteria for which the inhibition zone was 2 mm. On the other hand, the aqueous extract of the Safflower plant inhibited growth of both bacteria

Acinetobacter baumannii and *E. coli* where the inhibition zone was 5 mm and 3 mm, respectively. Generally, the methanol extract of the tested medicinal plants has more inhibition effect against the tested bacteria compared to the aqueous extract of this plant. The efficiency of Safflower as antibacterial for testing microorganisms goes in line with those of Son., *et al*. [12] who demonstrated that Safflower meal has an antimicrobial activity against a major foodborne pathogen, *L. monocytogenes*. It has been demonstrated that the antimicrobial action of plant extracts is because of phenolic compounds [13-16] the antimicrobial action of Safflower likely due to membrane disruption by polyphenols and leakage of cellular constituents. Consequently, membrane-disrupting compounds cause the spillage of cellular contents and interfere with metabolic enzymes, bringing about the inactivation of bacteria [17].

No.	Extract	Test organism	Inhibition zone (mm)	Control
1	A	*P. aeruginosa*	0	0
2	A	*K. pneumonia*	0	0
3	A	*E. coli*	0	0
4	A	*Acinetobacter baumannii*	0	0
5	A	*P. aeruginosa*	0	0
6	M	*P. aeruginosa*	0	0
7	M	*K. pneumonia*	0	0
8	M	*E. coli*	3	0
9	M	*Acinetobacter baumannii*	5	0
10	M	*P. aeruginosa*	0	0

Table 1: *Inhibition zone diameters of the tested bacteria against the tested plants. A: Aqueous Extract; M: Methanol Extract*

Safflower seed has been reported to contain various bioactive substances such as lignan and flavonoid, therefore, it has been used as a medicine and edible oil [18]. Moreover, it has been widely utilized for osteoporosis and other bone diseases [19]. Safflower seed meal is a by-product of production of safflower seed oil, and it contains bioactive polyphenols such as lignan, flavonoids, and serotonin derivatives [20]. However, few investigations on the antimicrobial activity of the safflower seed meal extract have been performed, and it has never been studied as a sanitizer on fresh-cut produce.

Minimal inhibitory concentrations (MIC) of Safflower against bacteria

Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will hinder the visible growth of a microorganism after overnight incubation, and least bactericidal concentrations (MBCs) as the lowest concentration of an antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media [21]. MICs are used by diagnostic laboratories principally to affirm resistance, yet frequently as a research tool to determine the *in vitro* action of new antimicrobials, and data from such investigations have been utilized to determine MIC breakpoints.

The minimum inhibitory concentration (MIC) test was performed against *S. aureus*, *K. pneumoniae*, *E. coli*, *S. aureus, A. baumanni*i and *P. aeruginosa*. The results of MIC are indicated in table 2. It is obviously that all tested bacterial species did not show visible growth. The table shows that Safflower plant extracts revealed antibacterial activity against *E. coli*, *S. aureus*, *K. pneumonia*, *A. baumannii* and *P. aeruginosa* with MIC values ranging from 2.25 - 25 mg/ml. The tested extracts showed various levels of prominent antibacterial activity depending on tested bacterial species. Suggesting that very small amount of the extracts is required to inhibit the growth of the bacteria.

No.	Extract	Test organism	MIC value (mg/ml)
1	A1	*S. aureus*	12.5
2	A2	*K. pneumonia*	6.25
3	A3	*E. coli*	25
4	A4	*A. baumannii*	12.5
5	A5	*P. aeruginosa*	12.5
6	M1	*S. aureus*	6.25
7	M2	*K. pneumonia*	5.125
8	M3	*E. coli*	12.5
9	M4	*A. baumannii*	12.5
10	M5	*P. aeruginosa*	6.25

Table 2: *Minimal inhibitory concentrations (MIC) of Safflower extracts against the tested bacteria. A: Aqueous Extract; M: Methanol Extract*

The results also indicated that that there is a reduction in MIC in case of aqueous extract of the Safflower plant against *K. pneumonia* aqueous and methanol extracts which were 6.25 mg/ml and 5.125 mg/ml respectively. The MIC values of the tested safflower plant extracts against *E. coli* were 25 and 12.5 mg/ml for aqueous extract and methanol extract, respectively. While MIC of aqueous extract and methanol extract against *S. aureus, A. baumannii, P. aeruginosa* were 12.5, 6.25, and 25, 12.25, 12.5 and 12.5, 6.25 mg/ml, respectively.

Synergism between the plant extracts and antibiotics

The synergism between the aqueous and methanol extracts of Safflower plant and antibiotics is shown in table 3. The antibiotic mix inhibited all tested bacteria with varying degrees and the most inhibited bacteria was *E. coli* where the inhibition zone diameter was 11 mm. On the other hand, upon adding the antibiotic mix to the plant extracts, the respective inhibition zone diameter of the various tested bacteria increased. This implies the synergism between brought about inhibition of tested bacteria. Besides, the methanol extract was more effective than aqueous extracts against all tested bacteria. *E. coli* was the most hindered bacteria where the inhibition zone diameter was 12 mm and 15 mm for the combination of Safflower aqueous extract plus antibiotic and Safflower methanol extract plus for antibiotics, respectively. However, Safflower aqueous and methanol extract had similar synergism values with antibiotics against each of *K. pneumonia* and *Acinetobacter baumannii* where the inhibition zone diameter was 10 mm and 11 mm, respectively.

No.	Extract	Test organism	Inhibition zone (mm)	Control (mm)	Antibiotic Inhibition zone (mm)	Synergism Antibiotic + plant extract (mm)
1	A	*S. aureus*	0	0	8	13
2	A	*K. pneumonia*	0	0	9	10
3	A	*E. coli*	0	0	11	15
4	A	*Acinetobacter baumannii*	0	0	9	11
1	M	*S. aureus*	0	0		12
2	M	*K. pneumonia*	0	0		10
3	M	*E. coli*	0	0		13
4	M	*Acinetobacter baumannii*	0	0		11

Table 3: *The Synergism between plant extract and antibiotics. A: Aqueous Extract; M: Methanol Extract*

The synergism between antimicrobial agents and medicinal plant extracts is a novel concept and has been recently reported [22-26]. Adwan., *et al.* [27] found that crude extracts from these plants increase the inhibition zones of oxytetracyclin HCl, gentamicin sulphate, and sulphadimethoxin, while combination between the plant extracts and enrofloxacin decreases inhibition zone.

The present study proposes the likelihood of simultaneous utilize of these antimicrobial medications and extracts in combination in treating diseases caused by harmful bacteria such as *S. aureus* and *E. coli* or at least the concomitant administration of these plants and antimicrobial medications may not debilitate the antimicrobial activity of these antibiotics.

Conclusion

The results of the present study support the traditional utilization of Safflower and it can be recommended for utilization as antimicrobial agents in new drugs for the therapy of infectious disease caused by pathogens. This could be ascribed to the plant active ingredients. In addition, the zones of inhibition of all tested bacteria came about because of plant extract-antibiotic combinations were more prominent than those resulted from antibiotics solely. Furthermore, the outcomes revealed that the methanol extract of the plant was more effective than that of the aqueous extract. Further work is expected to isolate the secondary metabolites from the extracts studied to test the particular antibacterial activity and the underlying mechanisms.

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