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# EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *CARTHAMUS TINCTORIUS* EXTRACTS AGAINST NOSOCOMIAL MICROORGANISMS

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***Abstract***

*Drug-resistant pathogens are the main cause of health-associated infections. They continue to significant infections, increase mortality of hospitalized patients and also have a negative financial impact. Taking into consideration those factors, there is a great need to find and develop new compounds with low toxicity, specific activity, and high bioavailability to combat them. In this way, have increased the attention on medicinal plants and their bioactive compound with antimicrobial properties. This review evaluates the antimicrobial activities of different Carthamus tinctorius (safflower) extracts from seeds, leaves, and flowers against nosocomial pathogens. The study concludes that safflower different extracts might be used against multidrug-resistant microbes that cause nosocomial and community-acquired infections.*

***Key words****: antimicrobial activity, nosocomial pathogens, phenolic compounds, safflower extracts.*

## INTRODUCTION

In the European Union, nosocomial infections affect approximately 3 million people each year, of which about 50,000 cases are fatal (data from European Centre for Disease Prevention and Control) (Miklasińska - Majdanik et al., 2018). The most frequently multidrug resistant (MDR) pathogens which are the main cause of nosocomial infections among hospitalized patients are: *Acinetobacter baumannii, Escherichia coli (E. coli)*, *Klebsiella pneumoniae (K. pneumoniae), Pseudomonas aeruginosa* (*P. aeruginosa*), *Pseudomonas* spp, *Enterococcus faecalis* (*E. faecalis*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Candida albicans* (*C. albicans*) (Chen et al., 2022; Iyer et al., 2022; Urusova et al., 2022; Dhasarathan et al., 2021; Luo et al., 2021; Mo et al., 2019; Othman et al., 2019; Périer et al., 2020; Wang et al., 2019; Moreno et al., 2013). The extensive use of synthetic antimicrobial agents can cause concern due to their development of microbial resistance and environmental pollution potential. Considering this issue, it is important to develop new antimicrobial agents able to prevent microbe's adhesion and proliferation on materials surfaces and reduce their negative effects (Ivanov et al., 2022; Khameneh et al., 2021; Mittal et al., 2019). The growing incidences of MDR pathogens have increased the attention on several medicinal plants and their active principles for antimicrobial properties (Moreno et al., 2013). According to Khameneh et al., 2021, phytochemicals like alkaloids, tannins, carbohydrates, glycosides, terpenoids, flavonoids, steroids, and coumarins have particular clinical value because their bioactivity does not lead to resistance. Safflower (*Carthamus tinctorius* L.) which belongs to the Asteraceae family, contains many bioactive compounds for which it has biological activities, involving antidiabetic, anticancer, antioxidant, anti-ageing, anticoagulant, hepatoprotective and anti-inflammatory, analgesic and antibacterial activities (Sura et al., 2020). According to Górniak et al, 2019, mechanisms of this activity still require more studies.

## PHENOLIC COMPOUNDS FROM SAFFLOWER WITH ANTIMICROBIAL ACTIVITY

Safflower has been identified to contain more than 200 compounds including flavonoids, alkaloids, lignans, alkane diols, riboflavin, steroids and quinochalcone C-glycosides (Sun et al., 2020; Hussain et al., 2021; Ozkan et al., 2022).

The pathogens have developed several mechanisms of antibiotic resistance, such as overexpression of the efflux pumps, destroying the antibacterial agents, structural modification of porins, and modification of antibiotics. Therefore, inhibition of them is an integral part of combating antibiotic resistance (Khameneh et al., 2021). The phenolic compound can modify the permeability of cell membranes, make changes in various intracellular functions induced by hydrogen bonding with the enzymes or by the modification of the cell wall rigidity with integrity losses due to different interactions with the cell. This may induce irreversible damage to the cytoplasmic membrane and coagulation of the cell content that can even lead to the inhibition of intracellular enzymes. All of these mechanisms are possible due to the lipophilic character of phenolic compounds. Polyphenols also can suppress biofilm formation and exert a synergistic effect with antibiotics (Bouarab - Chibane, 2019; Othman et al., 2019; Miklasińska - Majdanik et al., 2018).

Polyphenols can also produce cell membrane damage of bacteria, indicated by the release of intracellular K+, with greater effects observed with gram-negative bacteria strains than with gram-positive bacteria strains (Liu et al., 2021).

The interaction of flavonoids with lipid bilayers of bacterial cell membranes is made through two mechanisms: partition of the more non-polar compounds in the hydrophobic interior of the membrane, while the second includes the formation of hydrogen bonds between the polar head groups of lipids and the more hydrophilic flavonoids at the membrane interface (Górniak et al., 2019). Flavonoids also may act by inhibiting both energy metabolism and DNA synthesis thus affecting protein and RNA syntheses, in Gram-positive bacteria can modify intracellular pH, also can interfere with the energy (ATP) generating system. (Bouarab-Chibane et al., 2019; Xie et al., 2017). Long aliphatic chain substitution in flavonoids leads to an increase in their hydrophobicity and facilitates interactions with the bacterial cytoplasmic membrane, in this way resulting in an increase in antibacterial activity of these compounds. The presence of phenolic hydroxyl groups, which have a high affinity for proteins, and microbial enzyme-inhibition, also may enhance the antibacterial effects of flavonoids. Hydroxylation of flavonoids improves antibacterial activity even against Methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Othman et al., 2019; Miklasińska-Majdanik et al., 2018). The most important phenolic compounds from *C. tinctorius* and their antimicrobial mechanism of action are shown in Table 1. Daphnoretin, a flavonoid compound found in *C. tinctorius*, had a potent binding affinity with TLR4 and TLR8. These findings could be used as a basis for further investigations for daphnoretin as the possible inhibitors for hyper-inflammatory conditions in COVID-19 by targeting Toll-like receptors (TLRs) (Hansur et al., 2021). Terpenes exert antimicrobial activities against both the antibiotic-susceptible and antibiotic-resistant bacteria, having the ability to promote cell rupture and inhibition of protein and DNA synthesis, disruption in microbe multiplication and development, interfering with their physiological and metabolic activities. Like flavonoids, terpenoids make their effects by disruption of microbial membranes (Masyita et al., 2022). Besides terpenes, another compound found in safflower is trans-trans-3,11-tridecadiene5,7,9-triyne-1,2-diol, which has antifungal properties (Attia et al., 2021).

In safflower, were identified also anti-nutritional factors (ANFs) which are present in the form of tannins, luteolin, acacetin, and serotonin derivatives. ANF compounds can be used as antibacterial, anti-inflammatory, antioxidant and anticoagulant agents for various health and pharmaceutical applications (Singhal et al., 2018).

Table 1. The most important phenolic compounds from *C. tinctorius* and their antimicrobial mechanism

| Nr crt. | Phenolic compound | Antimicrobial mechanism of action | References |
|---|---|---|---|
| 1 | Ferulic acid | -Decrease the hydrophobicity of *P. aeruginosa*.<br>-Propylferulate can be used for the use of efflux pump inhibition of *Staphylococcus aureus* IS-58 strains. | Liu et al., 2020; Pinheiro et al, 2022 |
| 2 | Catechins | -Cause alteration in the membrane permeability and membrane damage.<br>-Interfere with the biosynthesis of the bacterial cell wall by binding with the peptidoglycan layer.<br>-Neutralize bacterial toxic factors from *Vibrio cholerae*, *S. aureus*, *Vibrio vulnificus*, *Bacillus anthracis*, and *Clostridium botulinum*.<br>-In the combination with reuterin, exhibits synergistic antimicrobial activity against *S. mutans* by repressing growth, biofilm formation, water-insoluble glucan production, and virulence genes expression.<br>-Damage to bacterial cells through binding to their surfaces and the bactericidal action of hydrogen peroxide generated through catechin autooxidation. | Miklasińska-Majdanik et al., 2018; Górniak et al, 2019; Zhang et al, 2021 |
| 3 | Quercetin | -Decrease in proton-motive force in *S. aureus.*<br>-Cell membrane disruption (membrane rigidification), DNA intercalation, DNA gyrase inhibition, Type III secretion inactivation, dehydratase inhibition (HpFabZ), protein kinase inhibition in *E. coli*, *Helicobacter pylori*.<br>-Inhibit the neurotoxin from *C. botulinum*<br>-Hyaluronic acid lyase (Hyal B) inhibitors in *Streptococcus agalactiae*. The inhibitory effect of the flavonoids increased with the number of hydroxyl groups present in the flavonoid structure.<br>-Antibiofilm agent against *Salmonella spp.* biofilm formation, as well as biofilm caused by other bacteria.<br>-Quercetin in combination with polymeric nanoparticles produces an alteration in membrane permeability, adsorption, and adhesion of nanoparticles with fusion with the cell membrane. | Rempe et al 2017; Miklasińska-Majdanik et al., 2018; Górniak et al, 2019; Kim et al, 2022; Kumar et al., 2016 |
| 4 | Morin | -Cause destabilization of the membrane structure by disordering and disorientation of the membrane lipids and induced leakage from the vesicle.<br>-Inhibit sortase—the enzyme presents in the cytoplasmic membrane of Gram-positive bacteria, which is responsible for anchoring protein virulence factors to the cell wall peptidoglycan. Morin presence makes that *S. aureus* to have a reduced affinity to fibrinogen<br>Binds to FtsA close to ATP binding site affecting the ATPase activity of FtsA and inhibiting its polymerization.<br>-Inhibition of FtsA polymerization affects the cell division of *Vibrio cholerae* altering its morphology to an elongated one.<br>-Promoting bacterial aggregation, leakage of the cell membrane, intervention in the biofilm growth, and the down-regulation of the PBP2a-mediated resistant mechanism. inhibit *S. aureus*' virulence.<br>-Inhibit the initial biofilm formation of *Listeria monocytogenes* strains. | Miklasińska-Majdanik et 2018; Nag et al., 2021; Rajput et al., 2021; Sivaranjani et al.2021 |
| 5 | Apigenin | -Cause destabilization of the membrane structure by disordering and disorientation of the membrane lipids and induced leakage from the vesicle.<br>-Dehydratase inhibition (HpFabZ) and protein kinase inhibition.<br>-Adherence and fusion of liposomal apigenin with the bacteria produce membrane perturbation through reactive oxygen species generation.<br>-DNA cleavage assay showed that API inhibited DNA gyrase harbouring the quinolone resistance mutation gyrA (Ser84Leu) of *S. aureus*. | Rempe et al 2017; Banerjee et al 2015; Miklasińska-Majdanik et 2018. |
| 6 | Naringenin | -Have antibacterial activity against MRSA, reducing the fluidity in hydrophilic and hydrophobic regions of both inner and outer cellular membrane.<br>-Change the membrane fluidity and fatty acid composition.<br>-Inhibit *S. mutans* growth and biofilm formation, increased *S. mutans* surface hydrophobicity, reduced bacterial aggregation, and downregulated the mRNA expression of gtfB, gtfC, comD, comE, and luxS. | Miklasińska-Majdanik et 2018, Wang et al., 2018;Yue et al., 2018 |
| 7 | Rutin (quercetin-3-O-rhamnoglucoside) | -Decrease the bilayer thickness, and disrupt the lipid monolayer structure.<br>-Decreased biofilm production. Down-regulated the expression of luxS gene and wabG gene in strong biofilm producing *K. pneumoniae*. | Miklasińska-Majdanik et al., 2018; Wang et al., 2021 |
| 8 | Kaempferol and kaempferide-3-O-glucoside | -Activity against amoxicillin-resistant *E. coli*, but also the ability to reverse the resistance via inhibition of peptidoglycan and ribosome synthesis.<br>-Against *L. monocytogenes*, could cause cell death through cell membrane damage and decrease the expression of the virulence factors.<br>-Inhibit DNA gyrase from *E. coli*. Inhibit the neurotoxin from *C. botulinum.*<br>-Damage to the *E. coli* ATCC 25922 membrane.interaction with the polar head-group of the membrane followed by penetration into the hydrophobic regions. | Miklasińska-Majdanik et 2018,.Liu et al., 2021; Górniak et al, 2019 |

## ANTIMICROBIAL ACTIVITY OF *CARTHAMUS TINCTORIUS*

### Evaluation of antimicrobial activity of safflower leaves

In the composition of leaf essential oil, was identified caryophyllene oxide as a chemotype. Other representative compounds were found such as methyl eugenol (16,79%), α-pinene (8,03%) and cinnamyl acetate (7,29%). Eugenol is a very important compound useful in the pharmaceutical field because of its antibacterial, antifungal, anti-inflammatory and antioxidant properties and its insecticide effect (Bhuiyan et al., 2010).

In the leaves during growth and processing, were also identified eight flavonoids, apigenin-6-C-β-D-glucopyranosyl-8-C-β-D-glucopyranoside (AGG), quercetin 7-O-β-Dglucopyranoside (QG), luteolin 7-O-β-D-glucopyranoside (LG), quercetin 7-O-(6”-O-acetyl)-β-D-glucopyranoside (QAG), luteolin 7-O-(6”-O-acetyl)-β-D-glucopyranoside (LAG), quercetin (Q), luteolin (L) and acacetin 7-O-β-D-glucuronide (AG).

Steamed and roasted safflower leaves are a rich source of flavonoids and may be a good source of bioactive components with antibacterial activity (Lee et al., 2006). Moneim et al. (2017) investigated the antibacterial activity of water and methanol extracts from the leaves of some medicinal plants and their synergistic effect with antibiotics.

They used various antibiotic-resistant bacterial species, including: *Escherichia coli (E. coli), Klebsiella pneumoniae (K. pneumonia), Acinetobacter baumannii (A. baumannii), Pseudomonas aeruginosa (P. aeruginosa), Staphylococcus aureus (S. aureus)* and *Salmonella* spp.

The results showed that methanol extracts from most of the plants tested inhibited the growth of the bacteria tested, however, the largest inhibition was by Senna, followed by Harmal and safflower.

The synergism between plants extract and antibiotics resulted in inhibition of the tested organisms (Moneim et al., 2017). Omidpanah et al. (2019) and Salem et al. (2014a), demonstrated that safflower methanolic and aqueous leaves and flower extracts have an effect on *Aspergillus flavus*, *Aspergillus carbonarus*, *Sclerotinia sclerotiorum*, *Penicillium digitatum* on the fruits.

### Evaluation of antimicrobial activity of safflower flowers

Safflower florets contain yellow and red quinochalcone natural dyes such as safflower yellow A, safflower yellow B, safflomin C, precarthamin, and carthamin. These chalcones are the main constituents of glycosylated flavonoids in safflower which were not detected in other natural products.

The antimicrobial activity of quinochalcones, precarthamin and carthamin, was demonstrated by Salem et al (2014 b), against three Gram-positive bacteria (*E. coli, S. aureus,* and *B. cereus*), one Gram-negative bacteria (*P. aeruginosa*), and one yeast strain (*C. albicans*). The potency of the chalcones against *C. albicans* depended on their ability to interact with sulphydryl groups. Extracts of *Carthamus tinctorius* L. flowers harvested at the last stage of flowering, were shown to have significant antimicrobial effects against a fungal strain (*Candida albicans*) and certain bacterial strains (*E. coli*, *S. aureus*, *P. aeruginosa*, and *Bacillus cereus*). The most intense activity was observed against *E. coli* (Attia et al., 2021).

Flowers contain also polysaccharides that had immunomodulating activities. The immune system being the most effective weapon for preventing the invasion of the pathogen (Yao et al., 2018). Oil extracts from flowers have activity on *E. coli* and *S. aureus* (Sabah et al., 2015).

The antibacterial activity of hydro - alcoholic extracts from 3 genotypes of dried safflower flower was demonstrated by Ozkan et al., 2021 against Gram-positive bacteria (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus*) and Gram-negative bacteria *(Escherichia coli, Salmonella typhimurium*). The antibacterial activity of the safflower genotype extracts was determined by using the agar diffusion method. In addition, safflower can be used as antioxidants and vitamin stabilizers in some food types (Adamska and Biernacka, 2021).

### Evaluation of antimicrobial activity of safflower seeds

The edible seed oil extracted by cold pressing it is an extraction method that does not use any chemical products during the extraction process and ensures the preservation of oil components (Khémiri et al., 2020). Phenolics, polysaccharides, flavonoids, alkaloids, lignans (tracheloside), steroids, carboxylic acids, quinochalcone C-glycosides and quinone-containing chalcones have been the chemical groups isolated from safflower seeds (Singhal et al, 2019; Mani et al., 2020). Safflower seeds contain 38–48% oil, 15–22% proteins, and 11–22% fibres (Mani et al., 2020). Safflower oil contains 70% polyunsaturated linoleic acid and 10% monounsaturated oleic acid. Seven antioxidative serotonin derivatives were isolated from the oil of safflower (Mani et al., 2020). *C. tinctorius* L. seed oil display high antioxidant and antimicrobial effects (Attia et al. 2021).

Khémiri et al., 2020 show the antimicrobial activity of safflower seed oil on 2 gram-negative bacterial strains (*E. coli* and *Enterobacter cloacae*), 2 gram-positive bacterial strains (*S. aureus* and *Streptococcus agalactiae*), 3 yeast species strains (*Candida albicans*, *Candida parapsilosis*, and *Candida sake*), and 3 fungi species (*Aspergillus niger*, *Penicillium digitatum*, and *Fusarium oxysporum*) and Padole et al. (2021) on *E. coli*. Their reports showed that the oil extracted from safflower seeds under cold pressing exhibited high antioxidant activities and notable antimicrobial effects against human skin opportunistic pathogenic bacteria, yeast, and fungi, which commonly are involved in altering the healing of skin wounds.

Safflower oil nanoemulsion and cumin essential oil combined with oxygen absorber packaging delayed the growth of mesophilic and psychrotrophic bacteria, *Enterobacteriaceae*, and lactic acid bacteria during the storage period of refrigerated lamb loins (Hasani-Javanmardi et al., 2021).

The **seed coat** has effect on *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumonia*, and *Salmonella typhi* as gram-negative bacteria and *S. aureus* (Tayebeh et al., 2021).

On the vegetables Son et al., 2017 revealed the antimicrobial activity of safflower **seed meal** extract on *Listeria monocytogenes* on fresh lettuce.

Yavuzer et al., 2021 and Kuley et al., 2019, showed that ethanolic safflower and bitter melon extracts have activity in controlling the growth of fish spoilage bacteria (*Acinetobacter lwoffii*, *Pseudomonas oryzihabitans*, *Enterobacter cloacae*, *Shigella spp*., *Morganella psychrotolerans* and *Photobacterium phosphoreum*) and food-borne pathogens (*S. aureus*, *Klebsiella pneumoniae* and *Salmonella paratyphi* A). As a result, safflower and bitter melon extracts could be used as antimicrobial agents to inhibit bacterial growth in food.

Aqueous extract from **waste** (mixture of stem and leaf obtained after the seed collection process) has antibacterial activity on *S. aureus* (Gram positive) and *Pseudomonas fluorescens* (Gram negative) (Rodríguez-Félix et al., 2021).

Aqueous extract from air dried powder plant has antibacterial activity on: *Proteus vulgaris*; *S. aureus*, *Erwinia carotovora*, *Bacillus subtilis*, *Klebsiella pneumonia*, and *C. albicans* (Ibrahim et al., 2019) and on *E. coli*, *Klebsiella pneumoniae* (*K. pneumonia*e), *Acinetobacter baumannii*, *P. aeruginosa*, *S. aureus* and *Salmonella* spp. (Moneim et al., 2018)

In the literature, there are more studies about safflower antibacterial action on human pathogenic bacteria and fungi action, including Gram-positive *B. cereus*, *L. monocytogenes*, *S. aureus* and Gram-negative bacteria *E. coli*, *P. aeruginosa*, *Salmonella enterica* (Bouarab-Chibane et al., 2019; Thornfeldt, 2018; Sulieman et al., 2017; Karimkhani et al., 2016; Bessada et al., 2015; Salem et al., 2014).

## CONCLUSIONS

This review reveals the importance of various extracts of *Carthamus tinctorius* (safflower) from seeds, leaves and flowers in the control of nosocomial pathogens. The bioactive components of *Carthamus tinctorius* L. may be considered a good alternative to natural therapeutic treatments. The health-promoting applicability of safflower increases with the progressing knowledge of its chemical composition. Subsequent investigation of the plant extracts to isolate and recognize the active ingredients is highly recommended.

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