Article

Antimicrobial and Anti-Breast Cancer Activity of *Hawthorn* Extracts

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Abstract: The existence of many active components in plants, including flavonoids, make the herbal medicine an effective treatment for many diseases that are resistant to conventional medicine. Flavonoids, a diverse class of naturally occurring chemicals, have received substantial research due to their potent biological effects. Hawthorn (*Crataegus*) is an extensively utilized plant by many populations worldwide due to its varied medical benefits. The study looks at the antimicrobial and anti-breast cancer (Mcf7) properties of flavonoid extracts isolated from leaves, fruit and seed of hawthorn. The activity of the extracts was evaluated for *Escherichia coli*, *Staphylococcus aureus*, and *Candida tropical* using agar well diffusion assay. Based on the findings, the extracts recorded a good activity towards the selected microorganisms in concentration 0.03 g /mL and the bacteria strains were more sensitive to the flavonoid extracts with inhibition zones ranged between (20-27mm) than fungus strain which recorded (0-15mm). The activity against Mcf7 cell line by MTT assay exhibited that the flavonoid extract of fruit was more effective in inhibiting breast cancer in concentration (1000)µg/mL with activity 37.90% compared to other flavonoid extracts. Therefore, according to the results and after requiring in *vivo* experiments, hawthorn flavonoid extracts can be used as antibiotics and anti-breast cancer agents.

Keywords: *Crataegus*, Mcf7,Plant, Antimicrobial.

Introduction

Various civilizations around the world have employed herbal remedies for the prevention and treatment of a wide range of health disorders. According to the

WHO, 80% of people in underdeveloped nations authenticate on traditional medicine for their basic medical needs(1). The prevalence of chronic ailments, the overall desire for health, the rising expense of conventional drugs, and the false public perception that herbal products are safer than synthetic drugs are just a few of the causes behind the increased usage of herbal products(2). With more than 250 species classified, hawthorn (*Crataegus*) belong to Rosaceae family that grows throughout Asia and other regions of the world (3). It is a thorny, bushy shrub with white flowers, light green foliage, and tasty red fruits (4). Many pharmacological effects inclusive of anti-inflammatory, antioxidant, anticancer, antimicrobial, antidepressant, anti-diabetic, anti-Alzheimer, anticoagulant, anti-aging, anti-HIV, anti-hypertension, wound healing effects, treatment of metabolic syndrome, cholesterol homeostasis regulation, neuroprotective effects, gastrointestinal motility regulating, cardioprotective, and hepatoprotective effects, have been verified by recent investigations on the extracts of hawthorn fruits, flowers, and leaves(5). These properties are mostly related to many active compounds comprising flavonoids , procyanidins, some phenolic acids, terpenoids, lignans, sugars, organic acids and steroids (6). Flavonoids are secondary metabolites that are ubiquitous in a variety of plant parts but mainly extracted from tinted fractions: vegetables, blossoms, fruits, grain, seeds and nut (7). These polyphenolic consist of two substituted benzene rings and aliphatic chain which comprises a pyran ring. They occur as aglycones with methoxyl or hydroxyl groups or as O- or C-glycosides. Flavonoids types include: flavonones, flavonols, isoflavones, flavanes, flavones, chalcones and biflavones. Flavonoids are well documented for their pharmacological effects, including antioxidant, anticancer, antimicrobial, anti-inflammatory and antiviral activities(8,9). Therefore, the present study administrate the in vitro activity hawthorn flavonoids extracts of leaves, fruit, and seed as antibacterial, antifungal and anti-breast cancer agents.

Materials & Methods

Plant Materials

The chosen plant was bought in the Basrah vegetable market. The plant was recognized and verified in the biology department of the University of Basrah.

After gathering, the suspended material was thoroughly cleansed under running water before the fruit's outer shell was gently removed. The leaves, seeds, pulp and fruit peels were separately dried away from sunlight, finely ground with an electric mill and kept in a tight glass container.

Chemicals

The highest purity (99.0%) chemicals were used, and they were bought from Sigma Aldrich.

Isolation of Anthocyanin Pigments from Fruit Peels

(5 gm) of red hawthorn peels powder treated with 100 ml of ethanol 70% with continuous stirring for five hr. in room temperature, the mixture filtered and the red extract was treated with 50 ml of 2% aqueous lead acetate solution. Then the solution was placed in a separating funnel, shaken well for few minutes and left for 4 hours for complete the precipitation. The obtained blue-colored precipitate was separated from the red-colored filtrate and washed with distilled water and acetone. After drying the precipitate, 20mL of acetone and 10mL of HCl (2%) were added together. The red filterate removed from the white precipitate and left to dry at room temperature to get red amorphous substance weighing 2.8 gm (10).

Preparation of Flavonoids Extracts from Plant Parts:

The flavonoids extracts of leaves, pulp and seeds extracted according to (11)procedure:

About 10.00 gm of deffated plant parts (leaves, pulp and seeds) mixed with 200 mL of 70% ethanol and ascended to hot reflex for 5 hours. The solution was filtered by vacuum pressure filteration, and the filtrate was dried in a glass dish. Table -1- shows the chemical analysis for each extract.

Antimicrobial Assay

In vitro testing was done on the seed, leaf, and pulp extracts to check for antibacterial and antifungal activity. The microorganisms used in this study were gotten from the biology section and included two bacterial strains, *Escherichia coli* ATTC 25922 and *Staphylococcus aureus* ATCC 25923 as well as one fungus, *Candida tropical* ATCC 750. The test was conducted using the agar diffusion method. On the surface of Sabouraud dextrose agar and Nutrient agar mediums, respectively, 0.2 mL of fungal inocula and bacterial inocula were added. The extracts were dissolved in DMSO and 80µL sited in central pore in plates and incubated at (37 ± 2°C), while incubating fungus plates on (25 ± 2°C) for 24 hr, each isolate's inhibitory zones were measured in millimeter units(12).

Cytotoxic Activity

Cytotoxicity of anthocyanin, leaves, pulp and seeds extracts towards Mcf7 cell line was firm by the (3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) test as termed by(13). Fetal bovine, penicillin, and streptomycin added to RPMI-1640 to sustain the Mcf7 cancer cell line that was acquired from the Basrah-based Iraq Biotech Cell Bank Unit.

Mcf7 cell line were treated with the tested extracts (1000 µg/ mL) and cell inhibition was measured after 72 hrs. 28 µL of MTT was added and incubating the cells for 2 h at 37 °C. The MTT assay measures the amount of mitochondrial succinate dehydrogenase or reductase that converts yellow MTT into a dark purple, insoluble formazan product that was measured at 590 nm.

Results & Discussions

Antimicrobial Activity

Antibiotic resistance, which threatens societal health and is to blame for millions of deaths annually worldwide, is one of the enormous public health concerns we are currently facing. The emergence and spread of novel resistance mechanisms internationally threatens the effectiveness of current infectious disease control strategies(14,15). To treat a variety of complex illnesses brought on by multidrug-resistant pathogenic microbes, there is an increasing interest in generating natural products (NPs) (16).

Flavonoids among of broad category of NPs that have long been acknowledged as essential natural substances in the preclusion and cure of many diseases like infectious ailments connected to a variety of pathogenic microorganisms(16,17). The effectiveness of seed, leaf, and pulp flavonoids extracts against the chosen microorganisms was shown in Table 2. In general, bacteria strains were more sensitive to extracts than fungus did. It is widely acknowledged that fungi are harder to treat than bacteria without damaging the host because they share more essential cell structures and machinery with eukaryotic animal cells than with bacteria. As a result, there may be substantial pharmacological adverse effects that patients experience that are off-target. According to the findings, all extracts exhibit noticeable effectiveness against harmful microorganisms. While leaf extract was more effective against *Escherichia coli* (27mm), pulp extract had the best activity against *Staphylococcus aureus* (27mm). Compared to the seeds extract, the leaves extract was more active (15 mm) against *Candida tropical*, whilst the pulp extract had no effect.

Many plant-derived flavonoids have antibacterial properties that differ from those of traditional antibiotics in their target sites and mechanisms. As a result, bacteria and other pathogens cannot easily develop resistance to these substances since resistance genes do not initially encode most natural substances, which makes them important for improving antibacterial therapy(18-20). The researchers identified a number of mechanisms for flavonoids' antibacterial activity, including inhibition of: ATP synthesis, the electron transport chain, nucleic acid synthesis, cell envelop production, the formation of biofilms, bacterial enzyme-dependent virulence, bacterial toxins, bacterial quorum sensors, and inhibition of bacterial motility(20). Furthermore, polyphenols' interaction with bacterial plasma membranes can result in a variety of outcomes that support their antibacterial power. A substantial body of research suggests that the bacterial plasma membrane's structure can be changed by plant extracts and polyphenols, which can result in the formation of pores, leakage, modifications to electrical charge and polarity, transformed fluidity, enhanced penetrability, and delocalization of membrane proteins and other antibacterial effects(19). Moreover, (21) showed that alkylamino and alkyl chains, prenyl groups and heterocyclic moieties containing nitrogen or oxygen as lipophilic substituents are an important element in the fight against gram-positive bacteria that can boost the flavonoids antibacterial action(22). The necessity to research innovative anti-fungal medications, particularly against the Candida spp. that are well-known as the causal agents of candidiasis in people, was raised by the developing resistance against existing antifungal drugs (polyenes, azoles, echinocandins, and flucytosine). The growth of fungus is typically inhibited by strong antifungal compounds like flavonoids through a number of mechanisms example: the stimulation of mitochondrial breakdown, rupture of the plasma membrane, and inhibition of cell walls production, cell division, the efflux-mediated pumping mechanism, too(23-25)

Cytotoxicity

An important health problematic that can result in cancer is unusual cell growth. Only a few anticancer medications have an activity to tumor cells but they have destructive side effects(26,27). Because they are widely regarded as safe, accessible, have few harmful effects when used for long time, and are frequently cytotoxic for cancer but not healthy cells, phytochemicals, such as flavonoids, terpenes, isoflavones, and caretonoids, are a wonderful choice for creating novel medicines. The effects of flavonoids against cancer are linked to cancer progression and dissemination to distant areas as well as early stages of the disease(28-30)**.** The results of an investigation of the in vitro growth inhibitory activity of pigments and flavonoid extracts against the human cancer cell line (Breast cancer mcf7) revealed that all of the tested extracts inhibited the tumor cell line, with pulp extract being the most active with an inhibition rate of 37.90%, followed by anthocyanin extract at 33.12% and leaves extract at 30.63%, and seeds extract recording the least inhibition at 28.04% (Table 3).

The inhibition of proteasome DNA topoisomerase I/II and fatty acid synthesis, accumulation of p53, cell cycle detention, modulation of survival/ proliferation pathways, modulation of ROS-scavenging enzyme activities, autophagy, suppression of cancer cell angiogenesis and invasiveness or metastasis are just a few of the many effects of flavonoids against cancer initiation and progression. Also, flavonoids decrease proliferation, enhance apoptosis, and/or interfere with numerous signal transduction pathways during the carcinogenesis process (31,32).

The presence of anthocyanin pigments was consistent with the red hue of Crataegus fruit. Most plant parts include anthocyanins, which are water-soluble flavonoid pigments that give plants distinct colors in the red, purple, or blue. Anthocyanins have significant antioxidant, antibacterial, anti-inflammatory, and anticancer characteristics that can be employed in the deterrence of many diseases, including diabetes, cancer, and obesity, according to new studies(33-36). Anthocyanins may suppress cancer development and tumor growth through two basic mechanisms: Modification of the redox status and interference with fundamental cellular processes (including metastasis, apoptosis, autophagy, invasion, cell cycle, angiogenesis, and biochemical metabolism).

Anthocyanins, an anticancer agents, block the signaling pathways in cancer cells, including AMP-activated protein kinase, nuclear factor κB, and mitogen-activated protein kinase (35,36).

Our findings suggest that hawthorn extracts are potentially effective treatments for breast cancer mcf7 because they exhibit antitumor activity due to the presence of flavonoids, which have a variety of anticancer effects.

Conclusion

The flavonoids extracts from seed, fruit, and leaves of hawthorn have been extracted and evaluated their activity as antimicrobial and anti-breast cancer agents. Hawthorn extracts have been found to be effective against bacteria with inhibition zones (20-27mm) and appreciable activity towards fungi (0-15mm). All extracts exhibited a good activity against Mcf7 breast cancer cell line especially pulp extract. These effects indicating that they could be used as potential compounds for further research on the creation of new antibiotic and anticancer agents after the in vivo required studies.

Table 1: The chemical analysis for leaves, pulp and seeds extract.

Extract Type	Color	Weight (gm)	Yield (%)
Leaves Extract	Dark Nutty	4.98	50
Pulp Extract	Dark Brown	2.35	24
Seeds Extract	Dark Brown	4.57	46

Table2: The inhibition zones(mm) of flavonoids extracts against the selected microbes

Microbe	Seeds Extract	Leaves Extract	pulp Extract
Staphylococcus Aureus	25	20	27
Escherichia Coli	20	27	23
Candida Tropical	13	15	0

Table (3): Rate of Mcf7 inhibition for three replicate for each extracts, at concentration 1mg/ml

Extracts	Fruit extract	Seeds Extract	Leaves Extract	Anthocyanin extract
Mean	37.90	28.04	30.63	33.12

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