

ORIGINAL ARTICLE

THE EFFECT OF FLAVONOIDS EXTRACTS FROM HAWTHORN (*CRATAGUS OXYACANTHUS*) AGAINST SOME GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA SPECIES

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Summary

The present study has shown the screening test of Hawthorn fruits (*Crataegus spp.*) using crude hydro-alcoholic extract and in vitro antimicrobial activity of extracted flavonoid which has shown more antibacterial activity than crude extract with minimal inhibitory concentration (MIC) values ranged from 5% to 2.5%. The amount of total flavonoid and antioxidant activity of Hawthorn fruits (*Crataegus spp.*) in various concentration extracts and antioxidant activities of different concentration extracts were determined by radical scavenging by 2, 2-diphenyl-1-picrylhydrazyl (DPPH[•]). The output showed that the contents of flavonoid were found to be 0.386 mg quercet in equivalents (QUE/g). Dried extract displayed remarkable antioxidant activity according to (DPPH[•]) assays.

Key words: Hawthorn fruits; DPPH; phytochemical; flavonoids; antibacterial

Introduction

Antibacterial agents are increasingly used by healthcare providers, in both hospital settings and community pharmacy (1). The availability of antibacterial agents for the community, even without prescription has made it easy to access antibiotics and this has been associated with the risk of antibacterial resistance (2). Currently, almost all antibacterial has shown some degree of resistance to the antibiotic at least with one bacterial species; this issue is applicable with currently infecting micro-organisms, such as *Staphylococcus aureus* and *Escherichia coli* (3). Therefore, searching for alternative methods for antibacterial agents from herbal-isolated drugs is greatly important.

The selective toxicity of an antimicrobial agent varies; some function in a non-selective manner, affecting all cell types in the same way. In the treatment of tuberculosis, selective toxic antimicrobials are especially useful as chemotherapeutic agents (4,5,6). Several studies on *Crataegus* species have been conducted and confirmed their effects as a bioactive substance (7,8,9). Antioxidant phytochemicals found in *Crataegus* extracts include procyanidins, flavonoids, flavonols, glycosylated flavanones, and triterpene pentacyclic acids. Procyanidins and triterpene pentacyclic acids are found at the highest concentrations in fruit (7). Leaves are the most popular

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source of flavonoids (10,11,12). The significance of phytochemical compounds as phenolic antioxidant compounds that aid in the prevention of chronic diseases by reducing oxidative stress is related to their multiple actions (13,14,15).

Flavonoids in *Crataegus* are biosynthesized in plants through the Shikimic Acid Pathway and to play important role in protection against pathogens (16,17,18). On the epidermis of the fruit, there are a large variety and high concentration of metabolites that protect against pathogenic agents, insect attacks, and scaring processes. As a consequence, the epidermis' high concentration of metabolites has a wide range of biological effects, the most prominent of which are antimicrobial, antifungicide, and antioxidant (10,19). Flavonoid concentration is primarily determined by environmental factors such as type of soil and solar exposure (11,20).

This study aimed to find specific phytochemicals and calculate absolute muck hydro alcoholic concentrate and flavonoids, as well as the antimicrobial activity of Hawthorn fruits extract against (*Escherichia coli* and *Staphylococcus aureus*) using susceptibility tests and minimum inhibitory concentration tests (MIC) (3,21).

Materials and Methods

Plant materials collection: Fresh fruits of hawthorns were collected from local suppliers. One kilogram of *Crataegus* fruit samples was peeled decanting the seeds, and the two pieces' peel and pulp were dried at room temperature.

Preparation of extract: The dried fruit was milled into powder, soaked in hydro-alcoholic solvent. The extraction was done using the soxhlet method, and the crude extract was held in an airtight container in the refrigerator at 4°C.

Phytochemical screening: Phytochemical screenings of Hawthorn fruits extract were assessed by standard methods. These include detection of the founding of alkaloids, terpenoids, phenols, tannins, carbohydrates, saponins, glycosides, flavonoids, quinines, and steroids.

Extraction of flavonoids: The Hawthorn fruit extract was mixed with 25 ml of 1% lead acetate for optimum precipitation, then stirred for 4 hours, filtered, and filtrated. The filtrate was combined with a mixture of 65 ml acetone and 7.5 ml hydrochloric acid, and the accumulated solution was deposited at 4°C. Various experimental precipitates were made from each deep brown precipitate.

Flavonoid chemical screening: 1% potassium hydroxide (KOH) and 1% ferric chloride ($\text{FeCl}_3 \cdot 2\text{H}_2\text{O}$) solutions were used to detect the presence of flavonoids and phenolic compounds. They were added separately to each 5ml of plant extract. The appearance of yellow and blue-green colors in the plant extract demonstrated the existence of flavonoids and phenolic compounds.

Total flavonoid content determination in the extracts: 1.0 ml of harvested Hawthorn fruit was combined with 1.0 ml of ethanol-containing aluminum trichloride (20 mg/ml) and glacial acetic acid increases, and total flavonoid concentration was determined by diluting with 25 ml ethanol. After 40 minutes, absorption was measured at 415 nm with a spectrophotometer (Jenway 6300, UK).

Effect of extract on DPPH radical: Established volumes (50-150L) of plant extract were applied to test tubes, then completed to 1.0 ml by distilled water, (1.0ml) of 2, 2-diphenyl-1-picrylhydrazyl was added.

Each tube sample was added to a (DPPH[•]) solution (0.2 mM in ethanol), properly mixed, and incubated for 30 minutes at room temperature. The control solution was made using the same procedure but without the plant extract. Vitamin C was obtained from ascorbic acid solution (0.03% w/v) (standard solution). The absorbance of the solution was measured at 517 nm with spectrophotometer (Jenway 6300, UK). Inhibition of DPPH free radical in present (I %) was calculated Bears-Lambert equation.

Identification of the compound: The plant extract was analyzed using FTIR spectrophotometer (Bruker-Alha, Germany).

Susceptibility test: A wire loop was used to pick a few colonies (3 to 10) of the organism to be examined. The cells were removed from the culture plate and placed in a test tube with 4 ml nutrient broth. The tubes were then incubated for 2–5 hours, yielding a cloudy bacterial suspension. For the susceptibility plates, Mueller-Hinton agar was used in Petri-dishes.

Results

Characterization of bioactive compounds: The samples were transferred to phytochemical analysis. This revealed the presence of the bioactive compound contained in the ethanol sample of the plant extract. Terpenoids, phenols, tannins, flavonoids, steroids, and carbohydrates were all detected in this study.

Table 1. The results of bioactive compound.

Phytoconstituents test	React	Result
Alkaloids	No formation of yellow color	Alkaloids are not present
Phenols	Formation black blue precipitate	phenols are present
Terpenoids	Formation reddish color	Terpenoids are present
Flavonoids	Formation greenish blue	Flavonoid are present
Steroids	Formation layer reddish color	steroids are present
Quinine	No Formation red color	Quinine is not present
Saponins	No Formation foam	Saponins are not present
Coumarine glycoside	No Formation yellow precipitate	Coumarine glycoside is not present
Tannins	Formation yellow precipitate	Tannins are present
Protein	No Formation reddish color	Protein is not present
Carbohydrate	Formation purple ring	Carbohydrate is present

Total flavonoid content (TF): TF contents in the ethanolic extracts of red Hawthorn fruit showed that TF was (0.386mg/g dry weights in 70% ethanolic extract) obtained in the ethanolic extract.

DPPH radical scavenging activity (RSA): The results obtained from scavenging activity of flavonoids in ethanolic extract of red Hawthorne fruit extract against DPPH activity were compared with the standard antioxidant as ascorbic acid and the potential of different concentrations of Hawthorne fruit extract to scavenge free radical varied by 32.77 % in concentration 250 ppm, 45.01% in concentration 500 ppm and 53.25 % in concentration 750 ppm. Hawthorne fruit extract clearly showed that as the concentration showed higher the antioxidant activity against DPPH radicals increased. The results are found in Figure 1.

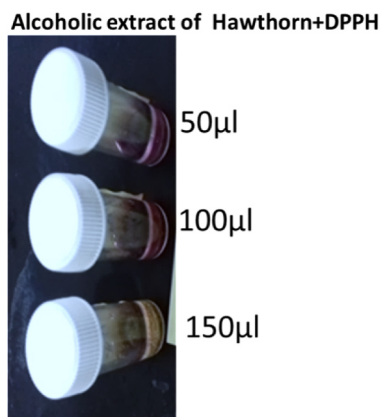
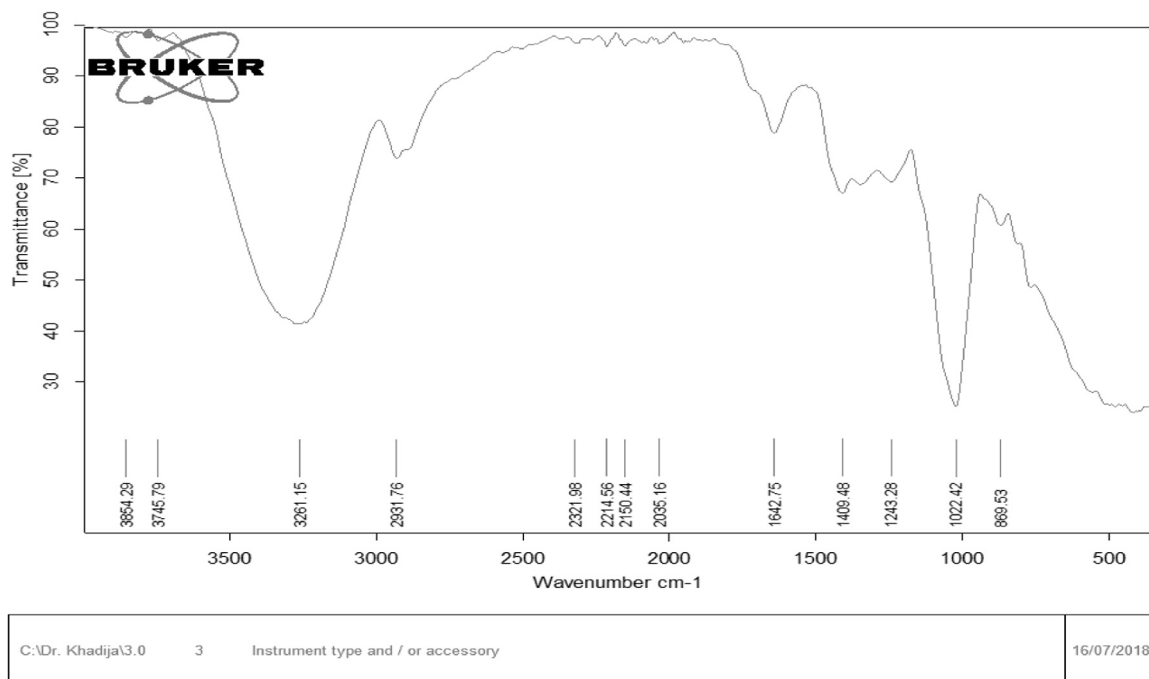


Figure 1. DPPH radical scavenging activity of alcoholic extract of Hawthorn.

FTIR: The solution was analyzed separately using FTIR spectrophotometer (Bruker-Alpha, Germany). The compound comprises multi-functional groups (Figure 2) such as phenolic –OH, carbonyl (–C=O), olefinic C=C, and so on, according to FTIR research (Table 2).



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Figure 2. The result of FTIR.

Table 2. The compounds' functional groups as determined by IR-spectrum.

Wave no.3	Band shape	Band	Functional group
3261.15	Band	OH	Alcohol
2931	Sharp	-CH ₂ -	Aliphatic stretch
1409-1642	Sharp	-C=C-	Stretching of olefinic
1100	Sharp	C-O	C-O Stretching

Antibacterial activity of Hawthorn extract yield:

Three kinds of harmful bacteria were used to assess the antibacterial properties of flavonoid and crude extract (*Escherichia coli*, and *Staphylococcus aureus*) (Table 3 and Figure 3). The potency of *Crataegus oxyacanthas* has antibacterial properties against bacteria was assessed by the presence or absence of inhibitory zones and zone diameter in this study (Table 3). The findings revealed that a Hawthorn extract (*Crataegus laevigata*) had some antibacterial activity.

Table 3. The effects of a flavonoid derived from hawthorn on *Staphylococcus aureus*, and *Escherichia coli*.

Concentration of Extracts (mg/ml)	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
200	26 mm	18 mm
100	21 mm	13 mm
50	16 mm	11 mm
25	12 mm	10 mm

The MIC values were in good agreement with the antibacterial patterns shown by the paper disc diffusion method (Table 4).

Table 4. Minimum inhibitory concentration (MIC) of flavonoids extracted from Hawthorn.

Bacterial Species Extracted compound	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
Flavonoid extract	0.5	0.25

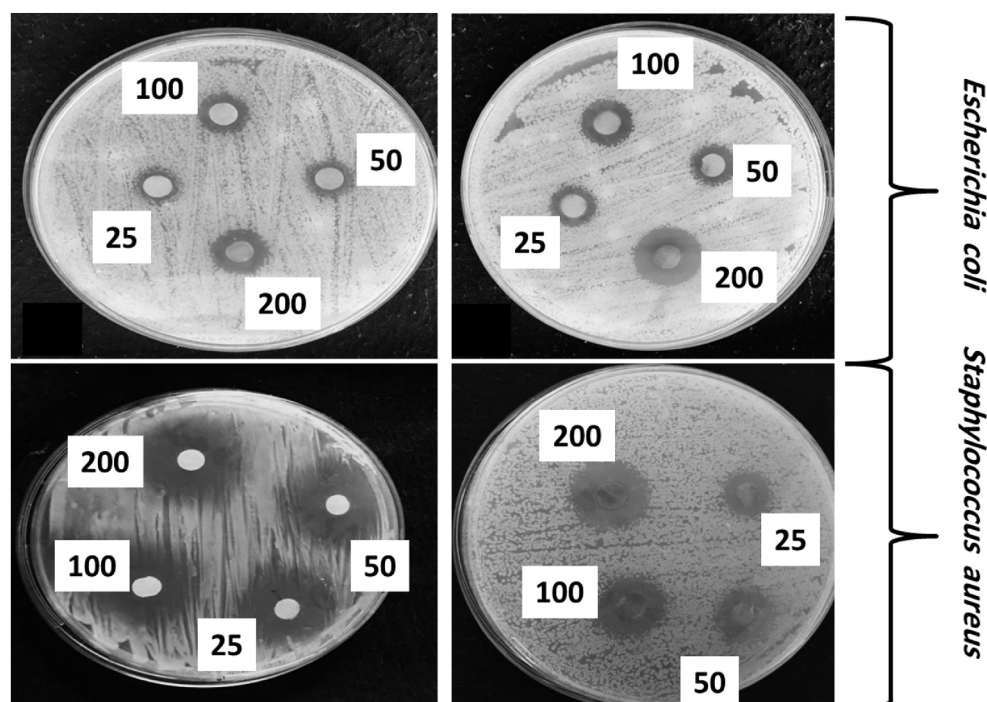


Figure 3. Zone inhibition of affected bacteria through agar disk diffusion method with, flavonoid and crud extract.

Discussion

The red Hawthorn fruit ethanolic extract has remarkable antioxidant activity against DPPH radicals from and the antioxidant activity is concentration-dependent¹⁹. The antioxidant activity of Hawthorn extract with 80% methanol was found to be the highest. Therefore, the results showed that the total flavonoid content was (0.386 mg QUE/g dry weight in 70% ethanolic extract) (20).

Flavonoids in fresh Hawthorn fruit extracts in 80% methanol ranged from 0.254 to 0.595mg RUE g⁻¹ (21). The total flavonoid content of the dried fruit was 55.89 mg quercetin/g. These differences can also be explained by soil and climate differences and different solvents. Antimicrobial drug effects on bacteria include protein synthesis inhibition, cell wall synthesis inhibition, and nucleic acid synthesis inhibition (22). Antimicrobial resistance is more common in nosocomially acquired *Pseudomonas aeruginosa* isolates than in community-acquired strains, with resistance to several groups of antimicrobials. Mutations in genes encoding purines, efflux pumps, penicillin-binding proteins, and chromosomal β -lactamase have been found to cause resistance in *Pseudomonas aeruginosa* (23).

As the number of multidrug-resistant *P. aeruginosa* strains increases, including those resistant to all β -lactams, carbapenems, aminoglycosides, and fluoroquinolones, the need for searching of new alternative therapy required (24).

The quest for antimicrobials with new modes of action has become more intense. Recently, there has been a fast increase in the number of multidrug-resistant clinical strains, limiting the therapeutic choices available (25). The present study agrees with used gram-positive bacteria, gram-negative bacteria, and fungi to test the antimicrobial activity of certain plant extracts (19). It was discovered that all of the plant extracts examined were ineffective against the gram-negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Serratia marcescens*, and *Citrobacter freundii* (26), and agree with in a study (20). The antibacterial activity of ethanol extract of *Crataegus oxyacantha* was tested against two Gram-positive and three Gram-negative bacteria. Bacteria with a positive gram are known as gram-positive bacteria. The extract was antimicrobial against all of the microorganisms tested. The current research contradicts with another study (20) by demonstrating that the extracts have antibacterial properties against Gram + and some Gram- bacteria. There were critical contrasts between the inhibition effects on various microorganisms; G+ bacteria are known to be more susceptible to Hawthorn extracts than G- bacteria (19). *Escherichia coli* was least susceptible to the Hawthorn extracts. The weak antibacterial activity against G- bacteria was attributed to the presence of their cell wall, lipopolysaccharide (3, 21). Results of this study showed that Hawthorn extracts have antibacterial activity against three tested bacterial strains. So the current work recommended that Hawthorn extracts could be used for the treatment of summer diarrhea caused by *Escherichia coli* as characteristic additives rather than counterfeit additives (3).

Conclusion

The present study has confirmed that Hawthorn fruit species (*Crataegus oxyacanthus*) has an important antibacterial and antioxidant effect which could be utilized in the treatment of infectious diseases that are related to infection from *Escherichia coli* and *Staphylococcus aureus*. The study outcomes were based on the extraction of components from *Crataegus oxyacanthus* and confirmation of the presence of candidate components by molecular *in vitro* techniques followed by confirmation of its microbiological activity on bacterial strains. These results are increasingly important and direct the orientation toward improving the antibacterial activity of these individual components through the motivation of industrial chemistry to modify the structure of these individual components based on structure-activity-relationship which could lead to the invention of a new generation of herbal-isolated antibiotics.

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Conflict of interest statement

The authors declare that they have no conflicts of interest regarding the publication of this article. The authors alone are responsible for the content and writing of the paper.

Adherence to ethical standards

This article does not contain any studies involving animals performed by any of the authors. This article does not contain any studies involving human participants performed by any of the authors.

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