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# *In vitro* Antimicrobial Activity of Some Phenolic Compounds (Coumarin and Quercetin) Against Gastroenteritis Bacterial Strains

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**Abstract:** In this work, antibacterial activity of coumarin and quercetin were compared. An *in vitro* study was carried out using the following bacterial strains involved in gastroenteritis diseases by using agar diffusion method and dilution method**:** *Escherichia coli 81nr.149 SKN541, Enterobacter aerogenes* CIP *104* 725, *Salmonella typhimurium* SKN533 and *Salmonella infantis SKN 557*. Only coumarin exhibited antibacterial activities against all the tested microorganisms. *Salmonella infantis SKN 557 and Salmonella typhimurium* SKN533 were more resistant to coumarin. Minimum inhibitory concentration of coumarin ranged between 0.625 and 5.0 mg/ml while the minimum bactericidal concentration was equal or more than 5 mg/ml.

**Key words:** Coumarin · Quercetin · Gastroenteritis · Bacterial Strains · Antibacterial Activity

## INTRODUCTION

Natural phenolic compounds are widespread in the plant kingdom. They are found in leaves, fruits, bark and wood and can accumulate in large amounts in particular organs or tissues of the plant [1]. Phenolic compounds are considered plant secondary substances because they are not involved in metabolic pathways. They are synthesized in response to stressful condition to protect plant against oxidative and bacterial aggression [2, 3].

Structurally, phenolic compounds comprise an aromatic ring, bearing one or more hydroxyl groups and range from simple phenolic molecules to highly polymerized compounds [4]. Polyphenols may be associated with various carbohydrates and organic acids [5]. These compounds exhibit a wide range of physiological properties, such as anti-inflammatory, antimicrobial and antioxidant effects [6, 7].

Several thousand molecules having a polyphenol structure have been identified in plants [3, 8]. Among these compounds there are coumarins and quercetins. Coumarins are phenolic substances made of fused benzene and á-pyrone rings [9]. Quercetins are phenolic compounds with several hydroxyl groups on aromatic rings [5].

The presence of a large number of phenolic hydroxyl groups enables phenolic compounds to form large complexes, mainly with proteins and to a lesser extent with other macromolecules like cellulose and pectin [10, 11]. Then, natural phenolic compounds have shown potential antiviral [12], antibacterial [13-15] and antiparasitic effects [16-18].

Commercial natural phenolic compounds are extracted from some trees rich in phenolic compounds or synthesized [19-21]. Then the most common commercial tannins are hydrolysable tannin (gallotannins and ellagitannins), condensed tannin (Catechol) and other simple phenolic compounds (Flavones, flavonoids and flavonols, coumarin etc) [3, 22, 23].

This study was carried out to evaluate the antibacterial properties of coumarin and quercetin against some bacteria involved in acute gastroenteritis diseases.

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## MATERIALS AND METHODS

**Phenolic Compounds:** Phenolic compounds used were coumarin and quercetin (sigma). Concentrated stock solutions were prepared by dissolving each extract in Dimethyl sulfoxide (DMSO) at 100 mg/ml and sterilizing by filtration (pore size 0.22 µm; Millipore).

**Microorganisms:** The antibacterial activity of phenolic compounds against referenced four human enteropathogenic bacteria was evaluated: *Escherichia coli 81nr.149 SKN541, Enterobacter aerogenes* CIP *104* 725, *Salmonella typhimurium* SKN533 and *Salmonella infantis SKN 557* (University of Copenhagen).

**Antimicrobial Test:** The antibacterial test was performed by following agar disc diffusion method [24, 25]. Bacterial strains were first grown on Muller Hinton medium (MHI) for 18 to 24 h at 37°C. The inoculums of the indicated bacterial strains were transferred into physiological suspension medium and adjusted to 0.5 Mac Farland turbidity standard [26]. A sterile 6 mm-diameter filter disc impregnated with 10 µl (1000 µg per disc) of each extract suspended in DMSO was placed on the infusion agar seeded with bacteria. Then, Petri dishes were kept at 4°C for 1 h and subsequently incubated at 37°C for 24 h. Gentamicin (10µg) and ciprofloxacin (5µg) (Liofilchem, Italy) standard discs were used as positive antibiotic controls. Discs impregnated with 10 µl of pure DMSO were used as negative controls. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs. All experiment was carried out in duplicate. The antibacterial activity was expressed as the mean of inhibition diameter zone produced.

**Determination of Minimum Inhibitory Concentration:** Minimum inhibitory concentration (MIC) of the extracts was determined using the broth microdilution method [27]. The inocula of bacterial strains were prepared as described above. The extracts were first dissolved in 10% DMSO and then sterilized as described previously. 100 µl of this stock extract solution were transferred into the first well of a 96-well sterile plate (Cellstar, Greiner Labortechnik, Germany) previously filled with 100 µl of nutrient broth. Serial twofold dilutions were made in to 11 consecutive wells. Into each well, 95 µl of nutrient broth and 5 µl of the bacterial inocula were added to achieve concentrations of extracts ranging from 5 to 0.0049 mg/ml. The final volume in each well was 200 µl. Growth control wells and sterility control wells were included in the plate. Each plate was mixed on a plate shaker at 300 rpm for 20 seconds and then incubated at 37°C for 24 h. Bacterial growth was indicated by the presence of turbidity and a pellet on the well bottom.

The least concentration of phenolic compound that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the minimum inhibitory concentration in each case [28].

**Determination of Minimum Bactericidal Concentration:** After culturing the test organisms separately in nutrient broth containing various concentrations of the active ingredients, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. 100 µl from each well demonstrating no visible growth were removed to spread onto Petri dishes filled with sterilized PCA medium. The culture was incubated at 37°C for 24 h [29]. The lowest concentration of extract which showed no bacterial growth on the solid medium after the incubation period was regarded as minimum bactericidal concentration [30].

## RESULTS AND DISCUSSION

In the present study, we focused on some phenolic compounds action against bacteria involved in acute gastroenteritis diarrhea.

The antibacterial activity of coumarin and quercetin against 4 bacteria was tested and compared to that of antibiotics ciprofloxacin and gentamicin (Table 1). The results showed that coumarin at 1000 µg per disc possessed high antibacterial activity against *Escherichia coli* and *Enterobacter aerogenes* whereas it was moderately active against *Salmonella typhimurium* and *Salmonella infantis*. On the other hand quercetin was inactive against all the tested bacterial strains (Table 2). Previous studies showed that strains of *Salmonella* genus are resistant to plants extract; this may be explained by frequent use of antibiotics against strains of this group [31].

It is known that polyphenols are bioactive molecules. These biological activities are related to the molecules structures; by their hydroxyl groups or by phenolic ring, phenolics compounds have capacity to link with proteins and bacterial membrane to form complexes [32]. Thus, several studies have reported the antimicrobial activities of plants extracts from various parts like leaves, seeds and flowers [14, 31]. These results often pointed

Table 1: Antibacterial activity of coumarin and quercetin against human pathogenic bacteria.

| | Diameters of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | Bacteria | | | |
| | *Enterobacter aerogenes* CIP *104* 725 | *Escherichia coli 81nr.149 SKN541* | *Salmonella typhimurium* SKN533 | *Salmonella infantis SKN 557* |
| Coumarin | 19.5±0.7 | 25.5±2.12 | 14±1.41 | 10.5±2.12 |
| Quercetin | NA | 8.5±2.12 | NA | NA |
| Ciprofloxacin | 28 | 29 | 39 | 26 |
| Gentamicin | 20 | 27 | 19 | 19 |

NA : No Activity

Table 2: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of coumarin

| Bacteria | MIC (mg/ml) | MBC (mg/ml) |
|---|---|---|
| *Escherichia coli 81nr.149 SKN541* | 1.25 | >5 |
| *Enterobacter aerogenes* CIP 104 725 | 0.625 | 5 |
| *Salmonella typhimurium* SKN533 | 2.5 | >5 |
| *Salmonella infantis* SKN 557 | 5 | >5 |

out that crud extract possessed low antibacterial activities against enteric bacteria [14, 32]. However our results are similar to other studies carried out with pure molecules of flavonoids or coumarin [20, 21]. These molecules showed antibacterial activities higher than crud extract. This difference may be explained by presence of some residue in crud extract which prevent direct contact between phenolic compounds and bacteria.

In fact Cowan [3] supposed that phenolic compounds without free hydroxyl groups have more antibacterial activity than those which are provided. That increases their chemical affinity to microbial lipid membrane. There are less hydroxyl groups in coumarin molecular structure than quercetin that might justify the difference of antibacterial activity between these two compounds in our study.

Minimum inhibition concentration of coumarin against the four studied bacteria ranged between 0.625 and 5 mg/ml. and the lowest minimum inhibitory (0.625 mg/ml) and bactericidal (5 mg/ml) concentrations were observed for *Enterobacter aerogenes* whereas the remaining strains survived over 5 mg/ml.

The increasing concentration of phenolic compounds enhanced antibacterial activities. Our results are in agreement with earlier investigations which showed that antimicrobial agents with high activity against an organism has a low minimum inhibitory concentration while a low active antimicrobial agent gives a high minimum inhibitory concentration [33, 34].

The present study showed that coumarin was active against the tested bacterial strains. Knowledge on antibacterial activity of simple molecules may help to develop new synthetic molecules to resolve bacterial resistance to ordinary antibiotics.

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