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Antimicrobial activity of synthetic quercetin analogue on *E. coli* and *S. aureus*

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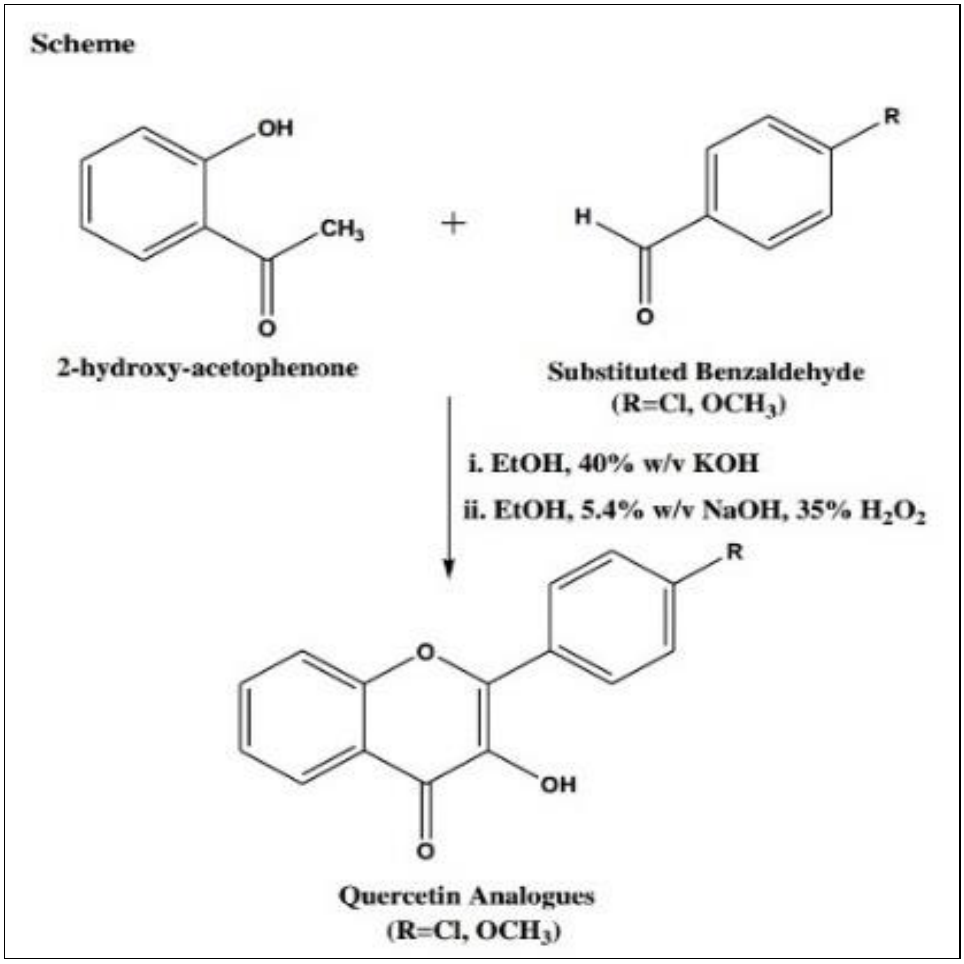
Abstract
Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Hence, new classes of antimicrobial drugs are urgently required and, in this regard, flavonoids represent a novel set of leads.
This study was done to evaluate antimicrobial activity of synthetic quercetin analogue alone and in combination with standard drug gentamicin on gram positive and gram-negative bacteria.
Quercetin analogue was synthesized fresh in the laboratory and was used to check antimicrobial activity by agar well diffusion method in gram negative and gram-positive bacteria.
The mean of zone of inhibition given by Quercetin analogue was 3.5cm against *E. coli* and 3.2cm against *S. aureus*. The mean of zone of inhibition shown by gentamicin is 2.5cm and 2.6cm for *E. coli* and *S. aureus* respectively. Gentamicin and quercetin analogue together showed more zone of inhibition (2.9 in both bacterial strain) as compared to the control gentamicin.
As per the statistical analysis, quercetin showed significant inhibition in gram negative (*E. coli*) bacterial growth ($p<0.001$). Gentamicin showed significant inhibition in gram positive bacterial growth ($p<0.035$). The combination of the two showed significant bacterial growth inhibition in both bacterial strains ($p<0.608$).
Synthetic flavonoid (quercetin) has a potent antimicrobial activity.

Keywords: quercetin, antibiotic resistance, bacteria, flavonoid

Introduction
The ability to identify novel compounds with the potential to treat the disease in a safe and effective manner is critical to drug discovery success [1]. Natural substances play an important role in medicine research and as lead structures for synthetic molecule creation. Natural products will continue to play an important role as active ingredients and model molecules in the future [2].
Fruits and vegetables contain quercetin, a polyphenolic bioflavonoid which belongs to the flavonol group [3]. It has the potential to be a potent bioactive component of the human diet, acting as a free radical scavenger and interacting with a variety of endogenous proteins (e.g. as an inhibitor of enzyme activity) [4]. The antioxidant capabilities, anti-inflammatory activity, antiproliferative, and antiplatelet actions of quercetin can all be ascribed to its anti-atherogenic activity. Antioxidant, anti-artherogenic, anti-inflammatory, anti-carcinogenic, neuroprotective, antibacterial, and antiviral effects have been discovered in it [5]. Quercetin also has GRAS (Generally Recognized As Safe) status.
It has been suggested that because flavonoids are widely distributed in edible plants and beverages and have previously been used in traditional medicine, they are likely to have minimal toxicity [6]. However, this family of compounds has a diverse range of activities in mammalian cells and in vivo confirmation of their side effects would be necessary for a full evaluation of their practical usefulness in the field of modern medicine [7].
Bacteria have developed antibiotic resistance as a result of chromosomal alterations or genetic material exchange via plasmids and transposons [8]. Antibiotic resistance among bacterial pathogens has reached alarmingly high levels. Indeed, according to a number of international organizations, the current "antibiotic resistance crisis" may soon return humanity to a "pre-antibiotic period."
In this background, it is evident that there is a pressing need to identify and develop novel antibiotics, as well as creative antimicrobial treatments that could potentially work in tandem with traditional chemotherapy [8, 9]. Even if interest in antibiotic research and development is increasing, there are still a number of unmet therapeutic needs that must be addressed as soon as possible [10].

In the present study, the antibacterial properties of quercetin was studied against *Staphylococcus aureus* and *Escherichia coli.*

Material and Methods



Synthesis of Quercetin Analogue

1. Synthesis of Quercetin analogues

A suspension of 4–benzaldehyde (1.20 g) and 2–hydroxy acetophenone (1.405 g) in ethanol (25 ml) was cooled to 10 °C and 7.5 ml of 40% w/v KOH solution was added drop wise. The reaction mixture was stirred for 18 h at room temperature.

Methonol (125 ml) was added and the organic layer was washed with H_2O, dried and concentrated in vacuo. The oily residue was dissolved in ethanol and 5.4% (w/v) NaOH solution followed by drop wise addition of 4 ml of 35% H_2O_2. The reaction mixture was stirred subsequently at room temperature for 15 hours resulting in a yellow suspension. After acidification with 2M HCl, the precipitate was filtered and dried.

2. Effect of Quercetin on suitable organism

The strain of suitable bacteria was isolated on sterile agar incubated at 30 °C under anaerobic condition. The purified culture was preserved on sterile agar slants. The inocula of the suitable bacteria was prepared as described above.

Effect of Quercetin on the bacteria was performed in the similar fashion as described for determination of Minimum Inhibitory Concentration (MIC) for other bacterial cultures. For confirmation of effect of Quercetin on growth of bacteria, agar well diffusion method was carried out.

Culture suspension was prepared in physiological saline. 0.6ml of culture was added to sterile agar and was allowed to set. Wells were punched and each dilution of Quercetin was put into the wells. The plates were incubated at 37 °C under anaerobic condition.

3. Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) of Quercetin was determined using the broth dilution method. Bacterial strains were first grown on Muller Hinton Medium for 18 to 24h at 37 °C. The inoculums of the indicated bacterial strains were transferred into physiological suspension medium. Quercetin stock of 10 mg/mL was prepared in 10% DMSO. St. Muller Hinton Broth was used as diluent.

Dilutions were prepared in the range of 10 mcg/mL to 500 mcg/mL. 0.1 mL of bacterial inocula was added in each dilution.

Growth control and sterility control were maintained. The tubes were incubated at 37 °C for 24h. Bacterial growth was indicated by the presence of turbidity. All tests were carried out in triplicates.

The least concentration of Quercetin that did not permit any visible growth of the inoculated test organisms in broth culture was regarded as the minimum inhibitory concentration in each case.

Result and Observation

43 mg of the crude compound was added with 1000 mcl of DMSO and water respectively. 400 mcl was taken from this mixture and then diluted up to 1000 mcl to get dilution of 400 mcg/ml in both DMSO and water.

10mcl was taken from the compound of dilution 400mcl/ml.

	E. coli	*S. aureus*
DMSO	No zone	No zone
H2O	3.5 cm	3.2
Gentamicin (4mcg)	2.5	2.6

20 mcl was taken from the compound of dilution of 400 mcl/ml.

	E. coli	*S. aureus*
DMSO	No zone	No zone
H2O	3.5 cm	3.3 cm
Gentamicin (4mcg)	2.9 cm	2.7 cm

10 mcl of compound was added with 10 mcl of 4mcl Gentamicin to check for its synergistic activity and following result was obtained.

	E. coli	*S. aureus*
DMSO + Gentamicin	2.4 cm	2.5 cm
H20 + Gentamicin	2.9 cm	2.9 cm

Conclusion and Discussion

For ages, various natural plants and their extracts have been utilized for medical purposes, and their biological and pharmacological impacts have drawn an increasing amount of research in recent years [11]. Polyphenols (flavonoids) are bioactive chemicals whose biological activities are linked to the structure of the molecules; phenolic compounds have the ability to link with proteins and bacterial membranes to form complexes thanks to their hydroxyl groups or phenolic rings [12].

As a result, antibacterial properties of plant extracts from diverse components, such as leaves, seeds, and flowers, have been found in multiple investigations [13]. These findings have consistently shown that crude extract has little antibacterial activity against intestinal microorganisms [14]. However, our findings are consistent with those of earlier investigations using pure flavonoids with antibacterial action. Quercetin also has a higher antibacterial activity than Gentamicin due to its larger inhibitory zone [15]. In crude extract, these compounds demonstrated antibacterial activity. This discrepancy could be explained by the existence of a residue in crude extract, which inhibits phenolic chemicals from coming into direct contact with bacteria [16].

Antibacterial activity is higher in phenolic compounds that lack free hydroxyl groups than in those that do [17]. This improves their chemical affinity for the lipid membrane of microbes. Gentamicin's chemical structure has fewer hydroxyl groups than Quercetin's, which could explain the difference in antibacterial effectiveness between the two drugs in our study [18].

The antibacterial properties of Quercetin can be used in preservation of food. Also, a carefully designed, mechanistic-based laboratory and clinical studies will help in developing functional foods containing flavanols and pro-biotics [19].

The present studies show effectiveness of Quercetin as antibacterial agent on selected organism *S.aureus* and *E. Coli*. This interaction between Quercetin and pathogenic bacteria can be utilized for development of functional foods; the same is evaluated further. Environmental factors and the genetic make-up of the host can modulate the distribution of microbial strains, diet appears to be a major factor in regulating the concentration of individual species of microorganisms that colonize the gut. Recent research has unveiled a potential therapeutic role of indigenous non-pathogenic microorganisms (probiotics) in maintenance of human health and treatment of various gastrointestinal diseases [20]. In addition, the use of plants for medicinal purposes has been, and remains, common practice throughout much of the world. Plants contain several bioactive compounds, including phytosterols, phytoestrogens, polyphenols and polyunsaturated fatty acids [21]. Many of these compounds have been investigated for their anti-inflammatory, antioxidant and/or anti-carcinogenic properties, and have been shown to modulate numerous immunological and cellular functions. It has been well documented that intake of flavanol rich foods can modify the composition of gut microbiota exerting pre-biotic like effect [22]. Unabsorbed dietary phenolics and their metabolites have been shown to exert antimicrobial or bacteriostatic effect. These metabolites selectively inhibit pathogen growth while favoring growth of commensals bacteria and in few cases, its rather found to favor growth of Lactobacillus and Bifidobacterium. It is clear that flavanol and probiotics have beneficial effect.

References

1. Kaushik R, Jain J, Mazumder A. Chromatographic fingerprinting of sarasvata churna-an ayurvedic polyherbal formulation for Epilepsy. Journal of Applied Pharmaceutical Science 2018;8(4):90–8.
2. Ernst E. The efficacy of herbal medicine - An overview. Fundamental and Clinical Pharmacology 2005;19(4):405–9.
3. Saraswathi K, Arumugam P, Sivaraj C. Pharmacological activities of differential parts of selected essential Indian spices. Journal of Pharmacognosy and Phytochemistry 2020;9(2):2024-33.
4. Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of the potential herbal sources of future drugs effective in oxidant-related diseases. Inflammation and Allergy - Drug Targets 2009;8(1):2-10.
5. Hazarika I, Das A. Anticancer and Antioxidant Property of Bunium bulbocastanum Fruits Various Fractions. Research & Reviews: A Journal of Pharmacognosy [Internet] 2016;3(1):9-13. Available from: www.stmjournals.com
6. Fatima Assistant Professor T, Beenish Ph ID, Kashmir S, Bazila Naseer I, Gousia Gani I, Tahiya Qadri I *et al*. Antioxidant potential and health benefits of cumin. ~ 232 ~ Journal of Medicinal Plants Studies 2018;6(2):232–6.
7. Sefidkon F, Gooshegir SA, Bahmanzadegan A, Golipour M, Meshkizadeh S. Chemical Composition of the Essential Oils of Five Iranian Bunium Species (B. lurestanicum, B. microcarpum, B. badghayzi, B. wolffi and B. carioides). Journal of Essential Oil-Bearing Plants 2014;17(1):13-7.
8. Ahmad H, Khan I. Antioxidation and Antiglycation Properties of Bunium Bulbocastanum Fruits Various Fractions and its Possible Role in Reducing Diabetes Complication and Ageing. Vitamins & Minerals 2014,03(01).
9. Vasil MG, Kljujkov EV, Pimenov MG. Plnnt §V. Stemutics nnd Evolution 1985;149(3):177–88.
10. Thippeswamy NB, Naidu KA. Antioxidant potency of cumin varieties-cumin, black cumin and bitter cumin-on antioxidant systems. European Food Research and Technology 2005;220(5–6):472-6.
11. Johri RK. Cuminum cyminum and Carum carvi: An update. Pharmacognosy Reviews 2011;5(9):63-72.
12. Zare D, Bakhshipour A, Chen G. Physical properties of cumin and caraway seeds. International Agrophysics 2013;27(4):491-4.

13. Degtjareva GV, Kljuykov EV, Samigullin TH, Valiejo-Roman CM, Pimenov MG. Molecular appraisal of Bunium and some related arid and subarid geophilic Apiaceae-Apioideae taxa of the Ancient Mediterranean. Botanical Journal of the Linnean Society 2009;160(2):149-70.
14. Tachjian A, Maria V, Jahangir A. Use of Herbal Products and Potential Interactions in Patients With Cardiovascular Diseases. Journal of the American College of Cardiology [Internet] 2010;55(6):515-25. Available from: http://dx.doi.org/10.1016/j.jacc.2009.07.074
15. Tiwari M, Pandey A, Mishra N. Dietetics for Healthy Life-an Ayurvedic Over View. 2013;(November):1044–56. Available from: www.wjpps.com
16. Ali I, Wani WA, Saleem K. Cancer scenario in India with future perspectives. Cancer Therapy 2011;8(IssueA):56-70.
17. Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. Side Effects of Chemotherapy in Cancer Patients and Evaluation of Patients Opinion about Starvation Based Differential Chemotherapy. Journal of Cancer Therapy 2014;05(08):817-22.
18. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: How are they linked? Free Radical Biology and Medicine [Internet] 2010;49(11):1603-16. Available from: http://dx.doi.org/10.1016/j.freeradbiomed.2010.09.006
19. Joshi SR. Diabetes Care in India. Annals of Global Health [Internet] 2015;81(6):830-8. Available from: http://dx.doi.org/10.1016/j.aogh.2016.01.002
20. Chakraborty M, Afrin T, Munshi SK. Microbiological quality and antimicrobial potential of extracts of different spices. Food Research 2020;4(2):375-9.
21. Khan I, Ahmad H, Ali N, Ahmad B, Tanoli H. Screening of Bunium bulbocastanum for antibacterial, antifungal, phytotoxic and haemagglutination activities. Pakistan Journal of Pharmaceutical Sciences 2013;26(4):787-91.
22. Aslam MS, Naveed S, Ahmed A, Abbas Z, Gull I, Athar MA. Side Effects of Chemotherapy in Cancer Patients and Evaluation of Patients Opinion about Starvation Based Differential Chemotherapy. Journal of Cancer Therapy 2014;05(08):817-22.