ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 2, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF CRUCIFEROUS VEGETABLES - CAULIFLOWER, BROCCOLI

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Received: 14 September 2022, Revised and Accepted: 11 November 2022

ABSTRACT

Objectives: This work aims to analyze the antibacterial and antifungal activities of cruciferous vegetables such as cauliflower and broccoli.

Methods: Cruciferous vegetables act as a good source of natural antioxidants due to their high levels of carotenoids, tocopherols, and ascorbic acid. In this study, two cruciferous vegetables, such as cauliflower and broccoli, were selected for antibacterial and anti-fungal studies. The stems, flowers of cauliflower, and broccoli were extracted with 125 mL of ethanol and water by Soxhlet's apparatus for 6 h. Mueller Hinton agar and Sabouraud's dextrose agar medium were used for antibacterial and antifungal activity, respectively. The antibacterial and antifungal activities of each cauliflower, broccoli stem, and flower extract were determined using a modified Kirby–Bauer disk diffusion method. Standard antibiotics, gentamicin (25 µg/mL), and fluconazole (25 µg/mL) served as positive controls for antibacterial and antifungal activity, respectively.

Results: Broccoli stem (100 µg/mL) ethanol extract produced higher antibacterial activity (13 mm) against *Escherichia coli*. Cauliflower, flower (100 µg/mL) ethanol extract produced higher antibacterial activity (13 mm) against *Staphylococcus aureus.* Broccoli flower (100 µg/mL) ethanol extract produced higher antifungal activity (14 mm) against *Candida albicans.* According to the results obtained from this project, broccoli stems and flower ethanol extracts show very good antibacterial activity against Gram-negative microorganisms such as *E. coli* and *Pseudomonas aeruginosa*. Similarly, cauliflower, flower ethanol extract shows excellent antibacterial activity against Gram-positive microorganisms such as *Bacillus subtilis* and *S. aureus.*

Conclusion: Further analysis is recommended for the identification of active constituents responsible for these activities.

Keywords: Cruciferous vegetables, Antibacterial, Antifungal, Agar disk diffusion.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i2.46346. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The popularity and consumption of vegetables from *Brassica* species are increasing because of their nutritional value. *Brassica* crops are used to reduce the risk of chronic diseases, including cancer and cardiovascular diseases. *Brassica* foods provide nutrients and health-promoting phytochemicals such as minerals, vitamins, carotenoids, soluble sugars, fiber, phenolic compounds, and glucosinolates [5]. Brassicaceae family vegetables are important sources of phenolic compounds in the human diet. They also contain derivatives of hydroxycinnamic, caffeic, chlorogenic, and ferulic acids, as well as flavonols (kaempferol derivatives, and quercetin derivatives), and anthocyanins (red cabbage) [6,7].

Cruciferous vegetables such as cauliflower, broccoli, cabbage, mustard, Chinese cabbage, carrot, Chinese kale, and turnip are edible plants that are low in calories, high in fiber, rich in vitamins and minerals, and have physiologic effects on humans [8]. Some important enzymes such as chitinase, glutathione transferase, and epoxide hydrolase are also found in cruciferous vegetables [9,10]. Indole and isothiocyanate, enzymatic products of myrosinase from glucosinolate found in cauliflower and cabbage, lower the incidence of tumor formation and have an antioxidative effect [11].

Broccoli is the richest source of different minerals, vitamins, and fiber. Broccoli contains potent antioxidants that support healthy cells and tissues in our body. Broccoli contains multiple active constituents that are responsible for an anti-inflammatory effect in animals. Multiple studies have shown that broccoli may have a cancer-preventive effect. Intake of broccoli may decrease blood sugar levels and control diabetes. This is due to its antioxidant and fiber content. Broccoli may help to reduce risk factors for various heart diseases and is used to prevent heart tissue damage. Broccoli contains Vitamin C, which is used to support a healthy immune response.

MATERIALS AND METHODS

Materials

Plant materials

The stems and flowers of cauliflower and broccoli used in this work were purchased in and around Vijayawada.

The common pathogenic microorganisms

The common pathogenic six microorganisms were used in this study, among these, two Gram-negative microorganisms, namely, *Escherichia coli* (National Collection of Industrial Microorganisms [NCIM] 2256), *Pseudomonas aeruginosa* (NCIM 2037), and two Gram-positive microorganisms, namely, *Bacillus subtilis* (NCIM 2710) and *Staphylococcus aureus* (NCIM 2794). All the tested strains were collected from the NCIM. Two other fungal organisms were also used in this study, namely, *Aspergillus niger* (ATCC 6275) and *Candida albicans* (ATCC 2091).

Instruments

The instruments used for this work are the Soxhlet apparatus, incubator (37°C), refrigerator (4–18°C), laminar airflow system, autoclave, hot air oven, precision electronic balance, grinder, micropipette (100–1000 µL), Bunsen burner, matches, and inoculating loop.

Chemicals

The chemicals used for this work are ethanol (research lab fine chemical industrial), dimethyl sulfoxide (DMSO) (research lab fine

chemical industrial), peptone, agar, sodium chloride, beef extract, and Mueller Hinton agar.

Methods

Preparation of plant extraction

Freshly collected plant materials, such as stems, flowers of cauliflower, and broccoli, were thoroughly washed. The plant materials were dried independently in the shade, followed by grinding them into a fine powder. The powdered plant materials of cauliflower and broccoli were stored in airtight jars and refrigerated separately at 4°C (Fig. 1).

15 g of dry powdered cauliflower, broccoli stems and flowers were extracted with 125 mL of ethanol by Soxhlet's apparatus for 6 h (or) till the plant material gets colorless. The leftover solvent was removed using a rotary vacuum evaporator to produce a concentrated extract. The same method was followed for the extraction of cauliflower and broccoli stems and flowers with water too. A concentration (100 µg/mL) of cauliflower and broccoli solvent extracts was prepared with DMSO (Fig. 2).

Screening for antimicrobial activity

Media for bacterial organisms

15.2 g of Mueller Hinton agar was added to 400 mL of sterile distilled water and autoclaved at 121°C for 15 min at 15 lbs, 0.1 g of dextrose was added to 10 mL of sterile distilled water and sterilized for 15 min. After cooling, the contents were mixed and poured into sterile Petri plates up to approximately 4 mm and allowed to be set at ambient temperature and used.

Media for fungal organisms

The media used for the antifungal test were Sabouraud's dextrose agar medium from HiMedia Pvt. Ltd., Bombay, India.

Preparation of inoculum

To prepare bacterial inoculum, the pure culture of the test organism was inoculated into 5 mL of sterile nutrient broth and incubated at 37°C for 2–8 h till moderate turbidity developed. The inoculum was standardized by matching it with the 0.5 McFarland turbidity standard, which corresponds to a cell density of approximately 10^8 CFU/mL.

Fig. 1: Powders of cauliflower, broccoli stem, and flowers

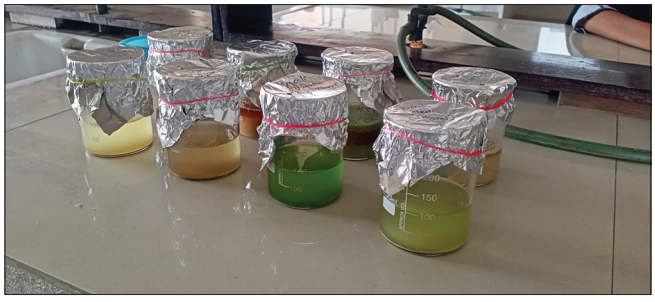

Fig. 2: Extracts of cauliflower, broccoli stem and flowers

Antibacterial and antifungal activity by agar disc diffusion method

The antibacterial and antifungal activities of each cauliflower, broccoli stem, and flower extract were determined using a modified Kirby–Bauer disk diffusion method [12]. Broth cultures of test bacterial and fungal organisms were spread on the Mueller Hinton Agar media and Sabouraud's medium in Petri plates under lab conditions. The extracts were tested using 5 mm sterilized filter paper discs impregnated with 100 µg/mL of ethanol, and water extracts of cauliflower and broccoli. After being allowed to dry for few minutes at room temperature, plates were incubated at 37°C for about 24 h for antibacterial activity and at 22°C for 72 h for antifungal activity.

Then, the zone of inhibition diameter was measured in mm, and the results were recorded. Discs with 7 mm diameter are considered to be with no bacterial activity. Diameter between 7 and 12 mm was considered moderately active and those with more than 12 mm were considered as highly active. For all solvent extractions using standard antibiotics, gentamicin (25 µg/mL) and fluconazole (25 µg/mL) served as a positive control for antibacterial and antifungal activity, respectively.

RESULTS AND DISCUSSION

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts was analyzed against Gram-negative microbes like *E. coli* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of water extracts at concentrations of 100 µg/ml was measured in millimeters and is tabulated below in Table 1.

Similarly, the antibacterial activity of cauliflower, broccoli parts such as flowers and seed extracts were analyzed against Gram-negative microbes like *E. coli* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of ethanol extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 2.

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against Gram-negative microbes like *P. aeruginosa* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of water extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 3.

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against Gram-negative microbes like *P. aeruginosa* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of ethanol extracts at various concentrations of 100 µg/ml was measured in millimeters and is tabulated below in Table 4

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts was analyzed against Gram-positive microbes like *B. subtilis* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of water extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 5.

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against Gram-positive microbes like *B. subtilis* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of ethanol extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 6.

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against Gram-positive microbes like *S. aureus* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of water extracts at various concentrations of 100 µg/ml was measured in millimeters and is tabulated below in Table 7.

Table 1: Antimicrobial activity of cruciferous vegetable water extract against *Escherichia coli*

S. No.	Cauliflower flower (100 µg/mL	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	8±0.2	10±0.4	9±0.3	12±0.8	21±0.7

*Each value was expressed as mean±SEM, where n=3 in each group

Table 2: Antimicrobial activity of cruciferous vegetable ethanol extract against *Escherichia coli*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	10±0.4	12±0.6	12±0.7	13±0.9	21±0.6

*Each value was expressed as mean±SEM, where n=3 in each group

Table 3: Antimicrobial activity of cruciferous vegetable water extract against *Pseudomonas aeruginosa*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	12±0.3	10±0.7	10±0.6	11±0.9	20±0.5

*Each value was expressed as mean±SEM, where n=3 in each group

Table 4: Antimicrobial activity of cruciferous vegetable ethanol extract against *Pseudomonas aeruginosa*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	10±0.4	9±0.7	13±0.8	12±0.6	20±0.6

*Each value was expressed as mean±SEM, where n=3 in each group

Table 5: Antimicrobial activity of cruciferous vegetable water extract against *Bacillus subtilis*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Std (gentamicin 25 µg/mL)
Zone of inhibition (mm)	9±0.2	8±0.7	9±0.6	10±0.5	22±0.3

*Each value was expressed as mean±SEM, where n=3 in each group

Table 6: Antimicrobial activity of cruciferous vegetable ethanol extract against *Bacillus subtilis*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Std (gentamicin 25 µg/mL)
Zone of inhibition (mm)	12±0.9	9±0.6	9±0.3	10±0.2	22±0.9

*Each value was expressed as mean±SEM, where n=3 in each group

Antibacterial activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against Gram-positive microbes like *S. aureus* by the disk diffusion method using gentamicin as a standard drug. The zone of inhibition of the ethanol extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 8.

Antifungal activity of cauliflower, broccoli parts such as flowers, and seeds extracts were analyzed against fungal microbes like *C. albicans* by the disk diffusion method using fluconazole as a standard drug. The zone of inhibition of water extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 9.

Antifungal activity of cauliflower, broccoli parts such as flowers, and seed extracts were analyzed against fungal microbes like *C. albicans* by the disk diffusion method using fluconazole as a standard drug. The zone of inhibition of ethanol extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 10.

Antifungal activity of cauliflower, broccoli parts such as flowers and seed extracts were analyzed against fungal microbes like *A. niger* by the disk diffusion method using fluconazole as a standard drug. The zone of inhibition of water extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 11.

Antifungal activity of cauliflower, broccoli parts such as flowers and seed extracts were analyzed against fungal microbes like *A. niger* by the disk diffusion method using fluconazole as a standard drug. The zone of inhibition of ethanol extracts at various concentrations of 100 µg/mL was measured in millimeters and is tabulated below in Table 12.

Broccoli stem (100 µg/mL) ethanol extract produced higher antibacterial activity (13 mm) against *E. coli*. Cauliflower, flower (100 µg/mL) water extract produced higher antibacterial activity (12 mm) against *P. aeruginosa*, Broccoli flower (100 µg/mL) ethanol extract produced higher antibacterial activity (13 mm) against *P. aeruginosa.*

Broccoli stem (100 µg/mL) water extract produced higher antibacterial activity (10 mm) against *B. subtilis*, and cauliflower, flower (100 µg/mL) ethanol extract produced higher antibacterial activity (12 mm) against *B. subtilis*. Broccoli flower (100 µg/mL) water extract produced higher antibacterial activity (10 mm) against *S. aureus.* Cauliflower, flower (100 µg/mL) ethanol extract produced higher antibacterial activity (13 mm) against *S. aureus*.

Table 7: Antimicrobial activity of cruciferous vegetable water extract against *Staphylococcus aureus*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	7±0.7	9±0.6	10±0.3	8±0.6	19±0.5

*Each value was expressed as mean±SEM, where n=3 in each group

Table 8: Antimicrobial activity of cruciferous vegetable ethanol extract against *Staphylococcus aureus*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard (gentamicin 25 µg/mL)
Zone of inhibition (mm)	13±0.3	12±0.9	9±0.2	10±0.7	19±0.6

*Each value was expressed as mean±SEM, where n=3 in each group

Table 9: Antimicrobial activity of cruciferous vegetable water extract against *Candida albicans*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard fluconazole 25 (µg/mL)
Zone of inhibition (mm)	9±0.4	10±0.7	8±0.5	10±0.4	20±0.6

*Each value was expressed as mean±SEM, where n=3 in each group

Table 10: Antimicrobial activity of cruciferous vegetable ethanol extract against *Candida albicans*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard fluconazole 25 (µg/mL)
Zone of inhibition (mm)	10±0.3	12±0.8	14±0.7	9±0.6	20±0.4

*Each value was expressed as mean±SEM, where n=3 in each group

Table 11: Antimicrobial activity of cruciferous vegetable water extract against *Aspergillus niger*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard fluconazole 25 (µg/mL)
Zone of inhibition (mm)	8±0.7	10±0.4	11±0.5	7±0.4	21±0.3

*Each value was expressed as mean±SEM, where n=3 in each group

Table 12: Antimicrobial activity of cruciferous vegetable ethanol extract against *Aspergillus niger*

S. No.	Cauliflower flower (100 µg/mL)	Cauliflower stem (100 µg/mL)	Broccoli flower (100 µg/mL)	Broccoli stem (100 µg/mL)	Standard fluconazole 25 (µg/mL)
Zone of inhibition (mm)	12±0.5	9±0.3	8±0.4	13±0.4	21±0.7

*Each value was expressed as mean±SEM, where n=3 in each group

Cauliflower and broccoli stem (100 µg/mL) water extracts produced higher antifungal activity (10 mm) against *C. albicans*, and broccoli flower (100 µg/mL) ethanol extract produced higher antifungal activity (14 mm) against *C. albicans*. Broccoli flower (100 µg/mL) water extract produced higher antifungal activity (11 mm) against *A. niger*, broccoli stem (100 µg/mL) ethanol extract produced higher antifungal activity (13 mm) against *A. niger*.

CONCLUSION

According to results obtained from this project, it is concluded that broccoli stems and flower ethanol extracts show very good antibacterial activity against Gram-negative microorganisms such as *E. coli* and *P. aeruginosa*. Similarly, cauliflower, flower ethanol extract shows excellent antibacterial activity against Gram-positive microorganisms such as *B. subtilis* and, *S. aureus.* Broccoli stem and broccoli flower ethanol extracts produced higher antifungal activity against *C. albicans* and *A. niger,* respectively.

ACKNOWLEDGMENT

The authors were thankful to the Department of Pharmacology and Microbiology, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Vijayawada, Krishna (Dist.), A.P., India for their kind encouragement and support.

AUTHOR'S CONTRIBUTIONS

The authors have contributed to the manuscript article preparation and editing.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR'S FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

REFERENCES

1. Sofowora A. Medicinal Plants and Traditional in Africa. New York: John Willy; 1982. p. 289-90.
2. Westh H, Zinn CS, Rosdahl VT. An International multicenter study of Antimicrobial consumption and resistance in *Staphylococcus aureus* isolated from 15 hospitals in 14 countries. Microbe Drug Resist

2004;10:169-76. doi: org/10.1089/1076629041310019
3. Hill AF. Economic Botany. A Textbook of Useful Plants and Plant Products 2nd ed. New York: Mcgraw-Hill Book Company Inc.; 1952.
4. Mithen R. Glucosinolates-biochemistry, genetics, and biological activity. Plant Growth Regul 2001;34:91-103. doi: org/10.1023/A:1013330819778
5. Jahangir M, Kim HK, Choi YH, Verpoorte R. Health affecting compounds in *Brassicaceae*. Compr Rev Food Sci Food Saf 2009;8:31-43. doi: org/10.1111/j.1467-789X.2010.00790.x
6. Vallejo F, Tomas-Barberan FA, Garcıa-Viguera C. Phenolic compound contents in edible parts of broccoli inflorescences after domestic cooking. J Sci Food Agric 2003;83:1511-6. doi: org/10.1002/jsfa.1183
7. Heimler D, Vignolini P, Dini MG, Vincieri FF, Romani A. Antiradical activity and polyphenol composition of local *Brassicaceae* edible varieties. Food Chem 2006;99:464-9. doi: org/10.1016/j.foodchem.2005.07.057
8. Goel U, Kawatra BL, Bajaj S. Nutrition evaluation of a cauliflower leaf protein. J Sci Food Agric 1997;28:786-90. doi: org/10.1002/jsfa.2740280903
9. Chang CT, Hseueh YL, Shung HY. Purification and properties of Chitinase from cabbage stems with roots. Biochem Mol Biol Int 1996;40:417-25. doi: org/10.1080/15216549600201922
10. Fahey JW, Zhang Y, Talalay P. Broccoli sprouts: An exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. Proc Natl Acad Sci U S A 1997;94:10367-72. doi: org/10.1073/pnas.94.19.10367
11. Nestle M. Broccoli sprouts in cancer prevention. Nutr Rev 1998;56:127-30. doi: org/10.1111/j.1753-4887.1998.tb01725.x
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized disk method. Am J Clin Pathol 1966;45:493-6.