Original Article

In-Vitro Assessment of Antibacterial Activity of Methanol Extract of Brassica Oleracae against Selected Bacterias

Tahira Zamir, Riffat Farooqui, Muhammad Ali Rajput, Khalid Mustafa

ABSTRACT

OBJECTIVE: To evaluate in- vitro antibacterial activity of methanol extract of Brassica Oleracae study against selected bacterias.

DESIGN: The study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi, Pakistan.

METHOD: The agar diffusion method was used to measure zones of inhibition in millimeter (mm) against organisms Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus, Escherichia coli and Proteus. Values of growth inhibitory zones are expressed as mean ± SD (standard deviation) of three triplicates i.e n=3.

RESULTS: The methanol extract of Brassica oleracae exhibited distinct zones of inhibition at all the concentrations i.e 10mg/ml, 20mg/ml, 40mg/ml, and 100mg/ml towards all bacterial strains under study i.e staphylococcus aureus, staphylococcus epidermidis, streptococcus, escherichia coli and proteus against the methanol control which did not show any growth inhibitory zone. Methanol extract of Brassica oleracae showed widest inhibitory zone toward E.coli (16.98±0.00) followed by proteus (13.10±0.55), streptococcus (12.00±0.00), staph epidermidis (11.10±1.3) and staph aureus (11.02±0.67) at the concentration of 100mg/ml of extract, in comparison to methanol control which did not manifest any growth inhibitory zone (0.00). The diameters of zones of inhibition were found to increase in size with increase in concentration of extract. However, the antibacterial activity against streptococci was almost same at 20mg/ml and 40mg/ml of concentration of extract (9.33±0.17 and 9.10±0.00 respectively). Results were also compared with the standard antibacterial agent against chloramphenicol at a concentration of 100 ug /ml.

CONCLUSION: These results suggest that of Brassica Oleracae may be use as anti-bacterial agent, thus providing ailing mankind a wonderful gift from nature.

KEY WORDS: Brassica Oleracae, Chloramphenicol , antibacterial activity, bacterial strains.

INTRODUCTION

Infectious diseases are still a major health issue, especially in developing countries, leading to the death of millions of people, despite enormous improvements in healthcare systems.[1] This is primarily due to acquired bacterial resistance to antibiotics.[2] These organisms trigger life threatening ailments and even may contribute to death.[3] Many bacterias have revealed resistance to synthetic antimicrobial drugs e.g resistance to penicillin by staphylococcus aureus.[4] Numerous mechanisms of resistance are identified in bacterias, but the process of active efflux performs the primary role.[5]

Attention is now being switch over to plants as they may present a new source of antibacterial, antifungal and antiviral activities.[6] Traditional use of various plants e.g, Delonix elata, Merremia tridentata, Mollugo cerviana, Enicostemma axillare and Solanum incanum is reported in the literature for the treatment of various ailments. Later on the extracts of these plants were found to have phytochemicals and antibacterial activity. The antimicrobial activity of plants may be due to their ability to synthesize several secondary metabolites of relatively complex structures having antimicrobial properties.[7] Compounds derived from natural products can lead to the discovery of novel drugs.[8] This necessitates to investigate biological activities of plants.[9]

Brassica oleracae var capitata (local name cabbage), member of cruciferous family, is a species of Brassica native to Coastal Southern and Western Europe. It is naturally confined to limestone sea cliffs. The chemical composition of Brassica Oleracae is similar to other Brassica vegetables.[10]

Many activities of Brassica Oleraae are established in literature e.g, anticancer, antioxidant, antiplatelet and antihyperlipidemic activities.[11,12] Compounds responsi-

ble for these activities of brassica oleracae include isothiocyanates, glucosinolates, and phenolics including flavonoids.[13] Glucosinolates have been reported to possess antimicrobial activity.[14] Fresh cabbage contain 300-1070 ug/g of total glucosinolate compounds which on hydrolysis produce isothiocyanates, nitriles and thiocyanates.

These evidences showed that Brassica Olerace may prove to be a promising antibacterial agent, and further exploration should be performed to determine its full therapeutic potential. In addition, its extract can also be used as a lead molecule in combating the diseases caused by the studied organisms. Hence keeping in mind the emerging resistance of pathogens to the available antimicrobial drugs and need of newer and safer agents from plant sources, an in-vitro study was designed to investigate the antibacterial activity of methanol extract of Brassica Oleracae against some selected bacterias.[15]

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, Faculty of Pharmacy, University of Karachi after getting approval from Board of Advance Study and Research (BASR) of the University.

1.2 Plant material and preparation of extract

Fresh cabbages were purchased from local market of Karachi and were identified by Prof. Dr. Anjum Parveen, Director Centre for Plant Conservation Herbarium and Botanic Garden, University of Karachi, Karachi-75270. The voucher specimen (H.No.BO-09-12) was deposited in the Department of Pharmacognosy, University of Karachi. The crude extract was prepared through cold extraction process.[16]After thorough washing, 5 kg of cabbage leaves were chopped into small pieces and dried under shade for about a week. The dried material was ground to coarse powder. This powder was soaked in 80% methanol for 10 days with occasional shaking and stirring. The solvent was filtered through cotton and then through filter paper (What-mann No.1). After filtration, the methanol extract was evaporated under reduced pressure in a rotary evaporator at 40℃ - 45℃ and then followed by freeze drying at -30℃, the extract was kept at -20 ℃ until further use. The resultant yield of extract was 19.3% of dry weight. The extract so obtained was then used for testing in-vitro antibacterial activity.

1.2 Test organisms

The bacterial strains used for evaluation of antibacterial activity were staph aureus, staph epidermidis, streptococcus, E.coli and proteus. Pure bacterial cultures were obtained from the Department of Microbiology, University of Karachi, Pakistan. The culture of organisms was sustained on stock culture agar. A loop of culture was inoculated in nutrient broth from the stock culture.

1.3 Inocula Preparation

Inocula were prepared by diluting twenty-four hours old cultures in normal saline (Nacl, 0.85% w/v) under aseptic condition. A dilution of 1:100 was used in all the tests. Bacterias were dislocated from their particular pure cultures by the help of flamed sterile wire loop.

1.4 Screening antibacterial activity by Agar well diffusion method

The antibacterial activity of methanol extract of Brassica oleracae was evaluated by agar well diffusion method.[17] Modified Soy agar Petri plates were prepared and 0.1 ml of diluted culture was poured on each plate. The plates were dried for thirty minutes at 37℃ for proper hardening of agar to make possible the homogeneous digging of wells. Wells of 7mm diameter were cut with the help of sterile cork borer in the inoculated agar. Different concentrations of the plant extract i.e 10mg/ml, 20mg/ml, 40mg/ml, 100mg/ml were filled in the wells. Methanol (1 ml) was used as control in one of the well and Chloramphenicol as standard drug was also filled in another one at a concentration of 100ug/ml. These plates were allowed to stand at room temperature for at least one hour for the even diffusion of poured components. The plates were incubated for twenty-four hours at 37℃. At the end of incubation period, the inhibition zones formed around the wells were measured in millimeters (mm) by using scale. Results were expressed as the net zone of inhibition which represented the subtraction of the diameter of well (7mm) from the measured zone of inhibition. Culture bacterias with halos equal to or greater than 7mm were supposed to have tendency to test extract for antibacterial activity.[18]

1.5 Statistical Analysis

Results obtained were analyzed on SPSS version 16. All the experiments were performed in triplicates with two independent trials and the results obtained were highly reproducible. Values of growth inhibitory zones expressed in mean$\pm$SD (standard deviation) of three triplicates i.e. n=3. Significance of differences compared to the control group was determined using student t test.

RESULTS

Zone inhibition assay results given in Table I revealed that the methanol extract of Brassica Oleracae exhibited distinct zones of inhibition at all the concentrations i.e 10mg/ml, 20mg/ml, 40mg/ml, and 100mg/ml towards all bacterial strains under study i.e staph aureus, staph epidermidis, streptococcus, Escherichia coli, and proteus, against the methanol control which did not manifest any growth inhibitory zone. Methanol extract of Brassica Oleracae showed widest inhibitory zone (shown by the diameter of zone of inhibition) toward E.coli (16.98±0.00) followed by proteus (13.10±0.55), streptococcus (12.00±0.00), staph epidermidis (11.10±1.3) and staph aureus (11.02±0.67) at the concentration of 100mg/ml of extract, in comparison to the methanol control which did not manifest any growth inhibitory zone (0.00). The diameters of growth inhibitory zones varied with the type of microbial strain involved in the study. The diameters of zones of inhibition (in mm) were found to increase in size as the concentration of extract was increased (in mg/ml) as shown in Table I. However, the antibacterial activity of brassica olerace extract against streptococci was almost same at 20mg/ml and 40mg/ml of concentration of Brassica Oleracae (9.33±0.17 and 9.10±0.00) respectively as shown in Table I. Results were also compared with the standard antibacterial agent Chloramphenicol at a concentration of 100 ug/ml.

TABLE I: EFFECT OF METHANOL EXTRACT OF BRASSICA OLERACAE ON ZONES OF INHIBITION (MM) AND COMPARISON WITH METHANOL (CONTROL) AND STANDARD DRUG

Bacterial Strain	Different concentrations of Methanol extract of Brassica Olerceae (mg/ml) and diameter of zones of inhibition (mm)				Standard Drug (ug/ml)	Methanol (Control)
	10mg/ ml	20mg/ ml	40 mg / ml	100mg/ ml	100 ug/ml	1ml
Staph aureus	5.00±0.00	8.10±0.00	9.00±0.15	11.02±0.67	15.33±1.4	0.00
Staphepidermidis	6.00±0.20	8.20±0.33	9.00±0.15	11.10±1.3	13.20±0.00	0.00
Streptococcus	5.03±0.12	9.33±0.17	9.10±0.00	12.00±0.00	11.00±0.78	0.00
E.coli	7.10±0.11	9.11±0.00	15.00±0.13	16.98±0.00	17.20±0.67	0.00
Proteus	7.13±0.00	8.00±0.44	12.00±0.67	13.10±0.55	13.00±0.00	0.00

Values of growth inhibitory zones are mean±SD (standard deviation) of three triplicates i.e. n=3. (Significance of differences compared to the control groups were determined using student t test)

DISCUSSION

Globally, there is widespread use of plants as ethnomedicines. After the pathogen attack, some plants may produce certain compounds such as secondary metabolites being known as phytoalexins. These compounds have shown remarkable in vitro antibacterial activity against potential pathogens.[19] There is immense need of antimirobial drugs developed from natural sources as plant derived products are safe in comparison to chemically synthesized products.[20] Some of the species of Brassica family like brassica nigram have revealed antibacterial potential against some food borne pathogens.[21] Furthermore, the leaf juice of brassica oleracae is also identified to have antibacterial activity against some food borne pathogens .[22]

Taking into account, the considerable potentiality of plants as a source of antimicrobial drugs, this study was carried out to explore the antibacterial activity of methanol extract of brassica oleracae against selected bacterias. Brassica olerace is a commonly consumed vegetable and a valuable source of glucosinolates, polyphenols and flavonoids. On hydrolysis by enzyme myrosinase, glucosinolates can produce d-glucose, sulfate, isothiocyanates, thiocyanates and nitriles.[23] Isothiocyanates have the largest bactericidal, bacteriostatic and antifungal potential among these bioactive products.[24] The medicinal properties of cruciferous vegetables can be related to the activity of isothiocyanates against several human pathogens.[25]

The results of this study have shown that the methanol extract of Brassica Olerace have displayed distinct antibacterial activity against E.coli, proteus, streptococcus, staph epidermidis and staph aureus, at various concentrations. This is mainly ascribed to the isothiocyanates compounds present in Brassica Ol-

eracae. This recommends the use of Brassica Oleracae as broad spectrum antibacterial agent to treat obstinate infections.

CONCLUSION

Results of present study indicated that methanol extract of Brassica Olerace effectively arrested the growth of selected bacterias. Further studies must be undertaken to assess the mode of action.

REFERENCES

1. York, H. de Wet, and S. F. Van Vuuren, "Plants used for treating respiratory infections in rural Maputaland, KwaZulu-Natal, South Africa," Journal of Ethnopharmacology, vol. 135, no. 3, pp. 696–710, 2011.s
2. Chopra I: New drugs for superbugs. Microbiology Today 2000; 47:4-6.
3. Daszak P,Cunningham AC, Hyatt AD. Emerging infectious diseases of wild life, threats to biodiversity and human health. Science. 2000:287:443-49.
4. Chain E, Abraham EP. An enzyme from bacteria able to destroy penicillin. Nature: 1940;146:837-42.
5. Pages J-M, Lavigne J-P, Leflon-Guibout V, Marcon E, Bert F, Noussair L, Nicolas-Chanoine M-H: Efflux pump, the masked side of ß-Lactam resistance in Klebsiella Pneumonia clinical isolates. PLoS ONE: 2009, 4:e 4817.
6. Maiyo ZC, Ngure RM, Matasyoh JC, Chepkorir R: Phytochemical constituents and antimicrobial activity of leaf extracts of three Amaranthus plant species. Afr J Biotechnol :2010, 9:3178-3182.
7. PS Pavithra, VS Janani, KH Charumathi, R Indumathy, Sirisha Potala, Rama S Verma. Antibacterial activity of plants used in Indian herbal medicine, international j of Green pharm,2010.,4:22-8.
8. Neroman, B.D,O Sala, and B.P Wilcox. Conference promotes study of echohydrops of Semiarid Landscapes.EOS. Trans, AGO: 2003, 84:13,17.
9. Bramwell D. How many plant species are there? plant talk:2002 28;32-34.
10. Gross D: Indole Phytoalexins from Brassica oleracea var. gongylodes. Planta Medica, 1994; 59: A-618.
11. Ang-Lee MK, Moss J, Yuan CS. Herbal medicines and perioperative care. JAMA,:2001, 286, 208-216.
12. Muhammad Anwar Waqar and Yasir Mahmood. Antiplatelet, Antihypercholesterolemic and Antioxidant activities of Brassica olerace in high fat diet provided Rats.World applied Sciences journal: 2010, 8(1):107-112.
13. Jeffery EH, Araya M. Physiological effects of broccoli consumption. Phytochemistry Reviews: 2009,8(1) 283-298.
14. Zsolnai,V.T. Die antimicrobielle wirkung von. Thiocyanaten and isothiocyanaten.1. Mitteilung, Arzneim, Forsch: 1966,16;8 70-876.
15. Cecil H, Van Etten, Melvin E, Daxenbichler,Paul H,Williams F,Kwolek. Glucosinolates and derived products in cruciferous vegetables.Analysis of the edible part from twenty two varieties ofcabbage.J.Agric.Food.Chem.,1976,24(3),pp 452-455.
16. Hossain MS, Ahmed M, Islam A. Hypolipidemic and hepatoprotective effects of different fractions of ethanolic extract of immature leaves of Mangifera indica (Linn.) in alloxan induced diabetic rats. IJPSR :2010, 1, 132-138.
17. CU Iroegbu., NKere. Evaluation of the antibacterial properties of the Picralima nitida stembark extracts Int.J.Mol.Med.Adv.Sci.:2005,1,182-189.
18. Brantner,A.Y. and Grein ,E. Antibacterial activity of plant extract used externally in traditional medicine.J.Ethnopharmacol:1994, 44:35-40
19. Chong, Mansfield, Bennet and J.F. Rossiter .Detection of antifungal compounds in Arabidopsis thaliana by Brassica oleracae by thin layer chromatography, ASEAN J.Sci and Tech for Development: 2006,123-131.
20. Singh D and Agrawal SK, Himachalol and β- himachalene insecticidal principles of Himalayan cedar wood oil J Chem Ecol. 1988 Apr;14 (4):1145-51.
21. Rameez F Indikar, Jyoti D Desale. Antimicrobial activity of oils of Brassica nigra and its formulation as an ointment , international journal of pharma research and development:2009 ISSN 0974-9446.
22. Brandi G, Amagliani G, Schiavano GF, De Santi M, Sisti M. Activity of Brassica oleracea leaf juice on foodborne pathogenic bacteria . J Food Prot: 2006, 69(9):2274-9.
23. Fahey J.W, Zalcamann A.T.,Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants, phyto-

chemistry: 2001 , 56 (1):5-51.

24. Sultana Tamanna,Savage G.P,MC Neil, D.C,Porter ,N.G. The yield of isothiocyantes in wasabi rhizomes stored at different temperatures, Science and Technology ,Food agriculture and environment,:2003, 1,39-45.
25. Smolinska U, Morra Mj, Knudsen GR and James RL. Isothiocyanates produced by species as inhibitors of Fusarium oxysporum, Plant Disease: 2003, 87,407-412.

AUTHOR AFFILIATION:

Dr. Tahira Zamir
Bahria University Medical and Dental College
Karachi, Sindh-Pakistan.

Dr. Riffat Farooqui
Bahria University Medical and Dental College
Karachi, Sindh-Pakistan.

Dr. Muhammad Ali Rajput *(Corresponding Author)*
Liaquat University of Medical & Health Sciences
Jamshoro, Sindh-Pakistan.
Email: ali_rajput2002@hotmail.com

Dr. Khalid Mustafa
Bahria University Medical and Dental College
Karachi, Sindh-Pakistan.