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Research Article

INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF THREE INDIAN SPECIES OF *BRASSICA* FROM VIDISHA DISTRICT

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ABSTRACT

The antimicrobial activity of several extracts of the seeds of *Brassica alba* L., *Brassica campestris* L. and *Brassica juncea* L. was investigated using disk diffusion method against seven reference microorganisms (five bacterial and two fungal strains). The methanolic extracts of all the three species were showed the maximum inhibitory whereas the ethyl acetate, chloroform and petroleum ether extracts of *B. alba*, *B. campestris* and *B. juncea* respectively exhibited a good antibacterial activity against four bacterial strains i. e. *S. aureus*, *B. cereus*, *P. aeruginosa* and *S. epidermidis* with the diameters of growth inhibition area in the range of 05 – 25 mm. Neither the n–hexane and benzene extracts nor the aqueous extracts of all the three species showed any antimicrobial activity against the tested microorganisms. No antifungal activity was seen with any of the extracts. The results of this study support the use of these species in Indian traditional medicine to treat skin infections.

Key Words: Antimicrobial activity, *Brassica* species, Bacterial strains, Fungal strains.

INTRODUCTION

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of new anti-infective agents (Agrawal *et al*., 2013; Saxena *et al*., 2005; Srinivasan *et al*., 2001; Perumal Samy *et al*., 1999; Sokmen *et al*., 1999; Perumal Samy *et al*., 1998). During the last few years, due to the increasing development of drug resistance to human pathogenic organisms as well as the appearance of undesirable side effects of certain antibiotics (Omololu-Aso *et al*., 2011; Poole, 2001; Mulligen *et al*., 1993; Piddock and Wise, 1989), antimicrobial properties have been reported more frequently in a wide range of plant extracts and natural products in an attempt to discover new chemical classes of antibiotics that could resolve these problems.

Brassica is a well-known genus in herbal medicine due to the therapeutic efficacy of its different species. *Brassica* is a genus of plants in the mustard family (Brassicaceae). The members of the genus are collectively known as cruciferous vegetables, cabbages or mustards. The genus is remarkable for containing more important agricultural and horticultural crops than any other genus. Most are annual or biennial, but some are small shrubs. Due to their agricultural importance, *Brassica* plants have been the subject of much scientific interest. *B. alba* L., *B. campestris* L. and *B. juncea* L. are most of the three species of this genus which have been used in herbal medicine externally for the treatment of various diseases. *Brassica* vegetables are highly regarded for their nutritional value. They provide high amounts of vitamin C and soluble fiber and contain multiple nutrients with potent anticancer properties. Boiling reduces the level of anticancer compounds, but steaming, microwaving, and stir-frying do not result in significant loss. Steaming the vegetable for three to four minutes is recommended to maximize sulforaphane. *Brassica* vegetables are also a good source of carotenoids, with broccoli having especially high levels. *Brassica* vegetables are a potent modulator of the innate immune response system with potent antiviral, antibacterial and anticancer activity; however, it also is an antiandrogen (Jain *et al*., 2011; Omar *et al*., 2009; Le *et al*., 2003).

On the basis of these results and because of the popular use of the different species of *Brassica* as an antibacterial and anticancer agent, the present work was undertaken to evaluate in vitro antimicrobial activity of different extracts of some *Brassica* species from the Vidisha district, Madhya Pradesh, India i. e. *Brassica alba* L., *Brassica campestris* L. and *B. juncea* L.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant seeds of *B. alba* L., *B. campestris* L. and *B. juncea* L. were collected randomly from the fields of Vidisha, M. P., India. The taxonomic identities of these plants were confirmed by relevant data and the voucher specimens of the plant seeds were preserved. Fresh plant material was washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles until extraction.

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Extraction of Plant Material

Organic extraction

10 g of air-dried powder was taken in 100 ml of different organic solvent i. e. n-hexane, benzene, chloroform, petroleum ether, ethyl acetate and methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours, the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Harborne 1984) and stored at 4 ºC in airtight bottles.

Aqueous extraction

10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through eight layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121 ºC and at 15 lbs pressure and stored at 4 ºC in airtight bottles (Harborne 1984).

Preparation of antibiotic discs

All the crude extracts each 10 mg, were dissolved in 1 ml of dimethyle sulphooxide (DMSO). These concentrations were filtered by using membrane (pore size 0.47 µm) and the discs of 4.5 mm diameter (Sterile blank, Whatman filter paper No. 1) were impregnated into the final concentration of the each extracts i. e. 10 mg ml^{-1}. The final impregnated discs used for the sensitivity test were 10 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 – 24 hours.

Microbial Strains

For the in vitro antimicrobial activity, microorganisms were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. Amongst seven microorganisms investigated, five bacterial strains *Staphylococcus aureus* NCIM 2079, *Bacillus cereus* NCIM 2459, *Escherichia coli* NCIM 2065, *Pseudomonas aeruginosa* NCIM 2200 and *Staphylococcus epidermidis* NCIM 2493 and two fungal strains *Aspergillus flavus* NCIM 535 and *Aspergillus fumigates* NCIM 902 were selected. All the microorganisms were maintained at 4 ºC on agar slants.

Media Preparation and Antibacterial Activity

The antimicrobial assay was performed by agar disc diffusion method (Bauer *et al*., 1966). The molten Mueller Hinton agar (Hi-media) for bacteria and Sabouraud Dextrose agar (Hi-media) for fungal strains were poured into the petri plate. For the sensitivity test, sterile impregnated discs (10 mg $disc^{-1}$) were placed on the petri plates. Discs of chloramphenicol (10 µg $disc^{-1}$, HiMedia), erythromycin (10 µg $disc^{-1}$, HiMedia), vancomycin (10 µg $disc^{-1}$, HiMedia) for bacteria and amphotericin-B (50 µg $disc^{-1}$, HiMedia), nystatin (50 µg $disc^{-1}$, HiMedia) were used for fungus, as a comparative and positive control and blank disc impregnated with DMSO were used as a negative control. All the bacterial test plates were incubated at 37˚C for 24 hours and the fungal test plates were incubated at 48˚C for 48 hours. Microbial growth was determined by measuring the diameter of zone of inhibition. The experiment was done at triplicate and the mean values are presented.

RESULTS

The results of the antimicrobial activity by the disc diffusion method of *B. alba*, *B. campestris* and *B. juncea* organic and aqueous extracts are presented in Table toward five bacterial and two fungal strains. The most significant activity was observed with the methanol extracts of all the species studied against *S. aureus*, *B. cereus*, *P. aeruginosa* and *S. epidermidis*. The diameters of growth inhibition area of extracts studied were in the range of 05 – 25 mm. No activity was seen against *E. coli*, *A. flavus* and *A. fumigates*. The ethyl acetate extracts of *B. alba* showed the good antimicrobial activity against *S. aureus*, *B. cereus* and *S. epidermidis.* Similarly, the petroleum ether extracts of *B. compestris* showed the good antimicrobial activity against *S. aureus* and *S. epidermidis* whereas the chloroform extracts of *B. juncea* exhibited a good antibacterial activity against *S. aureus*, *B. cereus* and *S. epidermidis*. Neither the n–hexane and benzene extracts nor the aqueous extracts of all the three species showed any antimicrobial activity against the tested microorganisms. No antifungal activity was seen with any of the extracts. DMSO showed no activity against any of the strain tested, whereas chloramphenicol, erythromycin, vancomycin, amphotericin-B and nystatin showed the activity of all the tested strains (Table 1).

DISCUSSION AND CONCLUSIONS

The antimicrobial activity of the total extracts of three *Brassica* species from the Vidisha district were studies by the disc diffusion method against seven microorganisms. Our results show a remarkable antimicrobial activity of the ethanol extract of all the *Brassica* species as well as the ethyl acetate, methanol and chloroform extracts of *B. alba*, *B. compestris* and *B. juncea* respectively. Among active extracts, it should be pointed out that the inhibition zones obtained in the disc diffusion method with the ethanolic extract from *Brassica* species against *S. aureus*, *B. cereus*, *P. aeruginosa* and *S. epidermidis* are comparable to those shown by chloramphenicol and gentamycin. This effect, however, was observed in the extract at a dose about 1000 times higher than that of the control antibiotics, but these results are interest as we are dealing with an extract and not a pure product. Moreover, from the results obtained it seems that the antimicrobial action of the extracts is more pronounced on bacterial strains than on fungal strains and these findings correlate with the observations of previous screenings of medicinal plants for antimicrobial activity (Shrivastava and Bhargava, 2012; Paul *et al*., 2012; Jain *et al*., 2011; Yasmin *et al*., 2009).

The potential for developing antimicrobials from higher plants appears rewarding, as it will lead to the development of a phyto medicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential (Agrawal *et al*., 2012a; Agrawal *et al*., 2012b; Agrawal *et al*., 2007; Chopra *et al*., 1992) as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of

the antibacterial compounds from within these plants and to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of *Brassica* species forms a primary platform for further phytochemical and pharmacological studies.

In conclusion, *Brassica* species extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial diseases. These promissory extracts open the possibility of finding new clinically effective antibacterial compounds. The data summarized above indicate that the three *Brassica* species under study showed good antimicrobial activity, specially *S. aureus*, *B. cereus*, *P. aeruginosa* and *S. epidermidis*, which may justify the use of these species in traditional medicine for treating skin diseases and infections and underline the selection of plants in the discovery of new bioactive substances. Further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these medicinal plants.

Table Antimicrobial activity of *Brassica* organic and aqueous extracts by the disc diffusion method

Plant Name	Solvent Name	Zone of Inhibition in mm						
		[a] 1	2	3	4	5	6	7
Brassica alba	n-Hexane	[b] --	--	--	--	--	--	--
	Benzene	--	--	--	--	--	--	--
	Chloroform	[c] +1	+1	--	+1	+1	--	--
	Petroleum ether	+1	+1	--	+1	+1	--	--
	Ethyl acetate	+2	+2	--	+1	+2	--	--
	Methanol	+3	+2	+2	+3	+3	--	--
	Distilled Water	--	--	--	--	--	--	--
Brassica Campestris	n-Hexane	--	--	--	--	--	--	--
	Benzene	--	--	--	--	--	--	--
	Chloroform	+1	+1	--	+1	+1	--	--
	Petroleum ether	+2	+1	--	+1	+2	--	--
	Ethyl acetate	+1	+1	--	+1	+1	--	--
	Methanol	+3	+3	+3	+3	+3	--	--
	Distilled Water	--	--	--	--	--	--	--
Brassica juncea	n-Hexane	--	--	--	--	--	--	--
	Benzene	--	--	--	--	--	--	--
	Chloroform	+2	+2	--	+1	+2	--	--
	Petroleum ether	+1	+1	--	+1	+1	--	--
	Ethyl acetate	+1	+1	--	+1	+1	--	--
	Methanol	+3	+3	+2	+3	+2	--	--
	Distilled Water	--	--	--	--	--	--	--
Chloramphenicol (10 µg disc^{-1})		+3	+4	+4	+3	+3	[d] NT	NT
Erythromycin (10 µg disc^{-1})		+4	+3	+3	+4	+3	NT	NT
Vancomycin (10 µg disc^{-1})		+4	+3	+3	+3	+4	NT	NT
Amphotericin-B (50 µg disc^{-1})		NT	NT	NT	NT	NT	+3	+3
Nystatin (50 µg disc^{-1})		NT	NT	NT	NT	NT	+3	+3
DMSO Blank Disc		--	--	--	--	--	--	--

[a] 1, *Staphylococcus aureus*: 2, *Bacillus cereus*: 3, *Escherichia coli*: 4, *Pseudomonas aeruginosa*: 5, *Staphylococcus epidermidis*: 6, *Aspergillus flavus*: 7, *Aspergillus fumigates* [b] --: No inhibition; [c] +1 : 05 – 10 mm, +2 : 10 – 15 mm, +3 : 15 – 20 mm, +4 : 20 – 25 mm. [d] NT: Not tested.

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