ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antibacterial efficacy of *Brassica campestris* Root, Stem and Leaves extracts

Milin K. Agrawal[1], Deepa Rathore[2], Surendra Goyal[3], Alok Varma[4], Alka Varma[5]

1. Division of Microbiology, Dept. of Botany, St. Mary's P. G. College, Vidisha, M. P., India.
2. Dept. of Zoology, St. Mary's P. G. College, Vidisha, M. P., India.
3. Dept. of Sericulture, Resham Project Centre, Seronj, Vidisha, M. P., India.
4. Dept. of Zoology, Govt. College, Mandideep, Raisen, M. P., India.
5. Dept. of Botany, Govt. Sarojini Naidu Girls P. G. College, Bhopal, M. P., India.

Manuscript Info

Manuscript History:

Received: 14 June 2013
Final Accepted: 28 June 2013
Published Online: July 2013

Key words:
Antibacterial activity,
Brassica campestris,
Root, Stem,
Leaves extracts,
Bacterial strains.

Abstract

The present investigation an attempt has been made to experimentally test the antibacterial efficacy of a common agricultural plant, *Brassica campestris* using disk diffusion method against five reference bacterial strains. The ethanol extracts of all the plant parts were found to be highly effective whereas the petroleum ether, methanol and ethyl acetate extracts of root, stem and leaves of *B. campestris* respectively exhibited a good antibacterial activity against all bacterial strains i. e. *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus epidermidis* with the diameters of growth inhibition area in the range of 05 – 25 mm. The benzene and chloroform extracts of plant showed least antibacterial activity against the tested microorganisms. The results of this study support the use of these species in Indian traditional medicine to treat skin infections.

Introduction

Natural products called secondary metabolites continue to be the major source of drugs and that to with greater structural diversity than drugs or compounds from standard combinatorial chemistry. *Brassica campestris* L. (syn. *Brassica rapa* L.), commonly known as turnip, turnip rape, fast plants, field mustard, or turnip mustard (Clive, 1997) is a plant widely cultivated as a leaf vegetable, a root vegetable, and an oilseed (but not normally rapeseed oil, from a different *Brassica* species). Almost all parts of some species or other have been developed for food, including the root, stems, leaves, flowers and seeds. *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).

The discovery of modern drugs such as quinine, vincristine, digoxin, emetine, artemisine, taxol etc., from medicinal plants signify the huge potential that still exists for the production of many more novel pharmaceuticals (Plokin, 1988). Thus, there has recently been a resurgence of interest in the development of drugs from plants, especially from those of the developing countries that have a rich heritage of botanical ethno-pharmacopoeia.

In the recent years, the development of resistance of pathogens against antibiotics has become a difficult issue caused by the indiscriminate use of modern antibiotics (Cohen, 1992; Kunin, 1993; Kunin, 1983; Neu, 1992). Therefore, it is important to find out newer, safer and more effective natural or synthetic antibacterial drug molecules. Considering the high cost of the synthetic drugs and their side effects, wide

varieties of natural plants can be considered as a vital source for anti-microbial agents (Geyid *et al.*, 2005). Therefore, the demand for new and effective anti-microbial agents with broad-spectrum of activity from natural sources is increasing day-by-day (Rahman *et al.*, 2008). Hence, the purpose of our present investigation was to evaluate the antibacterial activity of some Bangladeshi indigenous plants for the discovery of potential antibacterial agents that might be used for the management of bacterial infectious diseases.

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 *Brassica campestris* L. from the Vidisha district, Madhya Pradesh, India.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant parts of *B. campestris* L. were collected randomly from the fields of Vidisha, M. P., India. The taxonomic identities of these plants were confirmed by Dr. S. K. Jain, Department of Botany, S. S. L. Jain P. G. College, Vidisha, and the voucher specimen of the plant parts were preserved. Fresh plant parts were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles until extraction.

Extraction of Plant Materials

Organic extraction

10 g of air-dried powder of each part was taken in 100 ml of different organic solvent i. e. benzene, petroleum ether, chloroform, ethyl acetate, methanol and ethanol 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 of each part 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 different concentrations and antibiotic discs

All the crude extracts each 100 mg, were dissolved in 1 ml of dimethyle sulphooxide (DMSO) and prepared the different concentrations. The following doses were prepared to observe antimicrobial activity in vitro 100 mg ml^{-1} and 50 mg ml^{-1} by using the standard formula;

$$\text{X ml of the stock solution} = \frac{\text{Desired concentration X Final volume}}{\text{Concentration of stock solution}}$$

Where,

X ml of the stock solution = Quantity of the stock solution to make the desired concentration.

Desired concentrations = 100 mg ml^{-1} and 50 mg ml^{-1}

Final volume = 1 ml.

Concentration of stock solution = 100 mg ml^{-1}

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. 100 mg ml^{-1} and 50 mg ml^{-1}. The final impregnated discs used for the sensitivity test were 100 mg $disc^{-1}$ and 50 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 – 24 hours.

Bacterial Strains

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

Media Preparation and Antibacterial Activity

The antibacterial assay was performed by agar disc diffusion method (Bauer *et al*, 1966). The molten Mueller Hinton agar (Hi-media) was poured into the petri plate. For the sensitivity test, sterile impregnated discs (100 mg $disc^{-1}$ and 50 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), as a comparative and positive control and blank disc

impregnated with DMSO were used as a negative control. All test plates were incubated at 37˚C for 24 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 antibacterial activity by the disc diffusion method of *B. campestris* organic and aqueous extracts are presented in Table toward five bacterial strains. It was observed that the soluble active principles extracted in varying degree in the solvents used were effective in varying proportions against all bacterial strains. Two different concentrations of the extracts (100 mg ml^{-1} and 50 mg ml^{-1}) were used for testing the antibacterial efficacy. The diameters of growth inhibition area of extracts studied were very high to low in the range of 25 – 05 mm. The most significant activity was observed with the ethanolic extracts of all the parts studied against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *S. epidermidis*. In case of root, petroleum ether extracts being good effective against *S. aureus*, *E. coli* and *S. epidermidis* whereas benzene extract was least effective while in the case of stem, methanol extract being good effective against all the test bacterial strains whereas chloroform extract was least effective. Similarly, in the case of leaves, ZOI was observed with ethyl acetate extracts being good effective and chloroform being least effective. DMSO showed no activity, whereas chloramphenicol, erythromycin and vancomycin showed the activity against any of the bacterial strain tested.

Table: Antibacterial activity of different extracts of various plant parts of *Brassica campestris*.

Se. No.	Plants Parts	Extracts	Inhibition Zone in mm of Various Microbes in different concentrations (mg ml^{-1}) of extracts.									
			[a] 1		2		3		4		5	
			100	50	100	50	100	50	100	50	100	50
01	Roots	**Benzene**	[b]1+	--	1+	[c]--	--	--	1+	--	--	--
		P. Ether	**2+**	**2+**	**1+**	**--**	**2+**	**2+**	**1+**	**--**	**2+**	**2+**
		Chloroform	1+	--	1+	--	1+	--	--	--	1+	1+
		EtOH	1+	1+	2+	1+	1+	1+	2+	1+	2+	1+
		Methanol	1+	--	1+	1+	1+	--	1+	1+	2+	1+
		Ethanol	3+	2+	2+	1+	1+	1+	2+	1+	3+	2+
		Aqueous	1+	1+	1+	--	--	--	1+	--	1+	--
02	Stems	**Benzene**	1+	1+	1+	--	--	--	1+	--	1+	1+
		P. Ether	1+	--	1+	--	1+	--	1+	--	1+	--
		Chloroform	1+	--	--	--	--	--	1+	--	1+	--
		EtOH	2+	1+	1+	--	1+	--	1+	--	1+	1+
		Methanol	**2+**	**2+**	**2+**	**1+**	**1+**	**1+**	**2+**	**2+**	**3+**	**2+**
		Ethanol	3+	3+	2+	2+	2+	1+	2+	2+	3+	2+
		Aqueous	1+	1+	1+	--	--	--	1+	--	1+	1+
03	Leaves	**Benzene**	1+	1+	1+	--	1+	1+	1+	--	1+	1+
		P. Ether	1+	1+	2+	--	1+	--	1+	1+	1+	1+
		Chloroform	1+	--	1+	--	--	--	1+	--	1+	1+
		EtOH	**2+**	**2+**	**2+**	**1+**	**2+**	**1+**	**2+**	**2+**	**2+**	**2+**
		Methanol	2+	1+	1+	1+	1+	1+	2+	1+	2+	1+
		Ethanol	3+	3+	3+	2+	2+	2+	3+	2+	3+	3+
		Aqueous	1+	1+	1+	--	1+	1+	1+	--	2+	1+
04	**Chloramphenicol**		3+		3+		2+		3+		4+	
05	**Erythromycin**		4+		3+		3+		3+		3+	
06	**Vancomycin**		3+		4+		2+		3+		3+	
07	**DMSO**		--		--		--		--		--	

[a] 1, *Staphylococcus aureus*: 2, *Bacillus cereus*: 3, *Escherichia coli*: 4, *Pseudomonas aeruginosa*: 5, *Staphylococcus epidermidis*

[b] 1+ : 05 – 10 mm, 2+ : 10 – 15 mm, 3+ : 15 – 20 mm, 4+ : 20 – 25 mm;

[c] --: No inhibition.

DISCUSSION AND CONCLUSIONS

The antibacterial efficacy of the total extracts of all the three parts of *Brassica campestris* from the Vidisha district were studies by the disc diffusion method against five bacterial strains. Our results show a remarkable antibacterial efficacy of the ethanol extract of all the parts of *Brassica campestris* as well as the petroleum ether, methanol and ethyl acetate extracts of root, stem and leaves of *B. campestris* respectively. The wide variety of activity of the ethanolic extracts over the water extracts is significant because of the leaves of plants are of traditional uses. The antibacterial activity is passively because of the presence of secondary metabolites existed in the plant. Hence, it is difficult to explain the limited spectrum of activity of other extracts compared with the ethanolic extracts since all the extracts had had the secondary metabolites (Le *et al.,* 2003).

Ruhe *et al.*, (2005), very recently reported that the resistance mechanism developed by the *S. aureus* against tetracycline and methicilline. A wide range of antibiotics, which have been used in dermatological practice, show-increasing frequency of resistance. Therefore, today antibiotics can treat most bacteria causing skin diseases effectively. The indiscriminate use of antibiotics in some parts of the world in both human and veterinary medicine has led to the emergence of resistant strains of bacteria. Thus, the rational use of antibiotics is of utmost importance. This is why, the antibacterial therapy by medicinal plants will focus on a few problem areas.

For thousands of years, natural products have been used in traditional medicine all over the world and predate the introduction of antibiotics and other modern drugs. Plant materials or their extracts have been utilized as drugs since long in many parts of the world; India is the oldest among them (Chopra *et al.,* 1992). Over the last few years a large number of plant species have been evaluated for their antibacterial activity (Agrawal *et al.,* 2012a; Agrawal *et al.,* 2012b; Agrawal *et al.,* 2007; Perumal *et al.,* 1999).

In conclusion, Results of the present study were supported by the work done by various workers (Paul et al, 2012; Shrivastava and Bhargava, 2012; Jain *et al.,* 2011). *Brassica campestris* extracts possess a broad spectrum of activity against a panel of bacteria responsible for the most common bacterial infections of human. Further phytochemical studies are required to determine the types of compounds responsible for the antibacterial effects of these medicinal plants.

ACKNOWLEDEMENTS

The authors are very thankful to Dr. S. K. Jain, Dept. of Botany, S. S. L. Jain P. G. College, Vidisha, M. P., India for giving the valuable suggestions and authentication of plant. Thanks are also due to Dr. Ramkrishna Agrawal, Ret. Ayurvedic Medical Officer, Vidisha for providing authentic medicinal values of locally available and traditionally use of *Brassica* species for these investigations.

References

Agrawal, MK., A. Varma and S. Goyal. (2012). Antibacterial screening of extract of the leaves of the *Lantana camara*. *Indian Journal of Life Sciences*, **1 (2)**: 97-99.

Agrawal, MK., A. Varma, A. Varma and S. Goyal. (2012). *In vitro* and *in vivo* antibacterial activity of *Lantana camara* (leaves) against the pathogen causing skin diseases i. e. *Staphylococcus aureus*. *Indian J. Applied & Pure Bio.,* **27 (2):** 279-286.

Agrawal, MK., AK. Varma, S. Dwevedi and T. Saify. (2007). The antibacterial activity of the extracts of *Lantana camara* leaves against the skin disease causing bacteria. *Indian Journal of Applied Life Sciences,* **3 (2):** 15-16.

Bauer, RW., MDK. Kirby, JC. Sherris and M. Turck. (1966). Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Cl. Pathol.,* **45**: 493-496.

Chopra, RN., SL. Nayar and IC. Chopra. (1992). *Glossary of Indian Medicinal Plants (III* edition*)*, CSIR, New Delhi, 7-246.

Clive, S. (1997). New Flora of the British Isles. Cambridge: Cambridge. ISBN 978-0-521-58935-2.

Cohen, ML. (1992). Epidemiology of drug resistance: implications for a post-antibacterial era. Science, **257:** 1050-1055.

Geyid, A., D. Abebe, A. Debella, Z. Makonnen, F. Aberra, F. Teka, T. Kebede, K. Urga, K. Yersaw, T. Biza, B.H. Mariam and M. Guta. (2005). Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. *J. Ethnopharmacol.,* **97:** 421- 422.

Harborne, JB. (1984). Phytochemical methods - A guide to modern technique of plant analysis (II edition), Chapman and Hall, New York, 1-228.

Jain, B., A. Kanzarkar and VK. Jain. (2011). Comparative analysis of the anti-bacterial and anti-fungal activity of five selected Indian medicinal plants on human pathogenic microorganisms. *Asian Journal of Biochemical and Pharmaceutical Research,* **1 (2):** 437-442.

Kunin, CM. (1983). Antibiotic resistance - a world health problem we cannot ignore (Editorial). *Ann. Intern. Med.,* **99:** 859-860.

Kunin, CM. (1993). Resistance to Antimicrobial Drugs – A Worldwide calamity. *Ann. Intern. Med.,* **118:** 557-561.

Le, HT., CM. Schaldach, GL. Firestone and LF. Bjeldanes. (2003). Plant-derived 3,3'-Diindolylmethane is a strong androgen antagonist in human prostate cancer cells. *J Biol Chem.,* **278 (23):** 36-45.

Neu, HC. (1992). The crisis in antibiotic resistance. *Science,* **257:** 1064-1073.

Omar, IV., Lin Chia-Lei, GL. Firestone and LF. Bjeldanes. (2009). 3,3′-Diindolylmethane induces a G_1 arrest in human prostate cancer cells irrespective of androgen receptor and p53 status. *Biochemical Pharmacology,* **78 (5):** 469-476.

Paul, S., TB. Emran, D. Saha and SM. Zahid Hosen. (2012). Phytochemical and antimicrobial activity of the plant extracts of *Brassica oleracea* against selected microbes. *Asian Journal of pharmacy and medical science,* **2 (2):** 40-44.

Perumal, SR., S. Ignacimuthu and RD. Patric. (1999). Preliminary screening of ethnomedicinal plants from India. *J. Ethnopharmacol.,* **66:** 235-240.

Plokin, NJ. (1988). Conservation, ethnobotany and the search for jungle medicine: pharmacology comes of age … again. *Pharmacotherapy,* **8:** 257-262.

Rahman, MS., MZ. Rahman, MA. Wahab, R. Chowdhury and MA. Rashid. (2008). Antimicrobial Activity of Some Indigenous Plants of Bangladesh. Dhaka Univ. *J. Pharm. Sci.,* **7:** 23-26.

Shrivastava, N. and R. Bhargava. (2012). Antibacterial potential of selected medicinal plants against *Escherichia coli* and *Staphylococcus aureus*. *Acta Biologica Indica,* **1 (1):** 133-135.

Ruhe, JJ., T. Manson, RW. Bradsher and A. Menon. (2005). Use of long acting tetracyclines for Methicillin-Resistant *Staphylococcus aureus* infections: Case series and Review of literature. *Clinical Infectious Diseases.* **40:** 1429-1434.

****