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Evaluation of Anti-Bacterial Activity of Turnip Green Leaves Extracts

Jagwinder Kaur, Meenakshi Malhotra *, Ajeet Pal Singh, Amar Pal Singh

St. Soldier Institute of Pharmacy, Lidhran campus Behind NIT, Jalandhar-Amritsar Bye-pass, Jalandhar, Punjab 144011

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*Address for Correspondence:

Meenakshi Malhotra, St. Soldier Institute of Pharmacy, Lidhran campus Behind NIT, Jalandhar-Amritsar Bye-pass, Jalandhar, Punjab 144011

Abstract

The study aimed to assess the antimicrobial potential of aqueous and methanolic extracts from *Brassica rapa* L. leaves. Phytochemical analysis revealed the presence of various compounds including carbohydrates, fats, proteins, vitamins, minerals, electrolytes, sugars, soluble and insoluble fiber, and amino acids. Both extracts were evaluated for antibacterial activity against six microorganisms, including gram-positive and gram-negative bacteria, compared with the standard antibiotic Gentamycin. Methanolic extract exhibited higher antibacterial efficacy against gram-positive bacteria compared to the aqueous extract.

Although specific active components were not isolated, the plant extracts contained potential antibacterial agents such as soluble fiber, minerals, amino acids, isothiocyanate glycosides, and essential oils. Additionally, compounds like 3,3'-diindolylmethane, sulforaphane, selenium, indole-3-carbinol, and vitamins like C, E, A, and K were identified. The methanolic extract, rich in sulfur and nitrogen-containing compounds, showed promising antibacterial activity against gram-positive bacteria.

In conclusion, *Brassica rapa* L. leaves could serve as a valuable source for developing safe antibacterial agents. The observed spectrum of activity suggests the potential for further exploration in drug development.

Keywords: *Brassica rapa* L., antibacterial activities, agar disc diffusion method, agar well diffusion method, Gentamycin, phytochemical analysis, Turnip green

INTRODUCTION:

In recent years, there has been a growing interest in exploring natural sources for potential antibacterial agents due to the emergence of antibiotic-resistant bacterial strains and the limitations of conventional antibiotics. Plants, being a rich source of bioactive compounds, have attracted considerable attention in this regard[1]. Turnip greens (*Brassica rapa* subsp. rapa) are leafy vegetables widely consumed for their nutritional benefits, but their potential antibacterial properties remain relatively unexplored[2].

The genus Brassica belongs to the Brassicaceae family and encompasses various plant species with diverse pharmacological activities attributed to their bioactive constituents, such as phenolics, flavonoids, and glucosinolates. Turnip greens, a member of this genus, are known for their rich content of phytochemicals, which are believed to contribute to their potential therapeutic effects[3].

Studies have shown that certain plant extracts possess antibacterial properties against a wide range of pathogenic bacteria. These antibacterial activities can be attributed to the presence of secondary metabolites, including alkaloids, terpenoids, and phenolic compounds[4], which interfere with bacterial cell structures or metabolic processes, leading to their inhibition or death[5].

While some studies have investigated the antibacterial potential of extracts from various parts of turnip plants, including roots and seeds, limited research has focused on the antibacterial activity of turnip green leaves extracts. Therefore, exploring the antibacterial properties of turnip green leaves extracts could provide valuable insights into their potential as natural antibacterial agents[6].

In this study, we aim to evaluate the antibacterial activity of turnip green leaves extracts against clinically relevant bacterial strains. By employing different extraction methods and testing the extracts against a panel of bacterial species, we seek to elucidate the potential of turnip green leaves as a source of novel antibacterial agents.

MATERIAL AND METHODS

The whole study was divided into four Phases to generate the data as follows.

Phase I: To prepare various extracts of *Brassica rapa* (Methanolic and aqueous) by successive extraction technique.

<u>Phase II:</u> To find out the Percentage Yield of Various Extracts of *Brassica rapa.*

<u>Phase III:</u> To identify the type of phyto-constituents present in *Brassica rapa*.

<u>Phase IV</u>: To perform Antibacterial activity of each extract of *Brassica rapa* at various dose level (50&100mg/ml).

Collection and identification of plant

The leaves part of plant *Brassica rapa* was collected from the local dealer of medicinal herbs. This plant was identified and Morphological authenticated by Kuber Impex Limited, Indore, India.

Preparation of plant material

The leaves of plant *Brassica rapa* washed properly to remove dirt and powdered well by grinding and avoiding the moisture contamination. This powdered material passed through sieve for obtaining uniform particle size then this powdered crude drug was dissolved in several solutions to determine their optimum solubility.

Preparation & administration of crude extracts/standard drugs[7,8]

The commonly employed technique for separation of active constituents from the crude drugs is called extraction. Extraction involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. The purpose of standardized extraction procedures for crude drugs (medicinal plant parts) is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent known as menstrum. The extract thus obtained, after standardization, may be used as medicinal agent. These extracts contain complex mixture of many medicinal plant metabolites such as alkaloids, glycosides, terpenoids, flavonoids and lignans.

RESULTS AND DISCUSSION

The percentage yield of both extracts of *Brassica rapa* was calculated using the formula.

Percentage yield of both extract

Table 1: Percentage yield of both extracts of *Brassica rapa*

S. No.	Name of extract	Wt. of powder	Wt. of extract	Percentage yield (w/w)
1.	Aqueous extract	100 gm	6.44 gm	6.44%
2.	Methanolic extract	100 gm	5.86 gm	5.86%

Preliminary Phyto-profile for *Brassica rapa.*

Table 2: Preliminary Phyto-profile for *Brassica rapa.*

S. No.	Name of extract	Colour	Physical state	Percentage yield (w/w)
1.	Aqueous extract	Brown	Powder	6.44%
2.	Methanolic extract	Brown	Powder	5.86%

Phytochemical screening of *"Brassica rapa l."* :

Table 3: Phytochemical Screening of *Brassica rapa.*

S.NO.	TEST	OBSERVATION	INFERENCE
		Test for organic constituents	
1	**Test for carbohydrates** Molisch's reagent	violet ring is formed at the junction of two liquids	+ve
2	**Test for reducing sugars**: Fehling's test Benedict's test	first yellow then brick red color is observed. Green color is observed.	+ve +ve
3	**Test for Hexose sugars**: Selwinoff's reagent	red color is formed yellow to red color appears	+ve +ve

	Tollen's phloroglucinol tests for galactose		
4	**Test for non-reducing polysaccharides (starch) :** Iodine test Tannic acid test for starch	blue color precipitates obtained	+ve +ve
5	**Test for Gums**	no red color	-ve
6	**Test for mucilage**	no swelling in water	-ve
7	**Test for proteins** Biuret test Millan's test Xanthoprotein test Test for proteins containing sulphur Precipitation test	color change color change occur color change occur black brownish solution ppts. Formed	+ve +ve +ve +ve +ve
8	**Test for amino acids** Ninhydrin test Test for tyrosine Test for cysteine	black color light brown color white ppts obtained	+ve +ve +ve
9	**Test for fats and oils** sudan red III reagent Solubility test	no oil globoules appear Insoluble in water	-ve -ve
10	**Test for volatile oil**	solubility in water	-ve
11	**Test for cardiac glycosides** Baljet's test Keller -killiani test	yellow to orange color upper layer brown and lower layer bluish color	+ve +ve
12	**Test for anthra-quinone glycosides** Borntrager's test	ammonical layer turns pink	+ve
13	**Test for Saponin Glycosides** Foam test	persistent foam is observed	+ve
14	**Test for Coumarin Glycosides**	aromatic odour	+ve
15	**Test for alkaloids:** Dragendorff's reagent Mayer's test Murexide test for purine alkaloids	no ppts. no ppts. Obtained no change in solution	-ve -ve -ve
16	**Test for Tannins and phenolic compounds** 5% $FeCl_3$ solution Lead acetate solution Gelatin solution Bromine water Acetic acid solution Pottasium dichromate	deep blue color solution obtained white ppts. white ppts. decoloration of bromine water red color sol. red ppts.	+ve +ve +ve +ve +ve +ve

	Dilute iodine solution Dilute HNO_3 Dilute potassium permanganate solution	transient red color reddish to yellow color decoloration	+ve +ve +ve
17	**Test for Enzymes**	no changes	-ve
18	**Test for organic acid** Calcium chloride test	ppts.were not obtained immediately	+ve
19	**Test for Vitamins**	pinkish red color appears	+ve
	Test for inorganic constituents		
1	**Test for calcium**	white ppts.	+ve
2	**Test for magnesium**	white ppts.	+ve
3	**Test for sodium** Flame test	golden yellow flame	+ve
4	**Test for pottasium**	violet color flame	-ve
5	**Test for iron**	blood red color	+ve
6	**Test for sulphate**	white ppts	+ve
7	**Test for phosphate**	no crystal	-ve
8	**Test for chloride**	blue color ppts. Formed	+ve
9	**Test for carbonate**	white ppts.	+ve
10	**Test for nitrates**	brown color	+ve

Antimicrobial activity of *Brassica rapa*, leaves extracts at the concentrations of 50 mg/ml & 100mg/ml were tested against all microorganisms respectively. Standard antibiotic Gentamicin (10 μg/ml) was used against all microorganisms. Minimum concentration that showed inhibition was recorded as the MIC.

Antibacterial activity of aqueous and methanol extracts of *Brassica rapa*

Table 4: Antibacterial activity of aqueous and methanol extracts of *Brassica rapa*

S.N.	STRAIN	EXTRACT	MEAN DIAMETER OF ZONE OF INHIBITION (in mm)		
			50mg/ml	100mg/ml	Gentamicin (10mcg/ml)
I	S. epidermidis	Aqueous	12	13	26
		Methanolic	15	17	
II	M. luteus	Aqueous	13	15	21
		Methanolic	16	18	
III	B. pumilus	Aqueous	11	12	19
		Methanolic	15	17	
IV	P. fluorescens	Aqueous	13	12	22
		Methanolic	18	19	
V	P. vulgaris	Aqueous	11	12	20
		Methanolic	14	16	
VI	P. mirabilis	Aqueous	10	11	18
		Methanolic	12	14	

a: Inhibition zones are the mean including cup borer (8.5mm) diameter

b: Standard drug Gentamicin (10 μg/ml)

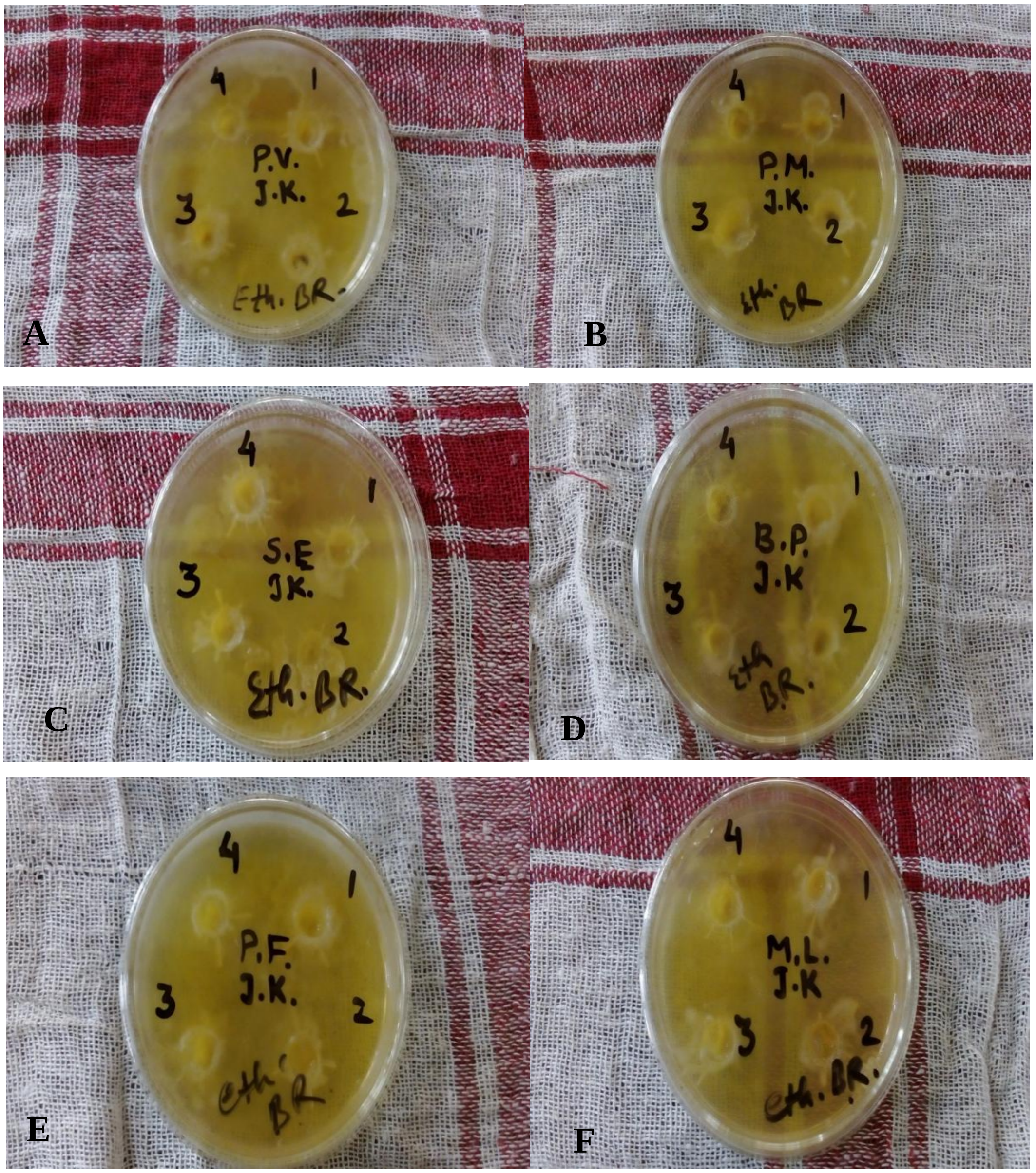

Figure 1: Different petridishes shows the zone of inhibition as well as growth of microorganism # 1=50mg/ml Eth. Ext. B.R., 2=100mg/ml Eth. Ext. B.R., 3= standard drug, Gentamycin 4=control (without any drug

DISCUSSION

The presence of antibacterial substances in the higher plants is well established.[1] Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of a drug.[2]Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study the plant extracts by methanol provided more consistent Antimicrobial activity compared to those extracted by water. The results of antibacterial activity of plant *Brassica rapa against* the investigated bacterial strains are shown in Table 4. The aqueous extract produced less zones of inhibition comparative to Methanolic extract in the Kirby-Bauer analysis. This might have resulted from the lack of solubility of the active constituents in aqueous solutions while methanol extract showed some degree of antibacterial activity. Further trials using solvents of various polarities will explore the effects of solvent composition on extract efficacy[9]. This study show that Gram negative bacteria are less susceptible comparative to Gram positive bacteria. On the other hand, the Gram-positive bacteria were the most susceptible bacteria. Various workers have already shown that Gram positive bacteria are more susceptible towards plants extracts as compared to Gram negative bacteria[10]. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram negative cell wall is multilayered structure[11]. Alternatively, the passage of the active compound through the Gram-negative cell wall may be inhibited. It is thought that observed differences may result from the doses used in this study. In addition, microorganisms show variable sensitivity to chemical substances related to different resistance levels between strains[12].

Preliminary phytochemical analysis of *Brassica rapa* revealed the presence of phenolic compound, Proteins, tannins, glycosides, Carbohydrate, Starch, Vitamins & Minerals etc.

It is not surprising that there are differences in the antimicrobial effects of plant species, due to the phytochemical properties and differences among species.

It is quite possible that some of the plants that were ineffective in the study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol or water. The drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants.[7] Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed.[8] Lack of activity can thus only be proven by using large doses.[9]

Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents.[10]With no antibacterial activity, extracts may be active against other bacterial species which were not tested.[11] The methanol extract of leaves of *Brassica rapa* showed the most remarkable activity. This plant can be further subjected to isolation of the therapeutic Antimicrobial and carry out further pharmacological evaluation.

CONCLUSION:

Plants have been utilized for medicinal purposes for millennia, offering safe and cost-effective alternatives to synthetic medications. *Brassica rapa*, commonly known as turnip or field mustard, is a biennial plant rich in medicinal potential. A field survey elucidated its various characteristics, while phytochemical analysis revealed compounds like phenolic compounds, proteins, tannins, glycosides, and essential nutrients. Investigating its antimicrobial properties against selected bacterial strains revealed significant activity, particularly in the methanolic extract, suggesting therapeutic potential in traditional medicine. The study advocates for further exploration into the identification and pharmacological evaluation of active compounds within *Brassica rapa*. Additionally, it underscores the importance of standardizing extraction methods and broadening screening techniques to unveil the full spectrum of plant-derived antimicrobial compounds. Such efforts hold promise for the development of novel, effective, and less toxic antimicrobial agents, addressing the pressing need for alternative treatments amidst increasing species extinction rates.

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