

Original Article

**EFFICACY OF AQUEOUS AND METHANOLIC EXTRACTS OF PLANT *BRASSICA RAPA* LEAVES
POTENTIAL ANTI-PSEUDOMONAL ACTIVITY**

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ABSTRACT

Objective: Efficacy of Aqueous and Methanolic Extracts of Plant *Brassica Rapa* Leaves Potential Anti-Pseudomonal Activity

Methods: Phytochemical analysis of *Brassica rapa* belonging to the family was examined using Aqueous and Methanolic extracts. Methanolic and aqueous extracts of leaves part of the plant were investigated individually for Anti-pseudomonal activity by agar well diffusion method. The susceptibility of three gram-negative bacteria to the aqueous and Methanolic extracts of this plant was measured and the results compared with standard antibiotic Gentamycin. Both the extracts were tested against selected species of *Pseudomonas aeruginosa* MTCC2295 (G-ve), *Pseudomonas fluorescens* MTCC2421 (G-ve), and *Pseudomonas alcaligenes* MTCC7095 (G-ve), to find the inhibitory activities of microbes at the dose level of 50 and 100 µg/ml.

Results: The phytochemical analysis of Aqueous and Methanolic extracts confirmed the presence of Carbohydrates, Fat, proteins, Vitamins, Minerals, Electrolytes, sugar, soluble and insoluble fiber and amino acids. Methanolic extract of *Brassica rapa* showed considerably high Anti-pseudomonal activities against all bacteria than Aqueous extract.

Conclusion: The leaves of *Brassica Rapa* were found to be rich in sulphur and nitrogen-containing compounds. *Brassicarapamethanolic* extract has anti-pseudomonal effects against bacteria.

The current study's range of activity can provide a new plant source for safe anti-pseudomonal drugs.

Keywords: *Brassica rapa*, Anti-pseudomonal activities, Agar well diffusion method, Gentamycin, Phytochemical analysis, Turnip green

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INTRODUCTION

Medicinal plants are the world's oldest method of healthcare. Throughout history, medicinal plants have been used by all civilizations. According to the World Health Organization (WHO), 80 percent of the world's population now uses herbal medicine for some form of primary health treatment [1, 2]. Medicinal plants are a native legacy of global significance. Natural substance extracts are a valuable source of novel medicines. Plant sections such as stem bark, root bark, aerial branches, vegetative buds, leaves, fruits, and latex were used to treat various illnesses in ancient medicine systems [3]. Current medication includes at least 25% medicines derived from plants, as well as many others that are synthetic drugs based on initial substances extracted from plants [4]. Multiple antibiotic tolerances has already emerged as a result of the indiscriminate usage of commercial antimicrobial medications widely employed in the management of the infectious disease [5]. The treatment of these infections is mainly based on the use of antibiotics; the antibiotics may be associated with adverse effects on the host, including immune suppression, hypersensitivity, and also allergic reactions [6, 7]. However, therapy using antibiotics is going through a crisis due to the development of resistance by pathogens. *Staphylococcus aureus* is one of the most important pathogens that have become resistant to almost all known antibiotics. Other examples for antibiotic-resistant bacteria are vancomycin-resistant enterococci, multidrug-resistant tuberculosis and others [8]. On the other hand; herbal plant products have got incredible success in serving as a guidepost for new antibacterial drug discovery. According to the World Health Organization, plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [9]. The spectrum of antimicrobial effectiveness of drugs varies greatly. *Brassicarapa* L. (turnip) is a biennial, outcrossing, mesopolyploid herbaceous plant in the genus

Brassica (Cruciferae). Turnip is native to Europe, Russia, Central Asia, and the Middle East, but it is now commonly grown as a vegetable and oil source all over the world [10]. *Brassica rapa* has historically been used to cure a host of infections, including hepatitis, jaundice, furuncle sore throats, as an antiscorbutic, antiarthritic, resolvent, stimulant, for gastrointestinal complaints, and as a laxative.

MATERIALS AND METHODS

Collection and identification of the plant

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

Aqueous extract

Approximately 100 g. of shade-dried powder of leaves of plant *Brassica rapa* was taken in a 1 L beaker and chloroform: water (1:99) was added up to a sufficient level to immerse the drug completely. Chloroform was added as a preservative to prevent microbial growth. This setup was placed aside for 72 h. with stirring at alternate intervals. Finally, the contents of the beaker were vacuum filtered to get a clear watery, greenish coloured extract. The extract was concentrated under a high vacuum and completely dried in a desiccator.

Methanolic extract

The leaves of the plant *Brassica rapa*, were shade-dried and powdered separately. The powder (100 g.) was packed in a Soxhlet apparatus and subjected to continuous hot percolation for about 8 h. with methanol (350 ml.) as solvent. The extract was concentrated to a semi-solid mass under vacuum and completely dried in a desiccator.

Preliminary phytochemical analysis

The Aqueous and Methanolic extracts of leaves of the plant *Brassica rapa* were subjected to different chemical tests separately for the identification of various active constituents. The tests are as follows [11-13].

Test for alkaloids

1. Dragendorff's test: 1 ml of Dragendorff's reagent was applied to the extract solution. A reddish-brown precipitate suggests the presence of alkaloids.

2. Wagner's test: Wagner's reagent (2 ml) was applied to the extract solution.

A reddish-brown precipitate suggests the presence of alkaloids.

3. Mayer's test: 2 ml of Mayer's reagent was applied to the extract solution.

The presence of alkaloids is shown by a dull white precipitate.

4. Hager's test: Hager's reagent (3 ml) was applied to the extract solution.

The formation of yellow precipitate confirms the existence of alkaloids.

Tests for steroids

1. Libermann-Burchard test: In a dry test tube, the extract was dissolved in 2 ml of chloroform. 10 drops of acetic anhydride and 2 drops of concentrated sulphuric acid were added. The presence of steroids causes the solution to turn red, then blue, and eventually bluish-grey.

2. Salkowski test: After dissolving the extract in chloroform, equivalent volumes of concentrated sulphuric acid were applied. The steroid components in the tested extract are represented by the formation of bluish-red to cherry red colour in the chloroform layer and green fluorescence in the acid layer.

Test for flavonoids

1. Shinoda test: Magnesium ribbons and 1-2 drops of concentrated hydrochloric acid were applied to the extract solution (1 ml). The existence of flavonoids is indicated by the formation of red/pink/green/blue dye.

2. Pew's test: Zinc (metal) dust was applied to the extract solution, along with a few drops of 5N HCl. The existence of flavonoids is indicated by the development of a red colour.

Test for saponins

1. Foam test: For 15 min, the test extract was dissolved in distilled water and shaken. Saponins were detected by a one-centimeter layer of foam.

2. Haemolysis procedure: One drop of blood on a slide was combined with a few drops of test extract; in the presence of saponins, RBCs rupture.

Test for glycosides

1. Legal test: The extract was dissolved in pyridine, and an alkaline sodium nitroprusside solution was added. The presence of glycosides is indicated by the formation of pink-red to red pigment.

2. Baljet Test: A 1 ml solution of sodium picrate was applied to a 1 ml solution of extract solution, and the shift in colour from yellow to orange indicates the presence of glycosides.

Test for amino acid

1. Ninhydrin Test: To the extract solution, two drops of freshly prepared 0.2 percent ninhydrin reagent were applied and heated. The existence of amino acids is revealed by the production of violet colour.

Tests for proteins

1. Millon's test: The extract solution was combined with Millon's reagent, resulting in a white precipitate. Warming causes the precipitate to turn a red brick colour.

2. Biuret test: Heat the extract solution with a 10% sodium hydroxide solution. Add a few drops of copper sulphate solution after that. The presence of proteins is indicated by the formation of a violet/reddish violet precipitate. The extract solution was combined with concentrated nitric acid for the xanthoproteic examination. The presence of proteins is shown by the formation of a yellow colour that turns orange when alkali is added.

Tests for phenolic compounds

1. Ferric chloride solution was applied to the extract solution the presence of phenolic compounds is indicated by a product that is dark blue or greenish-black in colour.

Tests for fixed oils

1. Spot test: A small amount of extract was pressed between two filter sheets the presence of fixed oils is indicated by oil stains on paper.

Tests for carbohydrates

1. Molisch's test: A few drops of Molisch's reagent were applied to the extract solution (2-3 ml.). When concentrated H₂SO₄ was added from the test tube's edges, a violet ring formed at the junction of the two liquids, signalling the presence of carbohydrate.

2. Fehling's test: In a test tube, 1 ml of Fehling's solution A and 1 ml of Fehling's solution B were combined, and an equivalent amount of extract solution was applied. Brick red precipitate was observed after being heated in a boiling water bath for 5-10 min.

3. Benedict's test: In the test tube, an equal amount of Benedict's reagent was combined with the extract solution and heated in a boiling water bath until the solution turned green, yellow, blue, or red.

4. Schiff's test: An equal volume of Schiff reagent was applied to the extract solution and shaken for two minutes, and the appearance of pink colour suggested the presence of an aldehydic group.

5. Tollen's test: Tollen's reagent was applied to the extract solution and heated in a boiling water bath. The presence of carbohydrates was suggested by the formation of a silver mirror.

Anti-microbial screening [14]

Both microorganisms were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India, including *Pseudomonas aeruginosa* MTCC 2295 (G-ve), *Pseudomonas fluorescens* MTCC 2421 (G-ve), and *Pseudomonas alcaligenes* MTCC 7095 (G-ve). The media used for microorganism activation followed the MTCC protocol. The Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR) of the Government of India jointly finance the Microbial Type Culture Collection and Gene Bank (MTCC), a national facility built-in 1986. The MTCC is a modern facility housed at Chandigarh's Institute of Microbial Technology (IMTECH). It is a member of the World Federation for Culture Collections (WFCC) and has a record with the World Data Centre for Microorganisms (WDCM). The primary goals of this national facility are to serve as a repository, to provide authentic microbial cultures, and to provide relevant services to scientists employed in research institutions, universities, and industries. On 4 October 2002, the World Intellectual Property Organization (WIPO), Geneva, Switzerland, approved the MTCC as an International Depository Authority (IDA), making it the first IDA in India, the seventh in Asia, and the 34th in the world. The deposit of microorganisms under the

Budapest Treaty is accepted in 55 member countries as satisfying the necessity of patent procedure. The MTCC currently has five sections: Actinomycetes, Bacteria, Fungi, Yeasts, and Plasmids, with a total of over nine thousand cultures. Cultures are usually preserved using liquid nitrogen and freeze-drying. Mineral oil is sometimes used to protect the fungi. The viability and some main characteristics of the species are tested on a regular basis to ensure that cultures continue to reflect the original deposits. Special properties such as metabolite synthesis and compound degradation are not usually tested. Relevant strain information is computerized for fast search, analysis, and retrieval.

Agar well diffusion method (cup plate/cylinder/pits method)

Autoclaving was used to prepare and sterilize standard glass Petridishes and nutrient agar media. Under aseptic conditions, nutrient agar media was poured into sterilized Petridishes to a depth of 3 to 4 mm. The prepared Petridishes were held in an incubator for 24 h to ensure that no microorganisms grew. Under aseptic conditions, standardized suspensions of selected bacterial strains were distributed on the surface of the Petridish agar media. Using a stainless steel borer with a diameter of about 5 mm, four cups were bored in each Petridish, one for standard antibiotic, one for test sample, one for solvent blank, standard antibiotic (corresponding to microorganisms) and test solutions were serially diluted, 100l of the serially diluted antibiotic solution, test solution, an antibiotic solution, These Petridishes were determined, and the zones of inhibition of the sample were compared to the standard zone of inhibition around the cup. The zones of inhibition of the

sample were compared to the standard zone of inhibition of the standard antibiotic used. 100 ml of sterilized 0.9 percent saline was also prepared and sterilized by autoclaving at 15 lbs per 1210c for 15 min. The subcultured organisms slant was shaken with 10 ml saline to achieve an organism suspension. The organism suspension was moved to a sterilized saline solution. The organism was shook into a uniform suspension in the solution. Throughout the experiment, aseptic conditions were maintained in a laminar flow environment. Approximately 0.6 ml of the test organisms' nutrient broth suspensions were applied to 60 ml of sterile nutrient agar that had been cooled to 45°C, blended thoroughly, and poured onto sterile plates. The agar was allowed to solidify before punching six wells (cups) per plate with a sterile cork borer (separate borer for each organism) to ensure proper distribution of wells in the periphery with one well in the middle. The agar plugs were removed, and 50 l test samples of each concentration were pipetted into the corresponding marked wells using micropipettes. Every organism was prepared in triplicate plates. The plates were left at room temperature for 2 h to enable sample diffusion before being incubated face up for 24 h at the corresponding temperatures of each organism. The diameters of the inhibition zones were determined down to the millimeter (the cup size also being included)15.

RESULTS AND DISCUSSION

The percentage yield of both extracts of *Brassica rapa* was calculated using the formula and shown in table 1. Preliminary Phyto-profile for *Brassica rapa* is shown in table 2. Phytochemical screening of "*Brassica rapa* l is shown in table 3.

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%

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%

Table 3: Phytochemical screening of *Brassica rapa* l

S. No.	Test	Observation	Inference
Test for organic constituents			
1	Test for carbohydrates		
	a) Molisch's reagent	Violet ring is formed at the junction of two liquids	+ve
2	Test for reducing sugars:		
	a) Fehling's test	First yellow then brick red color is observed.	+ve
	b) Benedict's test	Green color is observed.	+ve
3	Test for hexose sugars		
	a) Selwinoff's reagent	Red color is formed	+ve
	b) Tollen'sphloroglucinol tests for galactose	yellow to red color appears	+ve
4	Test for non-reducing polysaccharides (starch)		
	a) Iodine test	Blue color	+ve
	b) Tannic acid test for starch	precipitates obtained	+ve
5	Test for gums	no red color	-ve
6	Test for mucilage	no swelling in water	-ve
7	Test for proteins		
	a) Biuret test	color change	+ve
	b) Millan's test	color change occur	+ve
	c) Xanthoprotein test	color change occur	+ve
	d) Test for proteins containing sulphur	black, brownish solution	+ve
	e) Precipitation test	ppts. formed	+ve
8	Test for amino acids		
	a) Ninhydrin test	black color	+ve
	b) Test for tyrosine	light brown color	+ve
	c) Test for cysteine	white ppts obtained	+ve
9	Test for fats and oils		
	a) sudan red III reagent	no oil globoules appear	-ve
	b) Solubility test	Insoluble in water	-ve
10	Test for volatile oil	Solubility in water	-ve
11	Test for cardiac glycosides		

S. No.	Test	Observation	Inference
12	a) Baljet's test	Yellow to orange color	+ve
	b) Keller-killiani test	Upper layer brown and lower layer bluish color	+ve
13	Test for anthra-quinone glycosides		
	a) Borntrager's test	Ammonical layer turns pink	+ve
14	Test for saponin glycosides		
	a) Foam test	Persistent foam is observed	+ve
15	Test for coumarin glycosides		
	a) Mayer's test	aromatic odour	+ve
16	Test for alkaloids		
	a) Dragendorff's reagent	No ppts.	-ve
16	b) Mayer's test	No ppts. Obtained	-ve
	c) Murexide test for purine	No change in solution	-ve
16	d) alkaloids		
	Test for tannins and phenolic compounds		
16	a) 5% FeCl ₃ solution	Deep blue color solution obtained	+ve
	b) Lead acetate solution	Whiteppts.	+ve
16	c) Gelatin solution	Whiteppts.	+ve
	d) Bromine water	Decoloration of bromine water	+ve
16	e) Acetic acid solution	Red color sol.	+ve
	f) Pottasium dichromate	Redppts.	+ve
16	g) Dilute iodine solution	Transient red color	+ve
	h) Dilute HNO ₃	Reddish to yellow color	+ve
16	i) Dilute potassium permanganate solution	Decoloration	+ve
	Test for Enzymes	No changes	-ve
18	Test for organic acid		
	a) 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	Whiteppts.	+ve
2	Test for magnesium	Whiteppts.	+ve
3	Test for sodium		
	a) 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	Whiteppts.	+ve
10	Test for nitrates	Brown color	+ve

Antimicrobial activity of *Brassica rapa*, leaves extracts at the concentrations of 50 mg/ml and 100 mg/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 and Antibacterial activity is shown in table 4

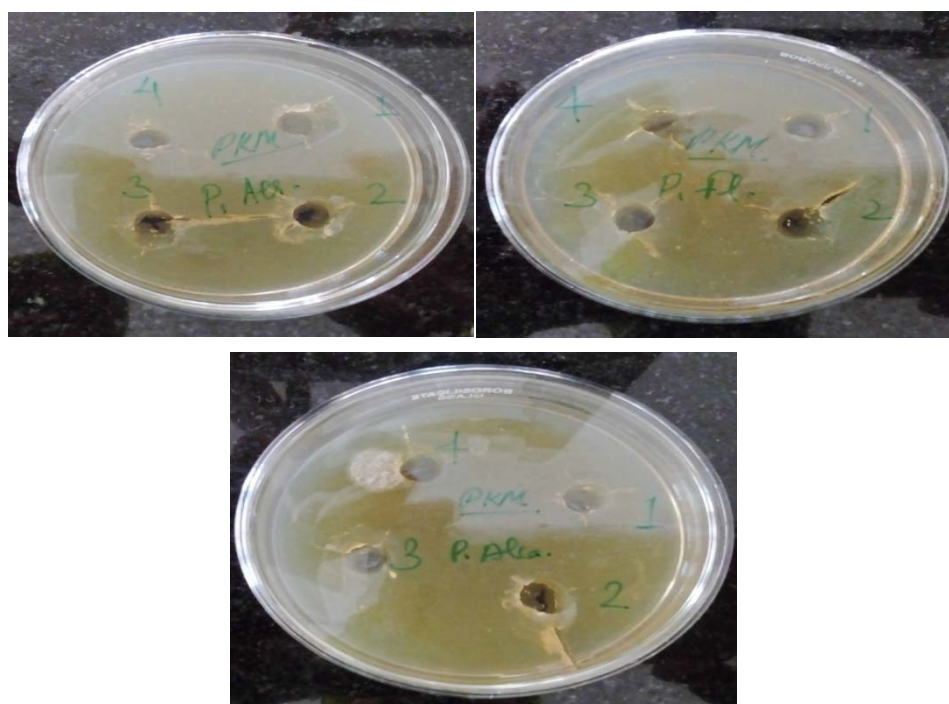


Fig. 1: Different petridishes shows the zone of inhibition as well as growth of microorganism # 1=50 mg/ml Aq. Ext. B. R., 2=100 mg/ml Aq. Ext. B. R., 3= standard drug, gentamicin 4=control (without any drug)

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

S. No.	Strain	Extract	Mean diameter of zone of inhibition (in mm)		
			50 mg/ml	100 mg/ml	Gentamicin (10mcg/ml)
I	Pseudomonas aeruginosa	Aqueous	13	14	27
		Methanolic	16	18	
II	Pseudomonas fluorescens	Aqueous	14	15	20
		Methanolic	17	18	
III	Pseudomonas alcaligenes	Aqueous	12	14	21
		Methanolic	16	18	

a: Inhibition zones are the mean including cup borer (8.5 mm) diameter, b: Standard drug gentamicin (10 µg/ml)

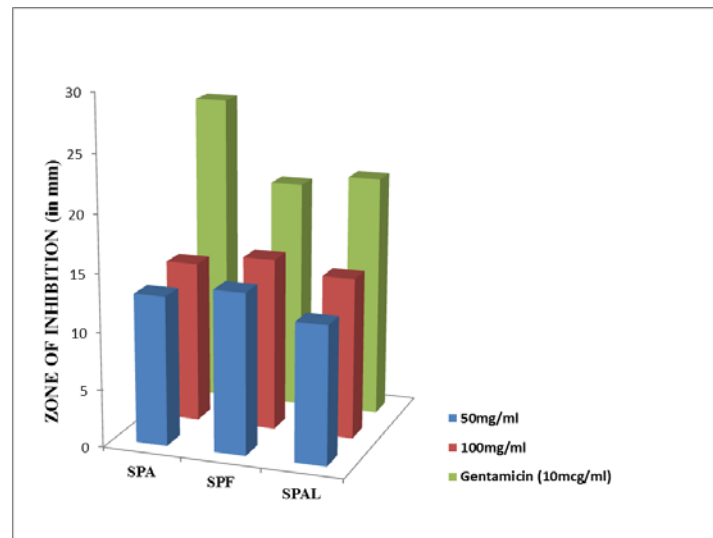


Fig. 2: Antibacterial activity of aqueous extracts of *Brassica rapa* (SPA = STRAIN *Pseudomonas Aeruginosa*, SPF = STRAIN *Pseudomonas Fluorescens*, SPAL = STRAIN *Pseudomonas Alcaligenes*)

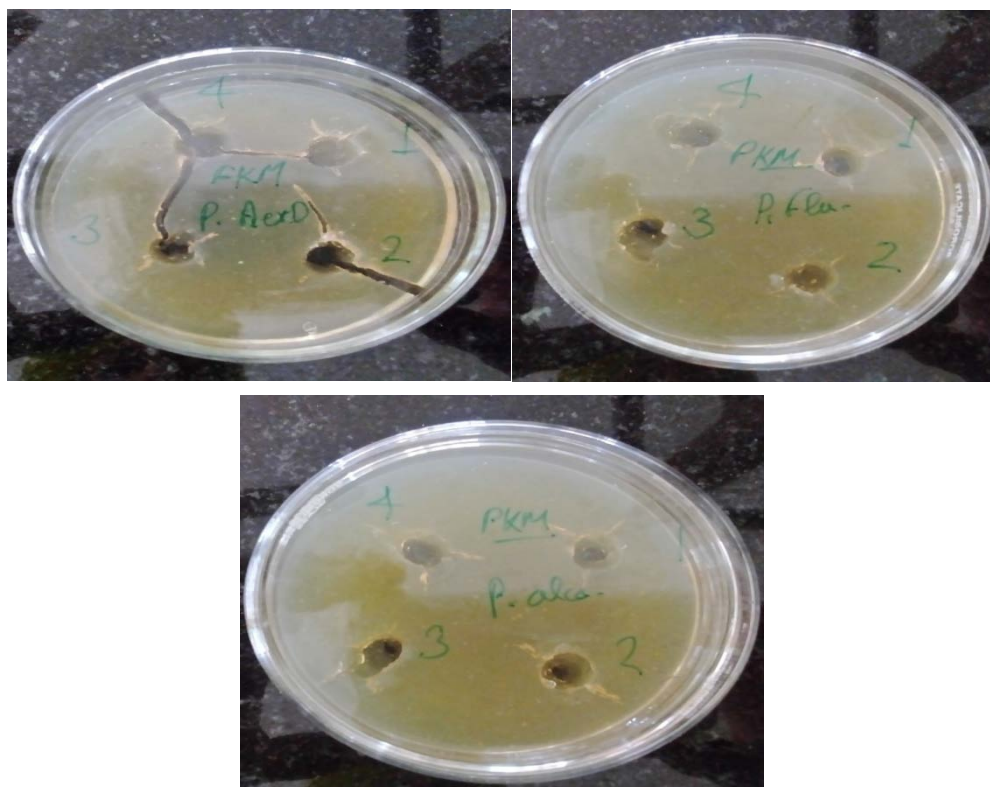


Fig. 3: Different petridishes shows the zone of inhibition as well as growth of microorganism # 1=50 mg/ml Eth. Ext. B. R., 2=100 mg/ml Eth. Ext. B. R., 3= standard drug, Gentamycin 4=control (without any drug)

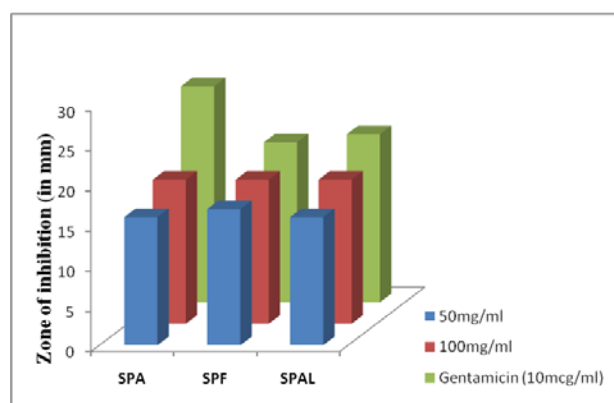


Fig. 4: Antibacterial activity of Methanolic extracts of *Brassica rapa* (SPA = STRAIN *Pseudomonas Aeruginosa*, SPF = STRAIN *Pseudomonas Fluorescens*, SPAL = STRAIN *Pseudomonas Alcaligenes*)

DISCUSSION

Antibacterial compounds are well known to exist in higher plants. Plants also inspired new drug compounds, and plant-derived drugs have made significant contributions to human health. Phytomedicine can be used to cure illnesses, as in the Unani and Ayurvedic systems of medicine, or it can be used as the foundation for the formulation of a medicine, a natural blueprint for the development of a drug. The type of solvent used in the extraction process has a significant impact on the subsequent separation of botanical compounds from plant material. Traditional healers mainly use water as a solvent, but we discovered in this analysis that plant extracts extracted with methanol had more consistent antimicrobial activity than those extracted with water. The antibacterial efficacy of the plant *Brassica rapa* against the tested bacterial strains. In the Kirby-Bauer study, the aqueous extract provided less zones of inhibition than the Methanolic extract. This may be due to the inability of the active constituents to dissolve in aqueous solutions, whereas methanol extract demonstrated some antibacterial activity. More experiments with different polarity solvents will be conducted to investigate the impact of solvent composition on extract efficacy. According to this analysis, Gram negative bacteria are less vulnerable than Gram positive bacteria. Gram positive bacteria, on the other hand, were the most vulnerable. Several studies have found that Gram positive bacteria are more sensitive to plant extracts than Gram negative bacteria. The observed variations are believed to be the product of the doses used in this research.

CONCLUSION

Anti-pseudomonal active plant principles include soluble fibre minerals, amino acids, isothiocyanate glycosides, essential oil (48 volatile components), 3,3'-di-indolylmethane, sulforaphane, selenium, indole-3-carbinol, Phyto, carotenoids, vitamin C, vitamin E, beta-carotene, manganese, vitamin K, omega-3 fatty acids. *Brassica rapa* leaves contain high levels of potassium, magnesium, pantothenic acid, vitamin B2, copper, phosphorus, vitamin B1, niacin, and calcium. The essential oil extracted from the leaves of *Brassica Rapa* was found to be rich in sulphur and nitrogen-containing compounds. *Brassica rapa* methanolic extract has anti-pseudomonal effects against bacteria. The current study's range of activity can provide a new plant source for safe anti-pseudomonal drugs. Alternatively, if the active theory is present in sufficient concentrations, other constituents can exert antagonistic effects or negate the beneficial effects of the bioactive agents. The most unusual occurrence was shown by a methanol extract of *Brassica rapa* leaves. This plant may be subjected to more isolation of the therapeutic antimicrobial and pharmacological evaluation.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

There are no conflicts of interest.

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