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Original Research Article

Phytochemical evaluation and investigation of anti-fungal activity of turnip top extracts

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ABSTRACT

Objective: To evaluate the activity of Ethanolic and Aqueous extracts of leaves of *Brassica rapa* against three fungal strains i.e. *Candida albicans* MTCC4748, *Candida glabrata* MTCC3814, and *Candida tropicalis* MTCC9038 in-vitro.

Material and Methods: Phytochemical analysis of *Brassica rapa* belonging to family brassicaceae was examined using Ethanolic and Aqueous extracts. Ethanolic and Aqueous extracts of leaves of *Brassica rapa* were investigated individually for antifungal activity by Agar well diffusion method. Both the extracts were tested against selected fungal strains i.e. *Candida albicans* MTCC4748, *Candida glabrata* MTCC3814, and *Candida tropicalis* MTCC9038 to find the inhibitory activities of fungal growth at the dose level of 50 and 100 µg/ml.

Results: The phytochemical analysis of ethanolic and aqueous extracts confirmed the presence of phenolic compounds, glycosides, tannins, carbohydrates, proteins, amino acids, tannins, reducing sugar, non-reducing sugar and inorganic compounds such as calcium, magnesium, iron, carbonate & sulphates. Ethanolic extract of *Brassica rapa* showed considerably high antifungal activities against selected microorganisms than aqueous extract.

Conclusion: Although the active components were not isolated but antifungal active plant principles such as flavonoids, glycosides and tannins were observed in the extract. Ethanolic extract of *Brassica rapa* possess effective antifungal properties for selected fungal strains i.e. *Candida albicans* MTCC4748, *Candida glabrata* MTCC3814, and *Candida tropicalis* MTCC9038.

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1. Introduction

Mycology is the branch of biology associated with the study of fungi, including its genetic and biochemical properties, taxonomy and its use to humans as a source for medicine, food, entheogens, as well its toxicity or infection.¹ Mycosis is a superficial, cutaneous, subcutaneous and systemic infections which effects humans and animals worldwide. Inhalation of fungal spores or localized colonization of the skin may kick off importunate infections; therefore, mycoses effects the lungs or the skin. Individuals with

weakened immune systems are also at high risk of developing fungal infections. This is the case of people with HIV/AIDS, people under steroid treatments, and people taking chemotherapy. Fungal infections of the skin was the 4th most common disease in 2010 affecting 984 million people. It is estimated that 1.6 million people die each year due to different types of fungal infections.²

Nowadays multiple drug resistance has developed in pathogens to common therapeutic agents due to the indiscriminate use of commercial antimicrobial drugs. In recent studies it is found that *Candida albicans* become resistant to fluconazole and amphotericin B.³ It has been troubling hospital services all over the world. In addition to

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this problem, antibiotics are sometimes allied with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation compels researchers to search for new antimicrobial drugs. Due to the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a regular need for new and effective therapeutic agents.⁴

Herbal drugs play a vital role in traditional and modern system of medicines in some regions of the world where the plants are often used as the therapeutic agents as antibiotics, antiseptics, anti-inflammatory and in the treatment of various skin infections. Nowadays plant extracts become more trustworthy to treat infectious diseases due to the side effects of allopathic drugs and drug resistance that microorganism put up against the antibiotics.⁵ Presently the isolation of biologically active compounds of plant extracts is become popular for the preparation of various herbal drugs. Herbal antimicrobials signify a enormous unexploited source of medicines so more exploration of plant based

antimicrobial drugs needs to take place because of their vast therapeutic potential. They are effective in the treatment of infectious diseases as well as diminish various side effects associated due to synthetic antimicrobial medicines. A drug which can kill pathogen or inhibit microbial growth without any harm to host and have selective toxicity is considered as successful chemotherapeutic agent.^{6,7}

In the present study, Ethanolic and Aqueous extracts of leaves of *Brassica rapa* were evaluated against three fungal strains i.e. *Candida albicans* MTCC4748, *Candida glabrata* MTCC3814, and *Candida tropicalis* MTCC9038 *in-vitro*. The group of *Brassica rapa* plants are used as a food in all over the world and there is evidence that this diet rich vegetables consist of high amount of flavonoids & strong antioxidant activity. *Brassica rapa* L. consisting of various widely cultivated subspecies including the turnip, mizuna, napa cabbage, bok choy, cime di rapa (leafy vegetable) and (*Brassica rapa* subsp. *oleifera*, an oilseed which has many common names, including field mustard, bird rape, keblock, and colza). *Brassica rapa* belonging to family brassicaceae is cultivated as a winter vegetable, & it is regarded as a typical product in many Italian regions. Turnip leaves are sometimes eaten as "turnip greens" and both leaves and root have a pungent flavor. Baby turnips come in yellow-, orange-, and red-fleshed varieties as well as white-fleshed. Their flavor is mild, so they can be eaten raw in salads like radishes and other vegetables. Turnip tops are an excellent source of vitamin K, vitamin A (in the form of beta-carotene), vitamin C, vitamin E, vitamin B6, folate, copper, manganese, potassium, dietary fiber, calcium etc.⁸ The turnip leaves were used traditionally for stomachic and as diuretic, in hemorrhage, tumors, carcinoma, fever and seed oil is used for fever, bronchitis, rheumatism. Turnip tops or turnip greens which many people not preferred to eat, is rich

source of beta-carotene that can relieve rheumatoid arthritis and other degenerative disorders. Turnip tops contain large amounts of lutein, which has been shown to help prevent cataracts, heart disease, stroke, various types of cancer specially lung and breast cancer. It has strong antioxidant, anti-inflammatory & antimicrobial activities.⁹

2. Material and Methods

The leaves of plant *Brassica rapa* L. was collected from the local dealer of medicinal herbs. Plant was authenticated by Kuber Impex Limited, Indore, India. The leaves of plant *Brassica rapa* washed properly to remove dirt, dried 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.

2.1. Preparation of extracts

1. A. *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 set up were 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 colored extract. The extract was concentrated under high vacuum and completely dried in a desiccator¹⁰.
2. B. *Ethanolic extract*: The leaves of 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 hrs. with methanol (350 ml.) as solvent. The extract was concentrated to a semi-solid mass under vacuum and completely dried in a desiccator.¹⁰

2.2. Percentage yield of both extracts

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

Percentage yield= (weight of extract in gram/weight of drug powder in gram) x100

2.3. Preliminary phytochemical analysis

Aqueous and Ethanolic extracts of leaves of plant *Brassica rapa* were subjected to different chemical tests separately for the identification of various active constituents i.e. alkaloids, steroid components, flavonoids, saponins, glycosides, amino acids, proteins, phenolic compounds, fixed oil, carbohydrates.

2.4. Anti-microbial screening

2.4.1. A. Species & MTCC protocol

All microorganisms *Candida albicans* MTCC 4748, *Candida glabrata* MTCC3814 and *Candida tropicalis* MTCC9038, were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. The media used for the activation of microorganisms were as per MTCC protocol. The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India.¹¹

2.5. Revival of cultures

2.5.1. Active cultures

Subculture the organism immediately to recommended medium and incubate at recommended temperature and at aerobic/anaerobic conditions as specified in the information sheet. Information about growth medium and conditions about each culture is also available in the MTCC catalogue of strains. Loop full of microorganisms were inoculated into nutrient broth and incubated at $35 \pm 2^\circ\text{C}$ for 24 h. The turbidity of the resulting suspensions was diluted with nutrient broth to obtain a transmittance of 25.0% at 580 nm. That percentage was found spectrophotometrically comparable to one McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0×10^8 CFU/ml. The Double beam UV-Visible Spectrophotometer 1700 (SHIMADZU Japan) was used to adjust the transmittance of working suspensions.^{11–13}

2.5.2. Freeze – dried cultures

- Care should be taken in opening the ampoule as the contents are in a vacuum.
- Mark on the ampoule near the middle of the cotton wool with a sharp file.
- Disinfect the surface around the mark with alcohol.
- Wrap thick cotton wool (or gauge) around the ampoule and break at the marked area.
- Gently remove the pointed top of the ampoule. Snap opening will draw the cotton plug to one end; hasty opening will release fine particles of dried organisms into the air of the laboratory.
- Carefully remove the cotton plug and add about 0.3 to 0.4 ml of specified medium to make a suspension of the culture. Avoid frothing or creating aerosols. For fungal cultures, suspension should be made in about 0.4 ml sterile water and let it stand for 20 minutes before transferring on to solid medium.
- Streak a few drops of the suspension to recommended liquid medium (solidified with agar) in a Petri plate. [Optional: Rest of the suspension may be transferred to 5 ml of recommended liquid medium in a test tube].

- Incubate at appropriate temperature and under conditions recommended for the culture.
- If all the instructions are strictly followed, growth of the culture should be visible within a few days.
- All the remains in the original ampoule should be sterilized before discarding. Common microbiological and safety procedures should be followed while handling cultures. All cultures should be handled carefully taking proper and established safety precautions.¹⁴

MICROBIAL TYPE CULTURE COLLECTION AND GENE BANK (MTCC) Instructions for opening the freeze-dried ampoule

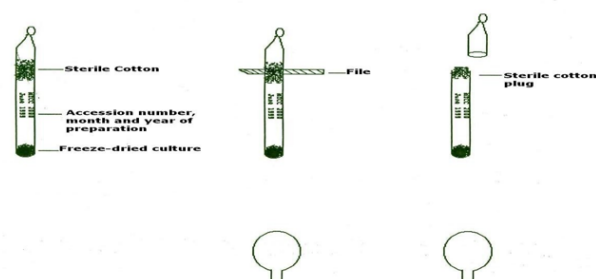


Fig. 1: Instructions for opening the freeze-dried ampoule

2.6. Preparation of nutrient agar media

Peptone -1g, Beef extract-1g, Sodium chloride -0.5 g, Agar -2 g and Distilled water 100ml was used for the preparation of media. PH was adjusted to 7.4-7.6. The media was sterilized by autoclaving at 15 lbs pressure at 125 0 C. The stock solutions of whole plant *Brassica rapa* extracts were prepared in separate at the concentrations of 100 mg/ml in water for injection; further concentration (50mg/ml) were prepared from stock solutions by serial dilution method. Standard antibiotic amino glycosides Gentamicin /Fluconazole injections were purchased from pharmacy and used at specific dose level (10mcg/ml).¹⁵

The antifungal activity of plant *Brassica rapa* was evaluated by Agar well diffusion method.¹⁶ Nutrient agar media was poured in to sterilized petridishes to give a depth of 3to 4 mm under aseptic condition & prepared petridishes were stored in incubator for 24

h. The standardized suspensions of selected bacterial strain were spread on the surface of the agar media of the petridish under aseptic condition. By using stainless steel borer around 5mm diameter cup were bored and residue from the bored area was removed, in each Petridish four cups were bored ,one for standard antibiotic ,one for test sample, one for solvent blank, Standard antibiotic (corresponding to microorganisms) and test solutions were serially diluted ,to each cup 100 μ l of serially diluted antibiotic solution , test solution, and solvent used for dilution were added separately under aseptic condition.

These Petridishes were measured, the zone inhibitions of sample were compared standard zone of inhibition around the cup measured, the zones of inhibition of sample were compared with standard zone of inhibition of standard antibiotic used. Sterilized 100ml of 0.9% saline was also prepared and sterilized by autoclaving at 15 lbs pr 1210c for 15 min.

The subcultured organisms slant was shaken with 10 ml saline so that the suspension of organism was obtained. This suspension of the organisms was transferred to sterilized saline solution & uniform suspension was prepared by shaking. The experiment was carried out under aseptic conditions in a laminar flow area throughout. Approximately 0.6

ml of the nutrient broth suspensions of the test organisms were added to 60 ml of sterile nutrient agar which had been cooled to 45°C, mixed well and poured into sterile plates.

The agar was allowed to solidify; six wells (cups) were punched per plate using a 8.5 millimeters diameter sterile cork borer (separate borer for each organism) to ensure proper distribution of wells in the periphery with one well in the center. Agar plugs were removed and 50 μ l test sample of each concentration poured in the corresponding marked wells by micropipettes.

Triplicate plates of each organism were prepared. The plates were left at room temperature for 2 h. to allow diffusion of the samples and incubated face upward, at corresponding temperatures of each organism, for 24 h. The diameters of the zones of inhibition were measured to the nearest millimeter (the cup size also being included).^{17–19}

3. Results & Discussion

According to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants.^{20,21} At least 7,000 medical compounds in the modern pharmacopoeia are derived from plants.²² Numerous herbal plants & shrubs have antibacterial activity and extracts of these plants are used for preparation of modern medicines.²³ *Brassica rapa* plant and its leaves were commonly used in traditional system of medicines.

A decoction of the leaves or stems of *Brassica rapa* is used in the treatment of cancer. It has strong antioxidant, anti-inflammatory & antimicrobial activities. It is effective against aphids, red spider mites and flies.²⁴

Brassica rapa, antifungal activity did not performed earlier on the following strains so in the present study it was decided to evaluate its aqueous and ethanol extracts antifungal activity by Agar well diffusion method (cup plate/ cylinder/ pits method) on the strains i.e. *Candida albicans* MTCC4748, *Candida glabrata* MTCC3814, and *Candida tropicalis* MTCC9038 in-vitro²⁶. Results revealed that Ethanolic extract of *Brassica rapa* showed considerably high antifungal activities against selected microorganisms than aqueous extract.

The media used for the activation of microorganisms were as per MTCC protocol. Antimicrobial activity of *Brassica rapa* leaves aqueous and ethanolic extracts at the concentrations of 50 mg/ml & 100mg/ml were tested against all microorganisms respectively. Standard antifungal Fluconazole (10 μ g/ml) was used against all microorganisms. The ethanol extract of *Brassica rapa* showed the most remarkable activity due to its rich chemical constituents. The percentage Yield of Various Extracts of *Brassica rapa* was calculated.

Present research reported the presence of phenolic compounds, glycosides, tannins, carbohydrates, proteins, amino acids, tannins, reducing suger, non-reducing suger and inorganic compounds such as calcium, magnesium, iron, carbonate & sulphates by preliminary phytochemical analysis. Plants containing phenolic compounds possess good antioxidant properties. Therefore *Brassica rapa* may also possess activity against a panel of fungus responsible for the most common fungal diseases like many other antifungal drugs.



Fig. 2: Petri plates showing zone of inhibition



Fig. 3: Petri plates showing zone of inhibition.

4. Conclusion

In conclusion, *Brassica rapa* extracts possess a broad spectrum of activity against a panel of fungus responsible for the most common fungal diseases. These promissory extracts open the possibility of finding new clinically effective antifungal compounds. The Ethanolic and aqueous extracts of *Brassica rapa*, investigated individually for Antimicrobial activity by Agar well diffusion method (cup plate/ cylinder/pits method). The Ethanolic extract of *Brassica rapa* showed considerably high activities than aqueous extract. These results were compared with standard antifungal Fluconazole. In conclusion, although active

Table 1: Percentage yield of aqueous & ethanolic extracts.

S. No.	Name of extract	Wt. of powder (In grams)	Wt. of extract (In grams)	Percentage yield (w/w)
1.	Aqueous extract	100 gm	6.45 gm	6.45%
2.	Ethanolic extract	100 gm	5.88 gm	5.88%

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	6.45%
2.	Ethanolic extract	Brown	Powder	5.88%

Table 3: Phytochemical screening of aqueous & ethanolic extract of plant *Brassica rapa*

S. No.	Class of compound	Aqueous extract	Ethanolic extract
1.	Alkaloids	-ve	-ve
2.	Glycosides	+ve	+ve
3.	Reducing - suger	+ve	+ve
4.	Non-reducing suger	+ve	+ve
5.	Steroids	-ve	-ve
6.	Saponins	+ve	+ve
7.	Flavonoids	+ve	+ve
8.	Carbohydrates	+ve	+ve
9.	Proteins	+ve	+ve
10.	Amino acids	-ve	-ve
11.	Phenolics	+ve	+ve
12.	Inorganic compounds i.e. calcium, magnesium, sodium, iron	+ve	+ve

Table 4: Antifungal activity of Aqueous and Ethanol extracts of *Brassica rapa*

S. N.	Fungus strain	Extract	Mean diameter of zone of inhibition(in mm)		
			50mg/ml	100mg/ml	Fluconazole (10µg/ml)
1	Candida albicans	Aqueous	14	15	28
		Ethanolic	16	18	
2	Candida glabrata	Aqueous	14	16	21
		Ethanolic	17	18	
3	Candida tropicalis	Aqueous	12	15	22
		Ethanolic	17	19	

mg- miligram; ml- mililitre; µg- microgram

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

components were not isolated, but antimicrobial active plant principles were observed in the both extracts. It would be advantageous to standardize methods of extraction and in vitro testing so that the search could be more systematic and interpretation of results would be facilitated. Attention to these issues is badly needed and new era of chemotherapeutic treatment of infection by using plant-derived principles can be useful for human being in the future.

4.1. Disclosure statement

There are no conflicts of interest.

5. Acknowledgment

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6. Source of Funding

None.

7. Conflict of Interest

None.

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