# Efficacy evaluation of ethanolic extract of *Brassica nigra* as potential antimicrobial agent against selected microorganisms

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**Abstract:**

Spices are used as additives to flavour, colour or preserve food from ancient time in India. These are pungent or aromatic substances obtained from by dried seeds, fruits, roots, bark or leaves. Spices are the potential source of natural products and naturally derived compounds. These compounds show the antioxidant, antimicrobial properties. The aim of this study is to evaluate the antimicrobial properties of *Brassica nigra*. In this study, antimicrobial activities of 70% ethanolic seed extracts of *Brassica nigra* were evaluated against different bacterial strains (*E.coli*, *Pseudomonas aeruginosa*, *Candida albicans*) by agar well diffusion method & MIC determination by broth dilution method. The extract showed a broad spectrum of antimicrobial activities by inhibiting the growth of respective microorganism in Agar well diffusion assay. The present study supports the immense medicinal properties of *Brassica nigra.* It may be helpful for future researches in area of new drug development of herbal origins.

**Keywords:** *Brassica nigra, Antibacterial; MIC; broad spectrum; medicinal properties*

## Introduction:

The World Health Organization (WHO) reported that about 80% of the world's population depends primarily on traditional medicine that mainly involves the use of plant extracts [1]. There are 2600 plant species of which more than 700 are noted for their uses as medicinal herbs [2]. In folk medicine, medicinal herbs and plant products were used in treating a wide spectrum of infections and other diseases. [3,4] Today, a great number of different medicinal tea and other plant products are available in market (including cosmetics and pharmaceuticals), which contains biologically active substances. In recent years, there has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as in developing countries, because plant-derived drugs have reported to be safe and without side-effects. A survey of literature reveals that there are many essential oils which possesses antifungal activity [5]. Satia-Abouta et al [6] in 2002 reported that the cuisines of Asia, Southeast Asia, and the Mediterranean are perceived by many to be healthier than the typical Western diet.

The traditional use of plants as medicines provide the basis for indicating which essential oils and plant oils may be useful for specific medical conditions. Historically, many plant oils and extracts, such as tea tree, myrrh, clove, Ajwain and *Asafoetida* (Heing) have been used as topical antiseptics and have antimicrobial properties [7,8,9]. It is important to investigate scientifically

those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [10]. Also, the resurgence of interest in natural therapies and increasing consumer demand for effective, safe, natural products means that quantitative data on plant oils and extracts are required.

*Brassica nigra (L)* (Black mustard) is an annual, erect, 2-7 degree high, high freely and widely branching, pubscent or glabrate. Lower leaves slender petioled, deeply pinnatifid, with 1 terminal large lobe and 2-4 smaller lateral ones, dentate all around; upper leaves shorter-petioled or sessile, pinnatifid or dentate, the uppermost reduced to lanceolateor oblong entire blades; flowers bright yellow, 3”-5” broad; pedicels slender , appressed,2” long in fruit; pods narrowly linear, 4 sided, 5”-7”long, ½ wide, a pressed against the stem and forming very narrow racemes; beak slender,1”-2”long;seeds dark brown [11].

It is cultivated in the states of Assam, Bihar, Gujarat, Haryana, Himachal Pradesh, Jammu & Kashmir, Madhya Pradesh, Orrissa, Punjab, Rajasthan, Uttar Pradesh etc.

The antimicrobial activity of *Brassica nigra* against different micro-organisms has been observed in few studies so it was thought worthwhile to assess the anti-microbial activity of *Brassica nigra* spice (Ethanolic Extract of seed) against different pathogens.

## Materials & Methods:

### Collection of sample:

The seeds of spice *Brassica nigra* (black mustard) were collected from local Gwalior market.

### Micro-organisms Used:

*1. Staphylococcus aureus*
*2. Escherichia coli*
*3. Candida albicans*

These micro-organisms were isolated from clinical samples by using standard microbiological procedures. Identification was done morphologically and biochemically following the standard of Bergey’s Manual of Determinative Bacteriology.

### Preparation of extracts:

The seed of black mustard were dried and broken into small pieces (powder) under sterile conditions, and 10 g of black mustard seed powder were dissolve with 100 ml of 70% ethanol, (Merck) and shaken at 160 rpm for 24 hours at ambient temperature. The mixtures were then filtered. The filtrates were evaporated and stored at 4°C for further use. Stock solutions of crude extracts were prepared by diluting the dried extracts with DMSO solution, to obtain a final concentration of 200 mg/ml.

### Collection of microorganism:

The bacterial strains such as *E. coli* , *Staphylococcus aureus and* Fungal strain *Candida albicans* were isolated from clinical samples and used for antimicrobial assay. Bacterial strain were grown in LB medium contains in water at pH 7.2 and incubated at $37^{o}$ C for overnight. Whereas fungal

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strain *Candida albicans* were grown in PDB medium containing potato infusion and dextrose at pH 5.6 incubated at 25$^{o}$ C for five day. The entire microorganism subcultured in 30 days.

## Screening for antimicrobial activities:

The antibacterial and antifungal activity studies were carried out by agar well diffusion method as given by Irobi et al [12]. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Staphylococcus aureus,* and *Escherichia coli* were spread over the nutrient agar plates by using separate sterile spreader. Then the fungal test organism like *Candida albicans* were spread over the potato dextrose agar plates. Wells were then bored into the plates of seeded organisms using sterile cork borer of 6 mm in diameter then 50µl extracts of concentration 200mg/ml were placed on the wells in different plates with control plates with solvent . All bacterial plates were incubated at 37°C for 24 hrs and fungal plates at 25°C for five days. The diameter of the minimum zone was measured in mm.

## Minimum inhibitory concentration (MIC):

The extracts which showed antimicrobial activity in agar well assay will be subjected to MIC assay. The antimicrobial MIC studies were carried out by broth dilution method.

## 6.Results:

In the present investigation the *Brassica nigra* (black mustard) spice were collected from local Gwalior market. The 70% ethanol solvent was used for the extraction of active agents from black mustard spices. Extracts were used for investigation of the antimicrobial potential. The results of *in vitro* antimicrobial activity of black mustard spices against pathogens are presented below:

Figure 1 shows the antimicrobial activity of 70% ethanol extract of Black mustard (*Brassica nigra*). The 70% ethanol extracts showed antibacterial & antifungal activity against *E.coli, S. aureus* & *C.albicans* respectively. The maximum zone of inhibition showed against *C.albicans* (30 mm) Whereas *S.aureus* (25mm) & *E.coli* (20.5mm) showed moderate activity.

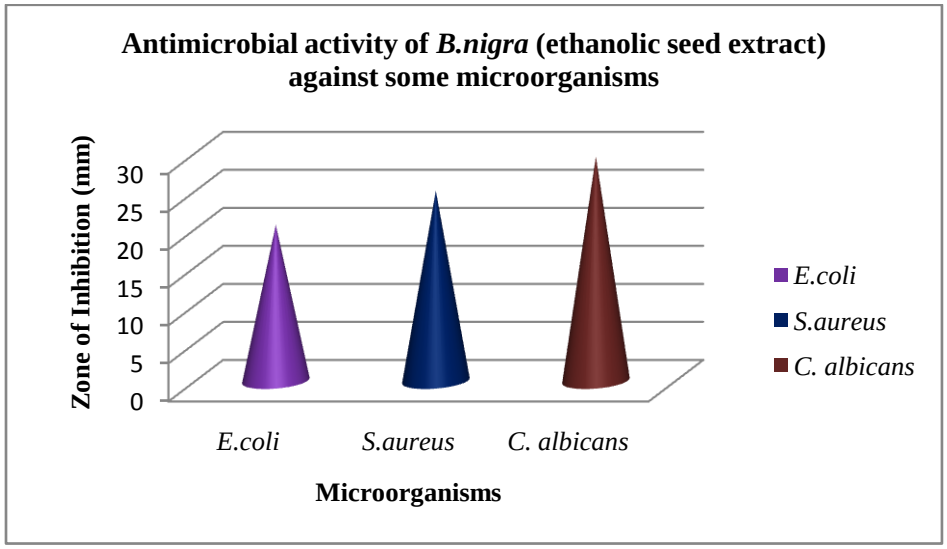


**Figure 1. Antimicrobial activity of 70% ethanolic extract of *B.nigra* against microorganisms**

The extract which showed antibacterial activity in agar well assay will be subjected to MIC assay. The antimicrobial MIC studies were carried out by broth dilution method.
Figure 2 shows the results of MIC assay, it indicates that the MIC value of crude extract against *E.Coli* was10 mg and for *S aureus* & *C. albicans* it was 8 mg.

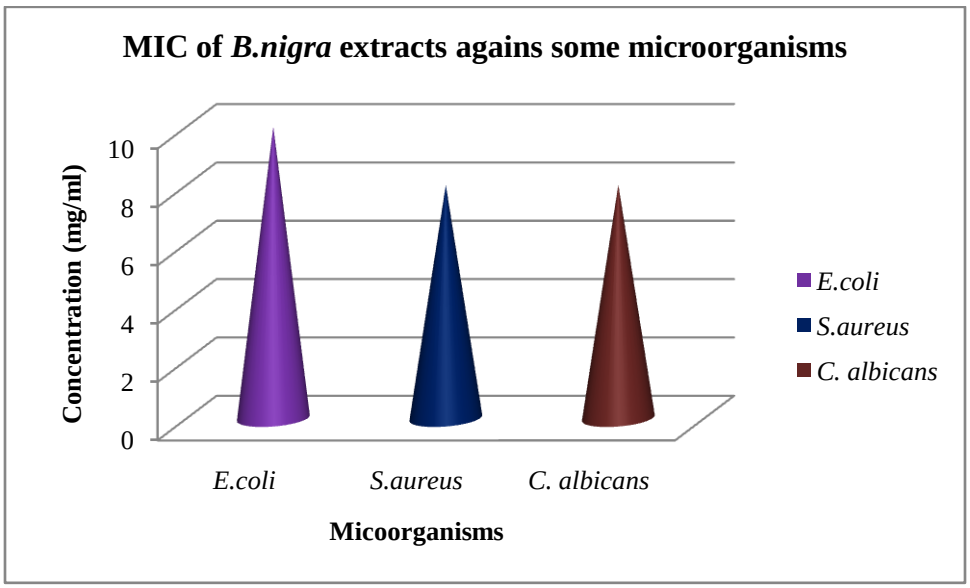


**Figure 1. Minimum Inhibitory concentration of extract of *B.nigra* against microorganisms**

## Discussion:

In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases [13,14]. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics [15]. Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens [16].

There is widespread use of plants as ethnomedicines. After the pathogen attack, some plants may produce certain compounds such as secondary metabolites being known as phytoalexins. These compounds have shown remarkable in vitro antibacterial activity against potential pathogens [17]. There is immense need of antimicrobial drugs developed from natural sources as plant derived products are safe in comparison to chemically synthesized products [18].

Brassica family is a commonly consumed vegetable and a valuable source of glucosinolates, polyphenols and flavonoids. On hydrolysis by enzyme myrosinase, glucosinolates can produce d-glucose, sulfate, isothiocyanates, thiocyanates and nitriles [19]. Isothiocyanates have the largest bactericidal, bacteriostatic and antifungal potential among these bioactive products [20]. The medicinal properties of cruciferous vegetables can be related to the activity of isothiocyanates against several human pathogens.

In addition phytochemicals investigation of Brassica family indicate oleic acid, phenolics, carotenoids, selenium, glucosinolates and vitamin C present in brassicaceae mustard seed [21,22].Mustard leaves have been reported to possess many bioactive substances and antioxidant properties [23]. Earlier reviews indicated the biocidal, bio-herbicidal, antioxidant, anticancer activities of glucosinolates and their products from Brassicaceae [24,25].

Mamta Bhatia et al [26] in 2012, reported that the *B. nigra*, at different concentration levels, displayed growth inhibitory effect towards three bacterial strains namely *B. cereus*, *P. aeruginosa* and *S. sonnei*. Moreover Senanayake et al [27] reported the activity against *Aspergillus niger & Rhizopus sp.* whereas Alyaa Sabti jasim [28] in 2012 showed the activity of oils extracts of *Brassica nigra* seeds against pathogenic oral micro flora.

The previous studies on ethanolic extract from *Brassica nigra* have shown that it has potent antibacterial activities. The extract was found to be active against *Streptococcus pyogenes* which is the cause of many important human diseases, ranging from mild superficial skin infections to life-threatening systemic diseases. The extracts were also active against *Salmonella typhimurium* which is the cause of gastroenteritis in humans and other mammals [29].

The results of our study indicate the effect of 70% ethanol extract of *Brassica nigra* against pathogens. I*n vitro* antimicrobial activity of black mustard spices against pathogens showed significant results in our study. The ethanolic extract showed broad spectrum activity since it is effective against both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria & *C. albicans*. Our results also extend and strengthen the previous research [29].

## Conclusion:

The results of the present study highlight the fact that 70% ethanol extract possessed good antimicrobial activity. This may be due to the presence of polar and non-polar antimicrobial principles in the extract. *Brassica nigra* contains phenol, falvonoids, alkaloids, sterols, terpenes etc., which may be responsible for the activity against microorganisms. Further study in this area may be helpful for finding of new principle compound which may be helpful in new drug development research.

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