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# IN VITRO ANTIMICROBIAL ACTIVITY OF BRASSICA PLANT'S SEED

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**ABSTRACT**

**Background:** Biological activity is a mirror telling the valuable or unhelpful possessions of crude drug or living substance. For the evaluation of biological activity of natural compound bioassays are performed. In this research antibacterial and antifungal activity of crude ethanolic and ethanolic extract was screened through bioassays.

**Objective:** The antimicrobial activity of the ethanol and methanol extracts of the plant's Brassica seed was investigated to verify its medicinal use in the treatment of microbial infections.

**Method:** The antimicrobial activity of the ethanol extract was tested against clinical isolates of some multidrug-resistant bacteria using the agar well diffusion method while antifungal activity is screened through agar tube dilution method. Commercial antibiotics were used as positive reference standards to determine the sensitivity of the clinical isolates.

**Results:** Both the ethanolic and methanolic extracts exhibited considerable antibacterial and antifungal activity. Maximum antibacterial activity was shown by methanolic extract of tested plant against Escherichia coli (17 mm), Enterobacter aerogenes (12mm) and Bacillus subtilis (11mm) inhibition zones at the 15 mg/ml concentration while the slandered antibiotics Doxycycline (DOX) showed 18mm inhibition zone. The Minimum inhibitory concentration (MIC) was found 1 mg/ml. The methanolic extract showed a highly significant results inhibition towards the tested fungal microorganisms compared to the ethanolic extract. Maximum antifungal activity was exhibited by the methanolic crude extract against A. fumigates and A. nigar and found to be 85.2% and 79%, respectively.

**Keywords: Antimicrobial activity,** Antibacterial, Antifungal, Ethanolic extract, Natural drugs, Traditional remedy, MIC.

**INTRODUCTION**

Medicinal plants nowadays have worth and lacking consequents as chemical medicines attracted in world. Plant have a great potential for producing new medicine of great benefit to mankind and remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicine for the treatment of common illnesses [1, 2]. Traditional remedies have a long-standing history in many locations in the Yemen and the Arabian Peninsula and continue to provide useful and applicable tools for treating patients [3].

In the recent days, one can observe an international drift of significance in the long-established structure of medicines [4]. Evaluation of therapeutic herbs has turn into a latent basis of biodynamic substances of curative value. Ethnomedicinal studies have been converted into more and more precious in the progress of fitness and protection programs in various parts of the globe [5]. Regardless of the outstanding progress in artificial organic chemistry of the twentieth century, more than 25% of prescribed medicines in developed countries have been derived from plants [6].
Microorganisms have enlarged resistance to several antibiotics and this has produced immense clinical difficulty in the treatment of infectious disorders [7]. The extend in resistance of microorganisms due to indiscriminate benefit of commercial antimicrobial drugs encouraged scientists to investigation for modern antimicrobial substances from several sources including medicinal plants [8]. Another driving factor for the renovated attention in the past thirty years has been the speedy ratio of plants species extinction. Over 12,000 plant secondary metabolites of antimicrobial significance have been isolated. These compounds drop in one of the superior groups of compounds such as alkaloids, tannins, flavonoids, phenols, terpenoids, quinines and other varieties [9]. This position forced to investigation for modern antimicrobial substances. Therefore, there is a demand to advance substitute antimicrobial drugs for the cure of contagious ailments from medicinal plants.

In the last two decades, Brassica Plant's seed has gained increasing attention from scientists and pharmaceutical companies to better define its medical effects and identify the constituents responsible for these effects. Consequently, several studies have been reported on the composition and its effectiveness in treating patients with various clinical complications such as diabetes, allergic reactions, dermatology [10], sickle cell anaemia [11], treatment of increased bone resorption [12], nasal and sinus dysfunction [13], treatment of angiogenesis and metastasis [14-15] and other [16].

The term "Brassica seed" or "Brassica seeds" refers to one or more whole seeds of Brassica genus within the family Brassicaceae (also known as Cruciferae family). The Brassica genus includes the particularly mustard group within the Brassica genus, Comprising B. Alba, B. Hirta, B. Juncea and B. Nigra. The genus and species-names provided above comply with the International Code of Plant Nomenclature.

To evaluate the biological antimicrobial activities of the present research work was attempted to evaluate the antibacterial and antifungal activity of Brassica plants' seed against five bacterial and two fungal strains. Ethanol and methanol were used as the solvent for the crude extraction of plant.

**Material and methods**

**Plant Materials**

The Brassica plant's seed were collected from different localities of Yemen and identified at the Pharmacognosy Department, Faculty of Pharmacy, Sana'a University. Part of the identification of the investigated plants was done by Dr. A. Wadieh, Department of Botany, Naser College, in Lahj Governorate, University of Aden, and Republic of Yemen. Voucher specimens were deposited at the Pharmacognosy Department, Faculty of Medicine, University of Science and Technology. Sana'a, Republic of Yemen. Voucher number allotted to Brassica seed is 123173; 38.

**Extraction of Plant material**
**Ethanol extract**

The method of extraction using ethanol was followed as stated by Randhir et al (2004). Two hundred and fifty grams of ground samples were soaked in 500 ml of 99.9% ethanol and homogenized in an electric blender for approximately 5 minute and incubated at room temperature for 7 days. The mixture was then filtered twice by Whatman No. # 1 filter paper using vacuum and then the solvent (ethanol) was allowed to evaporate at room temperature and then re-extracted with 250 ml of 99.9% ethanol by using a shaking water-bath at 70$^{o}$C for 6 hrs. The mixture was then filtered and ethanol was allowed to evaporate at room temperature. The residue (extract) was collected and kept at 4ºC. Semisolid crude extract was further evaporated in air and final extract was stored in refrigerator at 4$^{o}$C for further analysis. Dissolving 15 mg plant extract in 10 ml DMSO made stock solution. This stock solution was used for further dilutions i.e. 12.50mg/ml, 10 mg/ml and 5 mg/ml and 3 mg/ml etc. Doxycycline (DOX) was used as slandered antibiotic; dissolving 0.3 mg in 1 ml DMSO made solution.

**Methanolic extract**

To obtain a methanolic extract of Brassica seed, ethanol extraction protocol (as above) was followed with methanol replacing ethanol.

**Test Organisms**

Common pathogenic bacteria were used to test the antimicrobial activity of the extract. Tested microorganisms were Bacillus subtilis and Staphylococcus aureus (Gram positive), Vibrio cholera, Enterobacter aerogenes, Escherichia coli (Gram negative) which were routinely isolated, identified Rxn [18] and tested for antibiotic susceptibility in the microbiology diagnostic laboratory, Sultan Qaboos University Hospital (SQUH).

**Preparation of bacteria isolates:**

According to Kirby-Bauer [19] the organisms should be in the log phase of growth in order for the results to be valid. Therefore, fresh cultures (3 to 4 hour cultures) were used. All Gram-negative bacteria were sub-cultured on CLED media and incubated at 37ºC for 24hrs. All Gram-positive bacteria

were sub-cultured on blood agar and incubated at 37ºC for 24hrs. Each of the bacterial isolate used in this study was preserved in 2 ml of human blood at -80 ºC till ready to use.

**Bacterial susceptibility testing:**

Antibacterial activity was screened by disc diffusion method (Anonymous, 1996). Nutrient agar medium was prepared by dissolving 2.3g agar (MERCK) in 100ml of distilled water. Instrument was autoclaved along with the media and transferred to the laminar flow hood. Media was allowed to cool then poured in petri plates and allowed to solidify it. Bacterial strains were streaked in each plate and then wells were created by cork borer. The crude extract 100µl was inoculated in each well and these plates were incubated for 24 h to obtain the inhibition zones.

**Antifungal activity**

Antifungal activity was screened by following the agar tube dilution method (Choudhary et al.,1995). Aspergillus nigar and Aspergillus flavus were used as fungal strains. Test sample was prepared by dissolving 20 mg plant extract in 10 ml DMSO. 6.5g of sabouraud dextrose agar (MERCK) was dissolved in 100 ml of distilled water for media and pH was adjusted as 5.6 then autoclaved. Poured this agar in test tubes and allowed to cool in slanting positions. Each test tube was inoculated by fungal strain and positive control was adjusted by using terbinafin. Slants were incubated at 28$^{o}$C for seven days. Reading was calculated in mm and percentage inhibition was calculated by

$$\%\text{Inhibition of fungal growth } = 100\ [\text{Linear growth in test tubes (mm)}/\ \text{Linear growth in control (mm)}]\ \times 100$$

**Results**

Results of antibacterial activity are presented in figures 1 & 2. Results showed that the ethanolic extract exhibited almost significant activity against all the tested microbes. Maximum antibacterial activity was shown against Escherichia coli i.e. 17 mm inhibition zone at the 15mg/ml concentration while the slandered antibiotics DOX showed 18 mm inhibition zone. The MIC was found to be 1mg/ml. The Enterobacter aerogenes and Bacillus subtilis also exhibited maximum activity for ethanolic extract (12 and 11mm, respectively). Moreover, The ethanolic crude extract of tested plant's seed showed maximum activity against Vibrio cholera i.e. 8 mm inhibition zone at 15mg/ml concentration while its MIC was 5 mg/ml that is high. Against other tested bacterial strains ethanolic extract showed considerable antibacterial activity. Hence ethanolic extract was more potent against bacteria compared to the methanolic crude extract.

Results of antifungal activity are presented in the table 1. Both extracts i.e., the ethanolic and methanolic extracts, showed significant inhibition activity against fungal strains. However, the ethanolic extract of Brassica seed showed significant affects against both the fungal strains while the ethanolic extract showed a little bit more activity compared to the methanolic extract. Maximum antifungal activity was exhibited by the methanolic crude extract against A. fumigates and A. nigar and i.e., 85.2% and 79%, respectively. The methanolic extract was more potent against fungal strains compared to the ethanolic extracts.

**DISCUSSION**

Plant substances continue to serve as viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, enormous number of plants has been widely used for the treatment of various diseases due to their antibacterial activity. Therefore, screening of medically important plants for their antimicrobial activity is an important aspect as an alternative potential for therapeutic use. The first step towards this goal is the in vitro antibacterial activity assay.

Although many reports are available on the antiviral, antibacterial, antifungal, anthelmintic and anti-inflammatory properties of plants [22] not many reports are available on the exploitation of such plants' properties for developing commercial formulations for applications in crop protection.

Results obtained from this study revealed that there is more potential in the activity against the fungal strains compared to the bacterial strains. The Brassica seed has number of implications in the traditional medicines. This study was conducted in the past scenario of this valuable medicinal plant and the results of study supported the traditional values of this plant. Two extracts were screened i.e., ethanolic and methanolic. The methanolic extract was found more potent as antifungal agent compared to that of the ethanolic plant extract. In the contrary it is interesting to find out that the two solvents used here showed different magnitude towards different microbes i.e., the metholoic extracts showed a significant inhibitory activity towards the fungal species used, whereas the ethanolic extract showed its significant effects on the bacteria side.

It is common observation of scientists that the maximum bacterial infections are not being well treated by the available antibiotics. Microbes are now more resistant against the common practiced antibiotics (Mahmood et al., 2012). Now, it is dare need to discover new antibiotics that should be safe and less expensive and more progressive against pathogenic organisms. This study reports the potential of Brassica seed to be a good antibiotic in tested plant. This plant can be used for further analyses to screen its active constituents.

**CONCLUSION**

The results of the present investigation clearly indicate that the antibacterial activity vary with the concentration of the plant material used. Thus, the study ascertains the value of Saussurea lappa rootas antibiotic due to that it may possess a new source of antimicrobial agents with possibly novel mechanisms of action. Systematic screening of such molecules, i.e., antibacterial agents, may result in the discovery of novel active compounds.

It is suggested that there should be a more comprehensive research on Brassica seed active constituents as it has ability to kill pathogenic microbes. Pharmaceutical companies should focus on the natural resources of medicines that should be more reliable and safe in use.

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**Conflict of interest**

The authors declare no conflict of interest with other any person, institution or commercial establishment in the execution of this project.

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| Test plant extract | *Fungal strains used | *L.G.C. (mm) | *L.G.T. (mm) | % Inhibition |
|---|---|---|---|---|
| Methanolic extract | A.niger | 108 | 19 | 79% |
| | A. fumigates | 84 | 14 | 85% |
| Ethanolic extract | A.niger | 103 | 39 | 37% |
| | A. fumigates | 96 | 35 | 33.2% |

*Aspergillus niger – A. niger and Aspergillus fumigates - A. fumigates
L.G.C. (mm) = Linear Growth in Control (millimeter), L.G.T. (mm) = Linear Growth in Test (millimeter)

**Table 1:** Antifungal activity of ethanolic and ethanolic Brassica seed against Aspergillus niger and Aspergillus flavus

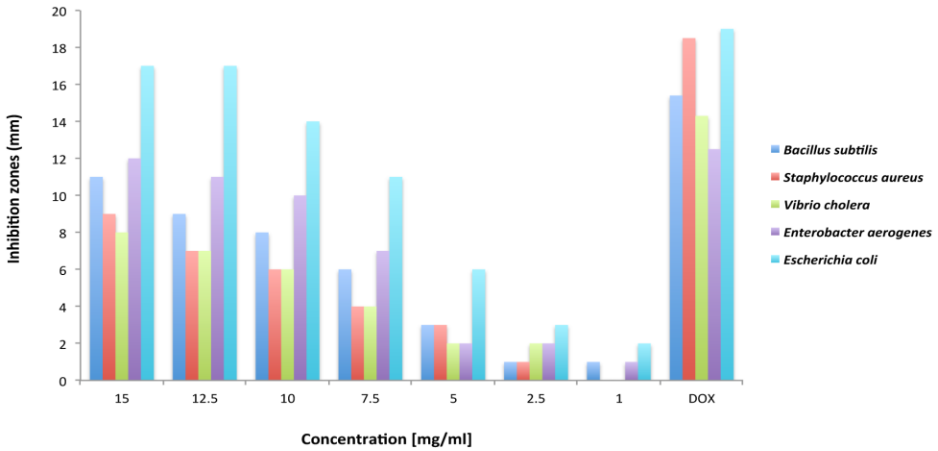


**Figure 1:** Antimicrobial activity of crude ethanolic extract of Brassica seed.

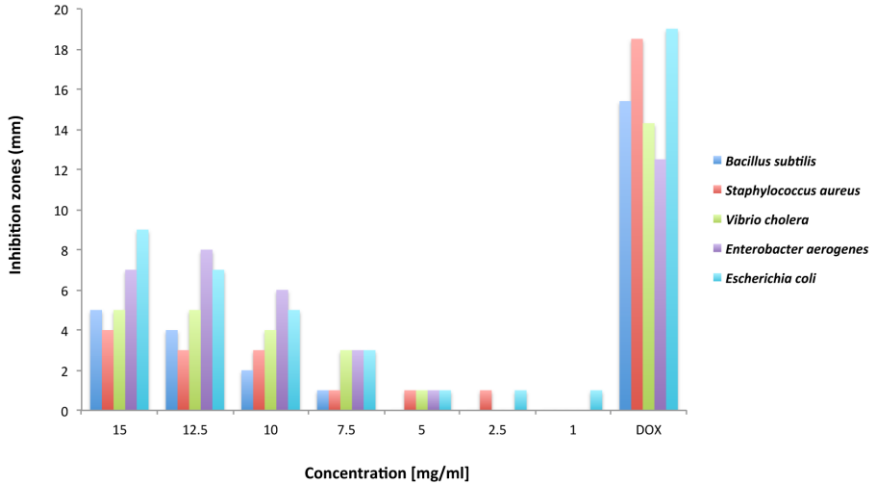


**Figure 2:** Antimicrobial activity of crude methanolic extract of Brassica seed.