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**Research Paper**

# Antibacterial activity of oil extracts of Black Mustard (*Brassica nigra*) seeds against bacteria isolated from fresh juice in selected areas of Axum town

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**ABSTRACT**

Plants are very useful and utilized as medicine due to their medicinal properties. Medicinal plants are great significance to the health of individuals and communities. To study antimicrobial activity of oil extracts of *Brassica nigra* seed against fresh juice pathogens. Isolation and identification of bacteria using selective and differential media. Oil extraction *B. nigra* was prepared to inhibit the fresh juice pathogens. Pathogens that was found in the fresh juice such as *B. cereus*, *S. aureus* and *Salmonella*. The inhibition zone of oil extract *B. nigra* by ethanol was dominated on oil extract *B. nigra* by acetone and positive control Ampicillin. Generally *B. nigra* is an effective medical seed by inhibiting the activity of the microbes.

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**1. Introduction**

Nature has wide source of medical agents and many drugs are isolated from nature. Plant based medicinal plants would be the best source to obtain variety of drugs. Traditionally man uses variety of drugs to cure disease. For example uses of turmeric (*Curcuma longa*) to treat flu, cough and nasal infection. With industrial development, chemical products are widely used to treat disease. But these chemicals products have some side effects. But people aware about these side effects and are now turned to traditional biological sources to cure disease. People try to reduce the use of chemical products/drugs and searching for natural products, which have no side effects compared to chemical drugs (Jadhve *et al.*, 2013).

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 (Low *et al.*, 2002). There are 2600 plant species of which more than 700 are noted for their uses as medicinal herbs (Ali-Shtayeh and Ghdeib, 1999).

Plant products are available in the market (including cosmetics and pharmacies), which contains biological active substance. In recent years they has been a gradual revival of interest in the use of medicinal and aromatic plants in developed as well as developing countries, because plant derived drugs have reported to be safe and without side effects. The traditional use of plants as medicinal provide the bases for indicating which essential oils and plant oils may be use full for specific medical condition (Tomar and Shrivastava, 2004).

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Medicinal plants are great significance to the health of individuals and communities (Hill, 1952). 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 (Shrivastava *et al.*, 2012).

*B. nigra* (black mustard) is an annual herbaceous plant in the Brassicaceae (the cabbage and mustard family) that organized in the Middle East and is now widely cultivated as a primary source of the mustard seeds used in making condiment sause, table mustard. Juices are often consumed for their perceived health benefits. For example, orange juice is rich in vitamin “C”, ferric acid, potassium, is an excellent source of bioavailable anti oxidant phyto chemicals and significantly improves blood lipid profiles in people affected with hyper cholesterol (Kurowska, 2000).

This research is mainly obtained to evaluate the anti bacterial activity of *B. nigra* against that contaminant found in the fresh fruit juice of some selected areas of Aksum.

Infectious diseases are still a major health issue, especially in developing countries, leading to the death of millions of people, despite enormous improvement in health care systems. Attention is now being switch over to plants as they may present a new source of anti bacterial, anti fungal and anti viral agents (Tahirazamir *et al.*, 2013).

The seed of *Brassica nigra* has been used to a spice and medicine since ancient times in the Middle East, India and Greece. Finely ground seeds of black mustard provide mustard meal, a neutral odor less powder which stores well if kept dry. This meal, mixed with vinegar, is the pungent condiment or table mustard known as French and England mustard (Fenw, 1982).

## 2. Materials and Methods

### 2.1 Description of the study area

The study was conducted in Axum town, central zone of Tigray. Axum is found at a distance of 1024 km from Addis Ababa, and 241 km from Mekelle. The annual rain fall of the area is about 400-700 mm and the town has annual mean temperature of 25<sup>o</sup>c. In absolute term, it is located at an approximate, geographical coordinates f 14<sup>o</sup>72’08” North latitude and 33<sup>o</sup>44’04” East.

### 2.2 Sample size and sampling technique

In Axum town there are about 20 juice houses. 10% of juice houses were selected by using sample random sampling technique. So, two juice houses were involved for the study.

### 2.3 Preparation of seed extracts

The seeds were properly washing and dried in hot air over for 2-3 days. These were grounded to a fine powder using a clean mortar and pestle and the powder was stored in a clean bottle at room temperature in dark place. The dried and powdered seed samples (20g) were weighed by using a sensitive balance and then were extracted with each 120 ml of ethanol and acetone in a separate flask. Then after, it was shaken for about 3 days. The extracts were filtered through Whatman No. 1 filter paper and then evaporated the alcohols. Each extracts were transferred to glass vials and labeled accordingly and then were stored at 4 <sup>o</sup>C until use (Shahidi Bonjar, 2004).

### 2.5 Antibacterial Activity Testing of Mustard (*B. nigra*) Using Paper disc diffusion Assay

The cultures were aseptically swabbed on the surface of sterile nutrient agar plates using a sterile cotton swab. Suspensions of the bacterial

isolates were made in sterile normal saline and adjusted to the 0.5 McFarland’s standard. Small volume (0.01mL) of bacterial suspensions were added to each nutrient agar plate and then evenly seeded and streaked by means of sterile swab on the agar plate surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum and finally, the rim of the agar was swabbed. Disc papers were used by immersing it in the Oil extracts of *B. nigra* seed in the plate in triplicate and the paper disc was dispensed with a dispensing apparatus (sterile pair of forceps) onto the surface of the inoculated agar plate and pressed down to ensure complete contact with the agar surface. Oil extracts and paper discs were allowed to diffuse for about 40 minutes before incubation and then the plates were incubated in an upright position at 37<sup>o</sup> C for 24 hours. After overnight incubation, the diameters of inhibition zones were measured in mm using a plastic ruler, which was held on the back of the inverted plate and the results were recorded. Antibiotic disc (Ampicillin) was served as positive control.



Fig. 1 - Oil extracted from *Brassica nigra* using Ethanol and Acetone

## 3. Results and Equations

### 3.1 Result

After 24 hour of incubation the bacteria grew in the bacillus and baird parker agar which are selective media and SS agar as the differential media. It was observed that the *S.aureus* and *B.cereus* bacteria. Also by used the bio chemical test from the SS agar we identify salmonella bacteria.

Table 1 Isolation and identification of bacteria from fresh juice mango

Place	Type of media	Status	Type of bacteria
Tele	Bacillus cereus	+	<i>B.cereus</i>
	Baired parker agar	+	<i>S.aureus</i>
	SS agar	+	<i>Salmonella</i>
Meneharia	Bacillus cereus	+	<i>B.cereus</i>
	Baired parker agar	+	<i>S.aureus</i>
	SS agar	+	<i>Salmonella</i>

NB: (+) presence, (-) absent

According to Table 1, there were a bacterial colony from samples taken from Tele and Meneharia. The identified bacteria were *B.cereus*, *S.aureus* and *Salmonella* using Bacillus cereus, Baird parker and SS agar respectively.

**Table 2 Isolation and identification of bacteria from fresh juice Avocado**

Place	Type of media	Status	Type of bacteria
Tele	Bacillus cereus agar	-	
	Baired parker agar	+	<i>S. aureus</i>
	SS agar	+	<i>Salmonella</i>
Meneharia	Bacillus cereus agar	-	
	Baired parker agar	-	
	SS agar	-	

NB: (+) presence, (-) absent

According to Table 2, there were bacterial colonies from samples taken from Tele. The identified bacteria were *S.aureus* and *Salmonella* using Baired parker and SS agar respectively.

When we added the antibacterial substance of oil extracts mustard (*B. nigra*) extracted by both ethanol and acetone solvents and after incubated 24 hrs the following inhibition zone (mm) was found in the form of table 3 and table 4.

**Table 3. The mean inhibition zone of ethanolic oil extract of *B. nigra* (mustard) against fresh juice pathogenic bacteria.**

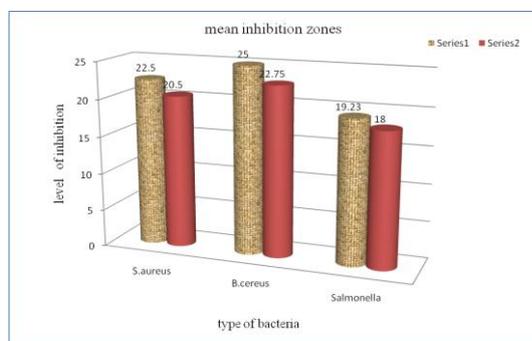
Test organisms	Inhibition zone (mm)
<i>S.aureus</i>	22.5 mm
<i>B.cereus</i>	25 mm
<i>Salmonella</i>	19.23

NB: Values were mean of triplicate determination.

**Table 4: The mean inhibition zone of acetone oil extract of *Brassica nigra* (mustard) against fresh juice pathogenic bacteria.**

Testing organism	Inhibition zone (mm)
<i>S.aurous</i>	20.5 mm
<i>B.cereus</i>	22.75 mm
<i>Salmonella</i>	18 mm

NB: Values were means of triplicate determination.



**Figure 1: comparison of the mean inhibition zone of ethanolic and acetone oil extract of *Brassica nigra* (mustard) against fresh juice pathogenic bacteria. NB: Series 1(ethanolic); series 2(acetone)**

**Table 5: Comparison of inhibition zone of ethanol and acetone oil extract of *B.nigra* (mustard) with commercially antibiotic disease against all test organisms.**

Test of organism	Solvent used for extraction	inhibition zone (mm)	
		Seed oil extraction	Positive control (Ampicillin)
<i>S.aureus</i>	Et	22.5 mm	20 mm
	Ace	20.5 mm	20 mm
<i>B.cereus</i>	Et	25 mm	22mm
	Ace	22.75 mm	22mm
<i>Salmonella</i>	Et	19.23	18.5
	Ace	18	17

Key words: Et = Ethanol; Ace = Acetone

According to Table 5, comparison of the mean inhibition zone of ethanol and acetone oil extract of *B.nigra* (mustard) with Positive control against fresh juice pathogenic bacteria. Oil extracted *B.nigra* using acetone and ethanol with positive control that tested organisms such as *S.aureus*, *B.cereus* and *Salmonella*.



**Fig. 2 - The inhibition zone of oil extracted *Brassica nigra* using Ethanol**

### 3.2 Discussion

When we compare and contrast the result of table-1 and table 2, mango juice was highly affected by the pathogens such as *S. aureus*, *B. cereus* and *Salmonella* in both Tele and Meneharia. Whereas avocado juice in Meneharia was free from *S. aureus*, *B. cereus* and *Salmonella* pathogens. But these pathogenic bacteria were present in Tele except *B.cereus*.

Mango juice was easily affected by the pathogens such as *S. aureus*, *B. cereus* and *Salmonella* than avocado juice. Both *S. aureus* and *B. cereus* were gram positive bacteria where as *salmonella* was gram negative bacteria. Gram negative bacteria were less inhibited by oil extract *B. nigra* than gram positive bacteria due to the presence of extra membrane lipopolysacride.

*S. aureus*, *B. cereus* and *Salmonella* were inhibited by the oil extract of *B.nigra*. There was more inhibition zone of oil extracted by ethanol as compared to the oil extracted by acetone. Because ethanol was high polar solvent and highly extract the active ingredient found in *B. nigra* than acetone. This indicated that as highly solvent extract increase the sensitivity of the antibacterial activity.

### 4. Conclusion

Based on the above result we can conclude that;

- *B. nigra* seed is an important for antibacterial activity.
- Mango juice was more contaminated than avocado juice in both Tele and Meneharia.
- More inhibition zone was observed in oil extracted by ethanol as compared to acetone.

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