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Antibacterial, Chromatographic, and Spectroscopic Characterization of Methanolic Extract of Brassica nigra Seeds

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Abstract

This study aims to investigate the methanol extract of Mustard (*Brassica nigra* L) seed, and to identify the chemical composition of the seed by chromatographic and spectroscopic methods based on the antimicrobial activity of the extract. Samples were taken from Khartoum local market, air dried, and cracked to be powder, in order to prepare the maceration methanol extract. The chemical properties of the Mustard (*Brassica nigra* L) Seed extracts were tested by phytochemical screening in our previous study which showed the availability of some components which were taken as an evidence for the presence of secondary metabolites. Results test of antimicrobial activity of methanol extract of seeds of *Brassica nigra,* proceeded by preliminary test using four bacteria (*Escherichia coli, Staphylococcusaureus Bacillus subtilis, Pseudomonas eruginos*). Our study showed that the methanolic extract (mac) of *Brassica nigra* have antimicrobial activity against all tested organisms. Thin Layer Chromatography (TLC) was applied using different solvents system. Intensive analytical investigations were carried out on the extract in order to identify and to isolate the effective components using column chromatography and preparative thin layer chromatography. The isolated pure fractions and analyzed by infra-red X-ray (IR) and ultra-violet (UV) spectroscopy. We recommend the benefit of medical plant such as Mustard (*Brassica nigra* L) Seed to explore possible future use of these extracts as alternative to common antibiotics and to determine their ability to enhance activities.

Keywords: Solvent extraction; Infra-red X-ray (IR); Column chromatography; Antibacterial activity

Introduction

Solvent extraction is a technique extensively utilized in both industrial applications and in the laboratory. The choice of the solvent for extraction is very important to achieve the goal of near-complete extraction of the solutes of interest. This may involve either the extraction of the analyte or the separation of specific matrix components. Extraction techniques have been widely investigated to obtain such valuable natural compounds from plants for commercialization [1-3].
Brassica species is increasing because of their nutritional value. *Brassica* foods are very nutritive, providing nutrients and health-promoting Phytochemicals such as vitamins, carotenoids, fiber, soluble sugars, minerals, glucosinolates and phenolic compounds. **Brassica** is one of the most ancient spices. It has 3 varieties namely black, brown and white/yellow. [4].
Brassica nigra (L), popularly known as black mustard, family *Brassicaceae* popularly known as black mustard has both edible and medicinal value. [5-9].

Mustard plant is highly rich in essential amino acids and protein contents. The protein is 25-30 % that making it excellent source of food used as oil in industrial and commercial purposes. Mustard oil has a special fatty acid composition, it contains about 20–28% oleic acid, 10–12% linoleic, 9.0–9.5% linoleic acid, and 30–40% erucic acid, which is indigestible for human and animal organisms. It is used in the condiment production at the large scale.

Mustard is used as medicinal plant because contains biochemical compounds that are used for the treatment of various diseases such as cancer, diabetes and inflammatory immune diseases. It is also used as a food all world the world because of its large scale sowing and production [10].

In this study 80%Methanol solvent was utilized in order to extract Mustard (*Brassica nigra* L) seeds by maceration method because it is favorable method process is carried out in isolation of natural compounds. The extract was subjected to antibacterial investigation; the active compounds were isolated and identified using various chromatographic and spectroscopic analytical techniques.

Materials and Method

Plant Samples:

The *brassica nigra* seeds were brought from Khartoum local market**.**

Micro-organisms used:

Pseudomonas aeruginosa	ATCC 27853(Gram -ve bacteria)
Escherichia coli	ATCC 25922(Gram -ve bacteria)
Bacillus subtilis	NCTC 8236(Gram +ve bacteria)
Staphylococcus aureus	ATCC 25923(Gram +ve bacteria)

Media: Nutrient Agar

Preparation of methanol extract:

The air dried black mustard seed were cracked by mortar in order to be powder, 40grams of the dried seeds powder were transferred into a beaker and a solution of 80% methanol (200ml) was added. The contents of the beaker were left at room temperature for three days with frequent shaking. The extract was filtered using a funnel. The clear solution was evaporated, and the residual extract was dried, weighed and the %yield was calculated.

Anti –microbial methods:

The plant extract was tested against two Gram-positive bacteria (*Bacillus subtilius* NCTC 8236 and *Staphylococcus aurous* ATCC 25923), two Gram-negative (*Escherichia coli* ATCC 25923, *Pseudomonas aeruginosa* ATCC27853). Antibacterial activity was assessed by the cup-plate agar diffusion method was adopted with some minor modifications to assess the prepared methanol extract (Kavanagh 1 972)[11].**ATCC:** American Type Culture Collection, **NCTC:** National Collection of Type Culture.

Column Chromatography (wet packing):

A glass column (1 60x4cm) was well packed with (40g) silica gel (1 00-200 mesh) in benzene. The methanol extract (1 g) were dissolved in methanol and mixed with the (5gm) silica gel and the methanol was completely evaporated. The silica gel containing the extract was well packed on the top of the column and covered with silica gel. The column was wet packed, eluted with petroleum ether, and the polarity increased gradually by adding definite percent of ethyl acetate till 50% ethyl acetate was used, then chloroform was added gradually till 50% chloroform was used. Then methanol was added gradually till 50% methanol was used. About 1 4 fractions were collected, pooled into groups depending on their TLC behavior.

Preparative Thin Layer Chromatography:

A concentrated methanol (mac) solution of fractions obtained from wet column chromatography was chromatographed on 0.5 mm thickness preparative TLC plates. The plates were developed in a mixture of chloroform: ethyl acetate (4:1) by examining plates under UV light, and detection of plates by 1 %vanillin H_2SO_4 spray reagent for (F1) different zones were located. Each was scrapped off in a separate container, and each zone was removed by washing with a mixture of Chloroform: methanol (8:2) several times, decanting the solvents and removed Silica gel by filtration. The resulting solutions of different zones were concentrated and subjected to Thin Layer chromatography.

Spectroscopic methods:

Fractions (1 and 2) from the Preparative TLC were analyzed using Infra- red (IR) and Ultra- violet (UV) spectroscopic methods.

Results and discussion:

In this study the 80% Methanol solvent used for extraction of active agents from *Brassica nigra* (black mustard) seed which were collected from local Khartoum market. The methanol extract (weight 1.92 g, yield percent 6.03) was used for investigation of the antimicrobial activity. The results of *in vitro* antimicrobial activity are presented below:

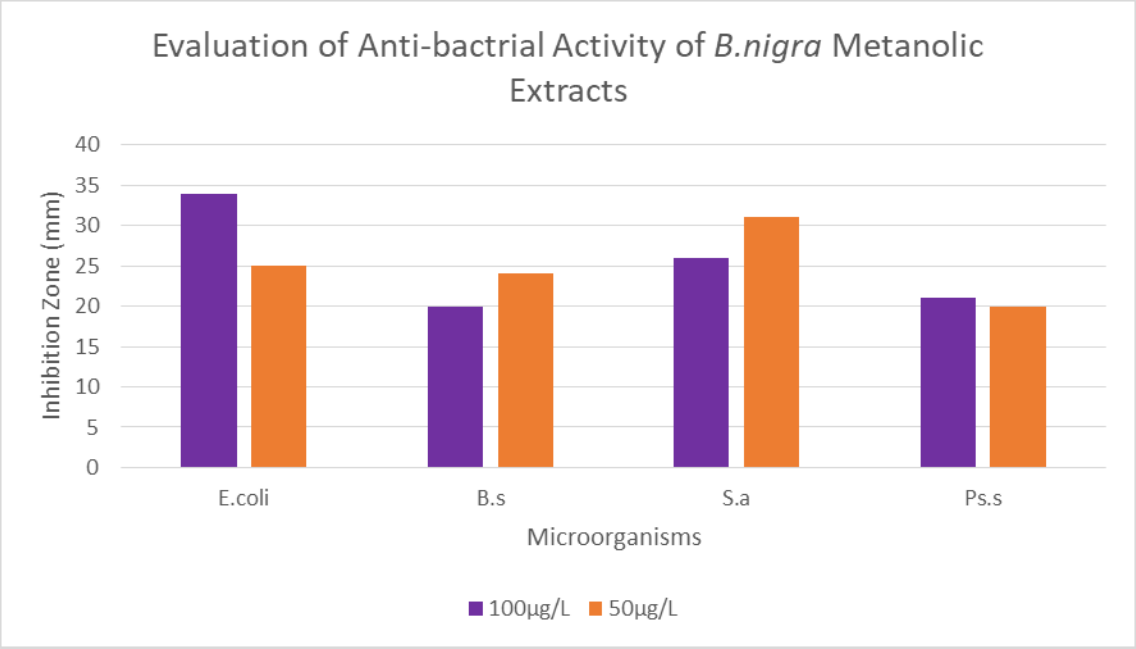

Figure 1: Anti-microbial activity of 80% methanol extract of *Brassica nigra* seed.

The results showed that the 80% methanol extract of *Brassica nigra* seed effective against all of the test organisms. The concentrations of extract of *Brassica nigra* used in inhibition growth and killing four bacteria (*Escherichia coli, Staphylococcusaureus Bacillus subtilis, Pseudomonas eruginos*) at the level (100mg/ml) then diluted to (50mg/ml), the result obtained at a concentration of (100mg/ml) show High activity against *E.coli* (34mm), the low concentration (50mg/ml) gave highest activity against Staphylococcus aureus (31mm).

(a) (b)

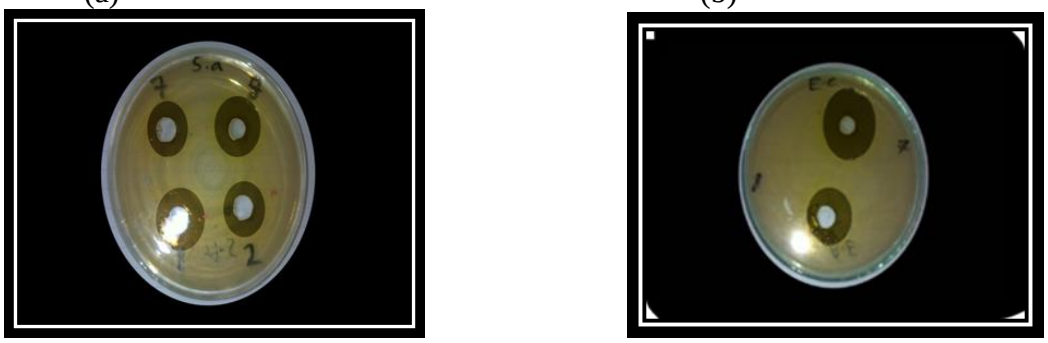

Plate (1): (a) Inhibition zone of Methanol 80% extract against *Staphylococcus aureus*
(b) Inhibition zone of Methanol 80% extract against *Escherichia coli*

Based on the Anti-microbial activity results in figure (1), the 80% methanol extract was subjected to various analytical thin layer trials using various solvent systems (Table1).

No	Color				
	R_f	N.E	UV (λ365 nm)	UV (λ 254 nm)	Spray reagent
1	0.985	-	Light	Violet	Violet
2	0.956	-	Light	-	Red
3	0.840	-	Light	Violet	Violet
4	0.691	-	Light	-	Grey
5	0.514	-	Light	-	Grey
6	0.294	-	Light	-	Grey

Table (1): TLC of Crude 80%methanol extract

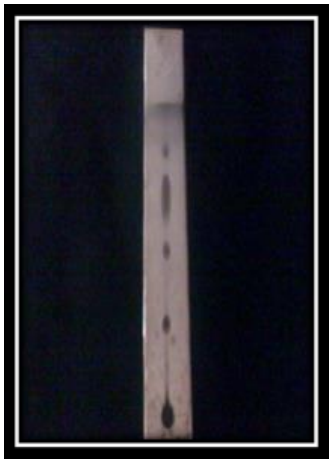

**Figure 2: Mobile phase (chloroform: ethyl acetate (4:1))
Detection: spraying with 1 %vanillin / sulphuric acid and UV lamp**

The active 80% methanol extract was carefully studied. In a keen and active attempt, to isolate the active component(s), the active 80% methanol extract was fractionated in a silica gel wet column using solvents (petroleum ether, ethyl acetate, chloroform, and methanol). Finally, 14 fractions were separated by column chromatography. The characteristic colors and Rf values of these fractions are tabulated in table (2).

Table (2): Wet Column fractions of the 80% Methanolic extract:

COLOUR						
No		**R.F**	**N.E**	**UV(λ365nm)**	**UV(254nm)**	**Spray reagent**
1	**F1**	**0.91**	**_**	**Light**	**Violet**	**Pink**
2		**0.87**	**_**	**Light**	**_**	**Violet**
3		**0.62**	**_**	**Light**	**_**	**Violet**
4	**F2**	**0.95**	**yellow**	**Yellow**	**_**	**Violet**
5		**0.91**	**_**	**_**	**_**	**Violet**
6		**0.73**	**_**	**Light**	**_**	**Violet**
7		**0.29**	**_**	**_**	**_**	**Violet**
8	**F3**	**0.93**	**_**	**Light**	**_**	**Violet**
9	**F4**	**0.84**	**_**	**Light**	**_**	**Violet**

10		**0.58**	**_**	**Light**	**Violet**	**Violet**
11		**0.50**	**_**	**Light**	**_**	**Violet**
12		**0.44**	**_**	**Light**	**_**	**Violet**
13	**F5**	**0.78**	**_**	**Light**	**_**	**Yellow**
14		**0.65**	**_**	**Light**	**_**	**Yellow**
15		**0.58**	**_**	**Light**	**_**	**Yellow**
16		**0.48**	**_**	**Light**	**_**	**Brown**
17		**0.41**	**_**	**Light**	**_**	**Brown**
18		**0.32**	**_**	**Light**	**_**	**_**
19		**0.25**	**_**	**Light**	**_**	**_**
20		**0.15**	**_**	**Light**	**_**	**_**

F1(1-7),F2(8-9),F3(10-11),F4(12),F5(13-14),NE=necked eye

Characterization and Identification of isolated fractions:

The first component eluted from the column was gave colorless amorphous substance from chloroform: ethyl acetate (4:1). F1 (1-7) from column chromatography and TLC ended up with isolation of one pure compound from fraction F1 (1-7).the second component eluted from the column given colorless amorphous substance from chloroform: ethyl acetate (1:4) and TLC ended up with the isolation of four pure compounds from fraction F2 (8-9) by using preparative. Identification of compounds was based on different spectroscopic techniques such as UV, IR spectroscopy.

UV-VIS Analysis:

The UV-VIS analysis preformed for identification of phyto-constituents present in methanolic extract of *B.nigra* seed.The UV-visible spectra were performed to identify the compounds containing σ-bonds, π-bonds and lone pair of electrons, chromophores and aromatic rings. The profile showed the peak at 296 nm with maximum absorption 0.100, 0262, 0.73 for compounds 1, 2, 3respectively .and peaks at 298,242 with maximum absorption 0.051and 0.061 respectively (Table 3):

Table (3): UV-VIS peak values of Isolated Compounds

	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
R_f	0.95	0.91	0.73	0.29	0.93
Absorbance	0.100	0.262	0.107	0.051	0.061
λ max	296	296	296	298	242

FTIR analysis:

Table (4): Characteristic Infra-red Absorption of the Isolated Compounds

Band assignments	Compound 1	Compound 2	Compound 3	Compound 4	Compound 5
C-H stretch Alkanes	2925.81-2854.45	2923.88-2852.52	—	2956.67	2925.81-2856.38
C-H stretch Aromatic	3081.39	—	3022.25	—	
C-H bending Aliphatic	—	—	—	—	1456.16
C=O stretch Carbonyl group	1749.32	1743.53-1654.81	1745.45	1645.17	—
C=C stretch Aromatic rings multiple bands	1514.02-1336.58	1515.94-1456.16	—	1546.80-1452.30	—
C-O stretch ethers (aromatic)	1217	—	1217	1218	—
C-O stretch Esters(two peeks)	—	1217-1022	—	—	—
N-H bending Amide	—	—	1515.94	—	—
O-H stretch Phenol	—	—	—	3438.84	—
O-H stretch Alcohol	—	—	—	—	3367.16

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation.
IR spectrum peaks of the isolated compounds in KBr pellet revealed the number of functional groups that have a great significant towards medicinal prospects of *B.nigra*.
The FTIR spectrum show various peaks associated with various stretching and bending can be assigned to different functional groups like C=O stretch Carbonyl group C-H stretch (Alkanes-

Aromatic),C-H bending Aliphatic, C=C stretch Aromatic ,C-O stretch ethers (aromatic),O-H stretch Phenol, C-O stretch Esters(two peeks)N-H bending Amide, O-H stretch Alcohol.

Our study showed that the methanolic extract (mac) of *Brassica nigra* have antimicrobial activity against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas eruginos* to inhibit the growth of pathogenic bacteria.The aim to explore possible future use of these extracts as alternative to common antibiotics and to determine their ability to enhance activities.

The results obtained in this study are in complete agreement with those reported by *S. Sujatha and Akila S 2013*that the petroleum ether, methanol and chloroform extracts of *Brassica nigra* seeds inhibit the growth of pathogenic bacteria. In this study Brassica nigra have antimicrobial activity against *S.aureus, P.vulgaris, P.mirabilis, E.aerogens, E.coli* which showed that the methanolic extract had antibacterial effect against all bacteria.

Rajesh S. T and Vikas S 2014 reported that 70% ethanol extract possessed good antimicrobial activity against *E.coli* (20.5mm) and *S.aureus* (25mm). This may be due to the presence of polar and non-polar antimicrobial principles in the extract.
The active methanol extract was fractionated in a silica gel wet column using solvents (petroleum ether, ethyl acetate, chloroform and methanol).Identification of the isolated compounds was based on different spectroscopic techniques such as UV, IR spectroscopy which indicate that *Brassica nigra* contains phenol, flavonoids, alkaloids, sterols, terpenes etc., which may be responsible for the activity against microorganisms.

Conclusion:

Mustard (*Brassica nigra* L) Seed extracted using methanol solvent. The methanolic (mac) extract exhibited high antimicrobial activity against all tested organisms. Total extracts and methanolic fractions from the column chromatography were studied using thin layer chromatography by different solvent systems.Column chromatography of 80% methanolic extract of *Brassica nigra* seed give five pure compounds. The isolated compounds were identified based on different spectroscopic techniques such as UV, IR spectroscopy. The result of FTIR analysis confirmed the presence of phenols, alcohols, alkanes, aromatics, carboxylic acids, esters, amide, aldehyde, ketones. The presence of various characteristic functional groups may be responsible medicinal properties of *B.nigra.*

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