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GC-MS Analysis, Physicochemical Properties and Antimicrobial Activity of *Brassica Juncea L* Seed Oil

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Abstract | **Original Research Article**

The purpose of this study is to assess the Physicochemical properties such as (Specific Gravity, Refractive Index, Acid Value, Percentage Free Fatty Acids, Peroxide Value, Saponification Value and Iodine Value), investigate the chemical constituents of the Fixed Oil from *Brassica Juncea L seed*s and to evaluate its potential antibacterial activity against five microorganisms (*Bacillus Subitus, Staphylococcus aureus, Escherichia Coli, Salmonella, Penicillium and Aspergillus Niger*). The chemical constituents of *Brassica Juncea L* seeds oil were identified and quantified by GC-MS, where disc diffusion assay were employed to evaluate the antibacterial activities and physicochemical properties using standard methods. Eight components that have been identified detected revealed the following major components: 13-Docosenoic Acid, Methyl Ester, (Z) (59.02%), 9,12-Octadecadienoic Acid, Methyl Ester (14.65%),10-Octadecenoic Acid, Methyl Ester (8.66%) and cis-11-Eicosenoic Acid, Methyl Ester (7.82%). The physicochemical properties of *Brassica Juncea L* seed oil showed (Specific Gravity (0.923), Refractive Index (1.469), Acid Value (7.62), Percentage Free Fatty Acids (1.1), Peroxide Value (12.4), Saponification Value (18.23) and Iodine Value (110.35). The antibacterial activity showed partial activity against both Gram-positive and Gram-negative bacteria and inactive against the fungi. In conclusion, the results showed that the Oil of *Brassica Juncea L* Seeds is a potential source of natural antibacterial, and justify its uses in folkloric medicines.

Keywords: Antimicrobial Activity, *Brassica Juncea*, Chemical properties, GC-MS analysis.

INTRODUCTION

Brassica is a genus comprising more than 150 species in the family *(Brassicaceae).* Different *Brassica* species are cultivated worldwide for their economic value (Manohar *et al*., 2009). Leaves of these plant species are edible and diverse medicinal uses of seeds are known in many communities. *Brassica Juncea* is species of mustard family of *Brassicaceae (Cruciferae) plants*. Its primary center of origin is central Asia. (Warwick, 2011) The plant is widely cultivated in many countries for its nutritive and medicinal values. For centuries *Brassica Juncea* has been used as a natural remedy. Seeds are traditionally used against muscular rheumatism, vomiting and jaundice. Seeds mixed with *moringa olefera* is a remedy for spleen and liver diseases (Singh 2005). Some *Brassica Juncea* preparations have been used as diuretic, liver –bile stimulators and laxative (Morningstar and Desai, 2003). Seeds are used by local healers against abscesses, cold, rheumatism and lumbago. A paste made from seeds is used as a treatment for arthritis, backache, styes, paralysis and edema of lungs and liver. Seeds are also used as stimulant and emmenagogue (Joy *et al*., 1998). Diverse pharmacological activities have been associated with seeds including hypoglycemic, anxiolytic, antidiabetic, goiterogenic, antioxidant and hepatoprotective activities (Grover *et al*., 2003). It has been reported that the seeds, leaves and stem may reduce the risk of heart attack and migraine diabetic heart diseases (Walia and Sumeet, 2011). *Brassica juncea* seed oil consists mainly of glycerides: erucic, eicosanoic, arachidic, nonadecanoic, behenic, oleic, and palmitic acids in addition to α-linolenic acid and arachidonic acid (Joardar and Das, 2007). In general, this oil is rich in erucic acid, which according to some authors could also have adverse effects in high doses. The economic value of the plant is mainly due to its widespread uses to produce fixed oils from its seeds, which still remains to be the main source of edible vegetable oils in many countries and cultural groups. Mustard seeds and oil are now also being explored for

producing bio-fuels and diverse other commercial purposes (Jham *et al*., 2009). Essential oils were found in the seeds of Brassica Juncea (L.) Coss, which are grown in Hebei, Shanxi and Shandong provinces.

MATERIALS AND METHODS

Plant material

Seed samples (5 kg) of species, Brassica Juncea L were purchased from the local market called Al-Anaqreeb in Omdurman (Sudan), The seed samples were further identified and authenticated by the Medicinal and Aromatic Plants Research Institute and ground to powder using a grinder prior to oil extraction. All chemicals used in the study were analytical grade and used without further purification.

Oil Extraction

A quantity (250 g) of the dried milled sample was put into the thimble and the materials were continuously extracted for 6 hours using n-hexane (76°C -80°C) as solvent, the end of the extraction, the thimble was removed and the solvent allowed to evaporate, the flask and the content were dried. The flask containing the oil was cooled in the desiccators, weighed and drying process repeated until a constant weight was obtained.

Determination of Physicochemical properties of the oil

Determination of Specific Gravity and Refractive Index

The test, Specific Gravity and Refractive Index, were determined by the manual of methods of analysis of food, fssai, 2015.

Determination of Acid Value

The Acid Value was determined using the method described by Ronald (1991). Equal volumes (25 ml) of diethyl ether and ethanol were mixed together and 1 ml of 1% phenolphthalein indicator solution was added and was then neutralized with 0.1M potassium hydroxide solution. The oil sample (between 1 to 10 g) was dissolved in the neutralized solvent mixture and titrated with 0.1 M potassium hydroxide solution with constant shaking until a pink color which persists for 15 seconds is obtained. The Acid Value is given as:

$$\text{Acid Value} \quad \frac{\text{Titer value (ml) X } 5.61}{\text{Weight of sample used (g)}}$$

Determination of Percentage Free Fatty Acids (FFA)

This was carried out using the method described by AOAC (1990). One gram of the oil sample was accurately weighed into a conical flask. This was followed by the adding 10 cm^3 of neutralized 95% ethanol and Phenolphthalein. This was then titrated with 0.1 M NaOH, with constant shaking until a pink color persisted for 30s. The percentage free fatty acid was calculated from Equation below:

$$\text{Free Fatty Acid (FFA)} = \frac{\text{V X M X } 2.82}{\text{Weighedof oil g}}$$

Determination of Peroxide Value

One gram of the oil was weighed into a clean dry boiling tube, 1g of powdered potassium iodide and 10cm3 of the solvent mixture were added. The mixture was allowed to boil vigorously for 30 seconds. The tube was washed twice with 25cm3 portions of water and the washings were added to the titration flask. This was then titrated with 0.002M Sodium thiosulphate using starch indicator.

The relation for peroxide value is given as;

$$\text{Peroxide value} = \frac{\text{V. Molarity of titrant X 100(meq KOH/g)}}{\text{Weight of oil}}$$

Determination of Saponification Value

This was carried out using the method described by AOAC (1998). Two grams of the oil sample was added to a flask with 30 cm3 of ethanolic potassium hydroxide solution and was then attached to a reflux condenser and heated on a water bath for 1 hour with occa-sional shaking to ensure the sample was fully dissolved. After the sample had cooled, 1cm3 of phenolphthalein indicator was added and titrated with 0.5M hydrochloric acid until a pink endpoint was reached. A blank determination was also carried out omitting the oil and saponification value was calculated using the equation:

$$\text{Saponification Value} = \frac{\text{(b – a) X M X } 56.1}{\text{Sample weight (g)}}$$

Where,
a = sample titre value
b = blank titre value
M = molarity of the HCl
56.1 = molecular weight of KOH

Determination of Iodine Value

The determination Iodine Value was carried out according to the IUPAC method (IUPAC 1979). With the aid of a dropping pipette, about 0.2 – 0.5 g of the oil was accurately weighed into a glass stoppered flat bottom flask and 10 ml carbon tetrachloride added to the oil to dissolve. Exactly 20 ml Wijs' solution was added and the stopper which had been moistened with potassium iodide solution inserted. The mixture was mixed and allowed to stand in a dark cupboard for 30 minutes. 15 ml of freshly prepared 10% potassium iodide solution and 100 ml water was added and mixed. The mixture was titrated with 0.1 M standard sodium thiosulphate solution and using starch as an indicator just before the end point. A blank titration was also carried out. The Iodine Value is given as:

$$\text{Iodine Value} = \frac{\text{(b – a) X } 1.269}{\text{Weight of sample used (g)}}$$

Where,
a = sample titre value
b = blank titre value

GC-MS analysis

The studied oils were analyzed by gas chromatography – mass spectrometry. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25 µm, thickness) was used. Helium (purity; 99.99 %) was used as carrier gas. Oven temperature program is presented in (Table-1), while other chromatographic conditions are depicted in (Table-2).

Table 1: Oven temperature program

Rate (min.$^{-1}$)	Temperature(C^{o})	Hold Time
-	150.0	1.00
4.00	300.0	0.00

Table 2: Chromatographic conditions

Column oven temperature	150 °C
Injection temperature	300 °C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3 KPa
Total flow	50.0 ml/min
Column flow	1.54 ml/sec
Linear velocity	47.2 cm/sec
Purge flow	3.0 ml/min
Split ratio	-1.0

Testing of antimicrobial Activity

Mueller Hinton (MH) agar and sabouraud dextrose agars were used as media for growth of bacteria and fungi respectively. They were prepared according to the manufacturer instructions.

The disc diffusion bioassay was used to assess the antibacterial potency of the oil. Bacterial suspension was diluted with sterile physiological solution to 108cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of the bacterial suspension were swabbed uniformly on surface of MH-agar and allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MH-agar and soaked with (20 µl) of the test solution. The inoculated plates were incubated at 37 °C for 24h. The diameters (mm) of the inhibition zones were measured in duplicates and averaged. The above procedure was also used for antifungal activity, but instead of Muller Hinton agar, Sabouraud dextrose agar was used. Samples were used here by the same concentrations used above.

RESULTS AND DISCUSSION

The physicochemical tests (Specific Gravity, Refractive Index, Acid Value, Percentage Free Fatty Acids, Peroxide Value, Saponification Value and Iodine Value) of *Brassica Juncea L.* seed oil was studied to evaluate the structural quality of the oils (Table-3), Mousavi *et al*., (2012). These parameters were used to monitor the quality of edible oil (Aremu *et al*., 2015).

Table 3: Physicochemical properties of *Brassica juncea L* seed oil

Parameter	Result
Specific gravity	0.923
Refractive index	1.469
Acid value	7.62
Free fatty acid	1.1
Peroxide number	12.4
Saponification value	18.23
Iodine number	110.35

The quality of the oil in the seeds was evaluated by parameters such as Free Fatty Acid Values, Acid Value, Peroxide number, Naponification number, and Iodine number (Nehdi *et al*., 2010). The Iodine Value is a measure of the degree of unsaturation of the fatty acids in an oil and can be used to determine the amount of double bonds present in an oil that reflects the oil's sensitivity to oxidation (Alireza *et al*., 2010). The Iodine Value of brown mustard seed oil is relatively high and this reflects the presence of a high percentage of unsaturated fatty acids in the oil Acid value is an important indicator of the physical and chemical property of an oil which is used to indicate the quality, age, edibility of the oil and its suitability for use in industries such as paint. The presence of free fatty acids (FFA) in the oil or fat is indicative of previous lipase activity, other hydrolytic effect or oxidation. According to Demian (1990), PH values are used to measure the extent to which glycerides in an oil are degraded by lipase and other physical factors such as light and heat (Michael *et al.*, 2014). Thus, the higher acidic value of brown mustard oil indicates that brown mustard oil is more susceptible to lipase action. The peroxide test is a predominant test for oxidative stress in oils and fats; This is a measure of the concentration of peroxides and aqueous peroxides formed at the initial stage of lipid oxidation. The peroxide value is also used as a measure of the extent to which rancidity reactions occur during storage. A higher peroxide value of brown mustard seed oil indicates a greater susceptibility to oxidation (Anyasor *et al*., 2009). The saponification value is used in the adulteration assay. The low saponification value obtained for brown mustard seed oil indicates that it is not industrially useful. The percentage of free fatty acids in the oil indicates their level of decomposition and their quality (Tagoe *et al*., 2012). In addition, seed duration and storage conditions are factors that may influence the value of free fatty acids (Fokou, and Meier, 2009).

The high content of free fatty acids in the oil may cause further oxidation and lead to the formation of an unpleasant taste and flavor in the oil. One of the main concerns in edible oil refining is the free fatty acid

content. Free fatty acid (FFA) is often used to indicate the quality of the oil and its suitability for eating.

GC-MS analysis

The GC-MS analysis of *Brassica Juncea* oil showed the presence of 8 components. Total ions chromatograms are depicted in Fig 1, while the different constituents of the oil are presented in Table (4). Fatty acids constituted major bulk of the oil. The GC-MS analysis revealed the following major components: 13-Docosenoic acid, methyl ester, (Z) (59.02%), 9,12-Octadecadienoic acid, methyl ester (14.65%),10-Octadecenoic acid, methyl ester (8.66%) and cis-11-Eicosenoic acid, methyl ester (7.82%).

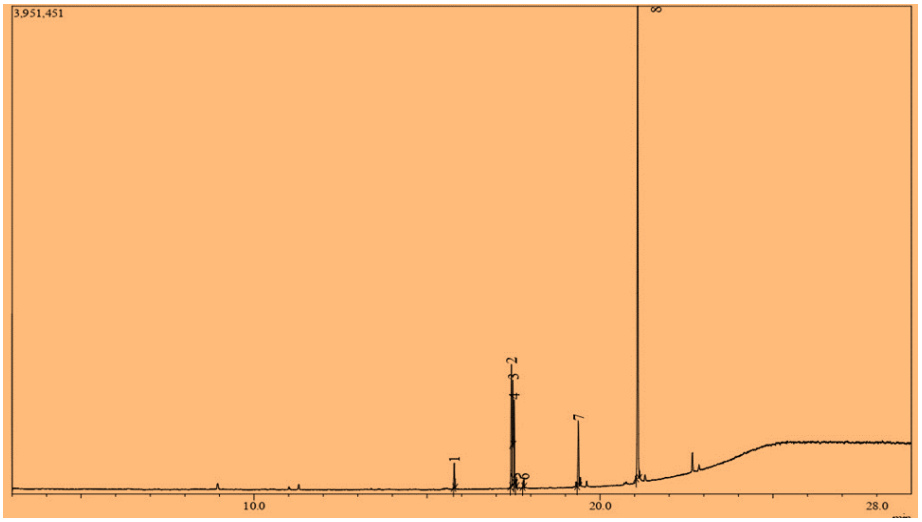

Fig 1: Total ions Chromatogram of *Brassica Juncea* oil

Table 4 Fatty acids constitutions of *Brassica Juncea* oils.

The EI mass spectrum of 13-Docosenoic acid, methyl ester is shown in Fig 2. The peak at m/z 352, which appeared at R.T. 21.085 in total ion chromatogram, corresponds: M+ [C23H44O2]. The peak at m/z 321 corresponds to loss of a methoxyl fu33nction.

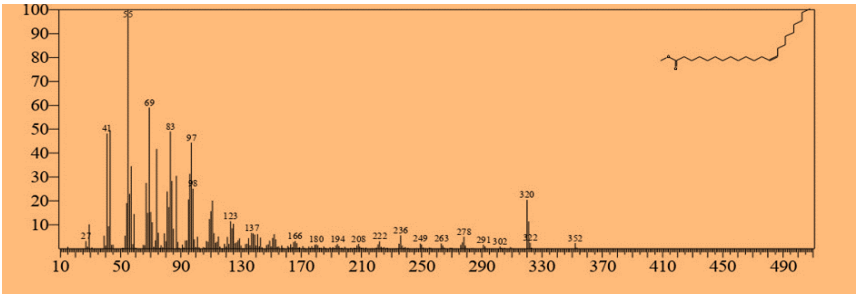

Fig 2: Mass spectrum of 13-Docosenoic acid, methyl ester

Fig 3 shows the mass spectrum of 9,12-Octadecadienoic acid, methyl ester. The peak at m/z 294, with R.T.17.437, corresponds the molecular ion: M+[C19H34O2]+, while the signal at m/z 263 is due to loss of a methoxyl.

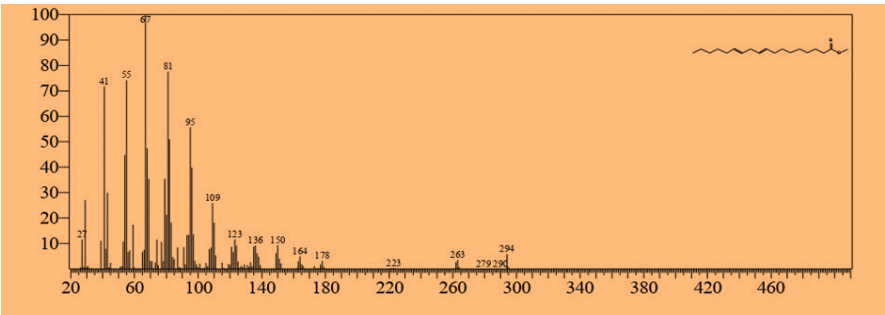

Fig 3: Mass spectrum of 9,12-Octadecadienoic acid, methyl ester

The mass spectrum of 10-Octadecenoic acid, methyl ester is presented in Fig. 4. The signal at m/z 296, which appeared at R.T. 17.515 is attributed to M+ [C19H36O2]+. The peak at m/z 265 accounts for loss of a methoxyl.

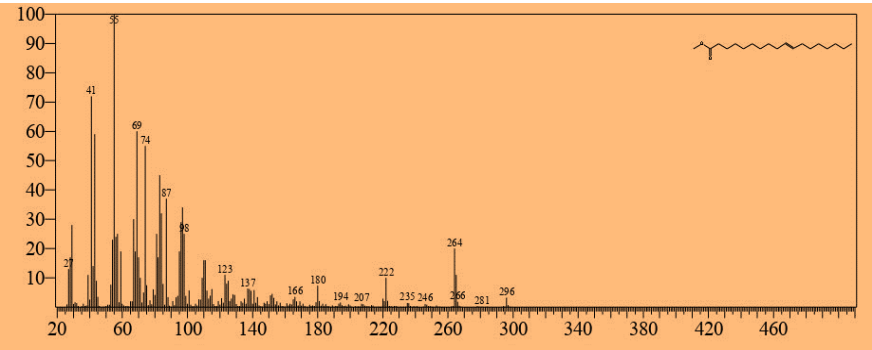

Fig 4: Mass spectrum of 10-Octadecenoic acid, methyl ester

The mass spectrum of cis-11-Eicosenoic acid, methyl ester is displayed in Fig. 5. The peak at m/z 324 (R.T. 19.370) is due to the molecular ion: M+[C21H40O2]+. The peak at m/z 293 accounts for loss of a methoxyl.

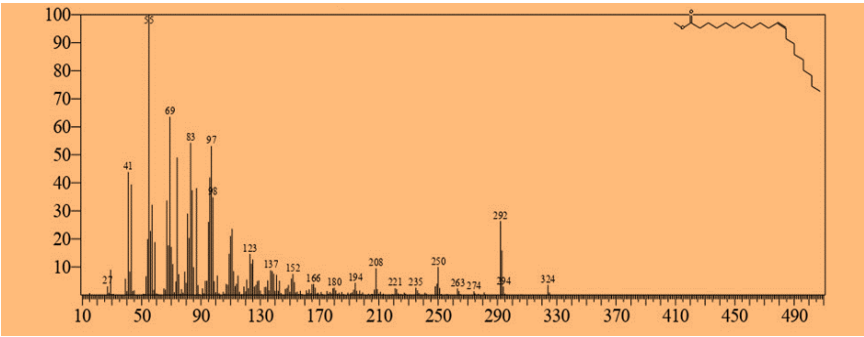

Fig 5: Mass spectrum of cis-11-Eicosenoic acid, methyl ester

Antibacterial activity

In cup plate agar diffusion assay, the oils were screened for antimicrobial activity against six standard human pathogens. The average of the diameters of the growth of inhibition zones are depicted in Table (5). The results were interpreted in commonly used terms (>9mm: inactive; 9-12mm: partially active; 13-18mm: active;< 18mm: very active). Tables (6) and (7) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table 5: Antimicrobial Activity of the isolated compounds

Organism		**Antibacterial activity**				**Antifungal activity**	
		Gram-positive		**Gram -negative**			
	Control Methanol	*B.Subtilis*	*St.Auerus*	*E. Coli*	*Salmonella*	*Penicillium*	*As. Niger*
Brassica Juncea oil	0.0	10	10.33	9.66	9.33	-	-

Brassica Juncea oil showed partial activity against both Gram-positive and Gram-negative bacteria and inactive against the fungi.

Table 6: Antibacterial activity of standard drugs

Drug	**Conc.(mg/ml)**	**Bs**	**Sa**	**Ec**	**Sa**
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 7: Antifungal activity of standard drug

Drug	Conc. (mg/ml)	Penicillium	Aspergillus Niger
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

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