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Biological Activity and Composition of the Essential Oil and Fatty Constituents of Petroleum ether Extract of *Brassica juncea* (L.)

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ABSTRACT

Brassica juncea L. (Brassicaceae) is widely used in making mustard oil. The petroleum ether extract of the seeds of this plant found to contain appreciable levels of secondary metabolites including phenolics, flavonoids, tannins and alkaloids that might be attributed to the good antioxidant activity of this extract. The extract exhibited broad antimicrobial activity against the tested pathogenic strains including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans*, *Escherichia coli* and *Proteus vulgaris*. 10 compounds were identified in the petroleum ether extract of *Brassica juncea* (L.) seeds using gas chromatography- electron ionization mass spectrometry (GC-EIMS) analysis. The identified constituents were 9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)- Cycloartenyl acetate (34.77%), 3-Methylpentane (25.26%), Methyl cyclopentane (15.44%), octadec-9-enoic acid (oleic acid) (9.02%), Allyl isothiocyanate (3.43%), 3-Butenyl isothiocyanate (3.38%), hexanal (Caproaldehyde) (3.14%) and nonacosane (0.95%). In conclusion, *Brassica juncea* seeds could be used as valuable source of active constituents that possess remarkable biological activity and could be used in the field of medicine.

***Keywords*: *Brassica juncea*(L.), mustard oil, antioxidant activity, antimicrobial activity, petroleum ether, GC-EIMS**

INTRODUCTION

The class Brassica includes in excess of 150 species that are developed everywhere throughout the world as oilseeds yields and vegetables. *Brassica juncea* is out of these significant plants that is known for its dietary and restorative impacts (Rahman *et al*., 2018; Nawaz *et al*., 2018). The leaves just as the seeds of this mustard assortment are consumable, and different therapeutic employments of its seeds are likewise notable.

Brassica juncea has a place with the cruciferae (Brassicaceae) plant family, ordinarily known as mustard family. It has light green foliage, with a couple of hairs on the main leaves and leaf sharp edges that end well up the petiole. Develop plants develop to a tallness of one to two meters. The lower leaves are profoundly lobed while the upper leaves are tight and whole. The inflorescence is a prolonged raceme and the blossoms are light yellow and open logically upwards from the base of the raceme. The seeds are round and could be yellow or darker (Flora of China, 2015; OECD, 2016). It is local to India, Cocos Islands notwithstanding China (PIER, 2018; Missouri Botanical Garden, 2019) and acquainted with most nations everywhere throughout the world including Egypt and Iraq (Warwick and Francis, 1994).

Brassica juncea is known to deliver a few classes of bioactive phytochemicals including glycosides, flavonoids, phenolic mixes, sterols and terpenoids, proteins furthermore, sugars (Ogidi *et al*., 2019). Together with glucosinolates, various polyphenolic auxiliary metabolites of *Brassica juncea* are frequently viewed as its significant treatment important bioactive segments (Barakat *et al*, 2009; Dubie *et al*., 2013). *Brassica juncea* seeds are broadly utilized in practically all customarily known frameworks of medication and its oils are engaged with their malignant growth preventive impacts (Ogidi *et al*., 2019).

This research was designed to determine the major secondary metabolites present in the petroleum ether extract of *Brassica juncea* (Brown mustard) seeds and to estimate their antioxidant and antimicrobial activity of this extract in addition to structural elucidation of the the components responsible for its activity using the appropriate spectroscopic technique.

MATERIALS AND METHODS

Preparation of the plant extracts:

15 grams of *Brassica juncea* seeds were extracted using 150 ml petroleum ether for 3 hours using a Soxhlet apparatus. The extracts were filtered and evaporated under vacuum to dryness using rotary evaporator. The crude extracts were kept in refrigerator to be ready for any further investigations.

Determination of the active secondary metabolites:

Total phenolics:

Phenolics content in the extract was measured using Folin Ciocalteu assay developed by Lin and Tang (2007) and expressed as milligram gallic acid equivalent/gram air dried seeds.

Total flavonoids:

Flavonoids content in the tested extract was measured using aluminum chloride colorimetric assay developed by Chang *et al*., (2002) and expressed as milligram quercetin equivalent / gram air dried seeds.

Total alkaloids:

Total alkaloids content in the studied plants were measured using 1,10-phenanthroline method described by

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Singh *et al*., (2004). and expressed as milligram colchicines equivalent / gram dried seeds.

Evaluation of antioxidant activity:

DPPH assay:

The antioxidant potential of the extracts was estimated using the DPPH radical scavenging assay described by Liyana-Pathirana & Shahidi, (2005). The remaining DPPH˙ percentage of each tested concentration of the studied extracts at the steady state was estimated as follows:

% DPPH˙ remaining = % DPPH˙ sample/% DPPH˙ blank × 100

These values were graphed against mg of seeds extract to show the concentration of the extract as antioxidant necessary to decrease the initial DPPH˙ concentration by 50% (IC_{50}). Ascorbic acid was used as reference.

Screening of the antimicrobial activity:

Disc diffusion assay:

The antimicrobial activity of the petroleum ether seeds extract was estimated using filter paper disc assay (Murray *et al*., 1995).

Tested organisms:

Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Escherichia coli and *Proteus vulgaris.*

GC-MS analysis:

The fatty content of the petroleum ether extract of *Brassica juncea* was isolated, identified and quantified on a Shimadzu GC-17A gas chromatograph (Shimadzu Corp., Kyoto, Japan), coupled with a Shimadzu mass spectrometer detector (GC-MS QP-5050A). The GC-MS system was equipped with a TRACSIL Meta X5 column**.** The relative concentration of each compound in the essential oil was quantified according to the peak area integrated by the analysis program.

RESULTS

This research was a trial to extract, characterize the essential oils components, to analyze the phytochemical constituents and to test the antimicrobial activity of *Brassica juncea* seeds petroleum ether extract.

The phytochemical analysis of petroleum ether extract of *Brassica juncea* seeds revealed the presence of medicinally active metabolites including alkaloids, tannins, phenolics and flavonoids. These active compounds were quantitatively analyzed and the results obtained revealed that the plant contains appreciable levels of these secondary metabolites. The extract also found to be furious with fatty content as it contains 11.5% fats as illustrated in Table (1).

Table 1. Secondary metabolites and total fats in *B. juncea* seeds extract.

Metabolites	*B. juncea* (Petroleum ether extract)
Alkaloids(mg colchicines equivalent/ gram dried seeds)	7.50
Phenolics (mg gallic acid equivalent/ gram dried seeds)	24.57
Flavonoids(mg quercetin equivalent/ gram dried seeds)	6.79
Tannins (mg gallic acid equivalent/ gram dried seeds)	4.85
Total fat (mg fats/gram dried seeds)	115

The petroleum ether extract exhibited good antioxidant scavenging activity as illustrated in table 2 and figure 1. Vitamin C was used as the reference compound. The antioxidants scavenging activities for DPPH are attributed to the hydrogen-donating capabilities of the antioxidant compounds present in the extract. It was recorded in literature that the presence of phenolic substances including flavonoids and tannins were responsible for the antioxidant activity of the plant extracts (Loganayaki *et al*., 2013; Mustafa *et al*., 2019). They may be of the main constituents that contribute to the antioxidant activity observed in this study.

Table 2. Antioxidant scavenging activity of the petroleum ether extract prepared from *Brassica juncea* using DPPH assay.

DPPH Conc. (mg/ml)	% remaining DPPH	% of scavenging	IC_{50}
0.625	44.58	55.42	
0.3125	60.93	39.07	
0.156	77.10	22.9	0.515
0.078	83.97	16.03	
0.039	87.18	12.82	
Ascorbic acid			0.024

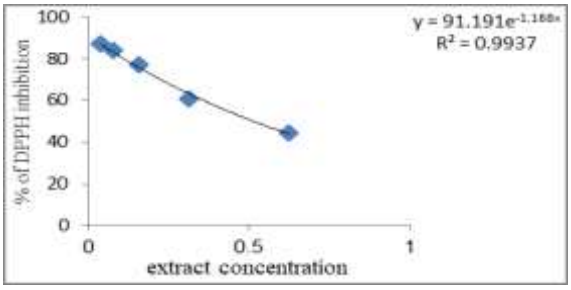

Figure 1. The exponential curve used for calculation of IC_{50}

The antimicrobial activity of the light petroleum ether extract was tested using *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Candida albicans, Escherichia coli* and *Proteus vulgaris*. The tested petroleum ether extract showed significant zones of inhibition in comparison with streptomycin as standard antibiotic in a dose-dependent manner against most of the tested microorganisms as illustrated in table (3).

Table 3. Antimicrobial activity of *Brassica juncea* petroleum ether extract using disc diffusion assay.

Microorganisms	*Brassica juncea* extract	Streptomycin
Staphylococcus aureus	18	11
Bacillus subtilis	17.5	15
Pseudomonas aeruginosa	12	11
Klebsiella pneumonia	9	-ve
Candida albicans	18.5	13
Proteus vulgaris	14	-ve
E. coli	9.5	-ve

"Values indicate zone of inhibition in mm and include filter paper disk diameter (6 mm); "-ve": no inhibition"

It has been reported in the literature that the major groups responsible for the antimicrobial activity of the plant extracts are phenolics, tannins, flavonoids, terpenoids, essential oils, alkaloids, lectins, and polypeptides (Mittal and Jaitak, 2019; Othman *et al*., 2019). This may approve that the phytochemical components present in *Brassica juncea* petroleum ether extract.

According to gas chromatography (GC)/EIMS analysis, 10 compounds were identified in the produced extract constituting 99.37% by weight of the *B. juncea* petroleum ether extract. All the identified components are presented in Table 4. The main components were 9,19-Cyclolanost-24-en-3-ol, acetate, 3.beta-Cycloartenyl acetate (34.77%), 3-Methylpentane (25.26%), Methyl cyclopentane (15.44%), octadec-9-enoic acid (oleic acid) (9.02%), Allyl isothiocyanate (3.43%), 3-Butenyl isothiocyanate (3.38%), hexanal (Caproaldehyde) (3.14%) and nonacosane (0.95%).

Table 4. Composition of the essential oil and fatty constituents present in *Brassica juncea* petroleum ether extract.

No.	Identified compound	Molecular weight	Area %
1	Methyl cyclopentane	84.16	15.44
2	3-Methylpentane	86.18	25.69
3	Allyl isothiocyanate	99.11	3.38
4	Hexanal (Caproaldehyde)	100.16	3.14
5	3-Butenyl isothiocyanate	113.18	3.43
6	Phenyl isothiocyanate	135.19	0.41
7	Octadec-9-enoic acid (oleic acid)	282.55	9.02
8	Nonacosane	408.79	0.95
9	2,4-Cyclohexadien-1-one,3,5-bis(1,1-dimethylethyl) - 4-hydroxy-	466.74	3.14
10	9,19-Cyclolanost-24-en-3-ol, acetate,3.beta-Cycloartenyl acetate	468.75	34.77
		Total	99.37

CONCLUSION

In conclusion, *B. iuncea* studied here can be seen as a potential source of useful drugs. The presence of these phytochemicals justifies the traditional medicinal uses of these seeds. The results from this study in addition to those from previous studies could be considered as a reference to the essential oils constituents, fatty content, antioxidant and antimicrobial activity of *Brassica juncea* with biologically active and stable components

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النشاط البيولوجي والتركيب الكيميائي للزيوت الأساسية والمحتوى الدهني لمستخلص الاثير البترولي لبذور نبات الخردل

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نبات الخردل من النباتات التي تستخدم على نطاق واسع في صنع زيت المستردة. وقد وجد من خلال تلك الدراسة أن مستخلص الإثير البترولي من بذور هذا النبات يحتوي على نسب من مركبات الأيض الثانوية وهي الفينولات والفلافونيدات والتانينات والقلويدات والتي يمكن أن يعزى إليها قدرة هذا المستخلص الجيدة كمضاد للأكسدة. كما أظهر هذا النبات قدرة مضادة للميكروبات واسعة المجال ضد مجموعة من الكائنات الممرضة والمتضمنة (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Escherichia coli, Proteus vulgaris*) حيث أدى معاملة تلك الكائنات بمستخلص الإثير البترولي لبذور الخردل إلى تثبيط نموها بمعدل 18 و 17.5 و12 و 9 و 18.5 و 14 و 9.5 مليمتر على التوالي مقارنة بمركب إستربتومايسن كمضاد حيوي قياسي. كما تم التعرف على عشرة مركبات في مستخلص الإثير البترولي لبذور نبات الخردل بإستخدام جهاز GC-EIMS والتي يمكن أن يعزي إليها نشاط وكفاءة هذا المستخلص وهي: 9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)- Cycloartenyl acetate (34.77%), 3-Methylpentane (25.26%), Methyl cyclopentane (15.44%), octadec-9-enoic acid (oleic acid) (9.02%), Allyl isothiocyanate (3.43%), 3-Butenyl isothiocyanate (3.38%), hexanal (Caproaldehyde) (3.14%) و nonacosane (0.95%). ومن ثم نستنتج من هذه النتائج إمكانية إستخدام بذور نبات الخردل كمصدر جيد جدا للمركبات التي تمتلك قدرة مميزة بصفتها نشطة بيولوجيا وكذلك في مجال العلاج.