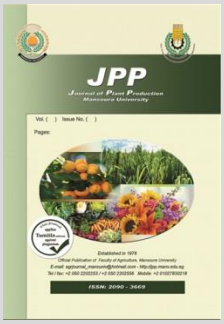
Journal of Plant Production

Journal homepage: www.jpp.mans.edu.eg
Available online at: www. jpp.journals.ekb.eg

Phytochemical Characterization, Antioxidant and Antimicrobial Activities of *Brassica juncea* (L.) Mustard Seeds Aqueous and Ethanolic Extracts

Sajida S. Aziz[1] ; Mustafa M. El-Zayat[2*] and Ayman Y. El-Khateeb[3]

[1]Department of Horticulture, College of Agricultural Engineering Sciences, Sulaimani University, Sulaimani, Kurdistan Region, Iraq.
[2]Unit of Genetic Engineering and Biotechnology, Mansoura University, Mansoura, Egypt.
[3]Department of Agricultural Chemistry, Faculty of Agriculture, Mansoura University, Mansoura, Egypt.



ABSTRACT

Brassica juncea (Brassicaceae) is used in folklore medicine and in local recipes as source of mustard oil. In the present study, phytochemical characterization, antioxidant activity, antimicrobial potential and structural elucidation using LC/MS-MS of the crude water and 30% ethanol *Brassica juncea* extracts were done. 30% ethanol extract exhibited antioxidant activity higher than water extract by using DPPH$^{\bullet}$ assay with IC_{50} value of 0.170 and 0.390 mg extract/mL, and by using $ABTS^{\bullet+}$ assay with inhibition percent values of 75.5% and 68.9% for 30% ethanol and water extracts, respectively. water extract was slightly higher than ethanol extract in alkaloids and phenolics content while that of ethanol was higher than water in the flavonoids content. The water extract expressed no antimicrobial potential against any of the tested pathogenic strains while that of 30% ethanol expressed broad antimicrobial potential against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Erwinia Carotovora, Proteus vulgaris Eterobacter cloacae, Shigella sp.* and *Streptococcus pyogensis*. LC/MS-MS profiling of the 30% ethanolic extract showed that caffeic acid, p-coumaric acid, epigallocatechin gallate, myricetin, apigenin, quercetin-3-*O*-(caffeoyl)-glucoside and quercetin present in this extract might be responsible for the antioxidant and antimicrobial activity of 30% ethanol extract.

Keywords: *Brassica juncea*, 30% ethanol, Antioxidant activity, Antimicrobial potential, Phenolics, Flavonoids, LC/MS-MS.

INTRODUCTION

Brassica juncea belongs to genus brassica is an economically important plant that possess nutritional and medical values (Rahman *et al*., 2018). It belongs to the Cruciferae (Brassicaceae) family and commonly known as mustard family with unique pungent and hot flavor (Kim *et al*, 2016). It is native to India, Cocos Islands and China (Missouri Botanical Garden, 2019).

Brassica juncea seeds contains many chemical constituents like proteins, fats, reducing sugars, non-reducing sugars, flavonoids, phenolics, saponins, tannins and terpenoids (Ogidi *et al*., 2019).

The active constituents present in *Brassica juncea* seeds and its oils possess anticancer activity (Bassan *et al*., 2018), hypoglycemic effects (Sharma et al, 2017) and used in cosmetics for hair control (Kumar *et al*., 2011).

Taking into consideration the edible and medicinal value of *Brassica juncea* L., This study aimed to study the antioxidant and antimicrobial activities of the seeds of this plant in addition to elucidation of the active compounds responsible for these activities.

MATERIALS AND METHODS

Preparation of the plant extracts:

Extraction of the target species was carried out using water and 30% ethanol. Water extract obtained just as these plants are administrated in the traditional uses where, 5 gm of the air dried aerial parts were extracted by shaking for 20 minutes at 70°C at 230 rpm with 100 mL deionized distilled water. While, for 30% ethanol extract, 5 gm of the air dried aerial parts were extracted by shaking for three hours at 230 rpm with 100 mL 30% ethanol. Subsequently, the extracts were filtered and evaporated under vacuum to dryness using a rotary evaporator, followed by lyophilizing. The crude extracts were then weighed and dissolved in dimethyl sulfoxide (DMSO) to be ready for any further investigations (EL-Demerdash *et al*., 2012).

Determination of the active secondary metabolites:

Total phenolics:

Phenolics content of different plant extracts was measured using Folin Ciocalteu assay developed by Lin and Tang, (2007). The total phenolics content was expressed as mg gallic acid equivalent (GAE) per100 gram air dried plant.

Total flavonoids:

Flavonoids content of different plant extracts was measured using aluminum chloride colorimetric assay developed by Chang *et al*., (2002). The total flavonoids content was expressed as mg catechin equivalent per100 gram air dried plant material.

Total alkaloids:

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

* **Corresponding author.**
E-mail address: mzayat2001@yahoo.com
DOI: 10.21608/jpp.2020.79099

Singh *et al*., (2004). The total alkaloids content was expressed as mg colchicine equivalent per100 gram dried plant material.

Evaluation of antioxidant activity:

Antioxidant activity of the studied plants extracts carried out according to the following assays:

Radical Scavenging Activity (RSA) Using 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 follow:

$$\%\ \text{Scavenging activity (RSA)} = \frac{(\text{A0 "control"}) - (\text{A sample})}{(\text{A0 "control"})} \times 100$$

Where A_0 is the absorbance of the control and A is the absorbance of the sample. Each sample was analyzed in triplicate.

These values were graphed against plant 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.

Radical Scavenging Activity (RSA) Using ABTS•+ Assay:

ABTS•+ (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was done according to the method described by Re *et al*., (1999) and ascorbic acid was used as reference compound for comparison. Percentages of inhibition were calculated from the following equation:

$$\%\ \text{Scavenging activity (RSA)} = \frac{(\text{A0 "control"}) - (\text{A sample})}{(\text{A0 "control"})} \times 100$$

Where A_0 is the absorbance of the control and A is the absorbance of the sample. Each sample was analyzed in triplicate.

Antimicrobial activity of *Brassica juncea* extracts:

The antimicrobial activity of the plant extracts were estimated using filter paper disc diffusion assay (Murray *et al*., 1995) using tested organisms namely: *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Erwinia Carotovora, Proteus vulgaris Eterobacter cloacae, Shigella sp*. and *Streptococcus pyogenesis* compared with streptomycin as standard antibiotic.

Liquid Chromatography/Mass-Spectrum (LC/MS-MS) Analysis:

Structural elucidation of the active compounds in 30% ethanol extract using LC/MS-MS spectroscopy. The system consisted of an HP-1100 HPLC and an LCQ Max mass spectrometer (Thermo Finnigan). using methanol (70%) and 0.1% formic acid (30%) as eluents for 55min. per each injection. The mass range m/z used 100-1000.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of water and 30% ethanolic extracts of the studied plant revealed the presence of medicinally active metabolites including alkaloids, tannins, phenolics, flavonoids, glycosides and terpenoids. These active compounds were quantitatively analyzed and the results obtained revealed that water extract was slightly higher than ethanol extract in alkaloids and phenolics content while that of ethanol was higher than water in the flavonoids content. Total phenolic compounds content was 536.7 and 491.58 mg gallic acid equivalent per100 g dried plant material for ethanol and water respectively. Total flavonoids content was 97.58 and 89.03 mg catechin equivalent per 100 g dried plant material for ethanol and water extracts, respectively as illustrated in Table (1).

Table 1. The estimated secondary metabolites in *Brassica juncea* extracts.

Secondary Metabolites	Water extract	30% Ethanolic extract
Alkaloids (gram colchicines equivalent per 100 g dried plant)	5.80	1.5
Total Phenolics (mg gallic acid equivalent per 100 g dried plant)	536.7	491.58
Total Flavonoids (mg catechin equivalent per 100 g dried plant)	89.03	97.58
Total Flavonoids : Total Phenolics Ratio	16.59	19.85

The ethanolic extract exhibited antioxidant activity higher than water extract by using DPPH assay with IC_{50} value of 0.170 and 0.390 mg extract/mL, and by using ABTS assay with inhibition percent values of 75.5% and 68.9% for 30% ethanol and water extracts, respectively as illustrated in Table (2).

Table 2. Radical Scavenging Activity (RSA) of *Brassica juncea* extracts using ABTS•+ DPPH• assays.

Extracts	DPPH• $IC_{50\%}$ (mg/mL)	ABTS•+ (% of Inhibition)
Water extract	0.390	68.9%
30% Ethanolic extract	0.170	75.5%
Ascorbic acid	0.022	95%

The antioxidants scavenging activities for DPPH• are attributed to their hydrogen-donating abilities (Rahman *et al*., 2015). Ascorbic acid was used as the standard compound. Since polyphenols and their subclass flavonoids present in plant extracts are well known compounds that are potent antioxidants (Panche *et al*., 2016; Karak, 2019). Flavonoids were of the main components present in the ethanol extract; they may be of the main constituents that contribute to the antioxidant activity observed in this study.

Antimicrobial activity of *Brassica juncea* extracts:

The antimicrobial activity of ethanol and water extracts were tested using *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Erwinia Carotovora, Proteus vulgaris Eterobacter cloacae, Shigella sp*. and *Streptococcus pyogensis*. The tested ethanol extract showed significant zones of inhibition in a dose-dependent manner against most of the tested microorganisms while that of water showed no activity against any of the tested microorganisms as illustrated in Table (3).

Table 3. Antimicrobial activity of *Brassica juncea* water and ethanol extracts using disc diffusion assay.

Microorganisms	Water extract	30% Ethanol extract	Streptomycin
Staphylococcus aureus	-ve	-ve	11
Staphylococcus epidermidis	-ve	-ve	20
Bacillus subtilis	-ve	11	15
Pseudomonas aeruginosa	-ve	-ve	11
Klebsiella pneumonia	-ve	8.3	-ve
Candida albicans	-ve	19.3	13
Erwinia Carotovora	-ve	15.6	18
Proteus vulgaris	-ve	15	-ve
Eterobacter cloacae	-ve	10	10
Shigella sp.	-ve	17.3	-ve
Streptococcus pyogensis	-ve	-ve	18

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

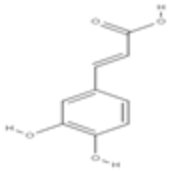
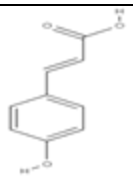
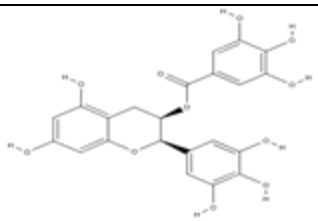
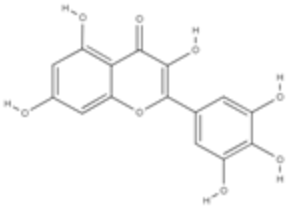
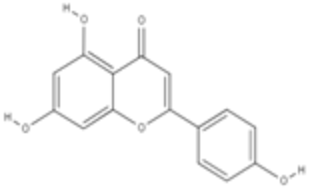
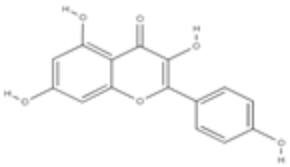
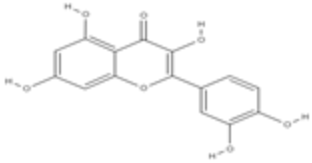
It has been reported in the literature that the major groups responsible for the antimicrobial activity of the plant extracts are phenolics, flavonoids, terpenoids, essential oils, alkaloids, lectins, and polypeptides (Erdem *et al*., 2015; Tulin and Kafkas, 2017; Othman *et al*., 2019).

Liquid Chromatography/Mass-Spectrum (LC/MS-MS) analysis:

The effectivity of 30% ethanolic *Brassica juncea* extract was subjected to more analysis for structural elucidation of the active compounds present using LC/MS-MS for determination of the phenolic profile of this extract. The structures of the compounds were identified or proposed based on comparison with standards (if applicable) and on MS-fragmentation. The compounds identified are shown in Table (4).

This extract might contain caffeic acid, p-coumaric acid, epigallocatechin gallate, myricetin, apigenin, quercetin-3-*O*-(caffeoyl)-glucoside and quercetin.

Table 4. The identified compounds in 30% ethanolic extract of *Brassica junceae* seeds.

Phenolic Compound	Chemical Structure	Molecular Weight	M-H	Fragments
Caffeic acid		180	179.0	179.1, 107, 135
p-Coumaric acid		164	162.9	162.9, 44, 118.9
Epigallocatechin gallate		442	441	441.1, 169.0, 441.1, 289.0
Myricetin		318	317	317.0, 150.9, 317.0, 178.9
Apigenin		270	269	269.0, 116.9, 269.0, 148.9
Quercetin-3-*O*-(caffeoyl)-glucoside		626	625	624.8 , 463.1 , 462.4, 341.5
Quercetin		302	301	300.9.0, 121.0

CONCLUSION

In conclusion, these rich phenolic profiles were probably responsible for the antioxidant and antimicrobial activities of *Brassica junceae* seeds. The studied plant can be seen as a potential source of useful drugs. The results from this study in addition to those from previous studies could be considered as a reference to antioxidant and antimicrobial activities of *Brassica juncea* seeds with biologically active and stable components. Thus, a scientific foundation to use these plants in medicine can be profound to improve the local users' healthcare

REFERENCES

Bassan, P., Bhushan, S., Kaur, T., Arora, R., Arora, S., & Vig, A. P. (2018). Extraction, profiling and bioactivity analysis of volatile glucosinolates present in oil extract of *Brassica juncea* var. *raya*. *Physiology and molecular biology of plants: an international journal of functional plant biology*, *24*(3), 399–409. https://doi.org/10.1007/s12298-018-0509-4

Chang, C. C.; Yang, M. H.; Wen, H. M. and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10, 178–182.

El-Demerdash M. A., El-Shahaby O. A., Khedr A., Kefalas P. and El-Zayat M. M. (2012). Antioxidant activity and phenolic profile of wild and cultivated endemic *Origanum syriacum var. Sinaicum* in Saint Catherine protectorate, southern Sinai.

Erdem, S. A., Nabavi, S.F., Orhan, I.E. *et al.* (2015). Blessings in disguise: a review of phytochemical composition and antimicrobial activity of plants belonging to the genus *Eryngium* . *DARU J Pharm Sci* 23, 53. https://doi.org/10.1186/s40199-015-0136-3

Karak P. (2019). Biological Activities Of Flavonoids: An Overview. Ijpsr, 10(4): 1567-1574.

Kim, H. W., Ko, H. C., Baek, H. J. *et al.* (2016). Identification and quantification of glucosinolates in Korean leaf mustard germplasm (*Brassica juncea* var. *integrifolia*) by liquid chromatography–electrospray ionization/tandem mass spectrometry. *Eur Food Res Technol* 242, 1479–1484. https://doi.org/ 10.1007/ s00217-016-2648-6

Kumar V., Kumar A., Dev Barothia N., Chatterjee S. (2011). Therapeutic potentials of Brassica juncea: an overview. 1(1): e2. doi: http://dx.doi.org/ 10.5667/ tang.2011. 0005

Lin, Jin-Yuarn and Tang, Ching-Yin (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101, 140–147.

Liyana-Pathirana M., Shahidi F. (2005) Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. J. of Agric. & Food Chemistry 53: 2433–2440.

Missouri Botanical Garden, (2019). Tropicos database. In: Tropicos database. St. Louis, Missouri, USA: Missouri Botanical Garden. http://www.tropicos.org/

Murray R., Rosenthal S., Kobayashi S., Pfaller A. (1998). Medical Microbiology. 3rd ed. St. Louis: Mosby, p.161.

Ogidi O., Omu O., Ezeagba P. (2019). Ethno pharmacologically active Components of *Brassica Juncea* (Brown Mustard) Seeds. International Journal of Pharmaceutical Research and Development, 1(1): 9-13.

Othman, L., Sleiman, A., & Abdel-Massih, R. M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Frontiers in microbiology*, *10*, 911. https://doi.org/10.3389/fmicb.2019.00911.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. *Journal of nutritional science*, *5*, e47. https://doi.org/10.1017/jns.2016.41.

Rahman, M. M., Islam, M. B., Biswas, M., & Khurshid Alam, A. H. (2015). In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. *BMC research notes*, *8*, 621. https://doi.org/10.1186/s13104-015-1618-6

Rahman, M., Khatun, A., Liu, L., & Barkla, B. J. (2018). Brassicaceae Mustards: Traditional and Agronomic Uses in Australia and New Zealand. *Molecules (Basel, Switzerland)*, *23*(1), 231. https://doi.org/ 10.3390/molecules23010231

Re R.; Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical. cation decolorization assay. *Free Radical Biology & Medicine*, Vol. 26, 1231–1237.

Sharma A., Kumar V., Kanwar M. K., Thukral A. K. and Bhardwaj R. (2017). Phytochemical profiling of the leaves of Brassica juncea L. using GC-MS. International Food Research Journal 24(2): 547-551.

Singh DK, Srivastva B, Sahu A (2004) Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals. Analytical Sci 20:571-573

Tulin A. and Kafkas E. (2017). Phytochemicals in Fruits and Vegetables, Superfood and Functional Food - An Overview of Their Processing and Utilization, Viduranga Waisundara and Naofumi Shiomi, IntechOpen, DOI: 10.5772/66987.

التركيب الكيميائي والنشاط المضاد للأكسدة والضد ميكروبي لمستخلصات بذور الخردل المائية والكحولية

ساجده سعيد عزيز[١] ، مصطفى محسن الزيات[٢] و أيمن يحيى الخطيب[٣]

[١]قسم البستنة ، كلية علوم الهندسة الزراعية ، جامعة السليمانية ، السليمانية ، إقليم كوردستان ، العراق

[٢]وحدة الهندسة الوراثية والتكنولوجيا الحيوية ، جامعة المنصورة ، المنصورة ، مصر

[٣]قسم الكيمياء الزراعية ، كلية الزراعة ، جامعة المنصورة ، المنصورة ، مصر

ينتمي نبات الخردل للعائلة الصليبية ويستخدم في الطب الشعبي وكمكون للأطباق الأساسية كمصدر لزيت المسترده. وقد تضمنت تلك الدراسة دراسة التركيب الكيميائي وكذلك النشاط المضاد للأكسدة والنشاط الضد ميكروبي بالإضافة إلى تفسير تركيب بعض المركبات النشطة للمستخلص الأكثر نشاطا بإستخدام جهاز LC/MS-MS. أظهرت النتائج أن مستخلص ٣٠% إيثانول كان له نشاط مضاد للأكسدة أعلى من المستخلص المائي حيث قدرت القدرة على خلب الشقوق الحرة مقاسة بتركيز المستخلص الذي يتمكن من خلب ٥٠% (IC_{50}) من الشق الحر DPPH والذي كلما قل تركيزه زادت كفاءة المستخلص كمضاد للأكسدة حيث وجد أنه بالنسبة للمستخلص المائي ٠,٣٩٠ مجم من المستخلص لكل ملليلتر وبالنسبة للمستخلص الايثانولي كانت ٠,١٧٠ مجم من المستخلص لكل ملليلتر وقد توافقت النتائج مع تجربة ABTS حيث كانت قدرة المستخلص الإيثانولي على خلب الشق الحر ٧٥% بينما كانت للمستخلص المائي ٦٨,٩% . وقد اتضح من خلال التحاليل الكيميائية أن المستخلص المائي إحتوى نسب من القلويدات والفينولات أعلى من المستخلص الكحولي بينما أظهر المستخلص الإيثانولي نسب أعلى من الفلافونيدات. أما عن مدى قدرة المستخلصات كمضدات ميكروبية فقد أظهرت النتائج عدم قدرة المستخلص المائي على التأثير على أي من السلالات الممرضة بينما أظهر المستخلص الإيثانولي مدى واسع من النشاط الضد ميكروبي ضد غالبية الكائنات المختبرة والتي تضمنت الميكروبات التالية (*Staphylococcus epidermidis, Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Candida albicans, Erwinia carotovora, Proteus vulgaris Eterobacter cloacae, Shigella sp* and *Streptococcus pyogensis*) وللتعرف على المكونات الفعالة المسئولة عن نشاط المستخلص الإيثانولى كمضد للأكسدة وكمضد ميكروبى تم تحليله بإستخدام جهاز LC/MS-MS وعليه فقد تم التعرف على المركبات التالية (caffeic acid, p-coumaric acid, epigallocatechin gallate, myricetin, apigenin, quercetin-3-*O*-(caffeoyl)-glucoside and quercetin).