

Original Research Article

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Proximate Composition and Biological Assay of Two Mustard Species (*Brassica nigra* (L.) K. Koch and *Brassica tournefortii* Gouan) in Libya

Salem Mousbah Khalifa *

Department of Biology, College of Education, University of Al-Jufra, Libya

*Corresponding author

ABSTRACT

People will still be looking for new sources of food, feed, and medicine to face the challenges of malnutrition and population growth. In this study, the proximate composition, minerals, bioactive-secondary metabolites, and biological assay (antioxidants and allelopathic activities) of two *Brassica* species collected from the coastal region of Misurata, Libya: *Brassica nigra*(L.) K. Koch and *Brassica tournefortii* Gouan were investigated. The shoot of *Brassica tournefortii* had the highest contents of moisture (22.19%), total ash (16.90%), crude fibers (25.40%), crude fat (5.5%), sodium (45.10 mg g⁻¹ dry weight), potassium (38.80 mg g⁻¹ dry weight), calcium (22.40 mg g⁻¹ dry weight), magnesium (13.50 mg g⁻¹ dry weight), total phenols (34.50 mg g⁻¹ dry weight) and flavonoids (18.78 mg g⁻¹ dry weight).While, the shoot of *Brassica nigra* attained the greatest values of crude protein (10.30%), tannins (10.4 mg g⁻¹ dry weight), alkaloids (2.09 mg g⁻¹ dry weight), and saponins (1.50 mg g⁻¹ dry weight). Regarding antioxidants activity, the extract of *B. tournefortii* shoots had a high IC50 value (0.32 mg ml⁻¹) than the shoot of *B. nigra* (0.37 mg ml⁻¹), but lower than catechol (0.18 mg ml⁻¹).The methanolic extracts of *B. tournefortii* shoots and roots had the highest germination inhibition percentages (88.1 and 82.32%, respectively) for *Chenopodium murale* seeds. The extracts of *B. tournefortii* shoots displayed the highest inhibition percentage for plumule and radicle growths of *Chenopodium murale*(93.17and 96.21 %, respectively).The highest allelopathic activity of *B. tournefortii* may be attributed to the high contents of total phenols and flavonoids as compared with *B. nigra*. The present work suggests that *Brassica tournefortii* is a good candidate as a nutritional, antioxidant source, and an eco-friendly bioherbicide against a noxious weed *Chenopodium murale*.

Keywords

Allelopathy,
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Introduction

Wild plants play a vital role in human life as sources of food, feed, dyes, fuels, ethnomedicine, phytoremediation, etc. (Zimdahl, 2007). The importance of these plants is attributed to their richness in proximate primary composition

(carbohydrates, proteins, fibers, fats, and minerals), and bioactive secondary metabolites (total phenols, flavonoids, tannins, alkaloids, saponins, etc.) (Newman and Cragg, 2007; Kumari and Kakkar, 2008). The biological activities of plants include many aspects, for example, antioxidant activity, allelopathic, antimicrobial, cytotoxic

effects, and other physiological effects on the living organisms.

Brassica is the most significant genus in Family Brassicaceae which includes 350 genera and ca. 3500 species. Species of genus *Brassica* had worldwide importance due to their medicinal, nutritional and pharmaceutical benefits. Globally, most *Brassica* species are cultivated for many purposes, for example, as oilseed crops, vegetables, medicinal and animal forage (Nawaz *et al.*, 2018). Following soybean and palm, *Brassica* spp. are the third supply for edible oils and vegetables. *Brassica* species are a rich source of phenolics, flavonoids, alkaloids, glucosides, and terpenoids. Therefore, these plants had antioxidant activities through free radicle scavenging activity, and metal-reducing and chelating. Among different species of the genus *Brassica*, *Brassi canigra* and *Brassica tournefortii* are also antioxidant and phytochemical-rich species. The different organs (root, stem, leaves, seeds) of *Brassica* plants are consumed as vegetables or oil, food, and for medicinal purposes (Nawaz *et al.*, 2018).

Brassica nigra (L.) K. Koch (black mustard) is an annual herbaceous plant with stiff hairs. This plant grows naturally as a weed within the winter field crops and along roads of the Mediterranean coastal region of Libya. It is widely distributed in Africa, China, Europe, and the USA (Al-Snafi, 2015).

The cultivation of *B. nigra* is attributed to its importance as a source of essential oil, mustard flour, condiment, honey producers, and various medicinal purposes (i.e. stimulant, diuretic, rubefacient, arthritis, edema, emetic, hair growth, colds, pneumonia, headaches, anticancer, anthelmintic, etc.) (Heidari and Abbsifard, 2002; Udin *et al.*, 2020). This plant is also

had antibacterial activities (Gomezde Saravia *et al.*, 1998; Danlami *et al.*, 2016).

Brassica tournefortii Gouan (Sahara mustard) is an annual erect herb native to the Mediterranean coast and arid deserts of the Middle East and North Africa (Trader *et al.*, 2006). Medicinally, it is a good source of antioxidants, polyphenols, nutrients and nutraceuticals (Rahmani *et al.*, 2020). Similar to other *Brassica* spp, *B. tournefortii* is widely used for medicinal, culinary and ecological purposes (Matthäus and Özcan 2009). Also, some previous studies (e.g. Minnich and Sannders, 2000; Curto *et al.*, 2006; Rahmani *et al.*, 2019) demonstrated the use of *B. tournefortii* in traditional meals, as anti-parasitic and as anti-breast cancer. On contrary, *B. tournefortii* becomes a problematic invasive weed in Australia (Mahajan *et al.*, 2020). Moreover, this plant is drought-tolerant and produces numerous long-lived seeds that can persist viable in the soil for several years (Mashaly *et al.*, 2015).

In this study, we assumed *Brassica rapa* and *Brassica tournefortii* are considered excellent candidates as nutritional and pharmaceutical sources. Therefore, the main objectives of the current study were to 1) estimate the proximate composition of the shoots and roots of two *Brassica* species, 2) know the concentration of bioactive secondary metabolites and 3) assay antioxidants and allelopathic activities.

Materials and Methods

Determination of proximate composition

Two *Brassica* spp. were collected from the coastal region of Misurata, Libya, then cleaned with running-water to remove debris, divided into shoots and roots, and finally air-dried before grinding. Moisture content, total ash, crude fiber, crude protein, crude fat and

total carbohydrates were estimated according to the protocol of the Association of Official Analytical Chemists (AOAC, 2000).

Minerals analysis

The macroelements (Na^+ and K^+) and (Ca^{++} and Mg^{++}) of shoots and roots of *Brassica nigra* and *B. tournefortii* were analyzed after heating with HNO_3 by using a flame photometer (Hanchen FP) and atomic absorption spectrometer (A Perkin-Elmer), respectively (AOAC, 2000).

Bioactive secondary metabolites

For both shoots and roots of the two studied *Brassica* spp, total phenols and tannins were estimated in ethanolic plant-extracts by using Folin and vanillin-HCl reagents, respectively (Sadasivam and Manickam, 2008). Flavonoids content was determined by using the AlCl_3 method (Boham and Kocipai-Abyazan, 1974). Alkaloids content was gravimetrically estimated using NH_4OH solution (Harborne, 1984). Saponins content was estimated according to Obadoni and Ochuko (2002).

Biological activities

Antioxidant activity using DPPH scavenging activity

The antioxidant activity of shoot and roots of two *Brassica* spp. were valued via scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH). The half inhibitory concentration (IC_{50}) of each extract to reduce DPPH was calculated (Lim and Quah, 2007). Catechol was considered for comparison.

Allelopathic activity

To study the allelopathic activity of the two studied *Brassica* species, seeds of a nuisance

weed *Chenopodium murale* L. was collected from field crops. Different methanolic concentrations of 8, 6, 4 and 2% from shoots and roots of *Brassica* species were prepared from stock extract (10% w/v). After sterilization of *Chenopodium murale* seeds, the methanolic extracts of *Brassica* species were tested against seed germination (germination inhibition percent), plumule and radicle lengths (growth inhibition percent) of *C. murale* until constant readings for germination and growth data (Hegazy, 1997; Uremis *et al.*, 2005).

Statistical analysis

The significance between data were tested using one-way ANOVA in XLSTAT program v. 2018.

Results and Discussion

Proximate composition, minerals and bioactive secondary metabolites

The primary proximate analysis is among the most important indicators that refer to the possibility of using any plant as a food or forage and categorizing nutritional facts (Keyata *et al.*, 2020). The proximate composition, minerals and bioactive secondary metabolites of shoot and roots of *Brassica nigra* and *B. tournefortii* are shown in Table 1.

The moisture contents for shoots of *B. tournefortii* and *B. nigra* were 22.19 and 21.10%, respectively. The value of moisture content is an indicator of food quality and degree of susceptibility for deterioration and storage period. The results showed that the shoots and roots of the study *Brassica* species had low moisture content as compared with aerial parts of related species Girgir and Figl (87.39 and 94%, respectively) (Keyata *et al.*, 2020). This finding addressed the resistance

of the aerial-parts of *Brassica* species against microbial spoilage.

Total ash showed a significant difference among shoot and roots of the *Brassica* species. Shoots of *B. tournefortii* had the highest value (16.90%) and roots of *B. nigra* had the lowest value (9.50%). This finding agreed with the ash content of leaves of radish from Egypt (Magied *et al.*, 2016) and proved the highest mineral content. The aerial parts of *B. tournefortii* had significantly higher fiber content (25.40%) similar to values in leaves of Fig.1. The consumption of fiber-rich plants reduces the risk of colon cancer,

improves digestion and removal of wastes (DeVries *et al.*, 2001). Based on dry matter, the protein content of the two *Brassica* species showed a significant variation at $p < 0.05$. Sufficient protein content in the diet keeps optimal growth.

The highest protein content was attained in the shoots of *B. nigra*(10.30%) but the lowest in the roots of *B. tournefortii* (7.11%). Similar results were also reported for dried leaves of Fig1 and *Moringa* (between 24 and 26 g/100 g) (Mikore and Mulugeta, 2017; Keyata *et al.*, 2020).

Table.1 Proximate composition, minerals and bioactive secondary metabolites of shoots and roots of *Brassica* species. Different letters in the same row are significantly different at $p < 0.05$

Parameter	<i>Brassica nigra</i>		<i>Brassicatournefortii</i>	
	Shoot	Root	Shoot	Root
Proximate composition (%)				
Moisture content	21.10±1.4 ^a	18.50±0.9 ^b	22.19±2.0 ^a	18.9±0.5 ^b
Total ash	13.40±3.0 ^a	9.50±2.4 ^a	16.90±1.0 ^b	10.18±1.5 ^b
Crude fiber	20.20±0.8 ^a	18.80±0.9 ^a	25.40±2.0 ^b	22.89±1.8 ^{ab}
Crude protein	10.30±2.5 ^a	9.67±1.3 ^a	8.58±0.5 ^b	7.11±1.2 ^{ab}
Crude fat	4.80±0.5 ^a	4.1±0.3 ^a	5.50±0.9 ^b	5.0±0.6 ^b
Total carbohydrates	50.4±1.5 ^a	58.23±1.2 ^b	46.83±2.1 ^a	58.81±2.0 ^b
Minerals (mg g⁻¹ dry weight)				
Sodium (Na ⁺)	38.90±0.6 ^a	28.60±0.8 ^b	45.10±2.5 ^c	34.20±0.1 ^d
Potassium (K ⁺)	29.20±1.5 ^c	18.23±0.5 ^b	38.80±0.4 ^a	29.70±2.0 ^c
Calcium (Ca ⁺⁺)	18.50±0.7 ^a	17.80±0.4 ^a	22.40±0.9 ^b	18.60±1.0 ^a
Magnesium (Mg ⁺⁺)	8.20±0.4 ^a	8.0±0.1 ^a	13.50±0.3 ^b	13.0±0.8 ^b
Secondary metabolites (mg g⁻¹ dry weight)				
Total phenols	26.12±1.5 ^a	18.4±1.3 ^b	34.50±2.6 ^c	20.50±1.3 ^{ab}
Tannins	10.4±1.8 ^a	6.40±0.5 ^b	9.11±1.0 ^a	7.60±1.9 ^{ab}
Flavonoids	12.90±2.0 ^c	8.21±1.0 ^a	18.78±2.0 ^b	16.80±1.5 ^d
Alkaloids	2.09±0.3 ^a	1.11±0.1 ^b	1.10±0.3 ^b	1.40±0.0 ^b
Saponins	1.50±0.1 ^a	1.03±0.0 ^{ab}	1.30±0.0 ^a	1.10±0.0 ^b

Fig.1 IC₅₀ values (mg ml⁻¹) of shoots and roots of *Brassica nigra* and *Brassica tournefortii*. IC₅₀ of catechol was 0.18 mg ml⁻¹

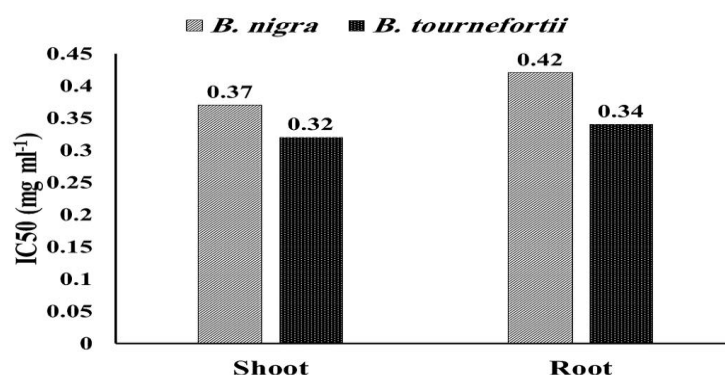
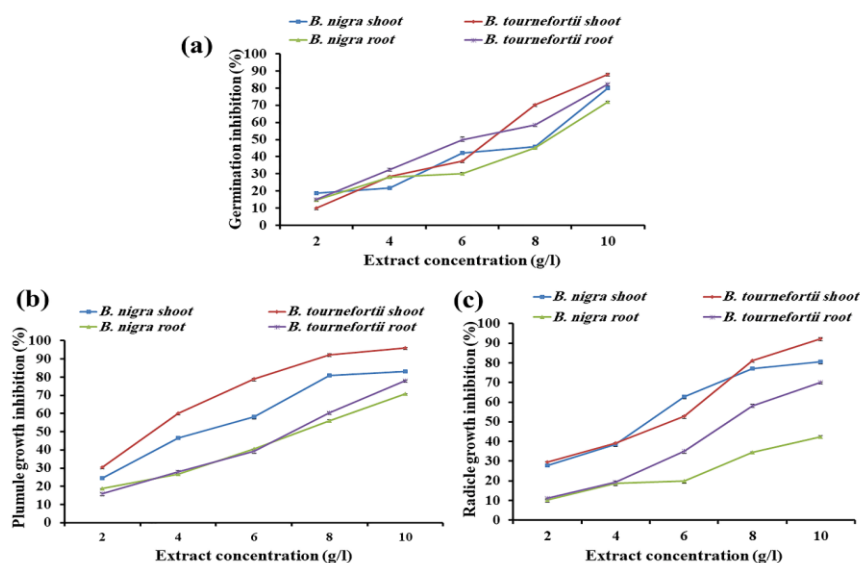


Fig.2 Allelopathic activity (mean values of inhibition percentages ±SE) of methanolic extracts from shoot and roots of *B. nigra* and *B.tournefortii* against a) seed germination, b) plumule growth and c) radicle growth of *Chenopodium murale*



The shoot and roots of *B. tournefortii* showed relatively great values of crude fats (5.50 and 5%, respectively) than shoots and roots of *B. nigra* (4.80 and 4.1%, respectively). Rios *et al.*, (2014) addressed the importance of raw fats and oils for modified-food products, such as bakery, sauces, margarine and confectionery. As compared with the study Brassica species, Kwon *et al.*, (2020) reported a low value of crude protein (1.5%) in *Brassica juncea* cultivars in Korea. The

percent of carbohydrates was significantly different between shoots and roots of the two *Brassica* species. Roots of *B. tournefortii* attained a comparable value (58.81%) with roots of *B. nigra* (58.23%). High levels of carbohydrates provide adequate energy for the human/animal and the plant itself.

The minerals content has a crucial role in the human body as it helps to build strong bones and performance of various metabolic

activities. The concentration of macroelements (Na^+ , K^+ , Ca^{++} and Mg^{++}) is significantly differed and displayed in Table 1. The shoots of *B. tournefortii* had the highest values of sodium (45.10 mg g^{-1} dry weight), potassium (38.80 mg g^{-1} dry weight), calcium (22.40 mg g^{-1} dry weight) and magnesium (13.50 mg g^{-1} dry weight). The minerals content in the studied *Brassica* spp is in close agreement with edible parts of Figl and Girgir (Keyata *et al.*, 2020). Sodium is an essential element for physiological activities and homeostasis, but an excess of sodium in a diet causes a rise in blood pressure (Farquhar *et al.*, 2015). Potassium-, calcium- and magnesium-rich-plants can help people who suffer from the deficiency of these elements in their body and also improve bone building (Trumbo *et al.*, 2001; Sodamade *et al.*, 2013).

The results of bioactive secondary metabolites contents of shoots and roots of *Brassica* species are shown in Table 1. The shoot of *B. tournefortii* had significantly high total phenols and flavonoids levels (34.50 and 18.78 mg g^{-1} , respectively) than the shoot of *B. nigra* (26.12 and 12.90 mg g^{-1} , respectively). On the other hand, the shoot of *B. nigra* attained the highest contents of tannins (10.4 mg g^{-1}), alkaloids (2.09 mg g^{-1}) and saponins (1.50 mg g^{-1}). Phenolics play a key role in eliminating cardiovascular diseases, diabetes and cancers (Santos-Buelga *et al.*, 2019).

This finding indicates that, the shoots and roots of two *Brassica* species had great phenolic compounds as compared to leaves of Figl and *Moringa* (26.3 and 2.53 mg g^{-1} , respectively) (Saha *et al.*, 2015; Keyata *et al.*, 2020). The flavonoids, alkaloids, tannins and saponins below toxicity level are known to have therapeutic activities such as anti-viral, anti-malarial and anti-tumor properties (Roy, 2017; Keyata *et al.*, 2020).

Biological activities

Antioxidants assay by DPPH free radicle scavenging activity

The free radicle 1, 1-diphenyl-2-picrylhydrazyl (DPPH) is widely used for an assay of the radicle scavenging activity of plant extracts. The scavenging activity on DPPH is expressed as IC₅₀ which denotes the concentration of the extract required to hinders the DPPH by 50% (Figure 1).

The extract of *B. tournefortii* shoots had high IC₅₀ value (0.32 mg ml^{-1}) than the shoot of *B. nigra* (0.37 mg ml^{-1}). A similar trend was observed for root extracts of both studied *Brassica* species. All extracts showed higher values than catechol (IC₅₀= 0.18 mg ml^{-1}). The antioxidant properties of *Brassica* species were confirmed by many previous studies (Heimler *et al.*, 2007; Fernandes *et al.*, 2007; Li *et al.*, 2012; Xiao *et al.*, 2018; Favela-González *et al.*, 2020). The antioxidant potential of *Brassica* species is attributed to phenolic compounds, flavonoids and glucosinolates (Aires *et al.*, 2011).

Allelopathic activity

The allelopathic activity of methanolic extracts of shoots and roots of *B. nigra* and *B. tournefortii* against germination and growth of *Chenopodium murale* is shown in Figure 2. In general, the inhibition percentage was concentration dependent, by increasing extract concentration, the inhibition percent for germination and seedling growth increases. The methanolic extracts of *B. tournefortii* shoots and roots had the highest inhibition percentages (88.1 and 82.32%, respectively) for *C. murale* seed germination after 15 days of treatment (Figure 2a).

Regarding plumule inhibition, the methanolic extracts take the following sequence: *B.*

tournefortii shoot (93.17%)>*B. nigra* shoot (83.25%)>*B. tournefortii* root (78.16%) >*B. nigra* root (70.89%) (Figure 2b). On the other hand, the methanolic extracts of *B. tournefortii* showed the highest inhibition percent (96.21%) for the radicle growth of *C. murale* (Figure 2c). The highest allelopathic of *B. tournefortii* may be attributed to the high contents of total phenols and flavonoids as compared with *B. nigra*. This finding in close agreement with Bones and Rossiter (1996) who reported the allelopathic potentials of *Brassica* species. Halkier and Gershenzon (2006) and Blum (2011) displayed that, *Brassica* species contain allelochemicals (phenolic compounds, flavonoids, tannins) that are released to the environment and can affect the germination and growth of other species. Twaha and Turk (2003) reported that, *B. nigra* had allelochemicals that minimize the germination and growth of other plant species. The mechanism of allelochemicals for germination and growth inhibition may be due to their effect on cell division, oxidative enzymes, protein synthesis or membrane permeability (Chou, 2006).

In conclusion, *brassica tournefortii* had high proximate constituents (moisture, total ash, crude fibers, crude fat, and carbohydrates), minerals (sodium, potassium, calcium, and magnesium) and bioactive secondary metabolites (total phenols and flavonoids) as compared with *Brassica nigra*. Moreover, *B. tournefortii* shoot had high antioxidant activity and allelopathic effect against the nuisance weed *Chenopodium murale*. The present study suggests that, *Brassica tournefortii* is a good candidate as nutritional, antioxidant sources and an eco-friendly bioherbicide.

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