DOI: http://dx.doi.org/10.32571/ijct.1146979 **E-ISSN: 2602-277X**

International Journal of Chemistry and Technology

http://dergipark.org.tr/ijct

Research Article

Nutritive value, antimicrobial and antioxidant activities of *Tchihatchewia isatidea* Boiss

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Received: 22 July 2022; Revised: 18 October 2022; Accepted: 24 October 2022

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Citation: Kırbağ, S.; Bircan, M. G.; Bircan, B. *Int. J. Chem. Technol.* 2022, 6 (2), 97-101.

ABSTRACT

Tchihatchewia isatidea Boiss (Brassicaceae) is popularly known as "Paint and Bridal Flower". Flower pieces and extracts can be used for painting, wound healing, and cough treatments. In this study, the nutritional and medicinal properties of *T. isidea* were determined. For this purpose, carbohydrate, crude oil, crude protein, crude cellulose, energy, crude ash, dry matter, moisture, element, vitamin, antioxidant capacity, DNA protective, and antimicrobial effects of *T. isatidea* were investigated. Crude ash, crude protein, crude fat, crude cellulose, carbohydrate, dry matter, energy, organic matter, K, Ca, Na, Fe, Cu, Zn, Pb, Mn, vitamin E and vitamin A paremeters of *T. Isatidea* were 14.37%, 20.60%, 1.82%, 29.02%, 63.21%, 91.57%, 351.62 kcal 72.20%, 42.5 mg/kg, 102.4 mg/kg, 260 mg/kg, 1.59 mg/kg, 0.121 mg/kg, 1.160 mg/kg, 2.16 mg/kg, 7.49 mg/kg, 22.95 mg/g and 2.82 mg/g, respectively. The DPPH (α, α-Diphenyl-1-picrylhydrazyl) radical scavenging activity of the plant extract was 82.34% at 100 µL, 68.20% at 50 µL, 30.80% at 25 µL and 11.68% at 10 µL. The total oxidant level was 69.96 µmol/L, the total antioxidant level of *T. isatidea* was 3.91 µmol/L and 133.56 nmol/g Malondialdehyde (MDA). In the existence of UV and H_2O_2, it was found that the *T. isatidea* plant extract protects scDNA. It has also been established that the flower extract of the plant hinders the growth of pathogenic microorganisms at varying rates. Because of its thorny structure, the plant which can not be used as a vegetable can be transformed into a dried powdered form of powder and can be consumed with water in the form of a decoction or infusion. The plant can be mixed with powdered petroleum jelly and used as an external wound healing agent.

Keywords: *T. isatidea*, paint flower, nutritional content, antimicrobial activity, antioxidant activity.

Tchihatchewia isatidea Boiss'in besin değeri, antimikrobiyal ve antioksidan aktiviteleri

ÖZ

Tchihatchewia isatidea Boiss (Brassicaceae) halk arasında "Boya ve Gelin Çiçeği" olarak bilinir. Çiçek parçaları ve özleri boyama, yara iyileştirme ve öksürük tedavilerinde kullanılabilmektedir. Bu çalışmada *T. isatidea*'nın besleyici ve tıbbi özellikleri belirlenmiştir. Bu amaçla *T. isatidea*'nın karbonhidrat, ham yağ, ham protein, ham selüloz, enerji, ham kül, kuru madde, nem, element, vitamin, antioksidan kapasite, DNA koruyucu ve antimikrobiyal etkileri araştırıldı. *T. isatidea*'nın ham kül, ham protein, ham yağ, ham selüloz, karbonhidrat, kuru madde, enerji, organik madde, K, Ca, Na, Fe, Cu, Zn, Pb, Mn, E vitamini ve A vitamini parametreleri sırasıyla 14.37%, %20.60, %1.82, %29.02, %63.21, %91.57, 351.62 kcal %72.20, 42.5 mg/kg, 102.4 mg/kg, 260 mg/kg, 1.59 mg/kg, 0.121 mg/kg, 1.160 mg/ kg, 2.16 mg/kg, 7.49 mg/kg, 22.95 mg/g ve 2.82 mg/g olarak bulundu. Bitki ekstraktının DPPH (a, a-Difenil-1-pikrilhidrazil) radikal süpürme aktivitesi 100 µL'de %82.34, 50 µL'de %68.20, 25 µL'de %30.80 ve 10 µL'de %11.68 olarak belirlendi. *T. isatidea*'nın toplam oksidan seviyesi (TOS) 69.96 µmol/L, toplam antioksidan seviyesi (TAS) 3.91 µmol/L ve Malondialdehit (MDA) seviyesi 133.56 nmol/g olarak belirlendi. *T. isatidea* bitki ekstraktının UV ve H_2O_2 varlığında scDNA'yı koruduğu tespit edilmiştir. Bitkinin çiçek özütünün patojenik mikroorganizmaların büyümesini değişen oranlarda engellediği belirlenmiştir. Dikenli yapısından dolayı sebze olarak kullanılamayan bitki, kuru toz haline getirilerek su ile kaynatma veya demleme yöntemiyle tüketilebilir. Bitki, toz vazelin ile karıştırılabilir ve harici bir yara iyileştirici ajan olarak kullanılabilir.

Anahtar Kelimeler: *T. isatidea*, boya çiçeği, besin değeri, antimikrobiyal aktivite, antioksidan aktivite.

1. INTRODUCTION

It is approximated that the number of plant kinds is between 250,000 and 500,000.[1] According to WHO data; 80% of the world population and 95% of the African population benefit from treatment methods based on medicinal plants. It is estimated that about 70,000 medical plant species are utilized for this purpose.

DOI: http://dx.doi.org/10.32571/ijct.1146979 E-ISSN: 2602-277X

Approximately, 21,000 of these plant species are being used in the pharmaceutical industry.[2] *T. isatidea*, is one of the plants used for medical targets and the textile industry in Turkey, which belongs to the Brassicaceae family and is publicly named Paint Flower, Reddish Bride, and Bridal Flower.[3-5] *T. isatidea*, is a paleoendemic species in Turkey, which is generally found in Giresun, Sivas, Gümüşhane, Tunceli, Erzurum, Elazığ, Kars and Erzincan regions of the country. It is known that *T. isatidae* naturally grows in the surroundings of Sivas and Divriği, and its flowers are used as a natural paint material.[3] The extract, which is made by mixing *T isatidea* and *Hesperis schischkinii* Tzvelev (Mus evening star) root parts, and *Pistacia atlantica* Desf. (chewing gum) resin, has been reported to be used in wound healing in Ovacik-Tunceli region.[6] In Eastern Anatolia, it was found that the roots of *T. isatidea* were pounded and used as wound medicine.[7] Around Maden-Elazığ, flowers and root parts of *T. isatidea* have been used as cough suppressants.[4] This study purposed to determine the nutrient content of *T.isatidea* (crude ash, dry matter, crude oil, carbohydrate, crude protein, organic substance, element and vitamin), as well as free radical scavenging activity (DPPH), total antioxidant and oxidant levels, DNA damage reducing effect, and antimicrobial activity.

2. MATERIALS AND METHODS

2.1. Sample collection

T. isatidea was gathered from the surroundings of Karakaş, Baskil-Elazığ, Turkey (’38°37’01.6"N 38°41’46.4"E, 38°34’26.4"N 38°46’37.6"E ve 38°34’34.1"N 38°45’24.6"E’). The plant was collected during the spring season (from April to May). Samples were cleaned, dried, and stored at room temperature.

2.2. Proximate analysis

The approximate compositions of *T. isatidea* containing crude protein, moisture, crude ash, dry matter, organic matter and crude fat were determined accordingly AOAC [7]methods. The determination of total nitrogen (N) were determined by Kjeldahl method. Crude protein was calculated as N×6,25. Crude fat was determined at 550°C using both the total ash combustion and the soxhlet extraction method with a solvent carried out as described by AOAC.

2.3. Element analysis

Previously air-dried samples (at room temperature) were re-dried at 105°C overnight and crushed with pestle and a mortar. Samples were then dissolved in a mixture of HNO_3: H_2SO_4: H_2O_2 (10:1:1, 12 mL for 1 g sample) and heated at 100°C for about 10-15 min. After cooling, 50 mL of deionized water was added to reach 50 mL and then the mixture was filtered. All the glassware was cleaned with deionized water in order to avoid contamination. While amounts of Fe, Zn, Mn, Cu, Cd, Co, Cr, Pb and Ni were determined by atomical absorption spectrometer, amounts of Ca, Na, and K was determined by atomic emission spectrometer.[8]

2.4. Analysis of A, E Vitamins

Plant extract was prepared accordingly literature to determine number of vitamins. Content vitamins were analyzed by HPLC (296 and 326 nm for vitamin E and A, respectively). [9-11]

2.5. Antioxidant assay by DPPH free radical scavenging activity

The DPPH radical scavenging property of plant extract was measured by method of Brand-Williams et al.[12] A solution of 25 mg/L DPPH in methanol was prepared and 4.0 mL of this solution was mixed with 500, 250, 100, and 50 µg/mL of extract. The absorbance of mixture was measured at 517 nm. The reduced absorbance, amount of DPPH remaining, was determined as free radical scavenging activity. Radical scavenging activity (%) = (Abs control – Abs sample) / (Abs control) × 100 where Abs control is absorbance of DPPH radical + methanol; Abs sample is absorbance of DPPH radical + sample extract/standard.

2.6. Determination of MDA, Total Antioxidant activity and Total Oxidant activity

MDA analysis were made according to method described by literatures[10,11,13]. MDA levels were determined by HPLC .TAS and TOS values were measured with Rel Assay brand commercial kits (Rel Assay Kit Diagnostics, Turkey). Trolox was used as a calibrator for TAS tests and results were expressed in mmol Trolox equiv./L.[15] Hydrogen peroxide was used as a calibrator for TOS tests and results were expressed in mmol H_2O_2 equiv./L.[16]

2.7. The Determination of DNA Protective Activity

pBR322 plasmid DNA (vivantis) was used to detect ability of the samples to protect DNA from UV and oxidative damage. Plasmid DNA was damaged with H_2O_2 and UV in the presence of extracts. Taking into consideration that working samples are studied by modifying the method determined by Russo et al.[16], a gel-closure technique was used to prevent them from dispersing into the TBE when mixed with TBE. After sample and control loads were applied on a 1% agarose gel, a 1.5% agarose gel coating was applied to close the wells once more. Then screening was performed.

2.8. Preparation of microorganism cultures

Bacterial strains (*Salmonella enterica typhimirium, Staphylococcus aureus* COWAN 1, *Enterococcus*

DOI: http://dx.doi.org/10.32571/ijct.1146979 E-ISSN: 2602-277X

faecium, Proteus mirabilis, Listeria monocytegenes, B. subtilis, Bacillus megaterium DSM32, *Klebsiella pneumoniae* FMC 5, *Staphylococcus cohnii, Enterobacter aeregenes* CCM 2531, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DMS50071, *Proteus vulgaris* FMC1) and yeast (*C. tropicalis* ATCC1380, *Candida albicans* FMC17, *C. glabrata* ATCC 66032, *Epidermophyton* sp. and *Trichophyton* sp.) malt extracts in nutrient buyyon were inoculated, respectively (48 h at 25±1°C for yeast, 24 h at 35±1°C for bacteria). Disc Diffusion Method was used in this study. Petri dishes prepared in this manner were incubated at 4°C for 1.5-2 h, then incubated for 24 h at 37±1°C with bacteria-inoculated plates and plates at yeast and dermophyte-grafted plates at 25±1°C for 3 days.[17] The inhibition zones (mm) formed on the feeder at the end of the period were evaluated. Standard antibiotic discs were used for the control group.

3. RESULTS AND DISCUSSION

3.1. Nutritional Content of *T. isatidea*

Crude ash, crude protein, crude fat, crude cellulose, carbohydrate, dry matter and energy paremeters of *T. isatidea* were 14.37%, 20.60%, 1.82%, 29.02%, 63.21%, 91.57%, 351.62 kcal, 72.20% and 351.62 kcal respectively (Table 1).

Table 1. Nutritional Content of *T. isatidea*.

Dry Matter (%)	Crude Ash (%)	Crude Cellulose (%)	Crude Fat (%)
%91.57	**%14.37**	**%29.02**	**%1.82**
Organic Matter (%)	Carbohydrate (%)	Crude Protein (%)	Energy (Kcal)
%77.20	**%63.21**	**%20.60**	**351.62**

3.2. Element Content of *T. isatidea*

Mn, Fe, Zn, Cu and Pb element contents of *T. isatidea* were 7.49 mg / kg, 1.59 mg / kg, 11.60 mg / kg, 0.12 mg / kg and 2.16 mg / kg. Cr, Co and Cd were not detected (Table 2).

3.3. Vitamin content of *T. isatidea*

The vitamin A and vitamin E content of *T. isatidea* were 2.82 µg/g and 22.95 µg/g respectively (Table 3).

Table 2. Element content of T. isatidea

Micro elements	mg kg^{-1}±SD
Mn	7.49±0.12
Fe	1.59±0.03
Zn	11.60±0.11
Cu	0.12±0.01
Pb	2.16±0.07
Cr	0
Co	0
Cd	0

(Study conducted as three replicates and means ± calculated as standard deviation)

Table 3. Vitamin A and vitamin E content of *T. isatidea*.

	Vitamin A	Vitamin E
T. isatidea	2.82 µg/g	22.95 µg/g

3.4. Free radical scavenging activity (DPPH) of *T. isatidea*

According to DPPH (α, α-Diphenyl-1-picrylhydrazyl) free radical clearing results; *T. isatidea* was detected to be effective from 10 µL, and continuing linearly up to 100 µL in a concentration dependent manner (Table 5).

Table 5. Free radical scavenging activity (DPPH) of *T. isatidea*.

DPPH	10 µL	25 µL	50 µL	100 µL
T. isatidea	11.68±0.27	30.80±0.35	68.20±0.56	82.34±1.06

(Study conducted as three replicates and means ± calculated as standard deviation)

MDA was 133.56 nmol / g, TAS was 3.91 mmol Trolox Eq/L and TOS was 6.96 µmol H_2O_2 Eq/L

Table 6. MDA, TAS and TOS values of *T. isatidea*.

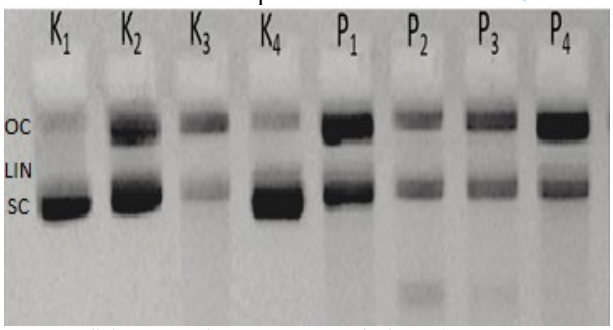
MDA	TAS	TOS
133.56 nmol/g	3.91 mmol Trolox Eq/L	6.96 µmol H_2O_2 Eq/L

3.5. DNA protective activity of *T. isatidea*

First 4 lines were used as controls. It was observed three bands (scDNA, linDNA and ocDNA) in 1st and 2nd lines of this study. In the third line, in the presence of H_2O_2, the interaction between UV and DNA resulted in OH radical to damage the DNA. We detected three bands in the 4th line as well (Figure 1). Under the presence of strong UV light and H_2O_2, protection potential of pBR322 plasmid DNA of the *T. isatidea* extracts were observed.

3.6. Antimicrobial activity of *T. isatidea*

Antimicrobial effect of plant is shown on Table 7.



(SC: Supercoiled DNA, LIN: Linear DNA, OC: Open-circular DNA)
C1: Plasmid DNA (3 µL) + distillate water (6 µL)
C2: Plasmid DNA (3 µL) + distillate water (6 µL) + ultraviolet
C3: Plasmid DNA (3 µL) + distillate water (6 µL) + ultraviolet+ hydrogen peroxide (1 µL)
C4: Plasmid DNA (3 µL) + distillate water (6 µL) + hydrogen peroxide (1 µL)
P1: Plasmid DNA (3 µL) + hydrogen peroxide (1 µL) + ultraviolet+ 1/10 Sample (5 µL)
P2: Plasmid DNA (3 µL) + hydrogen peroxide (1 µL) + ultraviolet+ 1/5 Sample (5 µL)
P3: Plasmid DNA (3 µL) + hydrogen peroxide(1 µL) + ultraviolet+ 1/2.5 Sample (5 µL)
P4: Plasmid DNA (3 µL) + hydrogen peroxide (1 µL) + ultraviolet+ 1/1.25 Sample (5 µL)

Figure 1. Gel image showing DNA protection pctivity of *T. isatidea*

DOI: http://dx.doi.org/10.32571/ijct.1146979 E-ISSN: 2602-277X

The development of microorganisms were inhibited best by the extract prepared with methanol, ethanol and water, in order. With the extract prepared by using methanol, the inhibition zone changed between 10-13 mm, and the largest zone was found on *B. subtilis* culture. Ethanol were investigated, inhibition zones between 9-11 mm were observed, and again the extract was most effective on *B. subtilis* culture. The plant extract prepared by using water was not effective on some microorganisms.

3.7. Discussion

Crude ash, crude protein, crude fat, crude cellulose, carbohydrate, dry matter, energy, organic matter, K, Ca, Na, Fe, Cu, Zn, Pb, Mn, vitamin E and vitamin A paremeters of *T. isatidea* were 14.37%, 20.60%, 1.82%, 29.02%, 63.21%, 91.57%, 351.62 kcal 72.20%, 42.5 mg/kg, 102.4 mg/kg, 260 mg/kg, 1.59 mg/kg, 0.121 mg/kg, 1.160 mg/kg, 2.16 mg/kg, 7.49 mg/kg, 22.95 mg/g and 2.82 mg/g, respectively (Table 1). It has been reported that crude ash, crude fat, protein and carbohydrate 0.28%, 0.40%, 2.88%, 8.28% in *E. sativa*[18], cooked broccoli protein 3.80 g/100 g and raw broccoli 4.40 g were determined.[19] In the other study reported Mn 7.49 mg/g, Fe 1.59 mg/g, K 42.05 mg/g, Na 2.60 mg/g, Zn 11.60 Cu 0.12 mg/g, Pb.16 mg/g of *T. isatidea*, Cr and Cd wasn't founded. In *E. sativa*, Mn 30.50 mg/g and Fe 30.89 mg/g, K 2.265 mg/g, Pb 0.22 mg/g, Cr 0.68 and Cd 0.84 mg/g were found.[18] In cabbage and cauliflower, Fe was 0.3 and 0.1 mg/g, respectively.[20] The amounts of Fe in raw broccoli, red cabbage, and white cabbage were determined to be 1.2, 0.6 and 0.4 mg/g, respectively.[21] The contents of element K in cabbage and cauliflower were determined to be 161 and 39 mg/g, respectively.[20] It was determined that the highest amount of Fe element belongs to *E. sativa* when all the data obtained are compared. The elemental amounts of the plant were found to be similar or different from other species. The differences are due to the different occurrence of species, the different habitats, and sometimes even the active substances in different organs of a plant. The amount of vitamin A and E in *T. isatidea* was determined to be 2.82 µg/g, be 22.95 µg/g. Vitamin A of the same plant was determined to be 1.75 µg/g vitamin E 3.96 µg/g.[21] The differences in vitamin amounts in the studies and the high vitamin content may be related to the time and location of the sample collection.

Table 7. Antimicrobial Activity of *T. isatidea.*

	E.coli	*P.vulgaris*	*P.aeruginosa*	*K.pneumoniae*	*C.albicans*
T. and Water	9	-	8	-	-
T. and Ethanol	10	9	10	9	9
T. and Methanol	12	10	11	10	10
Standard	13**	11**	11**	19**	9**
	B.subtilis	*B.megaterium*	*S.aureus*	*L.monocytogenes*	
T. Water	10	-	8	8	
T. Ethanol	11	9	9	10	
T. Methanol	13	10	11	11	
Standard	19**	9**	13**	18*	

It has been determined that the DPPH effect of *T. isatidea* is effective from 10 µL and this effect is linearly increased to 100 µL depending on the concentration. *D. virgate* was detected in DPPH 26.03 IC50 (µg/mL), *D. erucoides* 27.02 IC50 (µg/mL).[22] In the study, it was determined that the DNA protective activity of the plant was variable depending on the concentration of the plant. It has been determined that leaves of *Lepidium latifolium* (Brasicaeae) have DNA protective activity. It is thought that glucosinates detected in plants belonging to Brassicae can protect the cells from DNA damage and can inactivate carcinogens.[23-25] Studies have shown that *T. isatidea* methanol extract inhibits microorganism growth best. *Bacillus subtilis* 14 mm inhibition zone, *Staphylococcus aureus* 16 mm, *Pseudomonas aeruginosa* 17 mm, *Klebsiella pneumoniae* 31 mm, *Escherichia coli* 7 mm, *Enterobacter* sp., The methanolic extract of *Farsetia aegyptia* (Brassicaceae) 4 mm, *Salmonella typhimurium* 10 mm, inhibited their development at different rates, and did not prevent the development of *Candida albicans.*[26] *Diplotaxis virgata* (Cav.); *L. monocytogenes* 20 mm, *S. aureus* 19 mm, P. *aeruginosa* 15 mm, *E. coli* 22 mm, and *K. pneumoniae* 16 mm. An antimicrobial study of the *D. erucoides* (L.) extract was made; *L. monocytogenes* 16 mm, *S. aureus* 20 mm, *P. aeruginosa* 20 mm, *E. coli* 16 mm, and *K. pneumoniae* 17 mm.[22] In this study, it can be said that antimicrobial results are caused by plant species which are different from other studies.

Conclusion

In this study, it was determined that *T. isatidea* nutrient contains, antioxidant and antimicrobial properties as well

DOI: http://dx.doi.org/10.32571/ijct.1146979 E-ISSN: 2602-277X

as many other plants belonging to Brassicaceae. It is especially important to investigate the glucosinates of the plant. Because of its thorny structure, the plant which can not be used as a vegetable can be transformed into a dried powdered form of powder and can be consumed with water in the form of a decoction or infusion. The plant can be mixed with powdered petroleum jelly and used as an external wound healing agent.

ACKNOWLEDGMENT

We thank FUBAP for providing financial resources F. F.16.44.

Conflict of interests

I declares that there is no a conflict of interest with any institute, person, company, etc.

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