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Anticholinesterase, Antidiabetic and Antioxidant Activities of Chloroform Extract of *Genista carinalis*

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Abstract

Alzheimer's disease and diabetes have become increasingly public health issues in recent years. On the other hand, synthetic drugs are expensive, could be inadequate to treat diseases, cause irritation, and have side effects. Therefore, increasingly more researchis being done on plant-derived formulas and, bioactive ingredients, which can be an alternative to synthetic drugs for treatments to solve basic health problems. In this study the antioxidant, anticholinesterase and antidiabetic activities chloroform extract of *Genista carinalis* Griseb. (Fabaceae) were determined. Based on the results, the extract was not observed inhibitory activities of α-glucosidase and α-amylase. It showed better activity for acetylcholinesterase activity than butylrylcholinesterase activity. The antioxidant potential of *G. carinalis* chloroform extract was determined with different assays. The TEAC value was determined to be 0.484 mmol TE/g, FRAP value was found to be 1023.20 µmol Fe^{2+}/g for *G. carinalis* chloroform extract. The EC_{50} value of DPPH assays of the extract was found to be 0.101 µg/ml.

Key Words: *Genista*, *G. carinalis*, Anticholinesterase inhibitory activity, Butyrylcholinesterase inhibitory activity, TEAC, α-Amylase inhibitory activity, α-Glucosidase inhibitory activity.

1. Introduction

Alzheimer's, one of the essential chronic diseases associated with loss of consciousness, is considered the most common type of dementia. Fifty million people are struggling with this disease and unfortunately, it is estimated to will increase to 82 million in 2030 and 152 million in 2050 (World Health Organization 2020). Acetylcholine is a neurotransmitter that inhibits first acetylcholinesterase (AChE) and then butyrylcholinesterase (BChE), which is thought to play a role in the pathology of Alzheimer's disease (Helbert et al. 1995). It is suggested that low acetylcholine level is a reasons for the progression of this disease (Talesa 2001). It is stated that AChE and

BChE inhibitors could treat Alzheimer's disease. (Orhan et al. 2004). *Diabetes mellitus* is an increasing health problem (Shaw et al. 2010). There is a need for effective anti-diabetic agents with few side effects for human health.

In this context, effective and safe plant-based inhibitors have been searching to replace synthetic inhibitors (Hasan et al. 2001). Antioxidants can prevent some inflammations that cause health problems by using mechanisms such as inhibiting reaction initiating radicals, breaking the chain reaction, and reducing localized oxygen concentrations (Dorman et al. 2003).

Genista is a genus of the Fabaceae (Legumes) family with approximately 100 species generally grown in the Mediterranean and Western Asia (Noccioli et al. 2011). It is stated that many *Genista* species show biological properties against various diseases (Rauter et al. 2009; Rainova et al. 1988; Bomtempo et al. 2013)

Expensive synthetic drugs, their inadequacy in curing diseases and their side effects, there is a tendency towards plant-derived formulas in the world (Jain et al. 2019) and confidence in traditional medicine is increasing (Craig 1999). In this study, enzyme inhibitory activities (AChE, BChE, α-amylase, α-glucosidase) and antioxidant activity of chloroform extract of *G. carinalis* were determined.

2. Material and Methods

2.1. Plant material and Extraction

Genista carinalis (2048.56 g) was collected from the Thrace region of Turkey (Location: 41°52'47.8"N 27°34'42.9"E and 41°52'29.5"N 27°34'36.4"E). The specimens (EDTU-16811) were identified by Asst. Prof. Guler at Trakya University, Faculty of Science, Department of Biology. First, the dried whole plants were cut into small pieces and extracted with methanol (Merck-1070184000) by maceration method. After the methanol evaporation under vacuum, a small amount of water was added to the crude extract. Later, crude extract was continues to extraction with n-hexane (Merck-1043742500) (46.85 g), chloroform (Merck-1070242500) (15.50 g), ethyl acetate (Merck-1007892500) (45.66 g) and n-butanol (Merck-1019902500) (434.51 g) according to the polarity order. Solvents were evaporated in the evaporator under vacuum to obtain crude extracts and the crude extracts were obtained. (Sabudak et al. 2021).

In this study, antioxidant, anticholinesterase and antidiabetic activities were aimed at the chloroform extract.

2.2. Anticholinesterase activity

The spectrophotometric method was used to determine of acetyl- and butyrylcholinesterase inhibitory activities (Ellman et al. 1961). Electric eel AChE and horse serum BChE were used as enzymes, while acetylthiocholine iodide and butyrylthiocholine chloride were employed as substrates. Cholinesterase activity was monitored using DTNB (5,5′-dithio-bis(2-nitrobenzoic)acid). Test extract and the galantamine were dissolved in ethanol (Ozturk et al. 2011).

Acetyl- and butyrylcholinesterase inhibitory activities of extract and galantamine were given as IC_{50}. To calculate IC_{50} value 50.0, 100.0, 200.0 and 400.0 µg/ml extract concentrations and 3.125, 6.25, 12.5 and 25.0 µg/ml galantamine concentrations were used. The IC_{50} values were calculated from the curve plotted against the % iğnhibition versus cencentration graph.

2.3. Antioxidant activity

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid radical cations ($ABTS^{\bullet+}$) were used to evaluate the Trolox equivalent antioxidant capacity (TEAC) of G. carinalis chloroform extract. After $ABTS^{\bullet+}$ generation

in 2.45 mmol L-1aqueous sodium persulfate, reaction was carried out according Re et al. (1999) procedure. The results were estimated as mmol Trolox equivalents (TE) of per g extract.

The ferric-reducing antioxidant power (FRAP) of *G. carinalis* chloroform extracts were calculated as µmol Fe^{2+} equivalents per g of the extract by using Ferrous sulphate as standard and the absorbance values of the samples were measured at 593 nm (Benzie& Strain, 1996).

The DPPH (2,2-diphenyl-1picrylhydrazyl) capacity of *G. carinalis* chloroform was assayed by method against DPPH radical was monitored at 517 nm as described by Amarowicz et al. (2002). The radical scavenging capacity of extract for DPPH radical was given as EC_{50} values. EC_{50} values were calculated from the graph slope of absorbance versus extract concentration (ranging from 0.4 - 2.0 mg/ml concentration) and described as the µg/ml of extract needed to scavenge 50% of the $DPPH^{\bullet}$.

The CUPRAC assay was performed according to Apak et al. (2004). The method was based on the electron transfer to the media by the antioxidant sample. The extract was tested at four concentrations (25.0, 50.0, 100.0 and 200.0 µg/ml), while the positive antioxidant standarts at six concentrations (3.125, 6.25, 12.5, 25.0, 50.0 and 100.0 µg/ml). The results were given as $A_{0.50}$ (µg/ml), which corresponds to the concentration at 0.500 absorbance value. Absorbance was recorded at 450 nm.

2.4. α-Amylase and α-Glucosidase inhibitory activities

The α-amylase / α-glucosidase inhibitory activities of the chloroform extract of *G. carinalis* were investigated spectrophotometrically (Kim et al. 2010). The extract was tested in five concentrations (50.0, 100.0, 200.0, 400.0 and 800.0 µg/ml) to calculate the IC_{50}. Acarbose was used to compare both inhibitory activities.

3. Results and Discussion

The chloroform extract of *G. carinalis* was investigated for its bioactivities namely, antioxidant activity in four complimentary assays, anticholinesterase activity against Acetyl-butyryl-cholinesterase, and antidiabetic activity against α-amylase and α-glucosidase. Antioxidant activity, anticholinesterase activity and antidiabetic activity of chloroform extract of G. carinalis were studied herein for the first time.

Table 1 shows the α-glucosidase and α-amylase inhibitory activities. Based on the results, the extract inhibited α-glucosidase (IC_{50}: 117.9±14.5 µg/ml) better than that of acarbose (IC_{50}: 190.9±2.16 µg/ml) used as a positive standard. According to the α-amylase inhibitory activity test, the extract exhibited weak activity (IC_{50}: 418.9±14.5 µg/ml) and less than that of acarbose (IC_{50}: 85.6±1.56 µg/ml).

The results showed that the extract is slightly active against α-glucosidase but has less activity against α-amylase. According to the anticholinesterase activity the extract of *G. carinalis* exhibited weak activity (IC_{50}: 226.5±8.55 µg/ml and IC_{50}: 326.1±4.84 µg/ml) against AChE and BChE respectively (Table 2). The extract has a very weak capability to inhibit acetylcholinesterase and butyrylcholinesterase, the chief enzymes in Alzheimer's disease.

The antioxidant potential of *G. carinalis* chloroform extract was searched with different methods. The $ABTS^{\bullet+}$ scavenging activity of chloroform extract as shown in Table 3, explored that *G. carinalis* has 0.484 mmol TE/g TEAC activity. FRAP capacity (Ferric-reducing antioxidant power) of plant chloroform extract was found to be 1023.20 µmol Fe^{2+}/g.

Table 1. α-Glucosidase inhibition and α-Amylase inhibition activities of the chloroform extracts of *Genista carinalis*[a].

	Antidiabetic activity	
	α-Amylase Inhibitory Activity	**α-Glucosidase Inhibitory Activity**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
G. carinalis Extract	418.9±14.5	117.9±4.15
Acarbose[b]	85.6±1.56	190.9±2.16

[a] Values expressed herein are mean ± SEM of three parallel measurements p<0.05.
[b] Reference compounds.

Table 2. Anticholinesterase activity of the chloroform extracts of *Genista carinalis*[a].

	Anticholinesterase activity	
	Acetylcholinesterase inhibitory assay	**Butyrylcholinesterase inhibitory assay**
	IC_{50} (µg/ml)	**IC_{50} (µg/ml)**
G. carinalis Extract	226.5 ± 8.55	326.1 ± 4.84
Galantamine[b]	5.65±0.30	12.82±0.16

[a] Values expressed herein are mean ± SEM of three parallel measurements p<0.05.
[b] Reference compounds.

Based on EC_{50} value of DPPH assays, the EC_{50} value of *G. carinalis* chloroform extract was found as 0.101 mg/mL as shown in Table 3. The CUPRAC assay is a redox potential-based method, and according to results of the CUPRAC assay, the IC_{50} value of *G. carinalis* chloroform extract was found to be 99.81 µg/ml (Table 3). The EC_{50} value of DPPH of *G. carinalis* chloroform extract was found to be higher compared to previous studies, Meriane et al. (2014) found lower values in their study with the methanolic soluble fraction of *Genista saharae* (DPPH radical scavenging activity; IC_{50} = 8.27 µg /mL), and Boukaabache et al. (2013) obtained lower results (IC_{50} value of the extract against DPPH radical 61.64 µg/mL) in their study with ethyl acetate extract of *Genista quadriflora*. The difference could be explaining the difference between *Genista* species and extract types.

Table 3. Antioxidant activity of the chloroform extracts of *Genista carinalis*.

Antioxidant activity	
G. carinalis Chloroform Extract	
TEAC (mmol TE/g)	0.484 ± 0.11
FRAP (µmol Fe^{2+}/g)	1023.20 ± 3.45
EC_{50} value of DPPH capacity (µg/ml)	101.01 ± 0.02
IC_{50} value CUPRAC (µg/ml)	99.81 ± 3.24

4. Conclusion

Anticholinesterase, antioxidant and anti-diabetic activities among *Genista* species are available in the literatüre (Batista et al. 2015; Rauter et al. 2009). In this study, this plant was chosen because there is no biological activity study on *G. carinalis* in the literature. The antioxidant, anticholinesterase and antidiabetic activities of *G. carinalis* chloroform extract were studied in this report. Briefly, the extract exhibited weak anticholinesterase activity which is related with Alzheimer's disease. However, it inhibits α-glucosidase better than acarbose used as antidiabetic drug. Moreover, it has also low inhibitory activity against α-amylase which is also a chief enzyme for diabetic activity. What's more, the extract can be considered as powerful antioxidant activity. The TEAC

value was determined to be 0.484 mmol TE/g, ferric-reducing antioxidant power was found to be 1023.20 µmol Fe^{2+}/g for G. carinalis chloroform extract. The EC_{50} value of DPPH assays for extract was found to be 0.101 µg/ml.

This is the first report on the various bioactivities of *G. carinalis* chloroform extract. The results triggered us as a future study to isolate and elucidate the antioxidant compounds which may also have α-glucosidase inhibitory capacity.

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Author Contribution

TS, HHO and MO conceptualized the experimental procedures. NG identified the plant. HC, HHO, CC, MA, TS and MO conducted the experiments. HC, HHO, CC, MA and MO performed the data analysis. HC prepared the first draft of the manuscript. HC, HHO, CC, MA, TS and MO edited and revised the final draft of the manuscript. All authors approved the final version for submission

Conflicts of Interest

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