Evaluation of phytochemical content, antioxidant, antimicrobial activity and DNA cleavage effect of endemic *Linaria corifolia* Desf. (Plantaginaceae)

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*Cogent Chemistry* (2017), 3: 1337293
Evaluation of phytochemical content, antioxidant, antimicrobial activity and DNA cleavage effect of endemic *Linaria corifolia* Desf. (Plantaginaceae)

Melek Gul1*, Ilkay Ozturk Cali2, Arzu Cansaran3, Onder Idil3, Irem Kulu4 and Umut Celikoglu1

**Abstract:** The aim of this study is to isolate *Linaria corifolia* Desf. (*Plantaginaceae*) and identify new apigenine derivatives 6,3′-dimethoxyapigenin-7-O-[(rhamnosyl)-(1–2)-6″-acetyl glucoside]. Moreover, we analyzed biological activity and flavonoids by using HPLC methods from extracts. The *L. corifolia* Desf. samples were collected in Black Sea Region of Turkey. Ethanol (EtOH), ethyl acetate (EtOAc) and dichloromethane (DCM) extracts in aerial and under solid parts of plant were evaluated for antioxidant, antimicrobial activity and effect of DNA cleavage. Quercetin, gallic acid and catechin as flavonoids were identified in EtOH, EtOAc, DCM extracts.

**Subjects:** General Science; Natural Products; Organic Chemistry

**Keywords:** *Linaria corifolia* Desf.; antioxidant activity; antimicrobial; DNA cleavage effect

**Public Interest Statement**

• General aspects of endemic *Linaria corifolia* Desf. (*Plantaginaceae*).
• Antioxidant and antimicrobial properties of soil and under soil part extracts and volatile oil.
• Some flavonoids determination via HPLC.
• Isolated new apigenin derivatives.

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1. Introduction

The genus *Linaria* Mill. (Nevruzotu) has 200 species found in the wide Mediterranean basin and eastern Asia (1, 2). There are 20 *Linaria* species (29 taxa) in Anatolia and 34.4% of the taxa endemic in Turkey (3, 4). Several *Linaria* species are used in the treatment of various diseases in alternative medicine such as digestive problems, urinary disorders, hemorrhoids, and ulcus (5, 6). It is also known as the most anti-inflammatory and asthma agents (7). Recent developments in the field of *Linaria* genus have led to a renewed interest in other biological activity, diuretic and antiscorbutic effects (L. cymbalaria Mill.), purgative (L. japonica Miq.) (8–10).

The local name of the endemic *Linaria corifolia* Desf. is nevruzotu in Turkey. This species is endemic to Irano-Turanian region. Morphologically, *L. corifolia* Desf. has a perennial root system, the herbaceous stem is cylindrical, and leaves are filiform type. Moreover, this species has flowers with linear and lanceolate bracts (11). The plant was collected during the flowering period and natural populations in A5 Amasya (in the vicinity of Direkli village, steppe areas, at 1,300 m, 12 December 2012, Cansaran 3876) which is a city in the Black Sea Region of Turkey. Its taxonomical description was made according to Davis (1). Plants were identified and preserved in the herbarium of the Gazi University (GAZI).

Plant materials were separated as aerial and under soil part. Aerial part was extracted and isolated for essential oil and biological activity. Moreover, the under soil part of plant was used for the isolation of new apigenone derivatives and also activity analysis. For this purpose, *L. corifolia* aerial parts and under soil parts were dried. Air-dried aerial parts of (1.0 kg) were macerated with methanol at room temperature for three times. After filtration, a yellow-green precipitate was observed in the solution. Total extract was concentrated with n-hexane, ethyl acetate, dichloromethane, respectively.

The ethyl acetate extract was chromatographed on a silica gel column eluted gradient system of hexane:ethyl acetate (20:1, 15:1, 10:1, 5:1, 1:1, 1:2, 0:1) to give 7 fraction and end of column eluted with methanol (8 Fraction). Fraction 4 (Hexane:ethyl acetate/5:1) chromatographed silicagel 200 mesh different eluted system (with gradient of hexane dichloromethane, ethylacetate) and collected ten fraction. The fraction of hexane:dichloromethane:ethylacetate (1:1:1) was collected and readily identified as 6,3′-dimethoxyapigenin-7-O-[(rhamnosyl)-(1–2)-6″-acetylglucoside] (Figure 1; compound 1) by 1D-2D NMR techniques, elemental analysis, LCMSMS, FTIR, UV-vis methods.

This work seeks to address the following questions: chemical properties, isolation of flavonoid derivatives, biological activity of crude extract of endemic *L. corifolia*, quantitavive and qualitative analysis of flavonoids by HPLC, obtained essential oil and named content via GCMS-FID.

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**Figure 1. Isolated compounds of apigenin derivative.**

![Isolated compounds of apigenin derivative](image-url)
2. Results and discussion

Newly isolated apigenin derivative 1 was isolated as colorless solid. The $^{13}$C NMR (DMSO-d$_6$, TMS) spectral data provided supporting evidence for the structure of the apigenin derivatives and also rhamnosyl moiety. Due to carbonyl functional groups, the signals of C-4 and acetyl moiety appear at 182.33 and 169.97 ppm (Table 1). The HMBC correlations from H-C(3a) to C(10), from H-C(8) to C(10), from H-C(111) to C(7) were shown. $^1$H-$^1$H COSY correlations H-C(11) $\leftrightarrow$ H-C(211) and H-C(1111) $\leftrightarrow$ H-C(2111) established the crude structure of 1. The remaining one indicated the linkage of C(7) and C(1111) via oxygen due to the chemical shift of C(1111) ($^{13}$C $\delta$ 100.08 ppm), which was relatively upfield shifted compared to regular O-C-O groups. It was consistent with the skeleton of rhamnose-glucoside (12, 13).

We found the two different anomeric carbon and rhamnosyl characteristic CH$_3$ groups. Evidences of apigenin skeleton were existent in C2 positions of substitute phenyl ring and C4 positions carbonyl, C5 positions added OH groups (Table 2). Therefore, compound 1 was determined to be 3,5-dihydroxy-6-((5-hydroxy-6-metoxy-2-(4-methoxyphenyl)-4-oxp-4H-chromen-7-yl)oxy)-4-((3,4,5-trihydroxy-6-methyltetra hydro-2H-pyran-2-yl)oxy)tetrahydro-2H-pyran-2-yl)methy-lacetate, namely 6,3′-dimethoxy apigenin-7-O-[(rhamnosyl)-(1–2)-6″-acetylglucoside].

Essential oils have volatile, natural, and complex compound characterized by strong odors as secondary metabolites. Essential oils play an important role in the protection of the plants as antibacterial, antiviral, antifungal, insecticides and also against herbivores by reducing their appetite for such plants. They may also attract some insects to favor the dispersion of pollens and seeds or repel undesirable others (14). Essential component was determined GC/FID/MS. Tetracosane is a very high concentration of oil material (15) (Other essential oil components data are in the SI).

| Table 1. $^{13}$C NMR data for compounds 1 (at 150 MHz in CDCl$_3$, $\delta$ in ppm) |
|---------------------------------|---------------------------------|---------------------------------|
| Position (C)                   | Compound 1 $\delta$ (ppm)      | Compound 1 $\delta$ (ppm)      |
| Rhm-CH$_3$                     | 17.14                           | C$_1$ (C$_q$)                   |
| Acetyl-CH$_3$                  | 20.80                           | Acetyl-(C$_q$)                  |
| –OCH$_3$                       | 55.53                           | C$_1$ (C$_q$)                   | 164.01 |
| –OCH$_3^1$                     | 60.27                           | C$_1$ (C$_q$)                   | 162.39 |
| Acetyl-CH$_2$                  | 65.46                           | C$_1$ (C$_q$)                   | 152.47 |
| C$_3$ (CH)                     | 94.41                           | C$_1$ (C$_q$)                   | 152.14 |
| C$_3$ (CH)                     | 103.30                          | C$_1$ (C$_q$)                   | 132.68 |
| C$_1$–C$_5$ (CH)               | 114.69                          | C$_1$ (C$_q$)                   | 122.66 |
| C$_1$–C$_7$ (CH)               | 128.39                          | C$_1$ (C$_q$)                   | 105.82 |

| Table 2. $^1$H data for compound 1 (in CDCl$_3$, $\delta$ in ppm, J in Hz) |
|---------------------------------|---------------------------------|---------------------------------|
| Position (C)                   | Compound 1 $\delta$ (ppm)      | Compound 1 $\delta$ (ppm)      |
| 2 and 6                         | 7.90 (2H, d, J = 6.9)           | 6$^{11}$                       | 3.66 (2H, t, J = 6.4) |
| 3 and 5                         | 7.08 (2H, d, J = 7.0)           | AcMe                           | 2.08 (3H, s) |
| 8                               | 6.77 (1H, s)                    | Rhmn-CH$_3$                    | 1.06 (3H, d, J = 5.2) |
| 3                               | 6.64 (1H, s)                    | 5$^{11}$                       | 4.05 (1H, d, J = 9.2) |
| 1$^{11}$ (anomeric)            | 5.29 (1H, d, J = 6)             | 3$^{11}$                       | 3.92 (1H, m) |
| 1$^{11}$ (anomeric)            | 4.95 (1H, d, J = 6)             | 2$^{11}$                       | 3.89 (1H, m) |
| –OCH$_3$                       | 3.96 (3H, s)                    | 5$^{11}$                       | 3.83 (1H, dd, J = 2.8; 8.1) |
| –OCH$_3^1$                     | 3.91 (3H, s)                    | 5- OH (chelated)               | 12.94 (1H, brs) |
Plant secondary metabolites can have positive or negative effects for health. Flavonoids are polyphenolic secondary metabolite of plants with different biological activity (16, 17). Flavonoids play very important biological and medicinal roles (18–20).

Structural characterizations of flavonoids are one of the most important fields of the spectroscopy, which help to resolve issues of biological and pharmaceutical. Determination of flavonoid target compound is used in different types of instrumental analyses, such as, UV-vis, 1D-2D NMR, IR, MS, elemental analysis, chromatography, LCMS/MS. We have examined gallic acid, quercetin and catechin in L. corifolia by reverse phase HPLC (RP-HPLC) and diode array detector with wavelengths set at 280 nm (Figure 2).

Ethanol (LC1), ethyl acetate (LC2) and dichloromethane (LC3) aerial part plant extracts prepared by maceration method at room temperature were re-extracted with methanol solution (mg/mL). All extracts were filtered by 0.45 μm PTFE filter prior to HPLC analysis. All standards prepared five concentrations: 12.5, 25, 50, 100, 200 ppm in methanol solution. Each standards and mixed standard (from same concentration of gallic acid (GA), quercetin, catechin) were analyzed by different methods but we chose the gradient system to give more reliable results than others (21–23).

Catechin was found to have the highest concentration in LC1 extract, followed by quercetin in LC2 and also gallic acid concentration rose in LC1 in Table 3. It was interesting that gallic acid was not detected in LC3. LC1, LC2, and LC3 showed similar reactivity of antioxidant properties.

**Figure 2. HPLC using standard flavonoid derivatives.**

**Table 3. HPLC results of extracts for gallic, catechin and quercetin amount**

| Entry          | Gallic acid | Catechin | Quercetine |
|----------------|-------------|----------|------------|
| $R^2$          | 0.9933      | 0.9945   | 0.9875     |
| $t_{R}$        | 15.79 ± 0.8 | 35.21 ± 0.6 | 53.35 ± 0.7 |
| LC1 Area%      | 0.713       | 0.947    | 0.760      |
| LC1 Height%    | 0.675       | 0.766    | 0.551      |
| LC1 Concentration | 66,525     | 60,267   | 3.397      |
| LC2 Area%      | 0.274       | 0.024    | 1.015      |
| LC2 Height%    | 0.320       | 0.029    | 1.272      |
| LC2 Concentration | 32,189     | 3.749    | 21.729     |
| LC3 Area%      | ND          | 0.075    | 7.722      |
| LC3 Height%    | ND          | 0.109    | 6.551      |
| LC3 Concentration | ND         | 3.848    | 13.756     |
Phenolic compounds have shown lots of biological effects such as antioxidant activity (24). Also flavonoids have been used for preventing cancer and other very important human diseases such as heart effect and anti-inflammatory (25). Several types of plants include potential antioxidant compounds. Our studies included not only chemical composition but also flavonoid composition and antioxidant activity.

In this study, the antioxidant activity of aerial part of plant ethanol (LC1), ethyl acetate (LC2), dichloromethane (LC3) and under soil part of plant ethanol (LCS1), ethyl acetate (LCS2), dichloromethane (LCS3) extracts and also volatile oil (LCV) were selected for free radical scavenging activity (determined for DPPH), reducing activity (reduction of the Fe\(^{3+}\)/ferricyanide complex to its ferrous form), metal chelating (chelating activity capacity of ferrous ions), superoxide scavenging activity, total phenolic compound (Folin-Ciocalteu assay), and total flavonoid.

Antioxidant activity of *L. corifolia* Desf. various fraction (100–500 μg/mL) was determined using six different assays. Recently, there are very few researches related to antioxidant activity in *L. corifolia* Desf. However, there are lots of studies in literature about antioxidant activity in different *Linaria* derivatives (26, 27).

The free radical scavenging action is known as an important mechanism of antioxidants which was using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). When the antioxidant substance is added to DPPH radical solution, reactive species were generated to reverse the formation of DPPH\(^{•}\), and disappearance of the specific color. This method can determine structure-reactivity relationships. The results for all the compounds were shown in Figure 3. Scavenging activity of standards as BHA, BHT, and Trolox were higher than other extracts. However, the ethanol extract of aerial plant material LC1 was more reactive than other aerial and solid part of plant extracts. LCS1 was solid part of plant material ethanol extract which showed the highest radical scavenging activity of other solid part plant material but we could not observe strong activity in volatile oil.

Reduction activity results were obtained from reduction of the Fe\(^{3+}\)/ferricyanide complex to its ferrous form which indicated electron donating activity. Figure 4 showed five different highest reduction capacity of standard as BHT, BHA, EDTA, Trolox, Gallic acid but EDTA as a positive standard indicated the highest reducing activity. Gallic acid was used as a negative standard. Gallic acid is a kind of negative standard. All various concentrations of extracts had more reactivity than gallic acid. Reducing activity of all samples followed the order: EDTA > LC1 > LC3 > LC2 > LCS1 > LCS2 > LCS3 > LCV > gallic Acid.

The metal chelating activity of crude extracts were determined using FeCl\(_2\)/ferrozine reagent which include electron transfer to metal. Figure 5 showed metal chelating activity of all extracts, and methods standard as gallic and EDTA. LC1 as ethanol extract of aerial part had the highest metal chelating activity and also LCV volatile oil lower activity than other extracts.
Superoxide scavenging activity was dedicated to generate radicals from PMS/NADH system and reduction from NBT. This method showed correlation DPPH free radical scavenging activity. When BHT, ascorbic acid and tocopherol were used standards in this method. Ethanol extracts of aerial (LC1) and under soil part were showed the highest reactivity in Figure 6. Superoxide scavenging activity of volatile oil did not evaluate.

Total phenolic compounds showed free radical scavenging activity, superoxide anion scavenging reactivity and also metal chelating effect. Total soluble phenolic substances in EtOH, ETOAC, DCM extracts of air and under soil part of plant were determined by Folin-Ciocalteu reagent and calculated using gallic acid as standard.
Flavonoids compounds have antioxidant activity because of their ability of hydrogen and electron transfers. We calculated flavonoid content via quercetin calibration curve. In both part of plant extracts, the maximum content was calculated in ethanol extracts (Table 4).

Other parts of this study were carried out to determine the antimicrobial potentials of \textit{L. corifolia} as the part of under soil plant, aerial plant and essential oil. Microorganisms were obtained from the Faculty of Medicine, Ondokuz Mayis University. Among all samples, we investigated five microorganisms: two Gram-positive bacteria (\textit{Bacillus cereus} 7064 and \textit{Staphylococcus aureus} ATCC25923), two Gram-negative bacteria (\textit{Escherichia coli} W3110 \textit{Pseudomonas aeruginosa} ATCC 27853) and yeast (\textit{Candida albicans} ATCC1023). The antimicrobial assay was performed by using minimal inhibitory concentration method (MIC).

\textit{In vitro} antibacterial activities of extract were analyzed by MIC method. Plant extracts were prepared with five samples of aerial part of plant (LC-1, LC-2, LC-3, and acetone extract-LC-4, hexane extract-LC-5), three samples of under soil part (LCS-1, LCS-2 and hexane extract-LCS-4) and essential oil (LCV) of \textit{L. Corifolia}.

As shown in the Table 5, LC3, LCS, and LCS2 did not show a significant effect on microorganisms. LC1 crude extract was only effective \textit{B. cereus} between Gram (+) bacteria and yeast \textit{Candida albicans}. MIC values of \textit{B. cereus} and \textit{Candida albicans} were 500 μg/mL for both. LCS-4 has antimicrobial effect on all microorganisms except for \textit{S. aureus}. While MIC values were 750 μg/mL for \textit{E. coli} and \textit{Candida albicans}, these were 500 μg/mL for \textit{E. coli} and \textit{B. cereus}. Similar results were also reported in different studies by Duraipandiyan and co-worker (28). When we look at the LC2 and LCS1 samples, while entry LC2 affected only on \textit{Candida albicans}, entry LCS1 affected on \textit{S. aureus} (Table 5). These

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|}
\hline
\textbf{Plant extract} & \textbf{Total phenolics (GAE (mg)/g)} & \textbf{Total flavonoid (QE (mg)/g)} \\
\hline
LCS-1 & 48.56 & 47.65 \\
LCS-2 & 32.49 & 30.58 \\
LCS-3 & 33.75 & 32.47 \\
LC-1 & 72.39 & 71.94 \\
LC-2 & 52.63 & 48.63 \\
LC-3 & 42.25 & 41.56 \\
\hline
\end{tabular}
\caption{Total phenolic and flavonoid content of various extracts}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
\textbf{Extract name} & \textbf{Escherichia coli W3110} & \textbf{Pseudomonas aeruginosa ATCC 27853} & \textbf{Staphylococcus aureus ATCC 25923} & \textbf{Bacillus cereus ATCC 7064} & \textbf{Candida albicans ATCC10231} \\
\hline
LC-1 & 3000 & 1500 & 3000 & 500 & 500 \\
LC-2 & 6000 & 3000 & 1500 & 1500 & 125 \\
LC-3 & 3000 & 1500 & 3000 & 1500 & 3000 \\
LC-4 & 6000 & 6000 & 6000 & 6000 & 750 \\
LC-5 & 6000 & 3000 & 6000 & 6000 & 3000 \\
LCS-1 & 6000 & 3000 & 6000 & 500 & 1500 \\
LCS-2 & 6000 & 3000 & 6000 & 3000 & 1500 \\
LCS-4 & 500 & 750 & 3000 & 500 & 750 \\
LCV & 125 & 175 & 500 & 750 & 500 \\
\hline
\end{tabular}
\caption{Minimal inhibitory concentration (MIC) values of the complexes against wild-type microorganisms (μg mL⁻¹)}
\end{table}
MIC values were 125 μg/mL for *Candida albicans* at LC2 and 500 μg/mL for *S. aureus* at LCS1. The most effective for Gram (+) bacteria are entries 1, and 8 for *B. cereus*, LCS1 for *S. aureus*. Furthermore, the effective concentrations were 500 μg/mL. However, other extracts did not indicate any significant effect. This effect for Gram (−) bacteria were only for LCS4 and the effective concentrations were between 500–750 μg/mL for both *E. coli* and *P. aeruginosa*. Similar results were also reported by Parekh and co-worker and also Venkatesan groups (29, 30).

All the bacterial strains demonstrated sensitivity to the plant volatile oils. MIC values for volatile oils were 125 μg/mL for *E. coli*, 175 μg/mL for *P. aeruginosa*, 500 μg/mL for *S. aureus*, 750 μg/mL for *B. cereus* and 500 μg/mL for *Candida albicans*. It was observed that Gram-positive bacteria are more resistant than Gram negative bacteria in terms of antibacterial properties of plant volatile oils. The volatile oils (LCV) were more affected than others. It was demonstrated that volatile oils were more antimicrobial activity than other extracts. It is a well-known fact that hospital infections are quite important for the public healthy and quite resistant against standard antibiotics. Therefore, there is an urgent demand for new antibiotics and new classes of chemical formula that will efficiently inhibit the growth of pathogenic microorganism. Currently, chemotherapy using multiple drug regimens with isoniazid, rifampin, streptomycin, pyrazinamide is still a major concern and new generations of more effective antimycobacterial agents (31, 32).

DNA cleavage activity of the extracts was analyzed by monitoring the conversion of supercoiled DNA to nicked circular DNA and linked circular DNA in both H₂O and DMSO conditions for 2 h incubation. In the agarose gel electrophoresis, supercoiled DNA quickly migrates than other forms. If one strand is broken supercoil form will turn open circular form (33). If both strands are broken supercoiled DNA will generate linear form. In this study control groups showed no effect on the plasmid DNA, but increasing doses of LC1 and LC2, and LCS2, LCS3 extracts caused increasing the open circular form of DNA. Increasing doses of LCS was shown to completely break but LCS1 was shown having no effect on plasmid DNA. Small amount of essential oil (LCV) had a protective effect on plasmid DNA in both H₂O and H₂O₂ conditions (Figures 7 and 8).

Figure 7. Agarose gel electrophoresis diagram of essential oil which prevent cleavaging of pBR322 plasmid DNA

Notes: Lane 1: pBR322 plazmid DNA 2 h incubation; Lane 2: pBR322 plazmid DNA + H₂O₂ 2 h incubation. Lane 3: pBR322 plazmid DNA 24 h incubation. Lane 4: pBR322 plazmid DNA + H₂O₂ 24 h incubation. Lane 5: 0.50 μl Uçucu yağ + pBR322 plazmid DNA 24 h incubation. Lane 6: 1.50 μl Uçucu yağ + pBR322 plazmid DNA 24 h incubation. Lane 7: 1.50 μl Uçucu yağ + pBR322 plazmid DNA + H₂O₂ 24 h incubation. Lane 8: 0.50 μl Uçucu yağ + pBR322 plazmid DNA + H₂O₂ 24 h incubation.
3. Experimental

3.1. General
Mass spectra were analyzed by LCMS-MS/QTRAP ABSciex, spectrometer. NMR spectrums were recorded on Bruker 400 MHz and 500 MHz. Chemical shifts (in ppm) were referenced to tetramethylsilane ($\delta = 0$ ppm) in CDCl$_3$ as an internal standard. Elemental analysis determined LECO micro CHNS analyzer. Shimadzu Prominence Modular LC20A HPLC is used determination of flavonoids. Essential oil contents were recorded Agilent GCMSD/FID (7890B GC-5977MSD). All standard articles were purchased from Sigma–Aldrich and Merck in order for HPLC and antioxidant activity of plant material.

3.2. Preparation of essential oil
Drying aerial part of plant material was submitted for 5 h to water distillation using Clevenger apparatus (yield 1.74%). The obtained essential oil was dried over anhydrous sodium sulfate and after filtration, stored at +4°C until tested and analyzed. Essential oil material was used GC/MSD or FID analysis, and also biological activity. GC/MSD and GC/FID were carried out for analysis of the essential oil chemical compositions. Conditions of the method was split injection (25:1) 250°C, 1 μl injection volume in carrier gas helium (65 kPa) and oven program was started 60 to 240°C at 3°C/min. MS in scan mode (40–400 amu) FID detection was 300°C. Column was selected the HP-5MS (30 m × 0.25 mm id × 0.25 μm).

Figure 8. Agarose gel electrophoresis diagram showing cleaving activity of pBR322 plasmid DNA by the Linaria Corifolia Complex A (LC1), B (LC2), C(LC3), D(LC5), E(LC52), F (LC33).

Notes: Lane 1: pBR322 plasmid DNA 2 h incubation (H$_2$O). Lane 2: pBR322 plasmid DNA + DMSO 2 h incubation. Lane 3: pBR322 plasmid DNA 24 h incubation (H$_2$O). Lane 4: pBR322 plasmid DNA + DMSO 24 h incubation. Lane 5: 5 μg ekstrakt + pBR322 plasmid DNA 24 h incubation. Lane 6: 10 μg ekstrakt + pBR322 plasmid DNA 24 h incubation. Lane 7: 15 μg ekstrakt + pBR322 plasmid DNA 24 h incubation.
3.3. Determination of flavonoid content via HPLC
The 250 × 4.6 mm i.d., 5 μm column used as C18. The flow rate was 1 mL/min, injection volume was 30 μl and the column temperature was set at 27°C. Two solvent line gradient elution of was used as line A include formic acid–water (%0.1), line B methanol. The data were integrated and analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The *L. corifolia* plant samples, standard solutions, and mobile phases were filtered by a 0.45 μm pore size membrane filter. The amount of phenolic compounds in the extracts was calculated as μg/L *L. corifolia* using external calibration curves, which were obtained for each phenolic standard.

3.4. Antioxidant activity
α,α-Diphenyl-β-picryl-hydrazyl (DPPH), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol, 3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonicacid)-1,2,4-triazine (Ferrozine), polyoxyethylene sorbitan monolaurate (Tween-20) and trichloroacetic acid (TCA), Ferrozine, Folin Ciocalteu solution, nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), quercetin, were obtained from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). Ammonium thiocyanate was purchased from Merck. All other chemicals used were in analytical grade and obtained from either Sigma–Aldrich or Merck.

3.4.1. Free radical scavenging activity
The free radical scavenging activity was determined with 1,1-diphenyl-2-picryl-hydrazyl (DPPH) using methods of Brand-Williams et al. (34). Different concentrations of plant material were prepared and 0.75 mL of this extract was added the 1.5 mL of 20 mg/L DPPH solution in methanol. This solution was added to, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trolox (100–500 μg/mL). The mixture was shaken vigorously, and the decrease in absorbance at 517 nm was measured for 30 min at room temperature. Water (0.75 mL) in place of the sample was used as control. The percent inhibition activity was calculated using the following equation: free radical scavenging effect % = [(A₀ − A₁)/A₀]·100.

(A₀ = the control absorbance and A₁ = the sample solution absorbance).

3.4.2. Reducing activity
Measurements of reducing activity of extracted were according to the Oyaizu method (35). Various concentrations of the extract (μg) in 1 mL of distilled water were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL potassium ferricyanide [K₃Fe(CN)₆] (1%, w/v), and the mixture was incubated at 50°C for 30 min. Afterwards, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture and centrifuged at 3,000 rpm for 10 min. Finally, 2.5 mL of upper-layer solution was mixed with 2.5 mL distilled water and 0.5 mL FeCl₃ (0.1%, w/v), and the absorbance was measured at 700 nm in a spectrophotometer.

3.4.3. Metal chelating activity
The chelating activity of extract on ferrous ions (Fe²⁺) was measured according to the method of Decker and Welch (36). Aliquots of 1 mL of different concentrations (100–500 μg/mL) of extracts were mixed with 3.7 mL of deionized water. The mixture was incubated with FeCl₃ (2 mM, 0.1 mL) for 30 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the extract on Fe²⁺ was compared with that of EDTA at the same concentrations. Metal chelating activity (%) = [(A₀ − A₁)/A₀]·100.

3.4.4. Superoxide scavenging activity
Superoxide anion scavenging activity was measured in the method described by Liu (37). Firstly we need to generate superoxide radicals in PMS-NADH system by oxidation of NADH which was reduced to NBT. In this method 3 mL of 16 mM Tris–HCl (pH 8.0), containing 1 mL of 50 mM NBT solution, 1 mL 78 mM NADH and various concentrations of extracts in water reacted. When 1 mL PMS was added, reaction started. This mixture was incubated at room temperature for 5 min and measured absorbance...
at 560 nm. Measured absorbance was decreased though superoxide anion activity was increased. The percentage of inhibition of superoxide anion was calculated using the following formula.

\[ \% \text{Inhibition} = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 \]

### 3.5. Estimation of total phenolic content

According to the methods of Slinkard and Singleton (38), using Folin–Ciocalteu reagent were determined depending on phenolic standard gallic acid. 1 mL of the plant extract was introduced into test tubes followed by 1 mL Folin–Ciocalteu’s reagent. The solution was kept in the dark for 5 min and then 3 mL of sodium carbonate (2%) was added. The tubes were covered with parafilm and kept again in the dark for 1 h and were measured absorption at 765 nm with a spectrophotometer and compared to a gallic acid calibration curve. The results were expressed as mg gallic acid/g dried sample. Each assay was carried out in triplicate.

### 3.6. Estimation of total flavonoid content

Total flavonoid content was determined with quercetin standard solution using methods of Park et al. (39). The plant extract in 0.3 mL of was introduced into test tubes followed by 3,4 mL 30% methanol, 0.15 mL of 0.5 M NaNO2, and 0.3 M AlCl₃ reagent. After 5 min 1 mL of 1 M NaOH was added and measured absorption at 506 nm with a spectrophotometer and compared to a quercetin calibration curve. Each assay was carried out in triplicate. The total flavonoids were described as mg of quercetin equivalents per g of dried fraction.

### 3.7. Antimicrobial activity studies

The antimicrobial assay was performed by using minimal inhibitory concentration method (MIC). For the broth dilution method, cultures were grown in 5 mL nutrient broth (Merck) at 37°C for 18 h in shaking at 175 rpm. Bacterial and yeast cells were suspended in 50 mL nutrient broth at a concentration of approximately 106 cells/mL by matching with 0.5 McFarland turbidity standards. Nutrient broth containing microorganisms were transferred 1 mL in test tubes and were added complexes, and made 2-fold serial dilution; eventually the ranges were narrowed to define more exact values. All the test cultures were grown at 37°C in incubator. The incubation period was 24 h for bacterial strains and fungal strains. The minimum inhibitor concentration, at which no growth was observed, was taken as the MIC value (μg/mL), and represents the mean of at least three determinations.

### 3.8. DNA cleavage study

For agarose gel electrophoresis, extracts were dissolved in DMSO and then 0.25 ppm pBR322 plasmid DNA (0.5 μl) was treated with 1.5 μl of 5, 10 and 15 μg extract. After incubation at 37°C for 2 and 24 h 1 μl loading buffer was added then this mixture was loaded on 1% agarose gel for 90 min at 80 V in TBE buffer (pH 8). Gels were stained with ethidium bromide for 15 min and were washed with deionized water for 15 min. Then gels were photographed under UV light.

**Compound 1; MP: 167–169°C.**

RF: 0.37 (Hexane-MeOH-EtOAc, 3:1:2), IR (KBr): 3,378, 2,938, 1,720, 1,687, 1,511, 1,462, 1,039, 834 cm⁻¹, ¹H NMR and ¹³C NMR: (Detail in Tables 1 and 2), LCMSMS-QTRAP: m/z [M⁺], C₃₁H₃₆O₁₆: 664.60, Anal. Calcd for C₃₁H₃₆O₁₆: C, 56.02; H, 5.46. Found C, 55.89; H, 5.49.

### 4. Conclusion

In conclusion, we described a general approach on the *L. corifolia Desf.*, which is an endemic plant, chemical and some biological activity. We have isolated new apigenine derivatives (1) in EtOAc extract of aerial part of plant. We have determined antioxidant, antimicrobial activity and DNA interaction each part of plant. HPLC analysis and antioxidant activity results were also comparable. The high biological activities were dependent on the solubility of the active compound. Flavonoids and phenolic compounds are one of the most abundant bioactive molecules which are extracted from EtOH, EtOAC. LC1 (ethanol extract of aerial part of plant) had high concentration of catechin, gallic and also high total flavonoid, total phenolic content. LC1 antioxidant properties were also relatively high in
other extracts due to increased flavonoid contents. Biological activity of LCV material had a moderate effect but DNA preventing results were more potent than other extracts. Finally, this approach may also allow new kind of research in medicinal usage or development of drug research.

Supplementary material
Supplementary material for this article can be accessed here [https://doi.org/10.1080/23312009.2017.1337293].

Acknowledgments
The authors wish to thank Prof Dr Gulactı TOPCU, from the University of Bezmialem (Istanbul, Turkey) for the help in identification of Compound 1 NMR. All samples identified in the AUMAULAB central Laboratory in Amasya University in Turkey. I wish to thank all AUMAULAB staff for their very meticulous work.

Funding
I gratefully acknowledge financial support of this work by Amasya University Scientific Research Foundation [grant number FMB-BAP-12-02].

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Citation information
Cite this article as: Evaluation of phytochemical content, antioxidant, antimicrobial activity and DNA cleavage effect of endemic Linaria corifolia Desf. (Plantaginaceae), Melek Gul, Ilkay Ozturk Cali, Arzu Cansaran, Onder Idil, Irem Kulu & Umut Celikoglu, Cogent Chemistry (2017), 3: 1337293.

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