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Pharmacognostic, Physicochemical, Phytochemical Screening and *In-Vitro* Antioxidant Activity of *Chrysochamela Noeana,* Endemic in Türkiye

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ABSTRACT

The aim of the present study was to conduct preliminary phytochemical screening, pharmacognostical and physicochemical investigation of *Chrysochamela noeana*. The fresh and dried herbs were studied by morphology, microscopy, preliminary phytochemical screening and florescence analysis of powdered drug. In addition, physicochemical parameters were studied according to WHO guidelines. Physicochemical parameters and florescence analysis were also performed. The preliminary phytochemical analysis revealed the presence of steroids, flavonoids, phenolic compounds, and carbohydrates. Total phenol contents were 245 ± 3.67, 179.06 ± 4.52, and 15.98 ± 3.32 (GAE mg/g extract) and total flavonoid content were 180.85 ± 8.21, 146.41 ± 2.56, and 13.46 ± 0.23 (QUE mg/g extract) for ethyl acetate, methanol, and water extracts, respectively. The IC_{50} value for extracts in ABTS radical scavenging activity were calculated in order of 51.47, 122.26 and 1146.91 µg/mL, and in DPPH for 1.55, 1.13 and 27.16 mg/mL for ethyl acetate, methanol, and water extracts, respectively. The results of these studies could be useful for correct identification and detection of adulterants from this plant material.

1. Introduction

Microscopic characterization of medicinal plants is very important procedure for identity and quality assessment of herbal ingredients (Rajan et al 2011). It is well accepted by all national and international regulatory authorities as one of the four primary methodologies for the identification of crude drug materials including macroscopic appearance, organoleptic characters, microscopic characteristics and the phytochemical analysis (Manayi et al 2012).

The family Brassicaceae (= *Cruciferae*) consists of 350 genera and about 3500 species (Cartea et al 2011). This family includes important oilseed, forage, condiment and vegetable crops, are consumed by wild population from all over the world. The wild usage and beneficial effect of *Cruciferae* plants may be attributed to their phenolic compounds and flavonoids which are possessing antioxidant activity (Pereira et al 2009). Flavonoids are the most commonly found phenolic compounds, playing an important role in UV protection, pigmentation, and disease resistance (Crozier et al 2006).

The genus *Chrysochamela* (Fenzl) Boiss., includs four species, which are distributed in Turkey, Russia, Lebanon and Syria (Appel and Al-Shehbaz 2003). In flora of Turkey, *Chrysochamela* Boiss. species which has only three varieties so far identified, including *C. elliptica* (Boiss.) Boiss., *C. velutina* (DC.) Boiss. and *C. noeana* (Boiss.) Boiss (Sevindik et al 2019). Among them, two of these species are endemic to Turkey and show a very narrow spread. The third species is only spreads in the Syrian Desert except for Turkey (Davis 1965). *Chrysochamela noeana* (Boiss.) Boiss. (Brassicaceae) is an annual herb only grown in Sivas province of Turkey. The micromorphological and anatomical properties of three species of the *Chrysochamela* genus have been comperatively presented in a former study (Çakilcioğlu et al 2017). Literature review revealed that there is no report about the phytochemical investigations and biological activity studies except for phylogenetic and floristic studies. Despite the vast variety of the Brassicaceae family, only a few species, mostly from the Brassica genus, are consumed. The Brassicaceae plant are high in vitamins, minerals, and fiber while being low in fat. They are also rich in phytochemicals like as isothiocyanates and

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phenolic compounds, which play an essential role in the prevention of chronic illnesses (Fusari et al 2020).

Pharmacognosic investigation have not been reported for this plant. Therefore, the aim of the present work is to study the macro, microscopic and some other physicochemical and pharmacognostic characters of *C. noeana*, which could be used to prepare a monograph for the suitable identification of the plant

2. Materials and Methods

The plant materials of *C. noeana* were collected from Sivas (B6 Sivas: Center, gypsum areas in Seyfe Beli location, 1350-1400m, 1.5.2018) and identified by the Botanist XXX SJAFS. Each part of the plant dried under shade and powdered by laboratory-type blender. All the chemicals and reagents were analytical grade, obtained from Sigma chemical co. (St Türkiye) and Merck (Darmstadt, Germany)

Figure 1
Habitat image of *Chrysochamela noeana* (Photographed by E. Dönmez)

Pharmacognostic studies: Each plant parts have been morphologically observed by a microscope (Leica S6E) with magnifying lens for shape, size, surface characteristics, color, and odor etc. For anatomical investigation, parts of the fresh material were stored in %70 alcohol-water solution. Hand-made cross section of basal leaf, cauline leaf, stem, and root were taken by razor blade, then stained in 1% Alcian blue (Sigma) and 1% Safranin O (Sigma), in a ratio of 3/2. The sections were kept about 5 min in the dye. Semi-permanent slides were mounded using glycerin–gelatin (Tekin & Eruygur 2016). Photomicrographs were taken using Olympus BX51 light microscopy equipped with Olympus DP70 digital camera.

Quantitative investigation: The moisture content, total ash, acid-insoluble ash, water-soluble ash and hexane, chloroform, ethyl acetate, methanol and water extractive values of the powdered samples were determined by the method as described in WHO guidelines (WHO 1998).

Phytochemical screening: The phytochemical investigation of different extracts of *Chrysochamela noeana* herbs was carried out by the standard chemical tests.

Physicochemical investigation: Physicochemical parameters such as moisture content, loss on drying, water soluble ash, acid insoluble ash, and hexane, chloroform, ethyl acetate, methanol and water-soluble extractive values of the powdered samples were determined by the method as described in WHO guidelines (WHO 1998).

Fluorescence analysis**:** Fluorescence study of plant powder was conducted as previously reported procedure. The appropriate amount of powder is placed in a porcelain capsule, then a few drops of freshly prepared reagent is added and then allowed to stand for 2 minutes. Then the fluorescence properties were examined in day light and in the UV cabinet at 254nm and 366nm.

Antioxidant activity: For the DPPH radical scavenging assay, 20 μL of extract diluted appropriately in Dimethyl sulfoxide (DMSO) was mixed with 180 μL of DPPH in methanol (40 μg mL^{-1}) in wells of a 96-well plate. The plate was kept in the dark for 15 min, after which the absorbance of the solution was measured at 540 nm in a Microplate-reader. Appropriate blanks (only include the DMSO with free sample) and standards (quercetin solutions in DMSO) were run simultaneously. Extracts were first tested at a single concentration of 4 mg/mL, and those showing good evidence of antioxidant activity were tested over a range of concentrations to establish the EC_{50} (the concentration reducing DPPH absorbance by 50%) (Clarke et al 2013).

The ABTS radical scavenging activity assay was carried out via the ABTS cation radical decolorization with minor modifications (Chun et al 2005). The samples were prepared in the same procedure as the DPPH assay. The ABTS cation radical was prepared by reacting 7 mM aqueous solution of ABTS (15 mL) with 140 mM potassium persulphate (264 μL). The mixture was allowed to stand in dark at room temperature for 16 h before use. Prior to assay, the ABTS working reagent was diluted with methanol to give an absorbance of 0.70 ± 0.02 at 734 nm and was equilibrated at room temperature. The reaction mixtures in the 96-well plates consisted of 50 mL of sample and 100 μL of ABTS working solution in methanol. The mixture was stirred and left to stand for 10 min in dark, then the absorbance was taken at 734 nm against a blank. All determinations were performed in triplicate. The percentage scavenging effect was calculated as:

Scavenging activity %= $(Ac – A_s) / Ac \times 100\%$

Where Ac is the absorbance of the control (without sample) and As is the absorbance in the presence of the sample

The scavenging ability of the samples was expressed as IC_{50} value, which is the effective concentration at which 50% of ABTS radicals were scavenged. The IC_{50} value was calculated from the scavenging activities (%) versus concentrations of respective sample curve.

The total phenolics content of the extracts was determined by reaction with F-C reagent. Thus, 10 μL of extract diluted appropriately in DMSO was mixed with 100 μL F-C reagent freshly diluted 1/10 with distilled water. After five minutes, the solution was mixed with

100 µL 7.5% Na_2CO_3 solution, and the whole left for 60 min, before measurement of absorbance at 650 nm in a Multiscan plate-reader. Appropriate blanks (DMSO) and standard (quercetin in DMSO) were run simultaneously, after which the total phenolics content was calculated as mg gallic acid equivalents per g extract (Clarke et al 2013).

Total flavonoid contents were determined by Aluminum colorimetric method, using quercetin as the reference standard. Briefly, 150 µL of the test sample dissolved in ethanol was mixed with equal volume of 2% (w/v) $AlCl_3$ in 96-well plates. After 15 min of incubation at 25ºC, the absorbance was measured at 435 nm by spectrometer. All determinations were performed in triplicates. The content of total flavonoids was expressed as mg of quercetin equivalent per g of dry weight of the sample, using an equation obtained from the standard quercetin calibration (Jhade et al 2011; Yang et al 2011).

The chelation of ferrous ions by extracts was estimated by method of Dinis et al. (1994) and Ebrahimzadeh et al., (2008). Briefly, 50 µL of 2 mM $FeCl_2$ was added to 1 mL of different concentrations of the extract (0.2, 0.4, 0.8, 1.6 and 3.2 mg mL^{-1}). The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The percentage inhibition of ferrozine–Fe^{2+} complex formation was calculated as $[(A_0 - A_s)/ A_s] \times 100$, where A_0 was the absorbance of the control, and A_s was the absorbance of the extract/ standard. EDTA was used as positive control.

3. Results and Discussion

Macroscopic characteristics: The basal leaves are 0.5-3.5mm in length and 0.3-1.2mm in width. The leaf shape broadly or narrowly ovate, apex obtuse or acute, margin is entire, venation is pinnate, green above and beneath, leaf surface is covered with branched hairs or glabrous, leaf base is sessile, phyllotaxis is rosette. The stem leaves were 5-20mm in length and 3-5mm in width. The leaf is oblong-shaped, apex are acute to subobtuse, margin entire, venation pinnate, the bottom and top of leaf is green, leaf surface glabrous, leaf base is sessile and auriculate, phyllotaxy was alternate, have mustards' odor (Figure 2. a). Sepals are ovate, 1-1.5 × 0.4-0.6mm, entire or scarious, apex acute, bifurcate hairy. Petals are 1-1.5 × 0.4-0.6 mm, usually ovate-shaped, entire, obtuse, and glabrous. Inflorescence is zigzag, and the branches are glabrous or pubescent. The number of stamen is 6. Filaments are ascendens, adhered to the base and expanded in the lower part. Ovary elliptic, stilus is short, 0.1-0.15mm. Fruits are latiseptate siliqua, dehiscent, obovoid, outer side slightly reticulate, 3.5-4.5 × 1.8-2.5 mm, fruit stilus are 0.4-0.5 mm, reptum are 3.5-4.5mm. Seeds are 0.5-0.3mm.

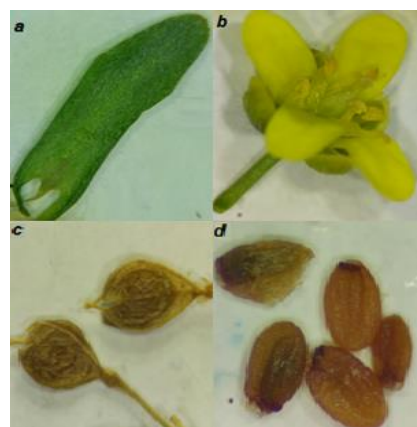

Figure 2

Macroscopic characteristics of *Chrysochamela noeana* leaf (a), flower (b), fruits (c) and seeds (d) (Photographed by E. Dönmez)

Anatomical Characteristics of roots: Transverse section of root is round shaped and in secondary structure. Outermost layer consists of 1–3 layered phellem cells. The phellem cells are 15–45 × 5–23 µm, depressed and sometimes fall broken. Phellogen is indistinguishable. There is 3–6 layered parenchymatic cortex under the periderm. Cortex cells are 15–63 × 5–38 µm, slightly depressed rectangular, oval, or irregular shaped and there are intercellular gaps between these cells. There is stele beneath the cortex. Phloem is located narrow, and xylem is located wide area of root cross section in stele. Cambium is indistinguishable between phloem and xylem. Xylem is composed of lignified xylem elements in close to cortex and unlignified xylem element in and around pith (Fig. 3 A, B).

Anatomical Characteristics of Stem: In the cross section of the stem, the epidermis is single layered, and cells have thin cuticle. The epidermis cells are 7–18 × 4–15 µm, squarish, rectangular, or oval shaped. The parenchymatous cortex with chloroplast is made up of 5–8 layers and placed just beneath epidermis. Cortex cells are 10–58 × 7–23 µm and oval or rounded shaped. The endodermis, innermost layer of the cortex, is distinct by having larger cells and thicker cell wall than other cortex cells. There is 7–14 layered pericycle which consist of sclerenchymatic fibers beneath endosperm. The stem has collateral type vascular bundles. Phloem is located between endodermis and sclerenchymatic fibers, and xylem is located in and under sclerenchymatic fibers surrounded by sclerenchyma fibers. The vascular cambium is indistinguishable between xylem and phloem. The pith is occupied by large, oval and thick-walled parenchyma cells and these cells are 12–63 × 5–50 µm in diameter (Fig. 3 C, D).

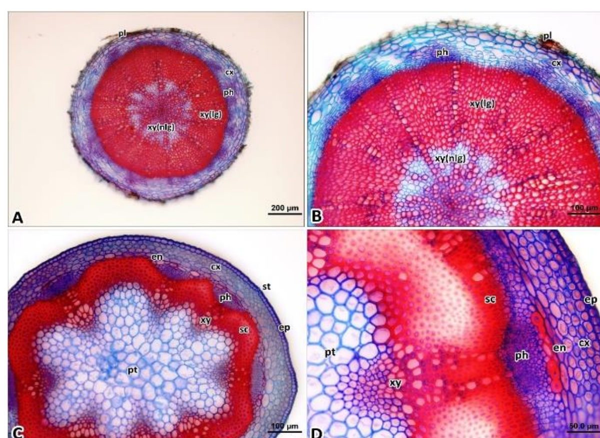

Figure 3

Photomicrographs of root cross section (A, B) and stem cross section (C, D) of *Chrysochamela noeana*. Abreviations: cx: cortex, en: endodermis, ep: epidermis, ph: phloem, pl: phellem, pt: pith region, sc: sclerenchymatic fibers, st: stoma, xy: xylem, xy(lg): lignified xylem, xy(nlg): non-lignified xylem.

Anatomical Characteristics of basal leaf: In cross section of basal leaf, adaxial and abaxial epidermises are uniseriate. The stomata are present on both epidermises. The stoma cells are located on the same level with the epidermal cells. Adaxial epidermis cells are 12–68 × 10–40 µm, oval or rectangular oval occasionally sguarish oval shaped. The mesophyll is bifacial and has 3–4 cell layers of palisade parenchyma below adaxial epidermis and 4–5 layers of spongy parenchyma below abaxial epidermis. Palisade parenchyma cells are 25–63 × 15–30 µm, and usually cylindrical, occasionally squarish shaped. Spongy parenchyma cells are 10–50 × 7–40 µm, usually oval, rarely roundish, or rectangular oval shaped. Mesophyll thickness is 180–230 µm. The midrib is obviously larger than the other bundles. Abaxial epidermis cells are 10-43 × 7-30 µm, oval or rectangular oval occasionally squarish oval shaped. (Fig 4 A, B).

Anatomical Characteristics of cauline leaf: In cross section of cauline leaf, adaxial and abaxial epidermises are uniseriate. The stomata are present on both epidermises. The stoma cells are mesomorphic. Adaxial epidermis cells are 7–43 × 7–28 µm, usually depressed rectangular, occasionally squarish shaped. The mesophyll is bifacial and has 3–4 cell layers of palisade parenchyma below adaxial epidermis and 4–5 layers of spongy parenchyma below abaxial epidermis. Palisade parenchyma cells are 22–58 × 17–30 µm, and usually cylindrical or oval rarely squarish shaped. Spongy parenchyma cells are 20–68 × 15–48 µm oval or rounded shaped. Mesophyll thickness is 210–300 µm. The midrib is obviously larger than the other bundles. Abaxial epidermis cells are 12–45 × 10–33 µm, oval or rectangular oval occasionally squarish oval shaped (Fig. 4 C, D).

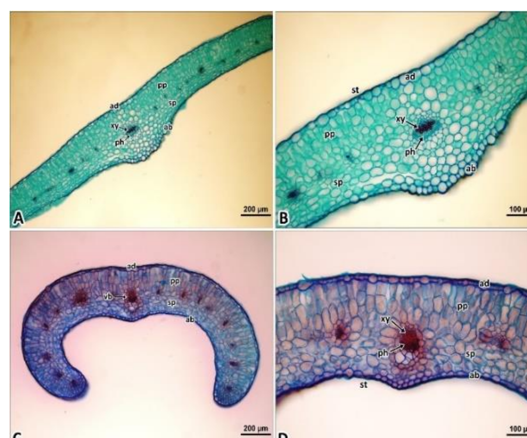

Figure 4

Photomicrographs of basal leaf cross section (A, B) and cauline leaf cross section (C, D) of *Chrysochamela noeana.* Abreviations: ab: abaxial epidermis, ad: adaxial epidermis ph: phloem, pp: palisade parenchyma, sp: spongy parenchyma, st: stoma, xy: xylem.

The physico-chemical evaluation of drugs is play an important role in detecting adulteration or improper handling of drugs. The total ash is indication of impurities in herbal drugs, to detect the presence or absence of inorganic components mixed with plant during harvesting coming from soil. The amount of moisture content effecting the stability of herbal drugs, it means that the lower moisture content, the lower proliferation of living microorganisms, therefore, the higher stability of the drug. Loos on drying is more used for detecting moisture content among the methods. The loose on drying value of the plant is not high, thus it is easy to storage or handling of the drug and lower than the general requirement for moisture content in drugs described in herbal pharmacopoeia. The total ash value is 18.7% (w/w), acid –insoluble ash value is 1.90% (w/w), most of the total ash is soluble in acid, therefore acid –insoluble ash value is low. In addition, the water-soluble extractive is higher in this plant, water extractive value is 8.75% (w/w) while other solvent extractive value is low, hexane, chloroform, ethyl acetate, methanol extractive value is, 0.65% (w/w), 1.03% (w/w), 0.38% (w/w), and 3.37 % (w/w), respectively. The results are presented in Table 1.

Table 1

Proximate parameters of *Chrysochamela noeana* herbs

Parameters	Value %
Loss on drying	5.2 ± 0.025
Total ash	18.7 ± 0.033
Acid insoluble ash	1.90 ± 0.020
Water soluble ash	3.72 ± 0.031
Hexane soluble extractive	0.65
Chloroform soluble extractive	1.03
Ethyl acetate soluble extractive	0.38
Methanol soluble extractive	3.37
Water soluble extractive	8.75

The results of fluorescence analysis of the entire plant powder of *C. noenana* are presented in Table 2, the fluorescence in day light, UV light (254 nm), and UV

light (365 nm) are different from each other when various chemical reagents are added. It indicates that there are various phytochemical groups existing in the plant.

Preliminary phytochemical screening: A different polarity solvent extracts namely hexane, chloroform, Ethyl acetate, methanol and water extracts prepared from herbs of *C. noeana* were investigated for their phytochemical profile. The extracts were subjected to various qualitative chemical tests for the identification of various plant constituents. The herbs show the presence of carbohydrates, steroid, flavonoid, phenolic compounds (Table 3).

Table 2
Fluorescence analysis of aerial part powder of *Chrysochamela noeana*

Reagents	Day light	UV light (254 nm)	UV light (365 nm)
Powder	Light yellow	Light yellow	Yellow
Powder + Water	Whitish yellow	Yellowish green	Light yellow
Powder + Methanol	Whitish yellow	Dark green	Bluish green
Powder + chloroform	Whitish yellow	Bluish yellow	Golden yellow
Powder + n-hexane	Whitish yellow	Bluish brown	Light yellow
Powder + CCl_4	Whitish yellow	Bluish green	Golden yellow
Powder + Xylene	Brown	Bluish brown	Whitish blue
Powder + Conc. Sulfuric acid	Dark Coffee	Golden yellow	Golden yellow
Powder + dil. Sulfuric acid	Pink	Whitish bluish green	Whitish blue
Powder + Conc. Hydrochloric acid	Yellowish orange	Brown	Dark yellow
Powder + dil. Hydrochloric acid	Pink	Greenish yellow	Whitish greenish yellow
Powder + Conc. Nitric acid	Reddish orange	Dark brown	Greenish brown
Powder + dil. Nitric acid	Light orange	Dark green	Dark green
Powder + Acetic acid	Light yellow	Whitish green	Whitish blue
Powder + Picric acid	Green	Greenish blue	Greenish brown
Powder + 1N NaOH	Greenish yellow	Brown	Greenish yellow
Powder + Ammonia	Green	Brownish yellow	Gold yellow
Powder + %5 Iodine	Dark	Brown	Dark brown
Powder + %5 $FeCl_3$	Dark green	Dark green	Dark green

Table 1
Phytochemical investigations of various extracts prepared from *Chrysochamela noeana**

Compounds	Tests	Hexane	Chloroform	Ethyl acetate	Methanol	Water
Alkaloids	Mayer	-	-	-	-	-
	Marquis	-	-	-	-	-
	Dragendorff	-	-	-	-	-
Carbohydrates	Molisch	+	+	+ +	+ + +	+
Saponins	Foam test	-	-	-	-	-
Coumarins	NaOH+ UV	-	+	-	-	-
Flavonoids	NaOH	-	+	+	-	-
	Shinoda	-	+ +	+	-	-
Lipids		-	-	-	-	-
Tannins, Phenolics	$FeCl_3$	+ + +	+ +	+	-	-
Anthraquinones	Borntrager's	-	-	-	-	-
Volatile oil	Sudan III	+	+	-	-	-
Protein	Ninhydrin	-	-	-	-	-
Steroids	Lieberman	-	+	+	+	-

* (+) means positive results, (++) is strongly positive results, (-) means negative results

Antioxidant activity: Figure 4 shows the dose-response curve of DPPH radical scavenging activity of the three different extracts of aerial parts of *C. noeana*, compared with quercetin. It was observed that the ethyl acetate and methanol extract have higher activity than water extract, the scavenging activity of the methanol extract was higher than ethyl acetate extract at the same concentration. However, ethyl acetate extract was more effective in ABTS radical scavenging than methanol extract, its activity was comparable with Trolox, water extract was lower than other extract. Results obtained in the present work revealed that the level of these phenolic compounds in ethyl acetate and methanol extract were considerable than water extract of *C. noeana*. In addition, the same extracts possessed higher level of total flavonoids (Figure 7). Excessive free irons have been implicated in biological system; it is used for antioxidant evaluation of plant extracts. In metal chelating assay, the extracts were tested in the concentration range of 10-1000 µg/mL, ethyl acetate and methanol extracts demonstrated strong chelating activities in concentration- dependent manners, while the lowest activity was detected in water extract.

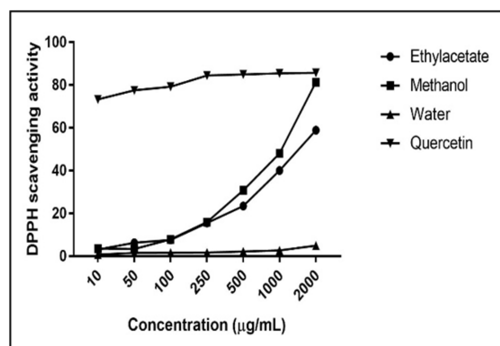

Figure 5
DPPH scavenging activity of different extracts of aerial parts of *C. noeana* and standard quercetin

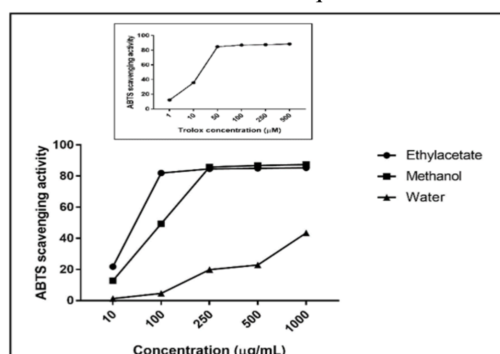

Figure 6
ABTS radical scavenging activity of different extracts of aerial parts of *C. noeana* and standard Trolox

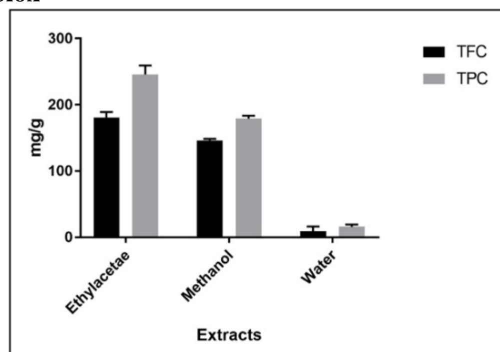

Figure 7
Total flavonoid (TFC) and total phenol content (TPC) of different extracts of aerial parts of *C. noeana*

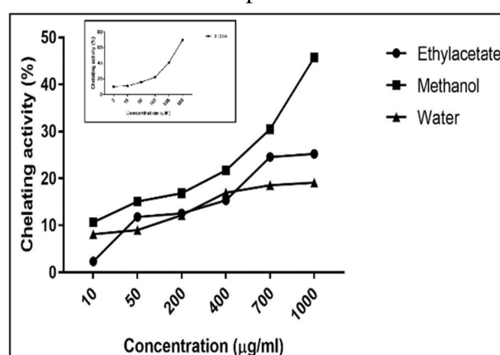

Figure 8
Metal chelating activity of extract prepared from herbs of *C. noeana*

The present study on the physico- and phytochemical investigation and pharmacognostic standardization of the *C. noeana* herbs can be useful to provide information in terms of its identification parameters. They can be used as proper quality control measures to ensure the quality, safety, and efficacy of this herbal drugs as well as may be helpful to preparation of monograph and herbal pharmacopeia standards of this plant.

The macroscopic and microscopic examinations revealed crucial traits that would aid in the identification of this plant. They are assumed significant for the acceptability of herbal drugs in the present case in Türkiye, which lacks regulatory laws to control the quality of herbal drugs. To the best of our knowledge, this is first study of its kind on *C. noeana*, therefore, it will be important to review and research on this plant. Despite the aforementioned research, more studies in phytochemical separation and in vitro screening in different enzymes and cell lines are needed to investigate the pharmacology of the uncommon growing indigenous species.

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