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Phytochemical composition, antibacterial and antioxidant activities of the ethanolic root extract of the endemic *Onosma*, northern Iran.

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// Abstract

This experimental study was performed for determining the antioxidant and antibacterial bioactivity of *Onosma dichroantha*'s root extract obtained using ethanol as our solvent. The results showed that phthalic acid, di(2-propylpentyl) ester (54.52%), .beta.-sitosterol (16.65%) and ethanol, 2-(butylamino)- (13.32%) were the major compounds of the ethanolic root extract of *O. dichroantha*. Total phenols was determined by the Folin-ciocalteu method and the flavonoid content was determined by the colorimetric aluminum chloride method. The maximum phenolic (139.2 ± 0.25 mg gallic acid equivalents/ g of root powder) and flavonoid (17.6 ± 0.12 mg quercetin equivalents/ g of root powder) contents were measured in the ethanol extract. The antioxidant capacity of the root extract was assessed by the scavenging of 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radicals. It was found that antioxidant activity elevated linearly with an increase in the concentration of the ethanol extract. Furthermore, the inhibition value was rather significant compared to the standard controls. The disk diffusion method was used to determine the antibacterial activity of this extract. Thereby, the ethanolic root extract of *O. dichroantha* exhibited great bioactivity against 11 bacterial species, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Staphylococcus epidermis* and *Streptococcus pyogenes*. In conclusion, the ethanolic root extract of *O. dichroantha* has antioxidant and antimicrobial activities.

Introduction

The *Boraginaceae* family has approximately 100 genera and 2000 species in temperate and tropical regions around the world (El-Shazly et al., 1998; Ozgen 2004). Iran is known to be a center of distribution of *Onosma* and has the maximum concentration of such species. This genus has 150 species distributed across dry, sunny and cliffy habitats, like the Mediterranean area and Central Asia (WILLIS 1973). High diversity centers of this genus include Iran, Anatolia and Central Asia (Tiwari et al., 2011). Many properties of different species of *Onosma* have been widely studied, especially their bioactive chemical compounds, since they have been applied for a wide range of diseases and injuries in Iranian traditional medicine. Ozgen *et al.*, 2003 extracted the roots of *Onosma argentatum* and found deoxy shikonin, 3 hydroxy-isovareyl shikonin, acetyl shikonin and 5, 8-O dimethyl acetyl shikonin. Afterwards, they did an in vitro antimicrobial and antioxidant test on the root extract and *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* were eventually found to be rather sensitive to this root extract (Ozgen *et al.*, 2003). Burns and wound healing is one of the paramount properties of the roots of *O. argentatum*. Two other species of *Onosma* that are also used for burns and wound healing are *Onosma sericeum* and *Onosma microcarpum*, common in the rural regions of Turkey (Ozgen et al., 2004). The circumvention of experimental oxidative stress, reducing tumor growth and skin carcinogenesis has been experienced when the extract of *Onosma echiodes* is applied (Sharma et al., 2004).

Several secondary metabolites have been extracted from this family that show antibacterial and antioxidant activities, such as alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids (Li et al., 2010). As we know, phenolic components, like flavonoids, phenolic acids and naphthoquinones like alkannin and shikonin have antioxidant, antiviral, anti-inflammatory and antibiotic

activities which could be the cause of its wound healing property (Li et al., 2010). Moreover, it has been well-known that treatment with the extracts from different portions of this plant can be beneficial to ameliorating rheumatism, kidney irritation, hemorrhoids, heart palpitation, stomach ulcers and burn wounds (Salman et al., 2009; Ahmad et al., 2009). Black seeds, red-white or yellow flowers and hairy leaves are some of the prominent recognition characters of *Onosma dichroantha*. The roots of this plant have been described as similar to a red-colored finger. It has been proven that the aerial parts of this plant have emmenagogue and anti-diarrhea activities. To mitigate the liver and spleen pain, and to also treat the different kinds of fever, one must dry and cook this plant before consumption (Safavi et al., 2019). The application of *O. dichroantha* for reducing lymph node inflammation, removing skin spots, injuries and burns is quite common. Some sort of reddish ointment is cooked and prepared by mixing this plant's dried powder with butter in Iranian traditional medicine. The roots of this plant always have a stronger pharmaceutical effect than the aerial parts. (Safavi et al., 2019). To the best of our knowledge, there have been no studies carried out on the evaluation of antimicrobial and antioxidant activities of *O. dichroantha*'s root extract collected from the Mazandaran province of Iran.

The aim of this work was to investigate the inhibition effects of the ethanolic root extract of *O. dichroantha* on 16 different species of pathogenic bacteria causing common diseases. At the same time, the antioxidant properties of the extract were determined. The phytochemical components in the extract were also identified for understanding the mechanism of antibacterial and antioxidant activities. Accordingly, to bridge the gap in studies run on the subject of investigation.

Materials And Methods

Extract preparation

In this experimental study, the roots of *Onosma dichroantha* were collected from Alasht, Mazandaran, Iran (36.0665° N, 52.8339° E) at the altitude of 1908 m. This plant was identified by Dr. Alireza Naghinezhad, Faculty member of university of Mazandaran. The roots were dried at room temperature and coarsely ground before extraction. Powdered roots (50 g) of *O. dichroantha* were extracted with 1000 ml of pure ethanol via the maceration method for 48 hours in a mechanical shaker at room temperature. Extracts were filtered with filter paper (Whatman No. 1) and were stored at 4°C. Eventually, the extract was concentrated over a rotary vacuum evaporator to yield a solid extract sample.

Determination of total phenol content

Folin Ciocalteu reagent (2.5 ml, 0.2 N) was applied to the extract samples (0.5 ml of different dilutions) and mixed for 5 minutes. Next, an aqueous Na_2CO_3 (2 ml, 1 $g.l^{-1}$) was prepared and applied to the mixture. The mixture was set aside for 2 hours before utilizing the colorimetry at 760 nm for the

determination of the phenolic compounds. Based on the solutions of gallic acid, the standard curve was made. Gallic acid equivalent ($mg.g^{-1}$ of dry mass) is the term used for expressing the values of total phenolic compounds (Ghasemi *et al.,* 2015).

Determination of total flavonoid content

The method chosen for flavonoid determination was the colorimetric aluminum chloride method. In summary, 0.1 ml of aluminum chloride (10 %), 1.5 ml of methanol, 0.1 ml of potassium acetate (1 M) and 2.8 ml of distilled water were applied to 0.5 ml of the ethanolic root extract of *O. dichroantha* and mixed. In the next step, the mixture was put at room temperature for about 30 minutes. Double beam Perkin Elmer UV/Visible spectrophotometer was utilized and set at 415 nm in order to determine the mixture's absorbance. Total flavonoid content was calculated as quercetin from a calibration curve. Quercetin solutions were prepared so that the calibration curve could be determined (Ghasemi *et al.,* 2015).

DPPH radical-scavenging activity

The ethanolic root extract's free radical-scavenging activity was investigated by making use of 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) (Tiwari *et al.,* 2013). Firstly, 1 ml of DPPH methonolic solution with a concentration of 0.1 mM was prepared and equal volumes of the ethanolic root extract with various concentrations were added to it. Afterwards, the mixture was set aside for 15 minutes at room temperature and the spectrophotometer was set at 517 nm for recording the absorbance of the mixture. The percentage of DPPH scavenging by the extract was calculated applying the formula:

$$\% \text{ Inhibition} = \frac{[\text{Absorbance of control} - \text{Absorbance of extract}]}{[\text{Absorbance of control}]} \times 100$$

We used butylated hydroxyanisole (BHA) and ascorbic acid as our standard controls. The test was repeated for three times. In order to scavenge 50 percent of the DPPH free radicals, IC_{50} values determine the concentration of the sample (Tiwari *et al.,* 2013).

Antibacterial activity

In order to determine the antibacterial activity of the ethanolic root extract of *O. dichroantha*, 16 different species of Gram-positive and Gram-negative bacteria were tested (Table 1). The bacteria were collected from the Iranian Research Organization for Science and Technology.

The antibacterial activity of the *O. dichroantha* root extract was investigated by taking advantage of the disk diffusion method (Somaranthna *et al.,* 2018). Firstly, concentrations of 25 $mg.ml^{-1}$, 50 $mg.ml^{-1}$, 75 $mg.ml^{-1}$ and 100 $mg.ml^{-1}$ of the extract were prepared with the usage of ethanol as our solvent. Then, 5 to 10 colonies of bacteria were grown in 1-2 ml Muller Hinton Broth at 37 °C for 24 h. The content was centrifuged at 1000 g for 10 min to yield the bacterial pellet. The supernatant was removed, and the bacterial pellet was re-suspended in 1 ml sterile 0.85 % NaCl. Then the content solution was serially

diluted by adding 9 ml of sterile 0.85 NaCl solution to obtain 5 × 10^5 CFU ml^{-1}, and 100 ml of diluted bacterial suspension was spread on Mueller Hinton Agar (MHA) plates. An aliquot (150 ml) of each concentration of the extract was pipetted onto a sterile paper disc of 6 mm diameter (Whatman No1) on the agar surface. The plates were inverted and incubated at 37 °C for 24 h.

Microbial inhibition was determined by measuring the clear zone of inhibition around each disc and recorded as the diameter zone of inhibition in millimeters. All assays performed independently three times with triplicates (Somaranthna *et al.,* 2018). Consequently, the yielded data were analyzed by taking advantage of SPSS software (version 19.0; SPSS Inc., Chicago, IL, USA) using the one-way ANOVA test and the p-value < 0.05 was considered to be significant difference.

GC.MS analysis

The chemical components of ethanolic root extract of *O. dichroantha* were investigated by gas chromatography−mass spectrometry (GC-MS). GC/MS was carried out using a HewlettPackard 5975B series instrument and an Agilent 19091J-433 HP-5 capillary column (30 m., 250 μm i.d., film thickness 0.25μm) which was set at 50°C for 10 min, then increased 4°C/min to 300°C; using helium as a carrier gas at a flow rate of 1 ml/min. The split ratio was 1:10; ionization energy was 70 eV; scan time was 1 s; acquisition mass range was m/z 40−400. The compounds were identified according to their retention indexes and by comparison of their mass spectra with those of a computer library or with authentic compounds.

Results And Discussion

Antimicrobial activity

The results of the present study shows that the ethanolic root extract of *O. dichroantha* originated from ethanol as our solvent has had a good potential for antibacterial activity. Nevertheless, the antimicrobial activity against bacterial organisms varied based on the different types of bacterial species. The results indicated that the extract has inhibitory effect on *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, Streptococcus agalactiae, Enterococcus faecalis, Escherichia coli,, Shigella sonnie, Shigella flexteriae, Listeria monocytogenes, Staphylococcus epidermis* and *Streptococcus pyogenes* was observed in the ethanolic root extract. Zarghami *et al*., (2012) in an in vitro study with various extracts of *Onosma dichroantha boiss*, showed that the ethanolic extract of *Onosma*'s aerial parts had the strongest antimicrobial activity compared to all other extracts. Nonetheless, Sezik et al., (1997) found out that three species of *Onosma* were active against different bacterial and fungal species. However, these authors demonstrated that the antimicrobial activities may be different according to both the *Onosma* species and the test organism. The antibacterial results of this study was in accordance with

the afore-mentioned studies and the extract in general showed more inhibitory effect on Gram-positive bacteria.

Phenolic compounds have tremendous morphological and physiological essentiality in plants and are considered prime phytochemicals (zlotek *et al.*, 2016). It has been reported that phenolic compounds have a key role in the biological activity and inhibitory effect on bacteria (Zain et al., 2012). Moreover, plants synthesize flavonoid compounds in response to bacterial infection. Many endophytes produce flavonoids, probably as antibacterial agents along with phenols (Anitha *et al.*, 2017). Therefore, the flavonoids and phenols, existing in the root and aerial parts of the plant, may contribute to the enhancement of the antimicrobial capacity of the O*nosma* extract.

Overall, the ethanolic root extract showed antibacterial activities on most of the bacteria and didn't have any effect on some of them, including; *Enterobacter aerogenes*, *Proteus mirabilis* and *Acinobacter buamannii*,. And it had slight inhibitory effect on *Klebisella pneumoniae* and *Salmonella typhi*.

Table 1
Zone of inhibition affected by *O. dichroantha* plant root extract using the disc diffusion method.

	Indicator organisms	ATCC	Pure ethanol (Mean ± SD)	25 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	50 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	75 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	100 $mg.ml^{-1}$ of the crude extract (Mean ± SD)
Zone of inhibition (DIZ)/(mm)	*Enterococcus faecalis*	29212	6 ± 0.00	8.7 ± 0.57	11.3 ± 1.15	12.3 ± 2.08	16.3 ± 1.52
	Streptococcus pyogenes	19615	6 ± 0.00	6 ± 0.00	6 ± 0.00	8.6 ± 1.15	12.6 ± 1.52
	Streptococcus pneumoniae	6305	6 ± 0.00	8 ± 0.00	10.6 ± 0.57	9.6 ± 2.31	12 ± 1.15
	Streptococcus agalactiae	12386	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	11.6 ± 0.58
	Staphylococcus aureus	25923	6 ± 0.00	11.3 ± 1.53	13 ± 1.00	15 ± 1.73	17.3 ± 1.15
	Staphylococcus epidermis	12228	6 ± 0.00	6 ± 0.00	7.3 ± 0.56	10.6 ± 1.15	14 ± 0.57
	Listeria monocytogenes	7644	6 ± 0.00	6 ± 0.00	8 ± 0.00	9.6 ± 1.53	15.3 ± 1.15
	Escherichia coli	25922	6 ± 0.00	6 ± 0.00	6 ± 0.00	6.6 ± 1.15	7.3 ± 1.15
	Klebisella pneumoniae	13883	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6.6 ± 1.15
	Enterobacter aerogenes	13048	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00
	Proteus mirabilis	43071	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00
	Pseudomonas aeroginosa	27853	6 ± 0.00	6 ± 0.00	8 ± 0.00	9.3 ± 1.15	15.6 ± 1.15
	Salmonella typhi	14028	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6.6 ± 1.15
	Shigella sonnie	9220	6 ± 0.00	6 ± 0.00	6 ± 0.00	7 ± 0.00	8.6 ± 0.58
	Shigella flexteriae	12022	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	7 ± 1.00

	Indicator organisms	ATCC	Pure ethanol (Mean ± SD)	25 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	50 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	75 $mg.ml^{-1}$ of the crude extract (Mean ± SD)	100 $mg.ml^{-1}$ of the crude extract (Mean ± SD)
	Acinobacter buamannii	BAA-747	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00	6 ± 0.00

In order to answer the research question of the study, the one-way ANOVA test was used for mean comparison. The descriptive statistics of the groups are shown below.

Table 2
The descriptive statistics for the comparison of the control and the experimental groups.

	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Pure ethanol	16	6.00	0.00	0.00	6.00	6.00
25 $mg.ml^{-1}$	16	6.62	1.48	0.37	6.00	11.30
50 $mg.ml^{-1}$	16	7.38	2.26	0.56	6.00	13.00
75 $mg.ml^{-1}$	16	8.16	2.73	0.68	6.00	15.00
100 $mg.ml^{-1}$	16	10.55	4.21	1.05	6.00	17.30
Total	80	7.74	2.95	0.32	6.00	17.30

The extracted means of the pure ethanol as the control group of study, 25 $mg.ml^{-1}$, 50 $mg.ml^{-1}$, 75 $mg.ml^{-1}$ and 100 $mg.ml^{-1}$ are 6, 6.62, 7.38, 8.16, 10.55 and 7.74, respectively. The result of the one-way ANOVA test for the comparison of the groups is presented below.

Table 3
The result of the one-way ANOVA test for the comparison of the control and the experimental groups.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	199.51	4	49.87	7.65	.00
Within Groups	488.50	75	6.51		
Total	688.01	79			

According to the Table 3 above, there was a statistically significant difference among the groups regarding their mean scores, $F(4, 75) = 7.65$, $p < .05$. Hence, the researcher safely rejects the null

hypothesis. The following table (Tukey) compares the groups individually.

Table 4
The result of the pairwise comparison of the groups.

(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	**Upper Bound**
Pure ethanol	**25** mg.ml^{-1}	-0.62	0.90	0.95	-3.14	1.89
	50 mg.ml^{-1}	-1.38	0.90	0.54	-3.90	1.13
	75 mg.ml^{-1}	-2.16	0.90	0.12	-4.68	0.35
	100 mg.ml^{-1}	-4.55*	0.90	0.00	-7.07	-2.02
25 mg.ml^{-1}	**Pure ethanol**	0.62	0.90	0.95	-1.89	3.14
	50 mg.ml^{-1}	-0.76	0.90	0.91	-3.28	1.75
	75 mg.ml^{-1}	-1.53	0.90	0.43	-4.05	0.98
	100 mg.ml^{-1}	-3.92*	0.90	0.00	-6.44	-1.40
50 mg.ml^{-1}	**Pure ethanol**	1.38	0.90	0.54	-1.13	3.90
	25 mg.ml^{-1}	0.76	0.90	0.91	-1.75	3.28
	75 mg.ml^{-1}	-0.77	0.90	0.91	-3.29	1.74
	100 mg.ml^{-1}	-3.16*	0.90	0.00	-5.68	-0.64
75 mg.ml^{-1}	**Pure ethanol**	2.16	0.90	0.12	-0.359	4.68
	25 mg.ml^{-1}	1.53	0.90	0.43	-0.984	4.05
	50 mg.ml^{-1}	0.77	0.90	0.91	-1.74	3.29
	100 mg.ml^{-1}	-2.38	0.90	0.07	-4.90	0.13
100 mg.ml^{-1}	**Pure ethanol**	4.55*	0.90	0.00	2.02	7.07
	25 mg.ml^{-1}	3.92*	0.90	0.00	1.40	6.44
	50 mg.ml^{-1}	3.16*	0.90	0.00	0.64	5.68

	75 mg.ml^{-1}	2.38	0.90	0.07	-0.13	4.90

As Table 4 shows, there was a significant difference between the pure ethanol and 100 mg.ml^{-1}groups ($p < .05$); there was a significant difference between the 25 mg.ml^{-1}and 100 mg.ml^{-1}groups ($p < .05$); there was a significant difference between the 50 mg.ml^{-1}and 100 mg.ml^{-1}groups ($p < .05$).

Total phenolic and flavonoid content

In the present experiment, the total phenolic and flavonoid content of *O. dichroantha*'s ethanolic root extract is 139.2 ± 0.25 mg.g^{-1}and 17.6 ± 0.12 mg.g^{-1}, respectively. According to the results, a quite high phenolic content was observed in the ethanol extract. Similarly, a high total flavonoid content was determined in the ethanol extract. Several studies demonstrated that the genus *Onosma* is a great source of phytochemical constituents like phenolic compounds (Lakenbrink et al., 2000)(Özgen et al., 2003)(Grzegorczyk *et al.*, 2006). The optimization of extraction factors of the phenolic content and antioxidant properties of plant-based foods are a major concern, as some studies have concluded. However, different plant material commonly have variety in an optimal procedure (Rababah et al., 2010). Data on the total flavonoid content of the *O. dichroantha*'s root extract are limited in the literature. Therefore, the comparisons were made with the other species of the *onosma* genus. The result of the present study confirms the one conducted by Zarghami Moghaddam et al., (2012) reporting that the ethanol root extract of onosma genus had a remarkable value of total flavonoid compounds (Zarghami Moghaddam et al., 2012). Moreover, the primary phytochemical screening of three species of *onosma* revealed that the total flavonoid compounds were a major part of their total chemical composition (Zarghami Moghaddam et al., 2012).

Antioxidant activity

In the present experiment, the antioxidant activity of the ethanolic root extract was determined by scavenging of the DPPH free radicals. The inhibition value as shown in Table 5 below, was found in the ethanol extract.

It is well-known that solvent polarity plays a key part in increasing phenolic solubility. Therefore, ethanol was chosen as the solvent (Naczk *et al.*, 2006; Alothman et al., 2009). Hence, the most appropriate solvent for extraction of the roots is ethanol or a solution based on water and ethanol (Tomsone et al., 2012). The IC_{50} of the standard control compounds, BHA and ascorbic acid were 106.82 ± 0.40 µg.ml^{-1} and 189.04 ± 0.02 µg.ml^{-1}, respectively. Since IC_{50} is inversely associated with the anti-radical activity of the compounds, the lower the IC_{50}, the higher the antioxidant activity (Noroozi et al., 2017). The IC_{50} of the DPPH radical-scavenging activity of the ethanolic root extract of *O. dichroantha* was 263.271 ± 0.02 µg.ml^{-1} which is higher than but close to both the IC_{50} of ascorbic acid and the IC_{50} of BHA. In summary, the ethanolic root extract of *O.* dichroantha has a high antioxidant activity.

Table 5
DPPH radical-scavenging activity of ascorbic acid, BHA and the *O. dichroantha* root extract

Concentration (μg.ml^{-1})	Radical scavenging activity (%) of ascorbic acid	Radical scavenging activity (%) of BHA	Radical scavenging activity (%) of the *O. dichroantha* root extract
800	96.4 ± 0.7	95.5 ± 0.001	76.12 ± 0.03
400	71.2 ± 0.2	93.2 ± 0.002	62.32 ± 0.04
200	55.1 ± 0.1	72.8 ± 0.01	47.74 ± 0.07
100	44.8 ± 0.4	43.7 ± 0.03	39.65 ± 0.02
50	38.7 ± 0.2	28.2 ± 0.02	19.62 ± 0.02

Chemical composition

In the present experiment, the chemical composition of the ethanolic root extract of *O. dichroantha* was determined and the results are presented in Table 6 The primary phytochemical screening showed that phthalic acid, di(2-propylpentyl) ester (54.52%), .beta.-sitosterol (16.65%), benzene, 1-hexyl-4-nitro- (4.89%) and cyclohexanone, (4-nitrophenyl) hydrazone (4.09%) were the major compounds of the ethanolic extract. Phthalic acid, di(2-propylpentyl) ester is known to be antimicrobial, Cytotoxic and anti-inflammatory (Cotter et al., 2005)(Habib *et al.*, 2009). Beta.-sitosterol is another major compound in the extract which has strong antioxidant activities and it also reduces thiobarbituric acid reactive substances (Gupta et al., 2011)(Upadhyay et al., 2012). However, the antimicrobial activities of this compound are still controversial and quite dubious (Sen et al., 2012)(Cota et al., 2003)(Gohari et al., 2009)(Mishra et al., 2010)(Bayor et al., 2009). A cyclohexanone derivative is another major compound in the extract which belongs to a paramount group of six-membered heterocyclic compounds that are known to have antibacterial activities against both Gram-Negative and Gram-positive bacteria (Asiri *et al.*, 2011). Consequently, several major compounds in the *O. dichroantha*'s root extract are responsible for its antibacterial activities against different bacterial species as shown in Fig. 1.

Table 6
The major compounds found in the ethanolic root extract of *O. dichroantha*.

No	Name of composition	RT	AREA (%)
1	Ethanol, 2-(butylamino)-	17.671	13.32
2	Phthalic acid, di(2-propylpentyl) ester	23.112	54.52
3	Acetamide, N-tricyclco [4.3.1.1(3,8)] undec-1-yl-	23.603	2.90
4	Benzene, 1-hexyl-4-nitro-	23.637	4.89
5	Cyclohexanone, (4-nitrophenyl) hydrazone	24.561	4.09
6	Pregan-12-one, (5.beta.)-	24.596	3.63
7	Beta.-sitosterol	31.024	16.65

Conclusion

The ethanolic root extract of *Onosma dichroantha* collected from the North of Iran has significant antibacterial and antioxidant activities, which could be because of its main chemical components such as phthalic acid and beta. -sitosterol. In fact, the ethanolic root extract of *O. dichroantha* can be a paramount source for pharmaceutical applications. *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus faecalis, Listeria monocytogenes, Staphylococcus epidermis* and *Streptococcus pyogenes* are some the bacteria that were highly sensitive to this extract and do not show any growth in its presence. Nonetheless, the ethanolic extract displayed high concentrations of phenol and flavonoid contents and a quite elevated scavenging of DPPH free radicals in different concentrations. Nevertheless, our knowledge of the many extraordinary properties of this plant at the molecular level is limited and needs further studies to see if it has anticancer, antifungal, anti-inflammatory or other properties as well.

Declarations

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Authors contribution

P.P & H.B ; Root sampling, phenol assay, flavonoid assay, DPPH assay, Disk diffusion antibacterial assay, GC.MS analysis. S.S.B ; Data analysis.

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Figures

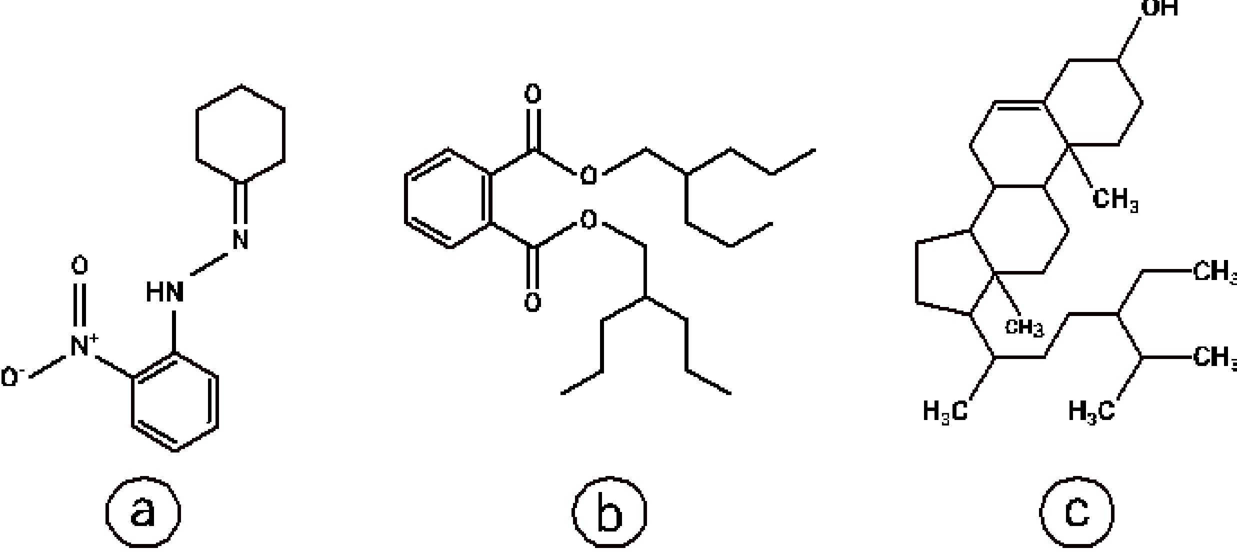

Figure 1

Chemical structures of the discussed compounds;

a) Cyclohexanone, (4-nitrophenyl) hydrazone

b) Phthalic acid, di(2-propylpentyl) ester

c) Beta.-sitosterol