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Original article

# Total phenolic and flavonoid contents and antioxidant activity of four medicinal plants from Hormozgan province, Iran

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## Abstract

**Background and objectives:** Hormozgan province is located in the south of Iran, bordering waters of the Persian Gulf and Oman Sea. Due to the antioxidant potential of plants which might be responsible for their medicinal properties, the antioxidant properties of four medicinal plants of the region were evaluated. **Methods**: The antioxidant properties of *Chrozophora obliqua*, *Daphne mucronata*, *Salvia aegyptiaca* and *Suaeda vermiculata* were evaluated by four different methods: free radical scavenging using 2,2-diphenyl-1-picrylhydrazyl (DPPH), metal chelating activity, inhibition of lipid peroxidation by the ferric thiocyanate method, and total reduction capability. The flavonoid and phenolic content of the plants were also analyzed. **Results**: Amongst the species, *C. obliqua* showed the best result in metal chelating activity test, and *S. vermiculata* showed the best antioxidant activity in the three other assays, and *S. vermiculata* and *S. aegyptiaca* had the highest amount of phenolic and flavonoid contents. **Conclusion**: The acceptable antioxidant activity of *S. vermiclata* as a halophyte plant, could justify the medicinal properties of the plant.

**Keywords:** antioxidant, flavonoid contents, halophyte, medicinal plant, phenolic contents

## Introduction

There is increasing scientific evidence indicating that reactive oxygen species (ROS) and free radical mediated reactions are involved in degenerative or pathological events, such as aging, cancer, coronary heart ailments, and Alzheimer's disease [1]. ROS, including superoxide anion, hydroxyl radical, and hydrogen peroxide, are generated in specific organelles of the cell under normal physiological conditions [2]. Excessive production of ROS, beyond the antioxidant defense capacity of the body can cause oxidative stress [3].

Hormozgan province is located in the south of Iran, bordering waters of the Persian Gulf and Oman Sea [4]. Since the plants have protective effects against some diseases, due to their antioxidant potential [5], and regarding the great role of the studied plants in the folklore medicine

 
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of the region, we were prompted to evaluate the antioxidant activity of four medicinal plants (*Chrozophora obliqua*, *Daphne mucronata*, *Salvia aegyptiaca* and *Suaeda vermiculata*) of the area by four different methods which have not been studied earlier. The phenolic and flavonoid contents of these plants were also evaluated because flavonoids are known to be the most important phytochemicals to be responsible for the antioxidant capacity of plants [6]. Three of these plants belong to the Geno protected area with a mountainous view and desert climate [7] and the other one (*Suaeda vermiculata*) is a halophyte (a diverse group of plants with tolerance to high salinity) [8] from a salt marsh area. Medicinal properties of these plants are shown in table 1.

**Table 1**. Medicinal properties of *Chrozophora obliqua*, *Daphne mucronata*, *Salvia aegyptiaca* and *Suaeda vermiculata* from Hormozgan province, Iran

| Scientific name and family | Therapeutic indication |
|---|---|
| *Salvia aegyptiaca* L. (Lamiaceae) | Flatulence, wound [7] |
| *Daphne mucronata* Royle (Thymelaeaceae) | Skin disorder [9] Warts, fever [10]. |
| *Chrozophora obliqua* (Vahl) A.Juss. ex Spreng. (Euphorbiaceae) | Hypoglycemia, hypolipidemia, cancer |
| *Suaeda vermiculata* Forssk. ex J.F.Gmel. (Amaranthaceae) | inflammation [11], difficult breathing [12], Jaundice [4] |

## Experimental

*Plant material*

Plant samples were collected from two different parts of the region: Geno protected (in 30 km north-west of Bandar Abbas, the capital of Hormozgan, (*Chrozophora obliqua*, *Daphne mucronata*, and *Salvia aegyptiaca*) and *Suaeda vermiculata* from a salt marsh between Minab and Bandar Abbas and they were identified by R. Asadpour (botanist, Hormozgan Agricultural and Natural Resources Research Center, Bandar Abbas, Iran). Voucher specimens were deposited in the Herbarium of Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran.

*Preparation of plant extracts*

The plants aerial parts were dried at room temperature. A sample (50 g) of the dried powdered plant material was extracted thrice in 100 mL methanol for 24 h. The extracts were filtered and the combined filtrates were concentrated using rotary evaporator.

*Free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH)*

Experiments were carried out according to the method of Blois [13] with a slight modification. Briefly, a 0.1 mM solution of DPPH radicals in methanol was prepared and thereafter 1 mL of this solution was mixed with the sample solution (3 mL) in methanol. After 30 min of maintenance in dark place, the absorbance was measured at 517 nm.

Decreasing the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. This activity was presented as percentage of DPPH radical-scavenging, calculated by the following equation:

$$\text{DPPH radical-scavenging (\%)} = (A - B / A) \times 100$$

Where, A is the control absorbance and B is the sample absorbance. The DPPH solution without sample was used as the control and butylated hydroxytoluene (BHT) was used as the standard.

*Ferric thiocyanate method (inhibition of lipid peroxidation)*

The antioxidant activities of the extracts were determined using to the ferric thiocyanate method as reported by Kikuzaki and Nakatani [14]. A mixture containing the extract (4 mL) in absolute ethanol, final concentration of 200 μg/mL, 2.51% linoleic acid in absolute ethanol (4.1 mL), phosphate buffer pH 7 (8 mL) and distilled water (3.9 mL), was placed in a vial with a screw cap, and then was incubated in an oven at 40 °C in the dark. To this solution (0.1 mL), 75% ethanol (9.7 mL) and 30% ammonium thiocyanate (0.1 mL) were added. Three minutes after adding $2\times10^{-2}$M ferrous chloride in 3.5% hydrochloric acid (0.1 mL) to the reaction mixture, the absorbance of the red color was measured at 500 nm, every 24 h

until one day after absorbance of the control (without sample) reached its maximum. BHT was used as the standard.
Percentage inhibition of lipid peroxidation was calculated by the following equation:

lipid peroxidation inhibition (%) = (Ac – As / Ac) × 100

Where As is the absorbance of the sample on the day when the absorbance of the control was maximum; Ac is the absorbance of the control on the day when the absorbance of the control was maximum.

*Total reduction capability*
The method of Oyaizu [15] was used to determine the reducing power. Various concentrations of the plant extracts (2.5 mL) were mixed with 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After addition of 2.5 mL 10% trichloroacetic acid (w/v), the mixture was centrifuged for 10 min. The upper layer (5 mL) was mixed with 5 mL deionized water and 1 mL 0.1% of ferric chloride. The absorbance was measured at 700 nm; higher absorbance indicates higher reducing power. BHT was used as the standard.

*Metal chelating activity*
The chelation of ferrous ions by the extracts was estimated using the method of Dinis *et al*. [16]. Briefly, 50 µl of 2 mM $FeCl_2$ was added to 1 mL of different concentrations of the extracts. Thereafter, the reaction was initiated by 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 [(A0- As)/As] × 100, where A0 is the absorbance of the control and As is the absorbance of the extract/standard. Ethylenediaminetetraacetic acid (EDTA) was used as the standard.

*Total phenolic and flavonoid contents*
The content of the total phenolic compounds in the plant extracts was determined using Folin-Ciocalteu method [17]. One milliliter of 1 mg/mL plant extract was mixed with 5 mL Folin-Ciocalteu reagent (diluted ten-fold with distilled water) and 4 mL (7.5 g/100 mL) sodium carbonate. The absorption of clear solutions was recorded at 765 nm, after 1 h at room temperature. Different concentrations of gallic acid solution were mixed with the same reagents as described above for plotting of calibration curve. After 30 min the absorption of the clear solutions was measured. The amount of total phenolics was expressed as gallic acid equivalent (GAE) in milligrams per gram of the dry plant extract.
The content of the flavonoids was determined using the aluminium chloride colorimetric method. One milliliter of 1 mg/mL of the plant extract was mixed with 1 mL of 2% $AlCl_3$ ethanol solution. After 1 h at room temperature, the absorbance was measured at 420 nm. The results were expressed in milligram rutin per gram of dry plant extract by comparing with the standard rutin treated in the same condition [18].

*Statistical analysis*
Data are presented as the mean ± standard deviation (SD) of each triplicate.

**Results and Discussion**
The plants were assessed for DPPH radical scavenging activity to obtain their concentration that would scavenge 50% DPPH radicals ($IC_{50}$). As shown in table 2, the highest DPPH radical scavenging activity (the lowest $IC_{50}$) was shown by *S. vermiculata* (75.30 ± 4.50 µg/mL), and the lowest activity was shown by *D. mucronata* (184.94 ± 7.85 µg/mL).
Figure 1 shows the inhibitory activity of the extracts against linoleic acid peroxidation. All extracts exhibited inhibition against linoleic acid peroxidation, especially *S. vermiculata* with 66.10±2.4% inhibition, on the seventh day of

incubation (table 2).

In total reduction capability evaluation Though, All the plant extracts increased the absorbance of the control solution (0.210 ± 0.098) in 700 nm as shown in figure 3, all the concentrations had lower absorbance and consequently lower reduction capability toward BHT.

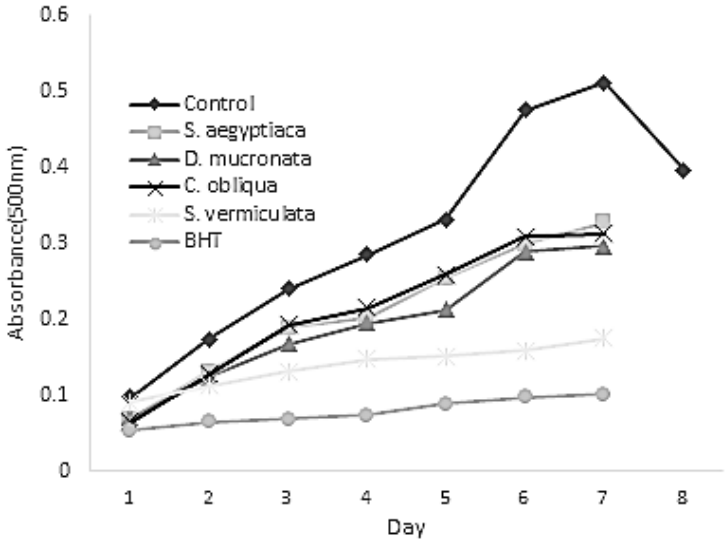


**Figure 1.** Inhibition of lipid peroxidation of plant extracts and BHT (standard). Final concentration 200 µg/mL. A low absorbance value represents a high level of antioxidant activity.

**Table 2**. Radical scavenging activity in the DPPH assay, iron chelating activity, total reduction capability, lipid peroxidation inhibition and the amount of flavonoids and phenolic compounds of *S. vermiculata, S. aegyptiaca, C. obliqua ,D. mucronata*

| **Plant extracts and standards** | **DPPH activity[(1)]** | **Iron chelating activity[(2)]** | **Total reduction capacity[(3)] (%)** | **Lipid peroxidation Inhibition[(4)]** | **Total phenolic compounds[(5)]** | **Total flavonoids[(6)]** |
|---|---|---|---|---|---|---|
| *S.vermiculata* | 75.30 ± 4.50 | 31.00 ± 3.41 | 28.44 ± 3.85 | 66.10 ± 2.4 | 18.56 ± 2.01 | 8.40 ± 0.98 |
| *S.aegyptiaca* | 135.91 ± 6.08 | 45.33 ± 2.43 | 22.09 ± 2.25 | 36.13 ± 1.08 | 15.34 ± 1.59 | 12.19 ± 1.60 |
| *C.obliqua* | 181.45 ± 6.54 | 61.09 ± 2.12 | 25.88 ± 3.45 | 37.90 ± 2.10 | 3.03 ± 0.89 | 3.85 ± 0.13 |
| *D.mucronata* | 184.94 ± 7.85 | 48.14 ± 3.41 | 23.52 ± 3.03 | 42.00 ± 0.05 | 10.93 ± 0.95 | 3.85 ± 1.20 |
| BHT | 41.80 ± 3.86 | - | - | 78.23 ± 3.90 | - | - |
| EDTA | - | 99.07 ± 0.49 | - | - | - | - |

[1]The concentration (µg/mL) of the plant extracts in inhibition of 50% DPPH Radical ($IC_{50}$)
[2]The percentage of iron chelating activity of the plant extracts in the concentration of 1000 µg/mL
[3]Increased control absorbance by the plant extracts (1000 µg/mL) / increased control absorbance by BHT (1000 µg/mL) × 100
[4] The percentage of the inhibition of lipid peroxidation by the plant extracts in the concentration of 200 µg/mL (the seventh day)
[5] mg/g plant extracts in gallic acid equivalent
[6] mg/g plant extract in rutin equivalent

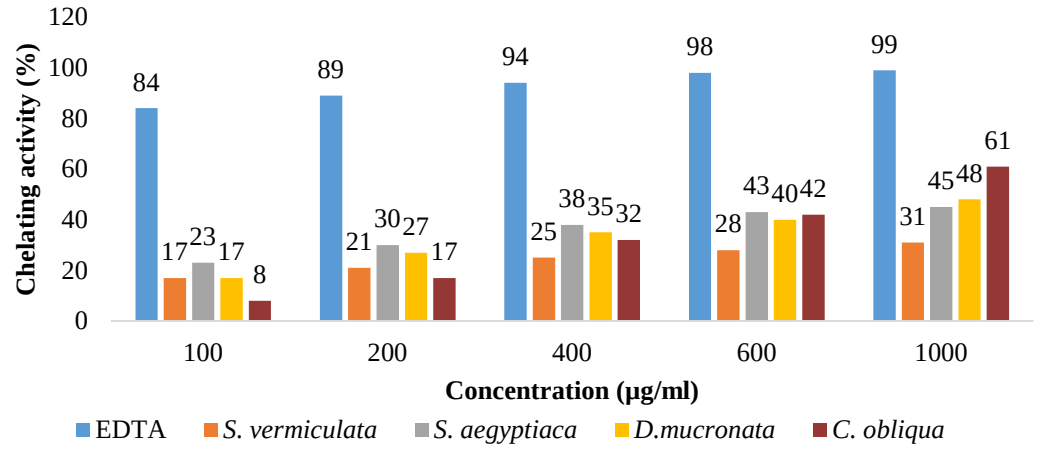


**Figure 2**. Iron chelating effect of *S. vermiculata*, *S. aegyptiaca*, *D. mucronata* and *C. obliqua* extracts (%). standard; EDTA.

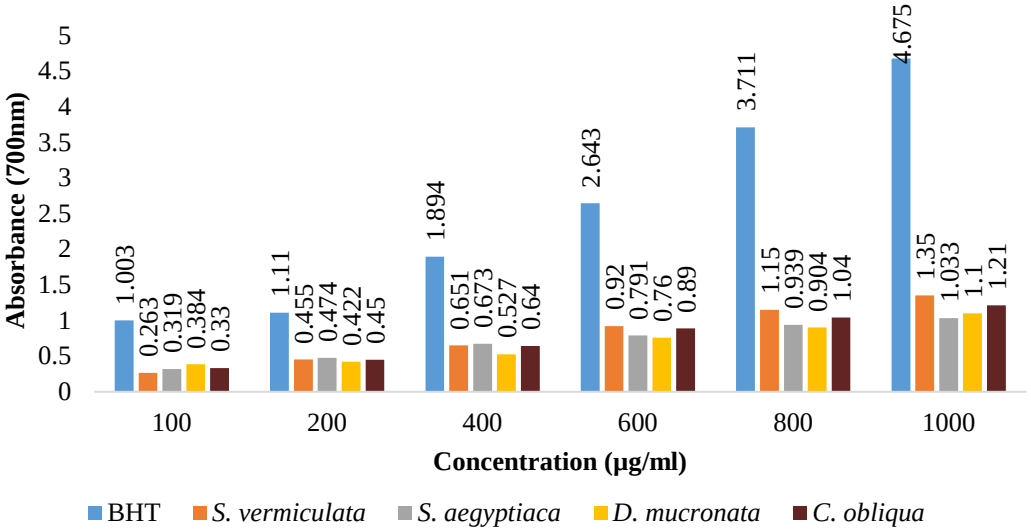


**Figure 3**. Total reduction capability of *S. vermiculata*, *S. aegyptiaca*, *D. mucronata* and *C. obliqua* extracts; Standard: BHT. All the extracts showed activity lower than BHT. Control absorbance: 0.210 ± 0.098.

Metal chelating activity (%) of the plant extracts and EDTA in concentrations of 100, 200, 400, 600 and 1000 µg/mL are shown in figure 2. The metal chelating activity of the plant extracts increased with increase in concentration. In concentration of 1000 µg/mL of the plant extracts, *C. obliqua* showed the strongest activity (61.09 ± 2.12%) while *S. vermiculata* showed the lowest activity (31.00 ± 3.41%) (table 2).

The content of phenolic compounds (mg/g) in plant extracts, determined from regression equation of calibration curve (y = 0.425x + 0.032, $R^2$ = 0.99) and was expressed per gallic acid equivalents (GAE), varied between 3.03 and

18.56 (table 1). The highest and lowest amounts were found for the extract of *S. vermiculata* (18.56 ± 2.01), and *C. obliqua* (3.03 ± 0.89), respectively.

The content of flavonoids in the plant extracts (mg/g) was expressed in rutin equivalents (regression equation of calibration curve, y = 0.966x + 0.030, $R^2$ = 0.99). The highest amounts were found for the extract of *S. aegyptiaca* (12.19 ± 1.60), and the lowest in *C. obliqua* (3.85 ± 0.13) and *D. mucronata* (3.85 ± 1.2).

Free radicals are produced by different mechanisms in the body. Superoxide anion radical is the first molecular species in the oxygen reduction pathway [19] which can be converted to hydrogen peroxide and hydroxyl radicals using Fenton reaction [20**]**. Hydroxyl ($OH^o$) is extremely active and dangerous and can attack body macromolecules, including DNA, lipids, carbohydrate and proteins, resulting in different diseases [21].

There is an ongoing demand for antioxidants to prevent oxidative stress [22]. Plants are considered as a source of exogenous antioxidants [23]. It is believed that most of the plant species possess medicinal properties, and many of them have excellent antioxidant potential [24]; it is therefore important to examine their antioxidant activity. Four different tests (DPPH method to evaluate radical scavenging activity, iron chelating activity and reduction capacity to evaluate the power of the extracts in inhibition of converting radicals to more active one ($OH^o$) and thiocyanate method to evaluate protective effect of the plant extracts in lipid peroxidation inhibition) were selected to cover the different aspects of the probable of antioxidant activity mechanisms of these plant extracts, because antioxidants act by different mechanisms.

Comparing the radical scavenging activity of the plant extracts (table 1) with BHT ($IC_{50}$: 41.8 ± 3.86 µg/mL), demonstrated acceptable effect of *S. vermiculata* (75.30 ± 4.50 µg/mL) and moderate activities for the three other plants in free radical inhibition. This species belongs to halophytes and is different from three other plants which exist in Geno mountain and are non-halophytes.

There are some reports that the accumulation of salts in halophytic plants can cause oxidative stress in the plant tissues. As a defense mechanism, halophytes have an antioxidant capacity with natural antioxidants occurring in all the plant organs, which include flavonoids, phenolics, carotenoids, alkaloids and vitamins [11,25,26].

It is believed that flavonoids and phenolic compounds have a great role in scavenging free radicals [27,28]. There are some reports concerning the presence of flavonoids [29] and phenolic compounds [30] in *S. vermiculata*, which might be responsible for DPPH radical scavenging activity. For further investigation, the content of flavonoids and phenolic compounds was also determined in the extracts (table 2).

As shown in table 2, *S. vermiculata* with the highest amount of phenolic contents showed the most potent effect on DPPH radical inhibition. Though, such correlation cannot be accurately attributed to other plants, but there is a significant correlation coefficient (R) between phenolic content and the data of DPPH scavenging activity of the plant extracts ($IC_{50}$) (R= - 0.82); such correlation with flavonoid content was - 0.62 which is moderate; for example *S. aegyptiaca* with the highest content of flavonoids, did not show the best activity in DPPH inhibition. Phenolic compounds are likely to contribute to the DPPH inhibitory activity of the plant extracts. Detailed examination of phenolics and other metabolites in the plant extracts is required for the comprehensive assessment of individual compounds showing DPPH scavenging activity (to evaluate probable synergistic effect of the plant metabolites on DPPH inhibition).

Cellular damage, due to lipid peroxidation, causes serious derangements, such as ischemia-reperfusion injury, coronary arteriosclerosis, diabetes mellitus and neurodegenerative diseases. This is also associated with aging and

carcinogenesis [31]. Each compound and extract which can inhibit lipid peroxidation, can be considered as a good antioxidant. In the ferric thiocyanate method, similar to the results of DPPH assay, *S. vermiculata* showed the highest inhibitory activity against lipid peroxidation (table 1 and figure 1). The correlation coefficient between the results of these plant extracts in lipid peroxidation inhibition test (on the seventh day of incubation) and phenolic and flavonoid contents were 0.62 and 0.09, respectively, which showed a medium correlation with phenolic compounds and weak correlation with flavonoids. Though flavonoids and phenolics can inhibit lipid peroxidation, but there are some reports that other compounds like terpenoids, xanthones and coumarins have more potent activity in lipid peroxidation inhibition [32,33]. It has been proven incidentally that tocopherols and carotenoids protect lipid membranes from oxidative stress, and prevent the propagation of lipid peroxidation [34]. These findings show the important role of lipophylicity of compounds in such effect [35].

Although, the three other plants showed lipid peroxidation inhibition, their activities were lower than *S. vermiculata*, which might be attributed to the fact that the carotenoid and polyphenolic content of halophytes are more than nonhalophytics [36].

The oxidation of ferrous to ferric ions using hydrogen peroxide forms a hydroxyl radical (Fenton reaction) [37]. The formation of free radicals may be inhibited by increasing the reduction of $fe^{3+}$ to $fe^{2+}$ or by sequestering metal ions through chelation reactions. Each compound can stop Fenton reaction, resulting in the inhibition of hydroxyl radical production and can be considered as a good antioxidant.

Figure 3 shows the reductive capabilities of the plant extracts. All tested concentrations showed higher absorbance compared to the control (0.210 ± 0.098), and the reducing power of all extracts increased with increase in the concentration. None of the extracts showed significant activity in stopping the change of $fe^{2+}$ to $fe^{3+}$ in comparison to BHT. Like the two previous tests, *S. vermiculata* showed better result (table 2). It has been reported that the presence of flavonoids and phenolic compounds in the plant extracts appear to function as good electron and hydrogen-atom donors and therefore could be considered as a good source of reductants [38,39]. There was no meaningful relationship between the reducing power of the extracts and phenolic and flavonoid contents in this research; thus, further studies are required to evaluate the probable antagonist effects of the plants metabolites in low reducing power of the extracts.

In iron chelating activity test, *C. obliqua* with 61.09±2.12% inhibition in the concentration of 1000 µg/mL showed the best result and the interesting point is that the phenolic content found in this plant was the least and *S. vermiculata* with the highest amount of flavonoids and phenolics showed the lowest result. It has been proposed that for a compound to be a chelator, the molecule must contain two or more atoms (such as oxygen or nitrogen) capable of interacting with a metal [40]. Some researchers have proven that compounds with structures containing two or more of the following functional groups: –OH, –SH, –COOH, $–PO_3H_2$, C=O, $–NR_2$, –S–, and –O–, can show metal chelation activity [41,42]. Several dolabellane diterpenoids have been isolated from *C. obliqua* [43]. These compounds with several hydroxyl and acetyl groups could be responsible for the chelating activity of the extract.

Heat stress and extreme salinity are known to trigger ROS production and increase the concentration of antioxidants in plants [44]. The acceptable antioxidant activity of *S. vermiclata* as a halophyte plant, could justify its medicinal properties. Therefore, further analysis of this plant and other halophytics of Bandarabbas is suggested.

**Declaration of interest**

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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