Phytochemical screening of *Allium Tuberosum Rottler. ex Spreng* as food spice

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**Abstract.** In Vietnam, *Allium Tuberosum Rottler. ex Spreng* (ATRES) was used in everyday meals as a seasoning, and as a common remedy for the prevention of diarrhea, asthma, and so on. In this study, We evaluated the phytochemical composition, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity of ATRES, which was harvested in Vietnam. The findings indicate that Vietnamese ATRES had a varied range of pharmacologically active compounds, including alkaloid, flavonoid, terpenoid, tannin, coumarin, saponin, and reduced sugar. Folin-Ciocalteu calculates the TPC and the TFC is derived using the aluminum chloride method. The TPC and TFC of ethanol extract in ATRES achieved 46.15 ± 1.26 mgGAE/g DW and 31.10 ± 1.12 mg QE/g DW, respectively. Taken together, we found that the antioxidant capacity in DPPH forms (2, 2-diphenyl-1-picyrlylhydrazyl) was assessed by spectrophotometric method and obtained an IC\(_{50}\) result of 97.46 µg/ml. These results indicate that Vietnamese ATRES is effective in increasing food use with its antioxidant and valuable activity.

**1. Introduction**

Nowadays, natural herbs have gained substantial media interest because of their benefits [1]. The use of plant-based antioxidants such as polyphenols, flavonoids and ascorbic acid is becoming increasingly popular and becoming an integral part of daily meals [2]. People have used many kinds of herbs to cure diseases. In the past, the alternative to traditional medicine has been replaced by a modern control system of medicine. Therefore, the current drugs are largely synthesized. Nevertheless, in recent years, traditional therapies are getting a lot of attention because the synthesis of drugs is reasonable price and their unwanted effects. The phytochemicals found in different plant species have been shown to be effective against common human diseases [3-8]. From the beginning of human civilisation, the *Allium* family has a special place in our society and literature as a perennial edible plant and herbal medicine. The *Allium* genus of the *Amaryllidaceae* family is reported to have about 750 species of plants, including *Allium chinense* (Kago), *Allium cepa* (onion), *Allium sativum* (garlic), and so on. Interestingly, *Allium Tuberosum Rottler. ex Spreng* (ATRES) has many valuable biological properties [9]. ATRES is a perennial plant grown in various countries in Asia and its aerial part is one of the popular edible green vegetables for the residents. Its leaves release strong odors, which is similar to the smell of garlic and other *Allium* plants [10], growing well in low light environments and do not require too much care [11]. Although there are different research of the globe for the *Allium Tuberosum Rottler ex Spreng*, they have not been researched in detail of ATRES in Vietnam. Therefore, phytochemical screening for ATRES should be carried out to be a source for contrast with similar ATRES produced in various world regions.
In traditional medicine, ATRES is commonly used to treat stomach aches, diarrhea, colds, asthma and is a typical spice used to increase libido [12], [13]. In India, this plant is also used to reduce cholesterol as well as tonics for hair [11]. The previous study reports that ferulic acid from ATRES can be used as a choline acetyltransferase activator to synthesize acetylcholine neurotransmitter [13]. The critical feature of ATRES is the inhibition of Reactive Oxygen Species (ROS). The control of the degenerative or pathological process of various serious diseases in the human body thanks to the ATRES extract’s ability to scavenge free radicals, including cancer, aging, or cardiovascular [14-15]. Moreover, due to its characteristic and robust taste, Allium Tuberosum Rottler Spreng in Vietnam is popularly used in traditional meals. Through day-to-day studies, they conducted a test for the presence of secondary and parallel plant chemical compounds that assessed the antioxidant potential of phenolic compounds present in AT extracts. Thereby providing additional scientific data on the biological activity of this plant locally to the general data table. In this study, ATRES extracts are used to assess the TPC and TFC and to test antioxidant function in various DPPH and ABTS models.

2. Materials and method

2.1. Chemicals and plant materials

Figure 1 illustrates the procedure of Allium ramosum L. extraction. 10g of dry leaf powder is extracted with ethanolic and water solvents for 1 hour at 70°C. The principle is to extract the mixture of substances in the material according to the increasing polarization. Then, the extract is filtered through a filter paper. The filtrate is evaporated in a vacuum in a rotary pad at 40°C and then dissolved in the corresponding solvent to carry out further evaluation tests. Extraction was performed in a sealed container under 40°C until use.

![Figure 1. Extraction scheme of the leaf of Allium ramosum L. in ethanolic and in aqueous media](image-url)
2.2. Phytochemical screening
The crude methanolic extracts were determined for the presence of flavonoid, alkaloid, terpenoid tannin, anthraquinone, anthraquinone, coumarin, saponin and reducing sugar. While the positive results are expressed as plus (+) for the presence, the minus (-) for the lack of phytochemicals. Alkaloid test with Mayer, Bouchardat, Dragendorff test. Flavonoid test with H$_2$SO$_4$ and Wilstatter test. Testing anthraquinones and tannins with color transfer reaction. Coumarin test with fluorescence reaction. Terpenoid test with Liebermann-Burchard and Salkowski test. Saponin test with foam test. Sugar reduction test with precipitation test [16].

2.3. Total Phenolic Contents (TPC)
The sample is proceeded with the extraction phase to obtain the appropriate concentration. Afterwards, 0.5ml of diluted sample solution is drawn into a test tube, then 2.5ml of 10% Folin-Ciocalteu solution was added. Homogenized using a Vortex machine for 5 minutes in the dark. Next, 2ml of 7.5% Na$_2$CO$_3$ solution was added and shaked well in the dark for 1 hour. Finally, measure the optical absorbance at 756 nm on the UV-Vis spectrophotometer. Using gallic acid as a standard and expressing poly phenol content in micrograms of gallic acid equivalent per 1 mg of extract (µgGAE/mg extract) [17].

2.4. Total flavonoids contents (TFC)
The method was conducted based on the method of N.Y.T. Tran (2020) improved [18]. First, dilute the extract obtained in the extraction test to the appropriate concentration, 0.5 ml of the diluted sample solution was then added to a test tube with 0.1 ml of 10% AlCl$_3$ solution. Next, 0.1ml CH$_3$COOK 1M solution and 4.3ml distilled water were mixed and shaked. Next, the solution was put in the dark for 30 minutes. Finally, measure optical absorption at a wavelength of 415nm on the UV-Vis spectrophotometer. Quercetin was standard for use. The TFC is expressed in micrograms of quercetin equivalent in 1 mg of extract (µgQE/mg extract).

2.5. Antioxidant activity
Free radical removal method DPPH (1,1-diphenyl-2-picrylhydrazyl). Dilute the extract obtained from the previous extraction to a reasonable concentration then draw a high 0.5ml of the diluted sample into a test tube. Control sample instead of ethanol extract (99.5%). Then, add a tube of 1.5 ml DPPH solution (OD517 nm = 1.1 ± 0.02) to a test tube and leave in the dark for 30 minutes. Measure optical absorbance at 517nm on UV-Vis spectrophotometer. Vitamin C (ascorbic acid) was applied as the reference standard [16]. DPPH (IC%) is determined by the following formula:

\[
\text{IC (\%)} = \frac{\text{Abs}_C - \text{Abs}_T}{\text{Abs}_C} \times 100
\]

Inside:
Abs$_C$: Optical absorbance of the control sample
Abs$_T$: Optical absorbance of the test sample
The result is reported based on the IC₅₀ value, which is the concentration at which the sample removes 50% of DPPH free radicals.

2.6 Free radical removal method ABTS (2,2'-azino-bis)
The free radical solution ABTS was prepared by adding 10 ml of ABTS solution of 7.4 mM into 10 ml of K₂S₂O₈ solution of concentration of 2.6 mM and incubating in the dark for 24 h, then diluting with ethanol and then adjusting the absorbance of the solution at a wavelength of 734 nm to 1.1 ± 0.02. Dilute the extract to the appropriate concentration, collecting 0.5 ml of diluted sample extract into a test tube. Control sample using ethanol (99.5%). Afterwards, add 1.5ml ABTS solution (OD517 nm = 1.1 ± 0.02) to a test tube and place in the dark for 30 minutes. Measure optical absorbance at 734nm on UV-Vis spectrophotometer. Vitamin C (ascorbic acid) is used as the reference standard [19]. The free radical scavenging activity of ABTS (IC%) is determined by the following formula:

$$IC(\%) = \frac{Abs_C - Abs_T}{Abs_C} \times 100$$

Inside:
Absₜ: Optical absorbance of the control sample
Absₜ: Optical absorbance of the sample

The result is reported based on the IC₅₀ value, which is the concentration at which the sample removes 50% of the ABTS free radicals.

2.7. Statistical Analyses
The results were evaluated by one-way ANOVA accompanied by using Fisher's Least Significant Difference (LSD) and Statgraphics Centurion XV version 15.0. Differences for both measurements were considered statistically relevant at P<0.05.

3. Results and discussion
Phytochemical extract analysis showed the existence of alkaloids, coumarins, tannins, flavonoids, sugars, phenols, saponins, terpenoids. Table 1 displays the findings of the Allium ramosum L phytochemical constituents in water and in ethanol 96%. Assessment of chemical components of Allium ramosum L indicated the presence of terpenoids, alkaloids, tannins, saponins, flavonoids, coumarins and reducing sugar.

This results in these solvents being effective in isolating biologically active compounds thanks to their high polarity. These chemical compounds have been played an important role in medicinal fields. Typically, alkaloids are reported to promote anti-inflammatory, antimalarial, antibacterial, and so on [16], [20]. Besides, tannins are widely known for their anti-cancer, antimicrobial and antiviral activities [21], [22]. Moreover, flavonoids are detected in both extracts. Flavonoids belong to the category of polyphenolic compounds and are widely recognised for their health-promoting properties, including antioxidants, anti-allergy, anti-inflammatory, antibacterial and anti-cancer [23]. They are often applied in medicines due to their famous biological activities.
Table 1. Phytochemical screening of the extracts of *Allium Tuberosum Rottler. ex Spreng*

| Chemical composition group | Ethanolic extract | Distilled water extract | Ethanolic react picture | Distilled water react picture |
|---------------------------|-------------------|------------------------|------------------------|-----------------------------|
| Alkaloid                  | +                 | +                      |                        |                             |
| Tannin                    | -                 | +                      | -                      |                             |
| Anthraquinon              | -                 | -                      | -                      |                             |
| Flavonoid                 | +                 | +                      |                        |                             |
| Terpenoid                 | +                 | +                      |                        |                             |
| Coumarin                  | +                 | -                      | +                      | -                           |
| Saponin                   | -                 | +                      | -                      |                             |
| Reducing sugar            | +                 | +                      |                        |                             |

According to the previously reported results, the higher TPC and TFC, the greater the antioxidant activity, the correlation is thought to be linear. Table 2 illustrates TPC, TFC, and antioxidant activities of ATRES extract. IC$_{50}$ values, which is the concentration at which the sample removes 50% of the ABTS free radicals. The results illustrated that TPC of ethanolic extract (46.15 ± 1.26 mg GAE/g) was higher than that of water extraction (32.01 ± 1.02 mg GAE/g). Our results are higher than reported by other researchers [24]. However, comparing the results of studies is not entirely appropriate to estimate the effect due to differences in geographic location, plant varieties, growing seasons and agricultural farming processes. In addition, the extraction technique and the type of solvent used is also a factor affecting the total polyphenol content and research results. Phenolic compounds are the most abundant plant chemicals of all plants and are rated as an important natural antioxidant. The biological activity carried out by polyphenols has an effect on preventing cardiovascular disease, cancer, and neurodegeneration, which has been shown to be due to their high antioxidant capacity [25]. Among polyphenols, the most studied subclass is flavonoids because of its small correlation with the antioxidant activity of cells. Evaluation results of flavonoid content of ATRES showed the effectiveness of ethanolic solvent than water in extracting with content of 31.10 ± 1.12 mg GAE/g and 16.74 ± 0.47 mg GAE/g, respectively. The quantitative results of the high phenolic and flavonoid content in ethanolic extract and the evaluation of their antioxidant activity once again confirmed their linear correlation.
DPPH is a free radical compound commonly used for checking different materials for free radical scavenging capability. Besides, ABTS assay relies on the capacity of the antioxidant compound to scavenge radical ABTS. The ability of a substance to capture free radicals is assessed by the IC\textsubscript{50} index, the concentration of a test substance that is capable of neutralizing 50% of free radicals at a given concentration. The smaller the IC\textsubscript{50} value of a substance, the higher the free radical capture activity is and vice versa. In this present, the IC\textsubscript{50} values in DPPH and ABTS free radical scans of ethanolic extract reached 97.46 µg/mL and 83.13 µg/mL, respectively. Its higher than water-based extract (141.68 µg/mL in DPPH test and 104.20 µg/mL in ABTS test) (Figure 3, 4). The DPPH free radical activity of ethanolic extract is 1.45 times higher than that extracted by water solvent and similar to ABTS free radical activity of 1.25 times. This shows that solvents play an essential role in preserving the biological value of the extraction of natural compounds.

Table 2. TPC, TFC and antioxidant activities (IC\textsubscript{50} values) of AT extract

| Sample       | Total polyphenol content (mg GAE/g) | Total flavonoid content (mg QE/g) | IC\textsubscript{50} value (µg/mL) | DPPH | ABTS |
|--------------|-------------------------------------|----------------------------------|-----------------------------------|------|------|
| Ethanolic extract | 46.15 ± 1.26                        | 31.10 ± 1.12                     | 97.46                             |      |      |
| Aqueous extract | 32.01 ± 1.02                        | 16.74 ± 0.47                     | 141.68                            |      |      |
| Ascorbic acid  | -                                   | -                                | 3.05                              |      |      |

Numbers in a column were statistically indifferent (p < 0.05)

**Figure 3.** DPPH free radical scavenging activity from *Allium Tuberosum Rottler. ex Spreng*; a) ethanolic extract, b) water extract and c) ascorbic acid.
Figure 4. ABTS free radical scavenging activity from *Allium Tuberosum Rottler. ex Spreng*; a) Ethanolic extract, b) Water extract and c) Ascorbic acid.

4. Conclusion

This research aimed to determine the TPC, TFC, and antioxidant activity of *Allium Tuberosum Rottler. ex Spreng*. Antioxidant activity was performed via ABTS and DPPH. Separately the phytochemical was processed with purified water and ethanol. The results concluded that *Allium Tuberosum Rottler. ex Spreng* have various chemical compositions, including alkaloids, tannins, flavonoids, terpenoids, coumarins, saponins and reducing sugar. These chemical components contribute to antioxidant and biological activity. The total phenolic and flavonoid content of ethanol extract in ATRES achieved 46.15±1.26 mgGAE/g DW and 31.10 ±1.12 mg QE/g DW, respectively. The study results also showed that the use of ethanolic solvent for phenolic content as well as higher resistance to oxidation than conventional extract with water solvent. Moreover, the extract obtained by aqueous exhibited the highest DPPH (141.68AAE/100g DW), ABTS (104.20mg AAE/100g DW) radical scavenging activity. This demonstrated the capacity of aqueous extracts to absorb free radicals is smaller than ethanolic extracts. Therefore, These results indicate that *Allium Tuberosum Rottler. ex Spreng* can be applied as a source of antioxidants and needs further research to further assess its other biological activities.

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