MALAYSIAN JOURNAL OF SCIENCE
MJS
ISSN 1394-3065

Regular Issue

Malaysian Journal of Science 43 (2): 30-37 (June 2024) https://mjs.um.edu.my

PHYTOCHEMICAL AND ANTIOXIDANT ASSESSMENT OF *ALLIUM HYPSISTUM*, *ALLIUM PRZEWALSKIANUM* AND *ALLIUM WALLICHII*

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Abstract: High altitude plants are tremendously used mostly as food and traditional medicine for their role on prevention and treatment of several diseases. Thus, this study focused on comprehensive analysis of the phytoconstituents and the antioxidant activities of three plants; *Allium hypsistum*, *Allium przewalskianum,* and *Allium wallichii.* Upon analysis, the phytoconstituents such as alkaloids, terpenoids, steroidal compounds, glycosides, carbohydrates and aminoacids were identified in ethanolic, n-hexane and aqueous extracts of the plant whereas saponins and quinones were not detected at all. Other phytoconstituents like flavonoids, phenolic compound, and tannins were found in ethanolic and aqueous extracts but not in the n-hexane extract. The antioxidant activities of the plant extracts were evaluated by total phenolic content (TPC) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. TPC was assayed by Folin-Ciocalteu reagent method and significant amount of phenolic content were found in all plant extracts; however, the highest TPC was estimated in ethanolic extract of *A. hypsistum* i.e. 172±6.53 mg GAE/100g, the least TPC was found in n-hexane extract of *A. wallichii* (12±5.72 mg GAE/100g). Similarly, DPPH assay showed that the highest DPPH free radical scavenging activity in ethanol extract of *A. hypsistum* with 59.44±1.20 % RSA while n-hexane extract of *A. hypsistum* showed the lowest DPPH activity with 21.29±0.64 % RSA. For reference, DPPH activity of ascorbic acid was estimated and found 75.11±0.31 % RSA. Therefore, among the plant extracts, the antioxidant activities were found to be higher in *A. hypsistum* followed by *A. przewalskianum* and *A. wallichii.* Furthermore, relationship between TPC and DPPH activity was established and found statistically to be non-significant but having weak to strong correlation. From this study, such findings may help to know chemical constituents and medicinal values of *Allium* species which may lead to develop new phytomedicines and use of these plants in herbal therapy.

Keywords: *Allium hypsistum, Allium przewalskianum, Allium wallichii, phytochemicals, antioxidant activities.*

1. Introduction

Plants are undoubtedly a major source for several therapeutic entities in the medical field. Around 80% of people prefer traditional medicine which has components extracted from medicinal plants. However, the efficacy, potency, and safety of such plants must be properly analyzed to promote their rationale use in the community (Roughani and Miri\ 2019). Plants produce various phytochemicals by primary or secondary metabolism and which in general play a key role in their growth, development, and defense against external factors. Secondary metabolites are usually bioactive molecules that comprise tannins, alkaloids, terpenoids, phenol compound, steroid compound and flavonoids. These bioactive components can elicit biological and therapeutic functions on the human body (Phan, Netzel et al. 2019).

Antioxidant properties of plants are mainly exerted by secondary metabolites like phenolics and flavonoids which are potent free radical scavengers which neutralizes free radicals and thereby inhibit the oxidative stress in the cells (Jalalvand, Zhaleh et al. 2019). Oxidative damage or stress is occurred due to an imbalance between antioxidant defense mechanism and generation of free radicals. As a result, an antioxidant decreases the harm that free radicals and reactive oxygen species do to cells (Chen, Krug et al. 2021, Chernukha, Fedulova et al. 2021). These damages include several degenerative disorders such as neuro-degenerative diseases, cancers, cardiovascular disorders, inflammation. Phenolic compounds are known to normalize oxidative stress in biological cells because of their ability to donate proton or electrons which then stabilize free radicals, delocalize the unpaired electrons and chelate metal ions. Mainly the phenol compounds found in plants are flavonoids, phenolic acids, tannins, xanthones and tocopherols (Chanda and Dave 2009, D'Sousa' Costa, Ribeiro et al. 2015). Thus, screening of the total phenolic content of the extracts may reveal their overall antioxidant property. Similarly, another reliable method for screening the antioxidant assay is using DPPH, as it is a free radical that is typically used to assess the antioxidant molecule's capacity to scavenge free radicals, as measured by the percentage of RSA.

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Received: December 31, 2022
Accepted: April 8, 2023
Published: June 30, 2024

DOI:https//doi.org/10.22452/mjs.vol43no2.4
Malaysian Journal of Science 43(2): 30-37 (June 2024)

Allium species are commonly used plants in households as spices and in traditional medicine as its component. Extracts of some *Allium* species have shown anti-inflammatory, antimicrobial, anticancer, antidiabetic, and also anti-HIV activities (Gross 2021). In the present study, commonly found high altitude *Allium* species such as *A. hypsistum*, *A. przewalskianum* and *A. wallichii* are chosen for phytochemical screening and *in vitro* antioxidant activity analysis. *A. hypsistum* is a popular plant that is frequently used as spice and herbal remedy in many rural Nepalese and Indian towns and villages. As a spice, it is used to flavor lentils, vegetables, salads, and pickles whereas medical uses of *A. hypsistum* include; high altitude sickness, diarrhea, stomach pain, flu, and common cold. Moreover, these are also used to cure lung and liver diseases of human as well as livestock. It is distinguished from other species by its purple color, reticulated fibrous bulb coatings, 4 to 6 narrow linear leaves, short pedicels, slightly dentate tepals, and simply combined filaments. *A. przewalskianum* is also consumed as medicinal plants to treat high altitude sickness, common cold, and diarrhea. It is bright red, regularly reticulate bulb coat, narrow leaves with tiny purple stamens. The three inner subulate filaments are present, whereas the three outer subulate filaments are broad at the base and acute on either side at the top. *A. wallichii* is popularly used in vegetables as spices and for the treatment of coughs, colds, altitude sickness, and even tuberculosis. It is a perennial plant and distributed in higher altitudes ranging from 2500 meter to 4500 meter (Bhandari, Muhammad et al. 2017).

To compute more therapeutical values of high altitude *Allium* species; *A. hypsistum*, *A. przewalskianum* and *A. wallichii*, this study emphasizes to screen their phytoconstituent and evaluate their *in-vitro* antioxidant potentials in ethanol, n-hexane and aqueous extracts.

2. Material and Methods

Plant Specimen Collection and Storage

A. hypsistum and *A. przewalskianum* were collected from Marpha, Mustang (28.8151°N, 83.6455°E), and *A. wallichii* was collected from Palungtar, Gorkha (28.05145°N, 84.4876°E) of Nepal. The plants were taxonomically identified with flora of Nepal provided by the Department of Plant Resources, National Herbarium and Plant Laboratories, Govt. of Nepal and deposited the voucher specimens with code as mentioned in Table 1. Aerial parts of the plants were taken and cleaned with refined water. Materials were first cut off into little pieces and shade-dried in open space for fortnight at room temperature (28°C to 30°C) (Phan, Netzel et al. 2019).

A. hypsistum

A. przewalskanum

A. wallichii

Figure 1. Selected *Allium* species under the study

DOI:https//doi.org/10.22452/mjs.vol43no2.4
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Extraction

The dried materials of *A. hypsistum, A. przewalskianum* and *A. wallichii* were ground to get a powder which was sieved with a mesh screen. After that, soxhlet extraction of the powdered materials with ethanol, n-hexane, and water was separately operated to get crude extract (Seino, Yamazaki et al. 2020). Then, the crude extract was fully dried and free from solvents by evaporating at 40 ^{0}C and kept in separate containers with proper labels. They were stored in the fridge at 4 ^{0}C until further use (Jeba Malar, Antonyswamy et al. 2020). Total percentage (%) extract yields were calculated for each extracts.

$$\% \text{ yield (w/w)} = x = \frac{\text{Dry weight of extract (g)}}{\text{Dry weight of a plant (g)}} \text{ X } 100$$

Phytochemical Testing

For phytochemical testing, the extracts were first prepared as 10 % extract solution by using 0.1 % dimethyl sulphoxide (DMSO) reagent. Then, following the standard protocols, occurrence of bioactive phytoconstituent in the extracts was analyzed (Bhandari, Muhammad et al. 2017, Batiha, Beshbishy et al. 2020).

- *Test for alkaloid:* With 2 ml of 1% HCl, 5 ml of the extract was dissolved, and then it was gently warmed. After that, the mixture was treated with Wagner's reagents (iodine in potassium iodide). Finally, presence of alkaloid could be confirmed by appearance of reddish-brown precipitation.
- *Test for Terpenoid:* 5 ml of the extract were diluted with 2 ml of chloroform, and then evaporated to remove the water. To create a grayish/reddish colored precipitate as proof of terpenoid content, 2 ml of concentrated H_2SO_4 was added to the mixture and heated for 2 minutes.
- *Tests for Flavanoid:* 5 mg of dry extract with 10 ml of ethyl acetate was heated on water bath for three minutes. After filtering, 4 ml of the filtrate was mixed with 1ml of diluted ammonia solution. A golden coloring indicated the access of flavonoids.
- *Tests for Steroid:* Extract was combined with 2 ml of chloroform and adding concentrated H_2SO_4 gently by sidewise. In the lower chloroform layer, a red color was appeared that confirmed the presence of steroid.
- *Tests for Phenol:* Extract was diluted with 2 ml solution of $FeCl_3$ (2 %). The presence of phenol was identified by a blue-green or black coloring solution.
- *Tests for Coumarin:* 3 ml of a 10% aqueous solution of NaOH was added to 5 ml of extract. The presence of coumarin was indicated by production of yellow color.
- *Tests for Tannin:* 5 mg dry extract was added to 20ml of water and boiled. A few drops of 0.1 % $FeCl_3$ were added to the filtrate after filtering. Brownish green or blue-black coloration revealed tannins were present by their appearance.
- *Tests for Glycoside:* 5 ml of the extract was mixed with 2 ml of each chloroform and acetic acid. Ice was used to cool the mixture and concentrated H_2SO_4 was added cautiously. It resulted in a color change from violet to blue to green, signifying the existence of glycone, a part of glycoside.
- *Tests for Carbohydrate:* Two drops of Molisch's reagent were added to 5 ml of extracts and mixed well. A few drops of conc. H_2SO_4 were placed along the test tube's sidewall. Development of a violet ring indicated the presence of sugar.
- *Tests for Aminoacid:* A few drops of ninhydrin reagent were added to 5 ml of extract. For a short while, the mixture was gently heated. The appearance of purple color indicated the presence of amino acids.
- *Tests for Saponin:* 5 mg of extract was directly diluted in 5 ml of water and shaken vigorously. Then, formation of frothing was indicator for presence of saponins.
- *Tests for Quinone:* Equal volumes of the extract sample and concentrated H_2SO_4 were mixed and observed for red color formation which indicated the presence of quinone.

Antioxidant Analysis

Assay of Total Phenolic Content

Total phenolic content (TPC) in the plant extracts was estimated by Folin-Ciocalteu (FC) reagent assay (Krishnaiah, Sarbatly et al. 2011, Ravipati, Zhang et al. 2012). Initially, 50 mg of plant extract was dissolved in 50 ml DMSO (10% v/v) and centrifuged at 2000 rpm for 5 minutes. Then, 0.5 ml of 50 % FC reagent was added to 0.5 ml of supernatant of each extracts which was taken in separate, labeled test tubes. The tubes were then allowed to stand for 15 min at room temperature. Immediately, 2.5 ml of 20% sodium carbonates were added, and the tubes were left for 30 minutes in a dark place. Optical density of all test solutions was recorded at 760nm against a reagent blank in a spectrophotometer (UV-1800 Shimadzu Spectrophotometer). TPC of all tests solution of plant extracts was calculated as mg gallic acid equivalent (mg GAE/100g) with ± SEM and data was obtained by using standard calibration curve of gallic acid with the equation y= 0.025x + 0.006 (R^2=0.983). Gallic acid's standard calibration curve was created using successive concentrations of 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml, and 10 µg/ml, and a graph between absorbance and concentration (µg/ml) was drawn (Iqbal, Salim et al. 2015, Szerlauth, Muráth et al. 2019).

Assay of DPPH Scavenging Activity

The stable free radical DPPH (2,2-diphenyl -1-picrylhydrazyl) interacts with molecules that can give off a hydrogen atom. It has been extensively employed in the antioxidant assay of most compounds. This approach relies on DPHH-scavenging by adding a radical species or an antioxidant that decolorizes the purple-colored methanol solution of DPPH. Antioxidant activities of plant extracts can be assayed by the following standard protocol (Krishnaiah, Sarbatly et al. 2011, Bhandari, Muhammad et al. 2017). DPPH reagent was prepared by dissolving 10mg DPPH (2, 2-diphenyl -1-picrylhydrazyl radical) in 100ml methanol i.e. 100 µg/ml. For DPHH control, 1 ml DPPH reagent was added in 4ml methanol. Simultaneously, test samples were prepared as; 50 mg

DOI:https//doi.org/10.22452/mjs.vol43no2.4
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of extracts were mixed in 50 ml methanol and further centrifuged at 2000 rpm for five minutes. After that, 2ml of supernatant of each extracts was taken in test tubes. 1ml of DPHH reagent was then added to each tube and solutions were kept in dark for exactly 30 minutes. Finally, absorbance of all test solutions and DPPH control were recorded at 517nm against reagent blank with a spectrophotometer (UV-1800 Shimadzu Spectrophotometer). Finally, DPHH free radical scavenging assay was calculated as %RSA (Radical scavenging activity).

% RSA= (Control absorbance – Extracts absorbance)/ Control absorbance x 100

Statistics

The obtained data were analyzed statistically in Microsoft Excel (windows 10) and SPSS (version 25). The Pearson's correlation (r) was considered to establish a relationship between TPC and DPPH activity of selected plants statistically.

3. Results and Discussion

Plant Extract Yield

The yield percentage of plant extracts varied, as shown in Table 1, with aqueous extract of *A. hypsistum* (23.70%) having the greatest yield, followed by extracts of *A. wallichii* (20.77%) and *A. przewalskianum* (20.43%). While the lowest percentage of yield was found in n-hexane extract of *A. wallichii* (7.2 %).

Table 1. Plants under study along with their voucher code, botanical name, common name, and extract yield %.

S.N.	Voucher code	Botanical name	Common name	Extracts	Percentage yield (%)
1	SMS-001	*A. hypsistum*	Jimbu, jamboo	Ethanol	15.03
				n-hexane	9.78
				Aqueous	23.70
2	SMS-002	*A. przewalskianum*	Lamboo, doona	Ethanol	16.87
				n-hexane	9.30
				Aqueous	20.43
3	SMS-003	*A. wallichii*	Banlasun, Himalayan garlic	Ethanol	12.73
				n-hexane	7.23
				Aqueous	20.77

Phytochemical Screening

Ethanol, n-hexane, and aqueous extracts of each plant *A. hypsistum, A. przewalskianum* and *A. wallichii* were analyzed for the presence of twelve phytoconstituents as shown in Table 2. The alkaloids, terpenoids, steroids, glycosides, carbohydrates, and aminoacids were detected in all three plants whereas coumarin was detected only in the extracts of *A. hypsistum*. Moreover, n-hexane extraction of the plants did not contain flavanoids, phenol, or tannins as one possible explanation being they may be poorly soluble in n-hexane. Overall, ethanol and aqueous extraction of all three selected plants showed a relatively higher chance of phytochemical screening than n-hexane extracts.

The presence of phytoconstituents in a plant is known to reveal medicinal as well as biological potentials. Many previous studies have described that the phenolic compounds present in the plants show remarkable antioxidant properties which are involved in anti-inflammation, cardiovascular protection, anti-aging, anti-carcinogen, and reduces apoptosis. Similarly, alkaloids exhibit analgesic, antispasmodic and antimicrobial properties. Likewise, glycosides are known to lower blood pressure in case of hypertension (Shrestha, Adhikari et al. 2015, B, D et al. 2016). A review by Lekshmi *et al* suggested that carbohydrates, flavonoids, saponins, steroids, and phenols are major constituents of the *Allium* species (Lekshmi, S et al. 2015) Nonetheless, our present study showed the absence of saponins and quinines in the selected plants. A similar report was made in a study by Bhandari *et al* (Bhandari, Muhammad et al. 2017), where they investigated the phytochemical composition of ethanol extracts of *A wallichii* and found the presence of flavonoids, glycosides, steroids, terpenoids but reported the absence of saponins, alkaloids, and tannins. Evidence from a previous study suggests that phytochemicals like quercetine and antioxidant activity were shown higher in fresh plant than the dried plant (Fredotović, Puizina et al. 2021, Yuasa, Kawabeta et al. 2021). Hence we can assume that the phytochemical screening might be affected by the types of solvent used and/or method chosen for plant extraction.

DOI:https//doi.org/10.22452/mjs.vol43no2.4
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Table 2 Result of phytochemical screening of selected plants

Phytochemicals	*A. hypsistum*			*A. przewalskianum*			*B. wallichii*		
	ethanol extract	n-hexane extract	aqueous extract	ethanol extract	n-hexane extract	aqueous extract	ethanol extract	n-hexane extract	aqueous extract
Alkaloid	+	+	+	+	+	+	+	+	+
Terpenoid	+	+	+	+	+	+	+	+	+
Flavanoid	+	-	+	+	-	+	+	-	+
Steroid	+	+	+	+	+	+	+	+	+
Phenol	+	-	+	+	-	+	+	-	+
Coumarin	+	+	+	-	-	-	-	-	-
Tannin	+	-	+	+	-	+	+	-	+
Glycoside	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+
Aminoacid	+	+	+	+	+	+	+	+	+
Saponin	-	-	-	-	-	-	-	-	-
Quinone	-	-	-	-	-	-	-	-	-

(Distilled water was as taken as a negative control. The symbols +; present and -; absent)

TPC and DPPH Free Radical Scavenging Assay

Total phenolics content (TPC) and DPPH free radical scavenging assay in selected plants were shown in Figure 2 and Figure 3. It is evident that *A. hypsistum* had higher TPC in each of the solvent extracts compared to that of *A. przewalskianum* and *A. wallichii.* More precisely, the highest phenolics content was observed in ethanol extraction of *A. hypsistum* (172±6.53 mg GAE/100g) and moderate levels of phenolic content were estimated in ethanol extraction of *A. przewalskianum* (122±0.82 mg GAE/100g), followed by *A. wallichii* (102±0.82 mg GAE/100g). Among the extracts, remarkable phenolics content was found in ethanol extracts followed by water and n-hexane extracts of the studied plants. The order of aqueous extracts, the highest value of phenolics content was found in *A. hypsistum* (90±10.61 mg GAE/100g), followed by *A. przewalskianum* (82±13.88 mg GAE/100g) and *A. wallichii* (72±1.63 mg GAE/100g). In contrast, n-hexane extracts showed the phenolics content more in *A przewalskianum* (44±1.63 mg GAE/100g), followed by *A hypsistum* (42±5.72 mg GAE/100g) and lesser in *A. wallichii* (12±5.72 mg GAE/100g). Moreover, Figure 3 shows a higher DPHH activity in ethanol extract of *A. hypsistum* (59.44±1.20 % RSA), followed by *A. wallichii* (55.20±0.53 % RSA) and *A. przewalskianum* (39.53±0.43 % RSA). In the case of n-hexane extracts, DPHH activity was found higher in *A. przewalskianum* (39.44±0.37 % RSA) than *A. wallichii* (35.02±4.31 % RSA) and *A. hypsistum* (21.29±0.64 % RSA). Subsequently, DPHH activity was found to be higher in aqueous extract of *A. hypsistum* (54.65±0.85 % RSA) than *A. wallichii* (48.11±0.05 % RSA) and *A. przewalskianum* (46.26±0.37 % RSA). While comparing with DPHH activity of reference ascorbic (75.11±0.31 % RSA), ethanol extract of *A. hypsistum* (59.44±1.20 % RSA) was found even lesser but found to be higher than that of *A. wallichii* (55.20±0.53 % RSA) and aqueous extract of *A. hypsistum* (54.65±0.85 % RSA).

The outcome presented in Figure 2 and Figure 3 from the prsesent study are in agreement with fact from a previous study that both TPC and antioxidant activity (DPPH assay) are noticeably varied in different species of *genus Allium* like *A. cepa, A. sativum, A. schoenoprasum* and *A. ursinum*. The total polyphenols were occurred in the range 444.3 to 1591 mg GAE/Kg and DPPH assay ranging from 12.29 to 76.57 % RSA (LenkovÁ, Bystrická et al. 2016). Another study carried out in different parts of *A. sativum* revealed that higher TPC and antioxidant activities in flowering parts rather than their bulbs. Another species *A. cepa* also showed significant antioxidant potentials in several forms (Yuasa, Kawabeta et al. 2021). Similarly, our present study also showed TPC and antioxidant activities are within the range but with variable values with specific plant extracts.

DOI:https//doi.org/10.22452/mjs.vol43no2.4
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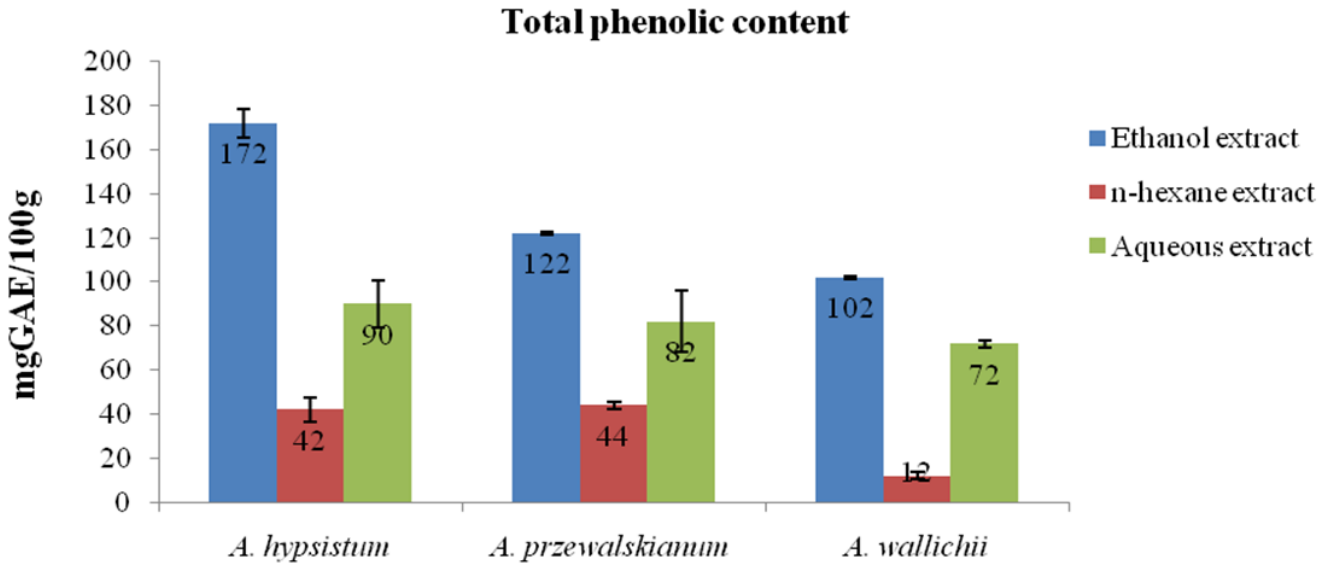

Figure 2 Total phenolic content (mg GAE/100g ± SEM) in the extracts of studied plants.

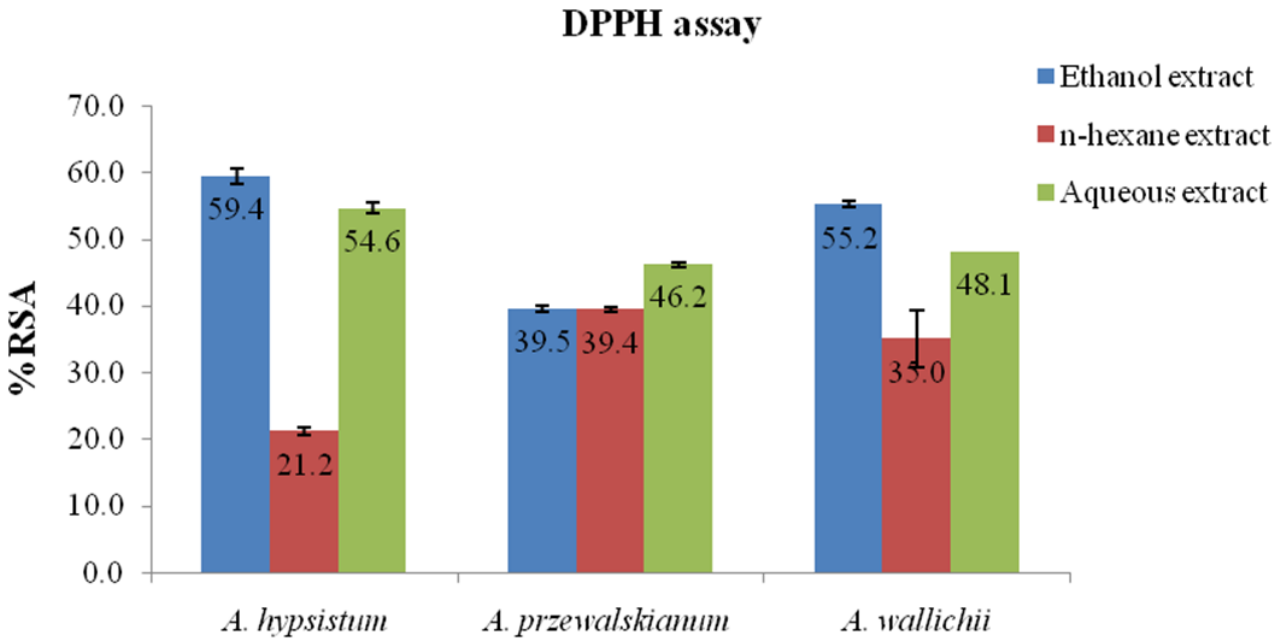

Figure 3 DPPH assay (% RSA ± SEM) of the extract of studied plants.

3.4 Correlation of TPC and DPPH free radical scavenging activity

To see whether TPC is associated with the DPPH activity, the statistical relationship between TPC and DPPH activity was established in the plant extracts by performing Pearson's correlation which has been shown in Table 3. An association of TPC and DPHH activity in ethanol extracts (r= 0.431) was found moderate and dependent whereas in n-hexane extracts (r= -0.230), it was found to be weak and negative. In case of aqueous extracts (r= 0.697), DPPH activity was found strongly and positively associated with TPC. In contrast, Despite the observed moderate to strong correlation, no any significant correlation (p>0.05) was seen between TPC and DPPH activity. A previous study (Ravipati, Zhang et al. 2012) on Chinese medicinal plants suggested phenolic content has a significant and strong positive correlation with DPPH scavenging activity. Similarly, a study on eight wild vegetables showed positively strong relationship between TPC and DPHH activity (Aryal, Baniya et al. 2019) and however, this study revealed no significant and found variable relationship between TPC and DPPH activity in plant extracts.

DOI:https//doi.org/10.22452/mjs.vol43no2.4
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Table 3 Correlation between TPC and DPPH activity in the following extracts of selected plants.

Plant extracts	Pearson's correlation (*r*)	Remarks
Ethanol	0.431	Moderate and positive
n-hexane	-0.230	Weak and negative
Aqueous	0.697	Strong and positive

From above-mentioned outcome, the plant extracts varies on their phytoconstituents however most of extracts have similar phytoconstituents. Subsequently, antioxidant activities of the plant extracts were found to be higher in ethanol extracts followed by aqueous extracts and n-hexane extracts. The study even though showed strong evidence of positive correlations between the TPC and DPPH activity but was too far from establishing a significant correlation between them. The lack of significant association could be attributed to the studied plant containing lower levels of phenolic content. However, further studies need to be conducted *in vivo* model for antioxidant property and should be implementing comprehensive research to develop phytomedicine extending the scope of *Allium species* in herbal therapy.

4. Conclusion

This study has demonstrated that the plant extracts under study exhibit significant *in vitro* antioxidant properties and contain promising phytochemicals. Therefore, these extracts might serve as potential antioxidant candidates which can be used to scavenge the radicals causing oxidative stress.

5. Acknowledgement

The authors are thankful to Department of Plant Resources, National Herbarium and Plant Laboratories, Nepal for identifying the plants. Also, we gratefully acknowledge Department of Chemistry, SALS, Uttaranchal University, India that providing laboratory facility to conduct this study.

5. References

Aryal, S., M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung and N. Koirala (2019). "Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal." Plants 8(4).

B, M., J. D, C. E, N. T, N. Kundal, M. Sarma and P. Kalita (2016). "Phytochemical Analysis of Traditional Medicinal Plants and their Antimicrobial Activity: An Experience from North East India." Open Access Journal of Pharmaceutical Research 1: 1-7.

Batiha, G., A. Beshbishy, L. Wasef, Y. Elewa, A. Al-Sagan, M. El-Hack, A. Taha, Y. Abd-Elhakim and H. Devkota (2020). "Chemical Constituents and Pharmacological Activities of Garlic (Allium sativum L.): A Review." Nutrients.

Bhandari, J., B. Muhammad, P. Thapa and B. G. Shrestha (2017). "Study of phytochemical, anti-microbial, anti-oxidant, and anti-cancer properties of Allium wallichii." BMC complementary and alternative medicine 17(1): 1-9.

Bhandari, J., B. Muhammad, P. Thapa and B. G. Shrestha (2017). "Study of phytochemical, anti-microbial, anti-oxidant, and anti-cancer properties of Allium wallichii." BMC Complement Altern Med 17(1): 102.

Chanda, S. and R. Dave (2009). "In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview." African Journal of Microbiology Research 3: 981-996.

Chen, X., L. Krug, M. Yang, G. Berg and T. Cernava (2021). "The Himalayan Onion (Allium wallichii Kunth) Harbors Unique Spatially Organized Bacterial Communities." 82(4): 909-918.

Chernukha, I., L. Fedulova, E. Vasilevskaya, A. Kulikovskii, N. Kupaeva and E. Kotenkova (2021). "Antioxidant effect of ethanolic onion (Allium cepa) husk extract in ageing rats." Saudi Journal of Biological Sciences 28(5): 2877-2885.

D'Sousa' Costa, C. O., P. R. Ribeiro, M. B. Loureiro, R. C. Simões, R. D. de Castro and L. G. Fernandez (2015). "Phytochemical screening, antioxidant and antibacterial activities of extracts prepared from different tissues of Schinus terebinthifolius Raddi that occurs in the coast of Bahia, Brazil." Pharmacognosy magazine 11(43): 607-614.

Fredotović, Ž., J. Puizina, M. Nazlić, A. Maravić, I. Ljubenkov, B. Soldo, E. Vuko and D. Bajić (2021). "Phytochemical Characterization and Screening of Antioxidant, Antimicrobial and Antiproliferative Properties of Allium × cornutum Clementi and Two Varieties of Allium cepa L. Peel Extracts." Plants 10(5): 832.

Gross, M. (2021). "All about Allium." Current Biology 31(22): R1449-R1452.

Iqbal, E., K. A. Salim and L. B. L. Lim (2015). "Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of Goniothalamus velutinus (Airy Shaw) from Brunei Darussalam." Journal of King Saud University - Science 27(3): 224-232.

Jalalvand, A. R., M. Zhaleh, S. Goorani, M. M. Zangeneh, N. Seydi, A. Zangeneh and R. Moradi (2019). "Chemical characterization and antioxidant, cytotoxic, antibacterial, and antifungal properties of ethanolic extract of Allium Saralicum RM Fritsch leaves rich in linolenic acid, methyl ester." Journal of Photochemistry and Photobiology B: Biology 192: 103-112.

Jeba Malar, T. R. J., J. Antonyswamy, P. Vijayaraghavan, Y. Ock Kim, A. A. Al-Ghamdi, M. S. Elshikh, A. A. Hatamleh, M. A. Al-

DOI:https//doi.org/10.22452/mjs.vol43no2.4
Malaysian Journal of Science 43(2): 30-37 (June 2024)

Dosary, S. W. Na and H.-J. Kim (2020). "In-vitro phytochemical and pharmacological bio-efficacy studies on Azadirachta indica A. Juss and Melia azedarach Linn for anticancer activity." Saudi journal of biological sciences 27(2): 682-688.

Krishnaiah, D., R. Sarbatly and R. Nithyanandam (2011). "A review of the antioxidant potential of medicinal plant species." Food and Bioproducts Processing 89(3): 217-233.

Lekshmi, P., V. S, J. S and R. J (2015). "PHYTOCHEMICALS IN ALLIUM SPECIES AND ITS ANALYTICAL METHODS – A REVIEW." International Journal of Institutional Pharmacy and Life Sciences 5: 38-58.

LenkovÁ, M., J. Bystrická, T. Tóth and M. HrstkovÁ (2016). "Evaluation and comparison of the content of total polyphenols and antioxidant activity of selected species of the genus Allium." Journal of Central European Agriculture 17: 1119-1133.

Phan, A. D. T., G. Netzel, P. Chhim, M. E. Netzel and Y. Sultanbawa (2019). "Phytochemical characteristics and antimicrobial activity of Australian grown garlic (Allium sativum L.) cultivars." Foods 8(9): 358.

Ravipati, A. S., L. Zhang, S. R. Koyyalamudi, S. C. Jeong, N. Reddy, J. Bartlett, P. T. Smith, K. Shanmugam, G. Münch, M. J. Wu, M. Satyanarayanan and B. Vysetti (2012). "Antioxidant and anti-inflammatory activities of selected Chinese medicinal plants and their relation with antioxidant content." BMC Complement Altern Med 12: 173.

Roughani, A. and S. M. Miri١ (2019). "Plants, Organic Farming, Natural and Pharmaceutical Ingredients."

Seino, Y., Y. Yamazaki and D. Yabe (2020). "The Asian Association for the Study of Diabetes: The first 10 years and the next 10 years." Journal of Diabetes Investigation 11(5): 1079-1084.

Shrestha, P., S. Adhikari, B. Lamichhane and B. Shrestha (2015). "Phytochemical Screening of the Medicinal Plants of Nepal." IOSR Journal of Environmental Science, Toxicology and Food Technology 1: 2319-2399.

Szerlauth, A., S. Muráth, S. Viski and I. Szilagyi (2019). "Radical scavenging activity of plant extracts from improved processing." Heliyon 5(11): e02763.

Yuasa, M., K. Kawabeta, M. Morikawa, M. Iwami and M. Tominaga (2021). "Antioxidant and taste properties of fresh onion (Allium cepa L.) leaves." Journal of Food Measurement and Characterization 15(2): 1083-1091.

DOI:https//doi.org/10.22452/mjs.vol43no2.4
Malaysian Journal of Science 43(2): 30-37 (June 2024)