

RESEARCH ARTICLE

## Phytochemical Constituents, Total Flavonoid and Phenolic Content of *Allium hookeri* Thw. Enum. Leaf Extracts and their Antioxidant Potential

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### Abstract

This study evaluated the phytochemical constituents, total flavonoid and phenolic content of *Allium hookeri* (Thw. Enum.) aqueous and solvent leaf extracts and their antioxidant potential. Qualitative phytochemical screening showed the presence of phytochemical constituents in the leaf extracts. It was noted that steroids, flavonoids and polyphenols were present in ethyl acetate extract whereas, steroids, flavonoids, tannins and polyphenols were present in ethanol extracts. It was noted that ethanol extract recorded highest flavonoid content (149.78±4.58) compared to ethyl acetate (134.4±12.09), chloroform (40.4±1.85) and aqueous (117.16±1.25) extracts. In case of total phenolic content, ethanol extract recorded highest phenol content (303.57±2.07) compared to aqueous (267.58±2.07), ethyl acetate (214.71±2.46) and chloroform (118.42±1.88) extracts. It was noted that ethanol leaf extract recorded the highest inhibition percentage of 72.86% followed by ethyl acetate (67.5%), aqueous (61.3%) and chloroform (50.2%) leaf extracts in the DPPH assay. Isolation and characterization of the phytoconstituents responsible for the antioxidant activities should be done in near future to formulate natural pharmaceutical products of high value from *A. hookeri*.

**Keywords:** Phytochemicals, *Allium hookeri*, flavonoids, phenols, antioxidant assay.

### Introduction

*Allium hookeri* Thw. Enum. (Alliaceae family) is an important ethnomedicinal plant native to the Himalayan region of Asia (Ayam, 2011; Pandey *et al.*, 2008). This plant has been utilized in the eastern Himalayan region of India as a vegetable and spice, and as a traditional medicine for cough, cold, painful swellings, and skin eruptions (Kala, 2005). However, preservation and cultivation of native *A. hookeri* germplasm has decreased in part due to the lack of fundamental research on this plant. After the recognition of the potent medicinal and nutritional value of indigenous *A. hookeri*, it began to be widely cultivated in fields and kitchen gardens in the mountainous regions of India and Myanmar (Ayam, 2011; Pandey *et al.*, 2008). Since this plant was introduced in 2010, its cultivation has expanded very rapidly in many parts of South Korea. *Allium hookeri* has recently gained significant attention because it produces many useful compounds, such as high amounts of phenolic antioxidants, phytosterols, fiber, ascorbic acid, flavonoids, and allicin, as well as unknown organic sulphur compounds distinct from those of *A. cepa* (Kala, 2005; Rhyu and Park, 2013). Allicins are sulphur containing compounds which have been demonstrated to have potent pharmacological properties, including antimicrobial activity, the ability to lower cholesterol levels, antiplatelet aggregation effects, the ability to lower the risk of heart attacks, anti-carcinogenic activity, and anti-inflammatory effects (Bae and Bae, 2012; Kim *et al.*, 2012; Rahman and Lowe, 2006; Rhyu and Park, 2013).

Fig. 1. *Allium hookeri* Thw. Enum. in its natural habitat.



However, the chemical structure of many novel compounds in *A. hookeri* and their biological effects on health remain to be elucidated. Considering the above facts in view, this study evaluated the phytochemical constituents, total flavonoid and phenolic content of *A. hookeri* leaf extracts and their antioxidant potential.

## Materials and methods

**Collection of plant material:** The plant material of *Allium hookeri* was collected from kitchen gardens of Khongman zone-2, Imphal east district, Manipur in the month of June–July in 2014 (Fig. 1). The plants were wrapped with plastic sheets during transportation to avoid any possible damage. Identification and authentication of the plant was done by Dr. Sunita Garg, Chief Scientist, NISCAIRE, CSIR-New Delhi. The sample species were submitted to NISCARE herbarium for future reference.

**Extraction of the leaf material:** The air dried leaf material was cut into small pieces and dried in shadow for at least two weeks for complete drying. Then it was grinded into a mixer and converted into fine powder. The 1000 g of powdered leaf materials were packed in soxhlet apparatus and successive extraction was performed using hexane, chloroform, ethyl acetate, ethanol and aqueous. The solution of the extract was filtered through Whatman No.1 filter paper and concentrated using rotary flash evaporator and dried under vacuum.

### Phytochemical screening

#### Test for flavonoids

**Lead acetate test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

#### Test for steroids

**Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

**Test for terpenoids (Salkowski test):** Five mL of each extract was mixed in 2 mL of chloroform and concentrated H<sub>2</sub>SO<sub>4</sub> (3 mL) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show the presence of terpenoids.

**Test for tannins:** About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

**Test for saponins-Foam test:** Small amount of extract was shaken with little quantity of water. If foam produced persists for 10 min, it indicates the presence of saponins.

#### Test for alkaloids

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

**Mayer's test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

**Test for polyphenols:** One mL of the leaf extract was added to 2 mL distilled water followed by few drops of

10% ferric chloride. Formation of blue/green colour indicates the presence of polyphenols

#### Determination of total flavonoids:

Total flavonoid content was determined by Dowd method described by Meda *et al.* (2005) with some modifications. Briefly, 1.5 mL of leaf extract was reacted with 2% (w/v) aluminium chloride for 10 min at room temperature and the absorbance was read at 415 nm. Total flavonoid content was determined by a standard curve of quercetin (0-50 mg/L) and was expressed as mg of quercetin equivalents (QE) per gram dry material.

**Estimation of total phenolic content:** The total phenolic content was determined by the spectrophotometric method using Park *et al.* (2008) with slight modifications. In brief, a 0.5 mL of the leaf extract was mixed with 2.5 mL of 0.2 N Folin-Ciocalteu's phenol reagent. After 5 min, 2 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture followed by the addition of 13 mL of deionized distilled water and mixed thoroughly. The mixture was kept in the dark for 90 min at 23°C, after which the absorbance was read at 750 nm. The TPC was determined from extrapolation of calibration curve which was made by preparing gallic acid solution. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per g dry material.

#### Antioxidant activity of leaf extracts

**DPPH radical scavenging activity:** The free radical scavenging activity of the leaf extracts were measured with DPPH, using the slightly modified method described by Yadhav *et al.* (2014). The leaf extracts (0.5 mL) at various concentrations namely 5, 10, 20, 50 and 100 µg were mixed with 2.5 mL of DPPH solution respectively. The reaction mixture was vortexed thoroughly and left for 30 min. The absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference drug. The ability of plant extract to scavenge DPPH radical was calculated from the following formula:

$$\text{DPPH scavenging effect (\%)} = \left( \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \right) \times 100$$

Where, OD Control is the absorbance of the control reaction and OD test is the absorbance of the extracts. The extract concentration providing 50% inhibition (IC 50) was calculated from the graph and inhibition percentage was plotted against extract concentration.

## Results and discussion

For the extraction of leaf extracts, 1000 g of *Allium hookeri* leaf powder were packed in soxhlet apparatus and successive extraction was performed using hexane, chloroform, ethyl acetate and ethanol. The solution of the extract was filtered through Whatman No.1 filter paper and concentrated using rotary flash evaporator and dried under vacuum.

Table 1. Weights of the plant leaf extracts of *Allium hookeri*.

Plant part	Extracts	Extracts weight
Leaf powder (1000 g)	Hexane	6.5 g
	Chloroform	4.2 g
	Ethyl acetate	2.8 g
	Ethanol	9.6 g
	Aqueous	11.2 g

Table 2. Phytochemical screening of *Allium hookeri* plant extracts.

Extracts	Steroids	Alkaloids	Flavonoids	Saponins	Terpenoids	Tannins	Polyphenols
Hexane	-	-	-	-	-	+	-
Chloroform	+	-	+	-	+	+	+
Ethyl acetate	+	-	+	-	-	-	+
Ethanol	+	+	+	-	-	+	+
Aqueous	+	-	+	-	-	+	+

'+' – Present; '-' Absent.

Table 3. Total flavonoid content of *Allium hookeri* leaf extracts.

Extracts	Total flavonoid content ( mg/g QE)
Chloroform	40.4 ±1.85
Ethyl acetate	134.4±12.09
Ethanol	149.78±4.58
Aqueous	117.16±1.25

Table 4. Total phenolic content of *Allium hookeri* leaf extracts.

Extracts	Total phenolic content ( mg/g GAE)
Chloroform	118.42±1.88
Ethyl acetate	214.71±2.46
Ethanol	303.57±2.07
Aqueous	267.58±2.07

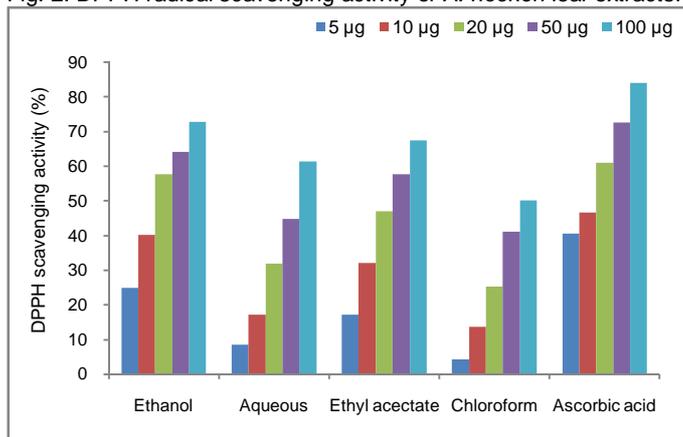
The respective weights after the soxhlet extraction are given in Table 1. Table 2 lists the phytochemical constituents of *Allium hookeri* leaf extracts. It was noted that tannins were only present in the hexane extract whereas, saponins and alkaloids were only absent in chloroform extract. Steroids, flavonoids and polyphenols were only present in ethyl acetate extract. Steroids, alkaloids, flavonoids, tannins and polyphenols were present in ethanol extract.

Flavonoids are a group of polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). Due to its antioxidant properties it can interfere with the oxidative process by reacting with free radicals chelating, catalytic metals and also by acting as oxygen scavengers (Harborne, 1973; Dreosti, 2000). Table 3 lists the total flavonoid content of *Allium hookeri* leaf extracts. It was noted that ethanol extract recorded highest flavonoid content (149.78±4.58) compared to ethyl acetate (134.4±12.09), chloroform (40.4±1.85) and aqueous (117.16±1.25) extracts. Srivastava *et al.* (2013) in his study recorded total flavonoid content of 18.50 mg catechin equivalents/100 g of fresh mass in stem of *Allium cepa*.

Phenolics hinder oxidative degradation of lipids and thereby enhance the excellence and nutritional value of food. Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability of modifying gene expression (Srivastava *et al.*, 2013). Table 4 shows the total phenolic content of *Allium hookeri* leaf extracts. It was noted that ethanol extract recorded highest phenol content (303.57±2.07) compared to aqueous (267.58±2.07), ethyl acetate (214.71±2.46) and chloroform (118.42±1.88) extracts. Kaur and Kapoor in their study recorded a total phenolic content of 1455.9 mg/100 g in aqueous extract of *Allium sativum*.

*In vitro* antioxidant activities of the *Allium hookeri* leaf extracts were evaluated at various concentrations using DPPH assay. It was noted that inhibition percentage increased in increasing concentrations of the extracts. The extract concentration providing 50% inhibition (IC 50) was calculated (data not shown) and inhibition percentage was plotted against extract concentration. It was noted that ethanol leaf extract recorded the highest inhibition percentage of 72.86% followed by ethyl acetate (67.5%), aqueous (61.3%) and chloroform (50.2%) leaf extracts (Fig. 2).

Fig. 2. DPPH radical scavenging activity of *A. hookeri* leaf extracts.



Cho *et al.* (2015) in his study has assessed the antioxidant activity of *Allium hookeri* root extracts and found a significant DPPH activity.

### Conclusion

Qualitative phytochemical screening showed the presence of phytochemical constituents in the leaf extracts. It was noted that ethanol extract recorded highest flavonoid content ( $149.78 \pm 4.58$ ) compared to ethyl acetate ( $134.4 \pm 12.09$ ), chloroform ( $40.4 \pm 1.85$ ) and aqueous ( $117.16 \pm 1.25$ ) extracts. In case of total phenolic content, ethanol extract recorded highest phenol content ( $303.57 \pm 2.07$ ) compared to aqueous ( $267.58 \pm 2.07$ ), ethyl acetate ( $214.71 \pm 2.46$ ) and chloroform ( $118.42 \pm 1.88$ ) extracts. It was noted that ethanol leaf extract recorded the highest inhibition percentage of 72.86% in the DPPH assay. Isolation and characterization of phytochemicals responsible for the antioxidant activities should be done in near future to formulate natural pharmaceutical products of high value from *A. hookeri*.

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