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A Comparative In Vitro Study on Potential of Anti-Diabetic and Anti-Oxidant Properties in Extracts of *Allium Hypsistum, Allium Przewalskanium* and *Allium Wallichii*

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Research Article

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Abstract

Allium hypsistum, Allium przewalskanium, and *Allium wallichii* are among the plants which are found at high altitude of Himalayan region. This *in-vitro* study has been focused on anti-diabetic and antioxidant properties of selected plants taken from Nepal region. Antidiabetic property was determined by in-vitro α- amylase inhibition method. Alcoholic extract of *Allium hypsistum* showed greatest α-amylase inhibitory action about 80% with an IC50 worth of 1.10 milligram/ ml followed by *Allium przewalskanium* which showed about 66% inhibition with an IC50 worth of 1.48 milligram/ ml. The antioxidant activities were evaluated using total phenolic content (TPC) and 2, 2-diphenyl -1-picrylhydrazyl (DPPH) radical scavenging assay method. Ethanolic extract of *A. hypsistum* showed the highest DPPH free radical scavenging property 67% as compared to reference ascorbic acid (75%). Among selected three plants, both TPC and %RSA revealing the antioxidant activities were found to be higher in *A. hypsistum* followed by *A. prsewalskanium* and *A. wallichii*.

Keywords: *Allium Hypsistum; Allium Przewalskanium; Allium Wallichi*i; Anti-diabetic; α-amylase Inhibition; Antioxidant

Abbreviations: TPC: Total Phenolic Content; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; RSA: Radical Scavenging Activity.

Introduction

Generally high altitude plants have good potential of pharmacological properties like antibacterial, antidiabetic, or anti-oxidant property. Plants selected in this study are *Allium hypsistum, Allium przewalskanium, Allium wallichii* which are found at higher altitudes areas of in himalayan region of India. Nepal or China. Majority of population prefer traditional medicine which has components extracted from medicinal or herbal plants [1]. Plants produce different phytochemicals by primary or secondary metabolism and such phytochemicals play a key role in growth, development and defense against external factors. Secondary metabolites are usually bioactive molecules that include tannins, alkaloids, terpenoids, phenolics, steroids, and flavonoids. These bioactive components can exhibit therapeutic functions on the human body [2].

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 [3]. Thus, analysis of total phenolic content (TPC) indicates potential of antioxidant nature. DPPH, as it is a stable free

radical and widely used to assess the radical scavenging activity of the antioxidant compound and is determined by percentage radical scavenging activity (%RSA). Diabetes mellitus is a non-infectious endocrine condition defined by a disruption in carbohydrate metabolism. More than 10% population in the world is suffering from diabetic problem and as such no allopathic medicine is full proof recommended for curing of diabetes [4].

In the present study, commonly found high altitude Allium species such as *Allium hypsistum*, *Allium przewalskanium* and *Allium wallichii* are selected for *in vitro* antioxidant and anti-diabetic activity analysis. *Allium hypsistum* is a popular herb which is widely used as spice and medicine in rural villages and towns of Nepal and India. *Allium przewalskianum* is also consumed as medicinal plants to treat cold fever at high altitude areas. *Allium 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 2500m to 4500m [5].

Material and Methods

Collection of Plant Materials

Allium hypsistum, Allium przewalskanium and *Allium wallichii* were collected from Mustang and Gorkha district 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. Aerial parts of the plants were taken and washed with distilled water. Materials were first chopped into small pieces and air-dried in shade for 15 days at room temperature (28°C to 30°C) [6].

Preparation of Plant Extract

The air dried plant material was grinded to get powder, extraction was carried out separately by the soxhlet method using ethanol, n-hexane, and distilled water [7].

Alpha Amylase Inhibitors Assay

Starch-Iodine Colour Assay: The starch- iodine assay was executed as defined by Xiao, et al. [8] for screenings alpha-amylase inhibitors, the combination was placed for incubation at 37°C. Then, each mixture received 870 µl of newly ready starch (1%, w/v) and kept for 17min for incubation at 37°C. To break the enzymatic reaction, 60 µl of muriatic acid. (10.0M) was transferred, before transferring of 300 µl of iodine reagent (5 mM I2 1+ 5 mM KI). At 620nm wavelength absorbance was noted and a color change was observed. There were no plant extracts added combination referred as control was used, which represent 100 percent enzyme activity. Appropriate control extracts without enzymes were also involved to abolish the absorbance produced by extracts. The chromogenic DNSA technique was also used to perform the inhibitory assay as Hara and Honda 1990 [9].

Absorbance of all mixture was taken at 540 nm using Uv visible spectrophotometer. No plant extract was poured in the control tube, which represented 100 percent enzyme activity. To exclude the absorbance caused by plant extract, appropriate extract controls were introduced in combination without the enzyme. The IC50 values were calculated as per the concentration of extract containing the α -amylase .Below given formula was applied to calculate Inhibition percentage. Inhibition (%) = Abs (control) – Abs (extract) ´X 100/ Abs (control) α Amylase Inhibition results are shown in Table 1.

Conc. (mg/ ml)	Std	A. hypsistum	A. przewal.	A. wallichii.
0	0	0	0	0
0.5	40.82±0.22	38.18±0.12	30.11±010	26.13±0.08
1	75.12±0.22	70.10±0.18	52.30±0.08	31.30±0.10
1.5	81.50±0.18	76.70±0.13	58.90±0.20	38.20±0.15
2	86.10±0.32	80.12±0.30	66.12±0.25	43.22±0.11

Table 1: α Amylase Inhibition (%) of Extracts Vs Standard Drug.

Antioxidant Analysis

Total phenolic assay & standard DPPH assay were used as per standard assay [10]. 50mg of each extract were dissolved in 50ml DMSO (10% v/v) and centrifuged at 2000rpm for 5 minutes. Then, 0.5ml of supernatant of each extract was taken in separate, labeled test tubes and 0.5ml of 50% Folin-ciocalteu reagent was added in it. Then, the tubes were allowed to stand for 15 minutes at room temperature. After that, 2.5ml of 20% sodium carbonates were added and the tubes were incubated for 30 minutes in a dark place. Absorbance of all test solutions was recorded at 760nm against a reagent blank in a spectrophotometer (UV-1800 Shimadzu Spectrophotometer). All test solutions were analyzed in duplicate. Total phenolic content was expressed as mg gallic acid equivalent (mgGAE/100g).

DPPH Assay

DPPH (2, 2-diphenyl -1-picrylhydrazyl radical) is a stable free radical that reacts with molecules that can donate a hydrogen atom.. The 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.

*Ascorbic acid was used as a reference compound which % RSA was found to be 75.0

Results

Many previous studies have described that the phenolic compounds present in the plants show remarkable antioxidant properties which are involved in anti-inflammation and anti-aging and antibacterial properties [11].

Among the selected plants, *a hypsistum* has shown a good potential of antidiabetic by showing α Amylase Inhibition from 38 to 80% with concentration of 0.5 to 2 mg/ml. This inhibition is comparable to that of std drug of anti-diabetic which showed 40 to 86% at conc. Of 0.5 to 2 mg/mL. While *a przewalskanium* has shown inhibition from 30 to 66% followed by *A wallichii* only 26 to 43% inhibition. This data is given in table 1. Overall by this study alcoholic extract has shown much better potential with IC50 of 1.10mg/ml as compared to IC50 value of 78 mg/ml of std reagent.IC50 values are shown in Table 2.

Alcoholic extract(Plant/std)	IC (50) value
Standard	0.78 mg/ ml
A. hypsistum	1.10 mg/ ml
A. przewal.	1.48 mg/ ml
A. wallichii.	1.85 mg/ ml

Table 2: IC 50 value of all plants vs Std.

Total phenolics content (TPC) and DPPH free radical scavenging assay in selected plants have been summarized in Table 3. From the table, it is evident that *A hypsistum* has higher TPC in each of the solvent extracts compared to that of *A przewalskanium* and *A wallichii.* More precisely, the highest phenolics content was observed in ethanol extraction of *A hypsistum* (180 mgGAE/100g). Moderate levels of phenolic content were estimated in ethanol extraction of *A przewalskanium* (128 mgGAE/100g) followed by *A wallichii* (105 mgGAE/100g). Among the solvents used for the plant extracts, significant phenolics content was found in ethanol extracts followed by water and n-hexane extracts. The order of phenolics content in aqueous extracts was *A hypsistum* (94 mgGAE/100g), Table 3 shows a higher DPHH activity in ethanol extract of *A hypsistum* (62.1 % RSA) followed by *A wallichii* (56.20 %RSA) and *A przewalskanium* (40 %RSA). In the case of n-hexane extracts, DPHH activity was found higher in *A przewalskanium* (42 %RSA) than *A wallichii* (39%RSA).. DPHH activity was found higher in aqueous extract of *A hypsistum* (58 % RSA) than *A wallichii* (50 %RSA). When compared with DPHH activity of reference ascorbic acid compound (75.0 %RSA), alcoholic extract of *A hypsistum* has shown significant antioxidant property which can be utilized in herbal medicine.

Plants	Total phenolic content (mgGAE/100g)			DPPH free radical scavenging assay (% RSA)		
	ethanol extract	n-hexane extract	aqueous extract	ethanol extract	n-hexane extract	aqueous extract
A. hypsistum	**180**	43	**94**	**62.1**	24.2	58.05
A.przewalskanium	128	**48**	84	40.5	**42.4**	48.2
A.wallichii	105	18	75	56.2	37.05	**50.1**

Table 3: Total Phenolic Content and DPPH Assays.

References

1. Agarwala M, Yadav RNS (2011) Phytochemical analysis of some Medicinal plants. Journal of Phytology 3(12): 10-14.

2. Vasu K, Vinayasagar J, Suryam A, Charya MAS (2009) Biomolecular and phytochemical analyses of three aquatic angiosperms. African Journal of Microbiology Research 3(8): 418-421.

3. Capasso A (2013) Antioxidant action and therapeutic efficacy of *Allium sativum* L. Molecules 18(1): 690-700.

4. Kumar A, Goel MK, Jain RB, Khanna P, Chaudhary V (2013) India towards diabetes control: Key issues. Australasian Medical Journal 6(10): 524-531.

5. Wawrosch C, Malla PR, Kopp B (2001) Micropropagation of *Allium wallichii* kunth, a threatened medicinal plant of Nepal. In Vitro Cellular & Developmental Biology - Plant

37(5): 555-557.

6. Abraham J, TD Thomas (2012) Antibacterial activity of medicinal plant *Cyclea peltata* (Lam) Hooks & Thoms. Asian Pacific Journal of Tropical Disease 2: S280-S284.

7. Malar TRJJ, Antonyswamy J, Vijayaraghavan P, Kim YO, Abdullah AAl-G, et al. (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.

8. Xiao Z, Storms R, Tsang A (2006) A quantitative starch-iodine method for measuring alpha-amylase and glucoamylase activities. Anal Biochem 351(1): 146-148.

9. Hara Y, Honda M (1990) The inhibition of alpha amylase by tea polyphenols. Agric Biol Chem 54(8): 1939-1945.

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

11. Lekshmi P, Viveka S, Jeeva S, Brindha RJ (2015) Phytochemical in Allium Species and its analytical methods – A Review. International Journal of Institutional Pharmacy and Life Sciences 5(1): 38-58.