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***In-vitro* antioxidant, antidiabetic and toxic effect of *Ageratum houstonianum* from Chitwan district Nepal**

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ABSTRACT

Medicinal plants are safe and the oldest natural products used for many years to conserve food, to treat health disorders and to prevent diseases. The active chemical compounds formed during secondary vegetal metabolism is usually responsible for the biological properties of some plant species used throughout the world for various purposes including treatment of diabetes, cancer, infectious diseases etc. The present study was undertaken to analyze the phytochemicals by colour differentiation method, to evaluate the toxic effect by phytotoxic assay, antidiabetic activity by α amylase enzyme inhibition and antioxidant potential by DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of methanolic extract of *Ageratum houstonianum*. Treatment of problem in carbohydrate uptake needed the inhibition of α -amylase plays a role in the digestion of polysaccharide and glycogen, is made a strategy for controlling diabetes. For this study whole plant was collected, dried and the powder was made. The extraction was carried out by cold percolation in which methanol was used as a solvent. The methanolic extract was subjected to *In-vitro* phytotoxic activity by adopting the standard protocol. The α -amylase enzyme inhibition activity of plant extract was carried out by using starch as substrate, pancreatic α amylase as the enzyme, and acarbose as standard. The result of *in-vitro* phytotoxic bioassay revealed that the plant extract showed moderate activity with percentage growth regulation 80 and 25 percent in a concentration-dependent manner. The α -amylase enzyme inhibition was 74.13 to 99.39 percent in a dose-dependent manner. The antioxidant potential of *Ageratum houstonianum* extract showed mild activity with IC_{50} 123.67 μ g/ml as compared to the standard ascorbic acid IC_{50} 5.38 μ g/ml. It is concluded from the present study that *Ageratum houstonianum* could be used as a natural source to isolate antioxidant, anti-hyperglycemic agent, herbicide and weedicide as it shows a good α amylase inhibition, radical scavenging and phytotoxic activity respectively.

Key Words: Phytotoxic, antioxidant, α - amylase, hyperglycemic, *Ageratum houstonianum*

INTRODUCTION

Medicinal plants have always had great importance in culture, medicine and nutrition of society in all over the world. Application of medicinal plants based on ethnobotanical knowledge has been increased recently at greater diversity not only in the national level but also at international level (Rajdoula, 2017). But, there is a significant gap that exists between scientific validations of using such medicinal plants as medicine (Rajdoula, 2017). Since many years plants have been using as food and medicine simultaneously (Etkin, 1982 & Totelin, 2015). Due to the wide diversity of plants around the world, the opportunity of using the plants as a medicine is huge. Medicinal plants can be used for the treatment of different diseases in different formulations like crude extracts, whole plants, a paste of plants, infusion etc. (Samuelsson, 2004). These medicinal plants can be used for the treatment of simple to life-threatening diseases in different communities of the world. Among the several diseases, diabetes mellitus is a metabolic disorder of the endocrine system. In most parts of the world, peoples are suffering from diabetes due to lack of insulin production in the body and its proper use in metabolism, so the blood has high glucose concentration (Li et al., 2004). There is no satisfactory effective therapy to cure diabetes mellitus in modern synthetic medicines. The drugs used in the treatment of diabetes are either too expensive or have lots of side effects (Pari & Amarnath, 2004). Different extracts from medicinal plants have also been used traditionally in the treatment of diabetes globally,

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and these are taken as relatively inexpensive, less toxic and with little or no side effects such as paranoia, extreme anxiety, hallucinations, homicidal behaviour etc. (Gupta et al., 2008). Oxidative stress is the major risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen and nitrogen species are known as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be cause for the human aging (Kanwar et al., 2009 & Chiavaroli et al., 2012). An antioxidant is the substance that delays or inhibits oxidative damage to a target molecule by trapping free radicals generated in the body of living organisms (Yamagishi & Matsui, 2011). The chemical compounds that act an antioxidant are phenolic acids, polyphenols and flavonoids, scavenge free radicals such as peroxide, hydroperoxide or lipid peroxy and inhibit the oxidative damage that lead to degenerative diseases (Wu et al., 2011).

Ageratum houstonianum weed is one of the important medicinal ornamental plants which is native to Nepal, India, Central and Southern America and has distributed in other parts of the world (Kumar, 2014). A large number of chemical compounds have been reported from this plant to possess insecticidal (Ravindran et al., 2012), antimicrobial (Kurade et al., 2010 & Tennyson et al., 2011) and antifungal (Pandey et al., 1984) effects. The essential oil of the plant leaves has antidermatophytic compounds which may not be toxic when used currently (Njateng et al., 2010). The plant *Ageratum houstonianum* is the excellent source of active natural antioxidant and anticancer compounds (Rizvi et al., 2014). Therefore, the present study provides the antioxidant, phytotoxic and α -amylase enzyme inhibition activity of *Ageratum houstonianum* extract that supports the partial scientific validation for using the plant against diabetes.

MATERIALS AND METHODS

Chemicals and reagents

The major chemicals used in this study were methanol, DPPH, ascorbic acid and porcine pancreatic alpha-amylase were of commercially available analytical grade.

Plant collection and extract preparation

Ageratum houstonianum was collected from Chitwan district of Nepal based on the ethnobotanical uses. The plant was identified by Rita Chhetri Research Officer, National Herbarium and Plant Resources, Ministry of Forests and Soil Conservation, Godawari, Nepal. 250 g powder of plant was extracted by cold percolation method in 500 ml methanol at 25 °C for 48 hours with frequent agitation. The mixture was filtered through clean cotton and filtrate was concentrated at 55 °C under reduced pressure by using a rotary evaporator to get the crude extract. Finally, the concentrated extracts were fridge dried to form a dry powder.

Phytochemical analysis

The preliminary qualitative phytochemical analysis was carried out to identify the plant secondary metabolites such as flavonoids, alkaloids, saponins etc. present in the methanol extract of *Ageratum houstonianum*. The analysis of the presence of main groups of natural compounds in the plant extracts was done by the colour differentiating reaction using different specific reagents (Jaykumar et al., 2018).

Alpha amylase enzyme inhibition assay

Alpha-amylase inhibition assay was performed using a standard protocol where the undigested starch due to enzyme inhibition was detected at 630 nm (blue, starch-iodine complex) (Kusano et al., 2011). The stock solution of the plant extract was made by dissolving 10 mg in 10 ml of dimethyl sulphoxide (DMSO) (1000 μ g/ml). The substrate was prepared by dissolving 200 mg starch in 25 ml of NaOH (0.4 M) by heating at 100 °C for 5 minutes. After cooling, pH was adjusted to 7.0 and the final volume was made up to 100 ml adding distilled water. Acarbose was used as a positive control. 400 μ l of substrate solution was pre-incubated at 37 °C for 5 minutes with 200 μ l of acarbose or plant extract at varying concentrations (40, 80, 160, 320, 640 and 1000 μ g/ml), followed by 200 μ l of 50 μ g/ml α -amylase (20 mM phosphate buffer with 6.7 mM NaCl, pH 6.9), and incubated at 37 °C for 15 min. Termination of the reaction was carried out by adding 800 μ l of HCl (0.1M). Then, 1000 μ l of iodine reagent (2.5 mM) was added, and absorbance measured at 630 nm using a spectrophotometer. Percentage of inhibition was

calculated as,

$$\% \text{ Inhibition} = (1 - [\text{Abs}_2 - \text{Abs}_1 / \text{Asb}_4 - \text{Abs}_3]) \times 100$$

Where,

Abs_1 is the absorbance of the incubated mixture containing plant sample, α amylase and starch. Abs_2 is the absorbance of an incubated mixture of sample and starch. Abs_3 is the absorbance of the incubated mixture of starch and α -amylase. Abs_4 is the absorbance of the incubated solution containing starch.

In-vitro phytotoxic bioassay (modified protocol of Prof. Mc Laughlin et al., 1991)

In phytotoxic assay E-Medium was prepared by mixing various constituents in 1000 ml distilled water and pH was adjusted 6.0 to 7.0 by adding KOH pellets (stock solution). Working E-medium was prepared by mixing 100 ml of stock solution and 900 ml of distils water. 30 mg crude extract was dissolved in 1.5 ml of solvent methanol serving as a stock solution. Three flasks were inoculated with 10, 100 and 1000 μl of solution pipetted from the stock solution for 10, 100 and 1000 $\mu\text{g}/\text{ml}$. The solvent was evaporated overnight. 20 ml of working E. medium was added and then plant of *Lemna minor*, each containing a rosette of two to three fronds, to each flask (total 20 fronds). Other flasks supplemented with E-medium and reference (standard drug) plant growth inhibitors and promoters serving as negative and positive controls, respectively. The flasks were placed in growth cabinet for seven days and the number of fronds were recorded per flasks on day seven. Results were analyzed as growth regulation in percentage calculated regarding the negative control (Atta-ur-Rehman 1991& Hideji 1982).

Antioxidant activity

The antioxidant potential was evaluated by the use of DPPH free radical. The ability of plant extract to scavenge free radical was performed by the protocol adopted (Jamuna et al., 2012). Ascorbic acid of the same concentration with plant extract was used as the standard in which the absorbance was measured at 517 nm. The percentage of DPPH free radical scavenging activity was calculated as,

$$\text{Radical scavenging (\%)} = [A_0 - A_s / A_0] * 100$$

Where, A_0 = Absorbance of the DPPH with methanol, A_s = Absorbance of plant extract. The IC_{50} value indicated as an effective concentration of sample/plant extract required to scavenge 50% of DPPH. IC_{50} was calculated graphically plotting concentration vs. radical scavenging.

Statistical analysis

Data were recorded as the mean of (\pm) standard deviation of three determinations of absorbance for each concentration, from which the linear correlation coefficient (R^2) value was calculated using MS Office Excel 2007. The linear regression for a straight line is, $y = mx + c$, using this regression equation, the inhibitory concentration of extract were calculated.

RESULTS AND DISCUSSION

Phytochemical analysis

The results of phytochemical analysis showed the plant extract was found to contain alkaloids, anthraquinones, phytosterols, flavonoid and carbohydrates as secondary metabolites.

Phytotoxic activity

The results of phytotoxic activity is shown in table 1.

Table 1. *In-vitro* toxic effect of *Ageratum houstonianum*

Name of the plant	Conc. of Plant extract ($\mu\text{g}/\text{ml}$)	No of fronds		% growth regulation	Concentration of standard drug ($\mu\text{g}/\text{ml}$)
		Sample	Control		
<i>Lemna minor</i>	1000	04		80	0.015
	100	11	20	45	
	10	15		25	
K Keys: Standard drug Paraquat				Incubation condition= (28 \pm 1 $^\circ\text{C}$)	

The phytotoxicity of the methanolic extract of *Ageratum houstonianum* investigated on *Lemna minor* was observed to have dose-dependent activity in which low activity was found in 10 $\mu\text{g/ml}$ (25% inhibition) and high activity was found in 1000 $\mu\text{g/ml}$ (80% inhibition) respectively. Moderate phytotoxic activity was found in the 100 $\mu\text{g/ml}$ (45% inhibition). Significant phytotoxicity was shown in 100 and 1000 $\mu\text{g/ml}$ with 45% and 80% growth inhibition.

Antidiabetic activity

The α -amylase inhibition activity was calculated by recording the absorbance against the different concentration of plant extract as shown in table 2. The result showed that plant extract was found potent α -amylase enzyme inhibitor as that of standard acarbose. The absorbance values of plant extract of different concentration are denoted as, Abs_1 = Absorbance of the incubated mixture containing plant extract, starch and α amylase, Abs_2 = Absorbance of the incubated mixture containing plant extract and starch, Abs_3 = Absorbance of incubated mixture starch and enzyme = 0.120 and Abs_4 = Absorbance of incubated solution containing starch only = 1.280.

Table 2: Concentration of plant extract and percent inhibition of α amylase

Concentration $\mu\text{g/ml}$	Plant extract + enzyme + starch Abs1	Plant extract + starch Abs2	% inhibition
40	0.517	0.817	74.13
80	0.547	0.797	78.44
160	0.590	0.771	84.34
320	0.663	0.754	92.15
640	0.650	0.688	96.72
1000	0.677	0.684	99.39

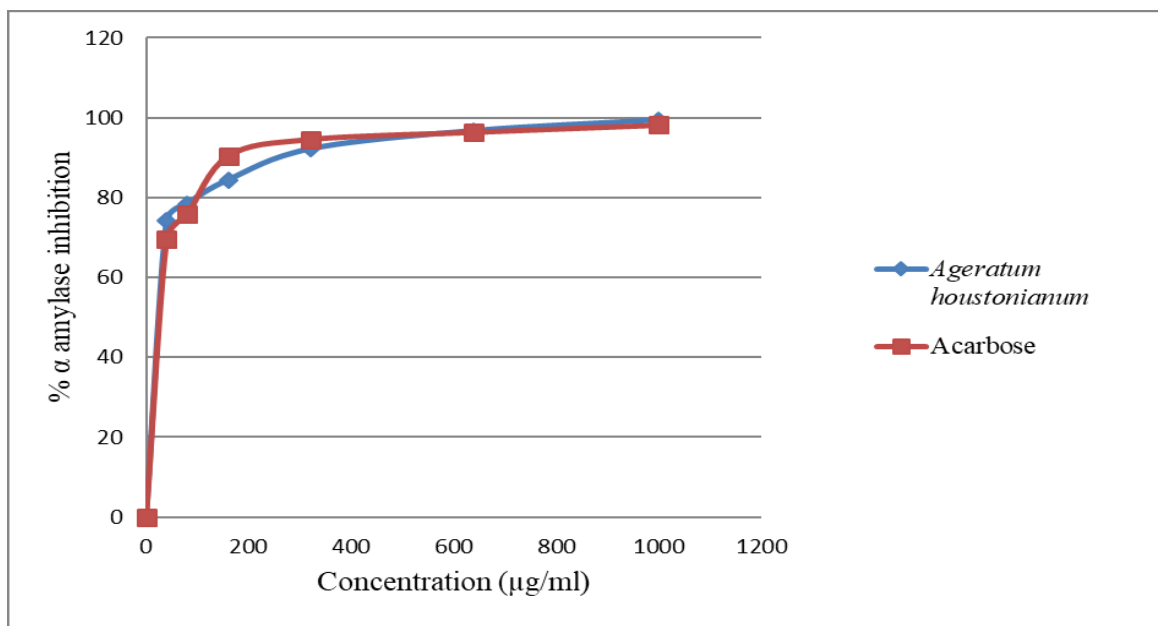


Fig1: Inhibition of α amylase activity by plant extract and standard acarbose

Diet rich in carbohydrate causes a sharp rise in the blood glucose level as the complex carbohydrate in food is rapidly absorbed in the intestine aided by the α -amylase and α -glucosidase enzyme which break carbohydrate to simple absorbable monosaccharides (Kwon et al., 2007). Inhibitors of saccharide hydrolyzing enzymes α -amylase and α -glucosidase have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type II diabetes mellitus (Gin et al., 2000). α -amylase inhibition activity of plant extract was determined using quantitative starch-iodine method. The result of α -amylase inhibition activity is shown in figure 1. The result showed the plant extract have the similar enzyme inhibition activity as that of standard drug acarbose. Hence, the

plant extract is the source of a potential α amylase enzyme inhibitory agent.

The result showed a dose-dependent increase in percentage inhibitory activity of α -amylase by the plant extract. Reddy et al., (2010) have analyzed the α -amylase inhibition activity by oral administration of ethanolic extract of *A. precatorius*. The serum glucose value of 131.16 ± 1.939 mg/dl was found whereas standard glibenclamide showed the value of 96.5 ± 1.607 mg/dl. This result showed few similarities to the result reported by a previous researcher. Many researchers had reported the phytotoxic activity exhibited by the medicinal plant extracts. Ali et al., (2009) has carried out the phytotoxic activity of the chloroform, ethyl acetate and n-butanol root extracts of *Euphorbia wallichii* and reported good phytotoxic (60-100%) effect at dose of 1000 μ g/ml. Atta-Ur-Rehman et al., (1991) and Malla et al., (2014) also reported similar phytotoxic potential of the various plants like *Chrozophora tinctoria*, *Fagonia Ricinus communis*, *Tribulus terrestris* and *Peganum harmala* showed phytotoxic activity at all the dilutions. Hence, here the phytotoxic and α -amylase inhibitory activity of the plant extract might be due to the presence of analogous phytoconstituents which were marked during phytochemical analysis.

Antioxidant activity

The result of DPPH radical scavenging activity is shown in fig 2 as compared to the standard antioxidant ascorbic acid. The antioxidant activity of *Ageratum houstonianum* extract subjected for the evaluation of antioxidant activity by DPPH radical scavenging assay was observed mild antioxidant activity with IC_{50} 123.67 μ g/ml.

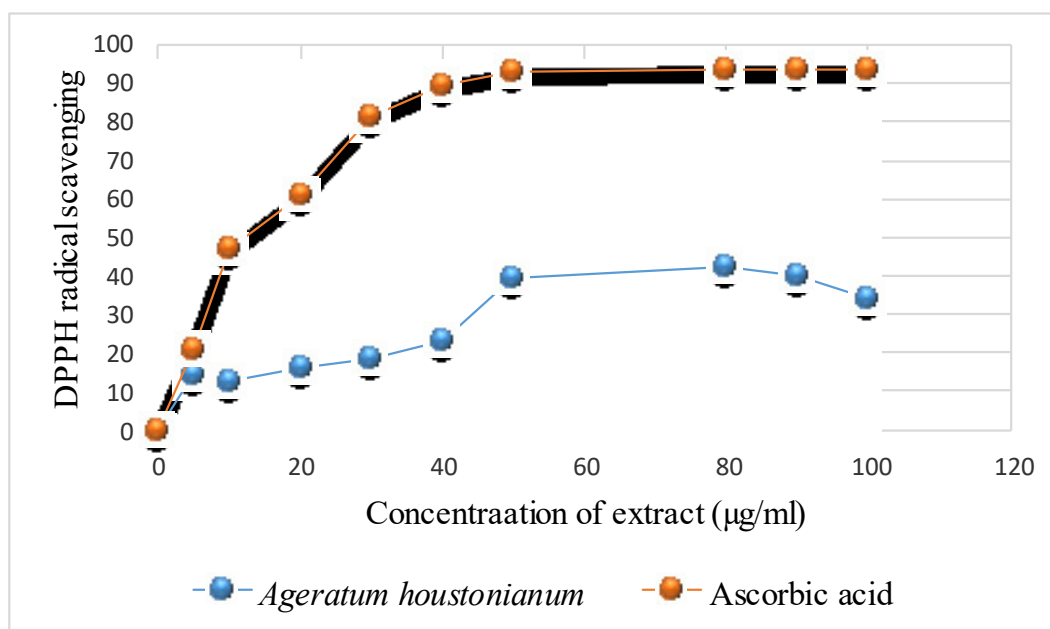


Fig 2: DPPH scavenging effect vs. concentration of *Ageratum houstonianum* extract and ascorbic acid

The antioxidant potential of plant extract was compared with the previously reported results and found mild antioxidant as compared to *L. camara* leaves revealed the best antioxidant properties with IC_{50} 16.02 μ g/ml. Similarly, the result compared to the antioxidant values of leaf, flower, stem, root and fruit extracts of *L. camara* of IC_{50} 16.02 \pm 0.94, 28.92 \pm 0.19, 46.96 \pm 2.51, 31.52 \pm 0.74 and 90.11 \pm 0.57 μ g/ml respectively (Pour et al., 2012). The previously reported result revealed that the extract of *Ageratum houstonianum* showed weak antioxidant potential. Antioxidants are tremendously important substances which possess the ability to protect the cell from oxidative damage caused by free radical induced oxidative stress. The antioxidant potential of *Ageratum houstonianum* methanol extracts was investigated in the search for new bioactive compounds from natural resources.

CONCLUSIONS

In summary, the present study firstly depicts the mild potential of the plant extract of *Ageratum houstonianum* on antioxidant, phytotoxic and α -amylase inhibition activity, which indicates that the plant might be considered as a source of antioxidant and alpha-amylase enzyme inhibition agent. This study provides some scientific support for the traditional use of *Ageratum houstonianum* in the treatment of diabetes and other ailments. But, further experiment is needed to find out antioxidant and antidiabetic activity under *in-vivo* conditions. The methanolic

extract of *Ageratum houstonianum* showed a significant effect of *Limna minor* fronds inhibition in the phytotoxic bioassay. Although the α -amylase inhibition and phytotoxic activity have studied, it is recommended that the activity guided isolation, purification, identification and quantification of the identified bioactive compounds are still needed.

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